ANTI-INFLAMMATORY RESPONSES OF BIOAVAILABLE SELENIUM FROM SELENIUM-RICH CEREAL GRAINS

A thesis submitted in fulfillment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

in

Department of Biotechnology

by

Noorpreet Inder Kaur Dhanjal
(Regd No. 901200008)

THAPAR INSTITUTE
OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

Patiala – 147004, India
January 2018

THAPAR INSTITUTE OF ENGINEERING & TECHNOLOGY

(Deemed to be University)

CERTIFICATE

It is hereby certified that the thesis **"ANTI-INFLAMMATORY RESPONSES OF BIOAVAILABLE SELENIUM FROM SELENIUM-RICH CEREAL GRAINS"** which is submitted by **Ms. Noorpreet Inder Kaur Dhanjal (Regd. No. 901200008),** in fulfillment of the requirement for the award of the degree of ***Doctor of Philosophy*** in the Department of Biotechnology, Thapar University, Patiala, India, is a record of the candidate's own independent and original research work carried out by her under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree in India or abroad.

Dr. N. Tejo Prakash
Supervisor
Professor
School of Energy & Environment
Thapar University
Patiala – 147004
India

Dr. Siddharth Sharma
Supervisor
Assistant Professor
Department of Biotechnology
Thapar University
Patiala – 147004
India

THAPAR INSTITUTE OF ENGINEERING & TECHNOLOGY

(Deemed to be University)

DECLARATION

I hereby declare that the work which is being presented in the thesis **"ANTI-INFLAMMATORY RESPONSES OF BIOAVAILABLE SELENIUM FROM SELENIUM-RICH CEREAL GRAINS"** submitted by me for the award of the degree of ***Doctor of Philosophy*** in the Department of Biotechnology, Thapar University, Patiala, India, is true and original record of my own independent and original research work carried out under the supervision of **Dr. N. Tejo Prakash** (School of Energy and Environment, Thapar University, Patiala, India) and **Dr. Siddharth Sharma** (Department of Biotechnology, Thapar University, Patiala, India). The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree in India or abroad.

Date: 05/02/2018
Place: Patiala

Noorpreet Inder Kaur Dhanjal

Noorpreet Inder Kaur Dhanjal

Acknowledgment

At the very onset and above all I must bow with all the humility before the divine power who has bestowed me with the requisite intelligence and health to carry out the stupendous task of investigation. I also thank him for bringing in contact with learned team of guides as well as loving and caring friends all of whom have contributed to my success.

I feel privileged to express my dense sense of gratitude, indebtedness to my respected and worthy supervisors ***Dr. N. Tejo Prakash and Dr. Siddharth Sharma*** *for their valuable guidance, keen interest, constant and vital encouragement, constructive criticism, patronage and dedication for research rendered to me during my work as well as in preparation of manuscript, which led me with perfection to achieve my goal. A special thanks to* ***Dr. Ranjana Prakash,*** *School of Chemistry and Biochemsitry, Thapar University and* ***Dr. K.S. Prabhu****, Professor, Department of Veterinary and Biomedical Sciences, Pennsylvania State University, USA, for valuable suggestions throughout the study. I extend my heartfelt thanks to them forever throughout my life.*

I would like to thank the rest of my doctoral committee, ***Dr. Moushumi Ghosh (****Professor and Head, DBT, TU),* ***Dr. Sanjai Saxena (****Professor, DBT, TU) and* ***Dr. S.K. Pandey****, (Assistant Professor and Head, SCBC, TU), for their encouragement, insightful comments, and relevant mid-course suggestion.*

My warm thanks are due to all faculty members of Department of Biotechnology and School of Energy and Environment, TU, Patiala, for their meticulous guidance, keen interest, invaluable suggestion and constant support during the course of my study. The personal and professional guidance that I received from them would be cherished lifelong.

I express my regards and grateful to my seniors ***Dr. Poonam Bhatia, Dr. Sumit Jaiswal, Dr. Avdhesh Kumar Gangwar*** *and my lab mates* ***Mr. Anirudh sharma*** *and* ***Ms. Rachana Pandey*** *for providing keen interest, unfailing support, inspiration, critical observations and ingenuous suggestions for my research work.*

I would like this opportunity to express my heartfelt thanks to my friends, ***Dr. Vineet Meshram, Dr. Neha Kapoor, Mr. Vagish Dwibedi, Ms. Parveer, Ms. Poornima, Ms. Jaishnu, Ms. Himadari, Ms. Palak, Mr. Rahil and Ms. Charu*** *for their invaluable smiles and friendship during the course, which has motivated and encouraged me for my research work. I also thank those who could not find a separate name but helped me directly or indirectly.*

For all the sacrifices that my dear mother ***Parminder Kaur Dhanjal*** *so selflessly made, words at my command are inadequate in form and spirit to convey the depth of feelings towards them for showering their blessings, flourishing, inspiration and support which brought me up to this level. Words are too less to express my love towards my husband* ***Satyajit Singh,*** *brother* ***Chanpreet Inder Singh*** *and sweet sister-in-law* ***Balraj Kaur*** *for their loving affection and encouraging support throughout.*

Date:
Place: Patiala ***Noorpreet Inder Kaur Dhanjal***

§ Dedicated To My Father,

Late Er. Preet Mohinder Singh Dhanjal§

TABLE OF CONTENTS

List of Figures

List of Tables

1.0 Introduction

Selenium (Se) was discovered by Jons Jakob Berzelius in 1817, a year which has initiated an immense research on its medicinal applications. It is a rare element on earth crust with atomic number 34 and atomic mass of 78.96. It belongs to group VIA of periodic table, located between sulfur and tellurium. It resembles with sulfur properties and follows the trans-sulfuration pathways (Birringer et al., 2002). It is a metalloid (Wilber, 1993) which possesses both metallic and nonmetallic properties (Wilber, 1993), with four states, i.e., +6 (selenate), +4 (selenite), 0 (elemental) and -2 (selenide) (Marian, 1984). Most of the inorganic species of Se contains selenium at the selenite and selenate states and the organic species are encountered by selenide state (H_2Se).

The global Se distribution varies significantly throughout the world, dividing the regions into seleniferous and non-seleniferous (McNeal and Balistrieri, 1989). Se deficient (SeDef) countries such as Africa, Australia, China and Finland have extremely low Se levels in soils with only 0.09 µg/g dry weight (Alfthan et al., 2000; Judson and Reuter, 1998; Koivistoinen and Huttunen, 1986). Plants and cereal crops grown on these soils do not provide sufficient Se intake in food meals (Fairweather-Tait et al., 2011). Thus, Se deficiency leads to numerous health diseases such as Keshan Disease (Chen, 2012), Kashin Beck Disease (Jirong et al., 2012), pancreatitis (McCloy, 1998; Kuklinski et al., 1991), arthritis (Peretz et al., 2009), asthma (Norton and Hoffmann, 2012), muscular dystrophy (Kurihara et al., 1990), osteoarthritis (Kurz et al., 2002) and many other diseases which due to long exposure, ultimately give rise to systemic inflammatory syndrome (Angstwurm et al., 1999).

In contrast, the extensive research and agri-extension activity of last two to three decades at Nawanshahr-Hoshiarpur districts of Punjab, India, found significantly highest Se concentrations in soil and cereal crops when compared with global averages (Dhanjal et al., 2016; Jaiswal et al., 2015). Soil of this region has Se levels as high as 10 μg/g (Dhillon and Dhillon, 1991). These soils can produce naturally fortified food crops without any artificial addition of Se fertilizers, containing 90% of organic Se (mainly SeMet), which is least toxic and have long term storage capacity in human body. Se content in cereal grains grown at said region was reported as highest as 670 μg/g (Dhanjal et al., 2016; Jaiswal et al., 2012a; Jaiswal et al., 2012b; Aureli et al 2012; Sharma et al., 2009). High Se content in these cereal grains influence the whole elemental profile with elevated mineral composition in comparison to non-Se crops (Boldrin et al., 2013). Se fortified crops have improved yield and nutrient quality of essential micro and macro elements (Souza et al., 2014).

The recommended daily Se allowance values of Se intake in adults is from 40 to 55 μg/g (Combs, 1988). The fraction of this intake available for absorption through intestinal lining is known as bioavailable fraction (Stahl et al., 2002) and the Se fraction that enters the blood circulatory pathway is known as bioaccessible fraction (Suzuki et al., 1998). Through blood plasma, when the absorbed Se enters hepatic portal system for selenoprotein synthesis is known as bioactive fraction (Van-Campen and Glahn, 1999). The bioactivity of Se is determined by its bioaccessibility after *in vitro* gastrointestinal digestion of seleniferous whole grains and its protein fractions. It quantifies the degree of Se absorption in ex-vivo conditions, which is a powerful screening technique to confirm the healthy Se intake as compare to normal Se intake. Cereal grains have higher Se bioavailability than from animal foodstuffs (Combs, 1988). The Se availability from dietary food (mainly as SeMet) is further recommendable over inorganic Se intake (Thiry et al., 2012). G/GI

fluids oxidize the SeMet into SeOMet forms, which have better bioaccessibility to conduct Se-dependent metabolic functions in body (Kapolna and Fodor, 2009).

These selenoproteins are metabolically active Se markers (Mugesh et al., 2001) present in body with strong antioxidant properties (Johansson et al., 2005). In our body more than 25 different selenoproteins are present (Gromer et al., 2005) including GPx, TRx, DIO, SelH, SelK, SelM, SelN, SelP, SelR, SelS, SelT, SelV, and SelW (Gandhi et al., 2013). These proteins are function and location specific with some mainly involved in suppression of the oxidative damage produced by free radicals. However, among all these selenoproteins, GPx maintains the over-all mammalian antioxidant network against RONS to mitigate the inflammation by down-regulation of pro-inflammatory enzymes such as COX-2, iNOS and mPGES-1 along with up-regulation of anti-inflammatory enzyme, H-PGDS. H-PGDS further catalyzes the synthesis of anti-inflammatory D an J series of prostanoids (Ricciotti and Fitzgerald, 2011). Thus, GPx regulates the switching-on process of "eicosanoid class mechanism" (Duntas, 2009; Vunta et al., 2007; Yui et al., 2015).

The Se levels in blood plasma accounts for the degree of up-regulation of selenoenzyme-gene expression in body (MacPherson et al., 1997). The supra-nutritional Se-supplementation increase the GPx activity upto 16-18 times within hours (Sun et al., 1998). In addition, the supra-nutritional intakes execute the anti-tumorogenesis properties (Sabichi et al., 2006), antibacterial and antiviral functions (Smith et al 2011), antistroke properties (Tapiero et al., 2003), anti-oxidant stress buster (Semchyshyn and Lushchak, 2012) and suppress inflammatory process via suppression of pro-inflammatory gene expressions (Vunta et al., 2008).

At physiological concentration, the free radicals (RONS) acts as secondary messengers in response to the external/internal stimuli. However, the persistent generation of these radicals oxidize the

cellular material which results in malfunctioning of proteins, enzymatic activities and DNA damage leading to cancerous stage or cell death (Kohen and Niska, 2002). The physical, chemical or thermal stress, trauma or auto-antibody generated immune response results in the primary infections-protective response of body i.e., inflammation. The inflammatory site start signaling its cardinal signs such as calor (heat), dolor (pain), robor (redness), tumor (swelling) and functio laesa (loss of function). These signs switch-on the inflammatory pathways with activation of cyclooxigenase mediators (COXs) and oxidant generating enzymes such as iNOS. The inducible enzyme complexes such as mPGES-1-COX-2 and iNOS-COX-2 are the key underlying basis of inflammation, the overproduction of which results in various cancer types such as breast cancer (Jana et al., 2014), colorectal cancer (Roelofs et al., 2014), lung cancer (Sandler and Dubinett, 2004), prostate cancer (Fenner, 2016), skin cancer (Zhu et al., 2015) and so on.

For timely resolution, macrophages play vital role in prevention of acute to chronic condition. These macrophages switch-on the arachidonic acid (AA) pathway to regulate the expression of pro-/anti-inflammatory genes (Vunta et al., 2008). Arachidonic acid is a 20-carbon fatty acid chain, which rearrange into multi-enzyme complexes called pro-/anti-inflammatory eicosanoids. There are four principle bioactive eicosanoids including PGD_2, PGE_2, PGI_2 and PGF2α, which are ubiquitously produced under homeostatic conditions. However, the lipopolysaccharide (LPS) induced chronic inflammation increase the expression of inducible COX-2 enzyme, which in turn activate the key product of AA pathway i.e., PGH_2. Depending upon the state of inflammation, PGH_2 differentiate the pathway into pro-inflammatory PGs (PGE_2 and TXA_2) and anti-inflammatory PGs (PGD_2 and their downstream products Δ^{12}-PGJ_2 and 15d-PGJ_2). PGD_2 activates PPARγ (Kapoor et al., 2007) dependent M_2-specific alternate macrophages anti-inflammatory response to resolve inflammation (Nelson et al., 2011; Gandhi et al., 2011).

The anti-inflammatory genes suppress the activation of key regulator of sepsis i.e., NFκB (Poli et al., 2004). In Se and GPx deficient conditions, prolonged NFκB activation results in neuro-inflammation by promoting the pro-inflammatory state (Lubos et al., 2011). NFκB, COX-2, iNOS, TNFα have inverse relationship with Se supplementation (Prabhu et al., 2002). Se molecules quench the acylation process of histone H4 at K12 and K16 position of COX-2, NFκB and TNFα promoters for down-regulation of inflammatory gene expression (Narayan et al., 2015). Therefore, Se supplementation is necessary to protect the cells from systemic manifestation of RONS. Se supplementation in the form of Se-rich natural dietary matrices facilitate the slow and steady release of Se through SeMet enzymatic-cleavage with better anti-oxidant and anti-inflammatory capacity in comparison to the inorganic Se-sources (Cao et al., 2014).

Numerous studies had been carried out to down-regulate the LPS stimulated COX-2, m-PGES-1 and iNOS expression with inorganic sodium selenite supplementation. However, the application of Se in the form of naturally grown seleniferous cereal grains has not been explored till present date to the best of our knowledge. Therefore, keeping this in view, the work presented here has been carried out to understand the growing importance of the natural source of bioavailable Se in edible crops as Se-neutraceuticals among SeDef population.

2.0 Review of Literature

Selenium ranks at 69th position, among its abundance in natural existence, ranging from 0.1 to 2.0 µg Se/g in soil (Swaine, 1955). The oxidised states i.e., selenite and selenate are present in well-aerated, humid and alkaline soils (Goh and Lim, 2004). Whereas, the elemental (Se^0) and reduced state (Se^{2-}) are characteristically present in poorly aerated and acidic soils (Sharma and Singh, 1983). Sedimentary (Tamari et al., 1990) and igneous rocks (Charter et al., 1995; Fordyce, 2005) contributes up to 0.08 µg/g to 1.0 µg/g Se to the soil. The formation of these igneous and phosphatic rocks during volcanic eruptions, add-on about 10% of total selenium in environment (Andren et al., 1975; Suzuki, 1965), while in limestone and granite rocks, the Se concentration is very low (Van Metre and Callan, 2001). However, the cretaceous shales and pyrites (Masscheleyn et al., 1991; Neal and Sposito, 1989) contain high Se levels up to 4.5 µg/g (Lakin, 1972). These rocks are the primary source of Se contribution in natural soil (Fleming, 1980; Christophersen et al., 1995). In addition to these rocks, about 60-80% of water bodies also contribute Se to the environment, even in remote areas like Antarctica (Zoller et al., 1974) and ice sheets of Greenland (Weiss et al., 1971), out of which 30-50 % is dimethylselenium (Doran and Alexander, 1977).

The availability of Se in nature, its utilization by livestock, ultimately depends upon the diversity of Se-species present in soil and its uptake by plants. In biological systems, it exists as inorganic and organo-selenium molecules such as selenoproteins, Se-amino acids: selenocysteine (SeCys) and selenomethionine (SeMet), low molecular selenosugars and other methylated Se-species (Table 2.1).

Table 2.1: The most common Se-species found in environmental and biological systems (Aureli et al., 2012; Huang et al., 2012; Behne and Kyriakopoulos, 2001; Dedina and Tsalev, 1995).

Type	Se-species
Inorganic	Selenate, Selenite, Elemental, Selenide
Organic	
Methylated species	Methylselenol (MeSeH), Dimethyl selenide (Me_2Se), Dimethyl diselenide (Me_2Se_2), Trimethylselenonium (Me_3Se^+), Dimethyl selenone (Me_2SeO_2), Dimethyl selenoxide (Me_2SeO), Selenourea ($Se=C(NH_2)_2$)
Amino acids	Selenomethionine ($C_5H_{11}NO_2Se$), Selenocysteine ($C_3H_7NO_2Se$), Se-methylselenocysteine ($C_4H_9NO_2Se$), Selenocysteic acid ($C_3H_7NO_5Se$)
Low molecular mass species	Se-methylselenomethionine ($C_6H_{14}NO_2Se$), Selenomethionine selenoxide ($C_5H_{11}NO_3Se$), Selenoniocholine ($C_5H_{11}O_2Se^+$), Selenobetaine ($C_4H_9O_2Se^+$), 2,3-dihydroxypropionyl-selenolanthionine ($C_{10}H_{18}N_2O_6Se$)
Other Se-compounds	Selenoosugars (Se containing monosaccharides and disaccharides), Selenoproteins (section 2.4.1), Se-metal metallothioneins (Se-Hg, Se-Zn, Se-Fe, etc.)

In India, Nawanshahr-Hoshiarpur districts of Punjab regions, the parent seleniferous material of soil is derived from Upper Shiwalik rocks (Dhillon and Dhillon, 2003) into run-off water through hills of Shiwalik range, significantly increase the Se levels of irrigation water (Bajaj et al., 2011), which get adsorbed on soil particles of low-lying areas of affected region (Dhillon and Dhillon, 1991). The soil irrigation with this water source can potentially convert normal area into seleniferous area. The extensive research and agri-extension activity at this site have resulted in Se levels in soils to as high as 10 μg/g (Dhillon and Dhillon, 1991; Dhillon et al., 1992; Dhillon and Dhillon, 2003a). Dhillon and Dhillon (1997) observed that the affected villages of this area were Barwa, Bhano Majra, Jainpur, Mahenpur, Nazarpur, Rakker, Sikandarpur and Simbli. Other

high Se-contaminated states of India includes Assam and Meghalata (Dey et al., 1999), West Bengal (Gosh et al., 1993), Haryana (Sharma and Singh, 1983; Arora et al., 1975). Sharma et al. (2009) reported Se concentration in soils of certain areas of Punjab ranges from 2.7-6.5 μg/g. In other countries like, the San-Joaquin valley of California, U.S.A. and Huleh valley of Israel, were also reported to accumulate high Se in soil samples up to 6 μg/g (Rosenfeld and Beath, 1964). The soluble and available fraction of Se in water was observed to be 30% of total Se present on these soil particles (Ravikovitch and Margolin, 1957). In contrast, the Se-deficient areas like Australia, Africa, China, Finland and others, the Se concentration in soil samples were observed only up to 0.09 μg/g (Alfthan et al., 2000; Judson and Reuter, 1998; Koivistoinen and Huttunen, 1986). Therefore, the Se distribution throughout the world varies from region to region dividing the global soils into seleniferous and non-seleniferous (McNeal and Balistrieri, 1989).

In earlier times, when the application of this important metalloid was not known, the highly Se-affected areas of Western U.S. (Rosenfield and Beath, 1964) were considered to be significantly contaminated with an unknown toxic element causing poisoning effect to the human health (Alarcon and Martinez, 2000). In those days, it was known as alkali disease, named after "more alkalinity soil concept" (Sharma and Singh, 1983). Later it was recognized as "selenosis" due to high Se content. However, after 156 years of its discovery, the first case study on appropriate doses of Se dietary intake acknowledged Se as the key regulator of antioxidant enzyme glutathione peroxidase (Gromer et al., 2005). In the following years to the present day, Se continues to be a hot topic of research and application due to its nutritional requirements in Se-deficient areas.

2.1 Ingestion, absorption, utilization and excretion of dietary selenium

Se absorption and utilization depends upon the chemical form of ingested selenium. Both organic and inorganic species present in food samples have variable degree of bioavailability (Meltzer et al., 1990; Amoako et al., 2009). The organic forms are more retained (Zeng et al., 2008; Rider, 2009) and less toxic than inorganic ones (Ammar and Couri, 1981). The Se-retention level of dietary Se in blood or specific organ can be examined through selenoenzymatic activity. The excess levels account to its toxicity or excretion (Whanger and Butler, 1988; Rider et al., 2010). Upon ingestion, any chemical species of Se ultimately gets transformed and stored in organic forms like selenomethionine, selenocysteine and methylated selenocysteine (Dumont et al., 2006).

2.1.1 Selenium in foods and diets

Se concentration in plant depends upon the quantity of Se present in soil and its capacity to accumulate, which segregates them into three categories i.e., non-accumulators, Se-indicators and accumulators (Brown and shrift, 1982; Dhillon and Dhillon, 2003a; Rosenfeld and Beath, 1964). The non-accumulator plants are resistant to Se exposure, as they exhibit toxic symptoms even at concentrations less than 100 μg/g (White et al., 2004). The Se-indicators can tolerate concentration up to 100 μg/g (Rodriguez et al., 2005), whereas the Se-accumulators can absorb significantly higher Se-concentrations above 1000 μg/g (Mayland et al., 1989). Among these Se-accumulators, allium species accumulate significantly high Se concentration from soils in non-toxic, methylselenocysteine and γ-glutamyl methylselenocysteine forms (Huerta et al., 2006; Ogra et al., 2004; Wrobel et al., 2004). Crops like wheat, maize, rice, mustard and their residues grown on Se-rich soils can accumulate significant concentrations up to 670 μg Se/g dry weight (Dhanjal et al., 2016; Jaiswal et al., 2012a; Jaiswal et al., 2012b; Sharma et al., 2009). In these grains, the most abundant and non-toxic Se-form present is selenomethionine (Cubadda et al., 2010; Guzman et al.,

2009; Stadlober et al., 2001), with efficient retention capacity (Behne et al., 2009; Levander et al., 1983; Meltzer et al., 1993).

Se deprived populations are necessary to have Se-rich diets either through intake of Se-rich edible crop products or through artificially Se-fortified food products or via direct ingestion of inorganic Se tablets available in markets to elevate the selenium levels in their bodies. The Se-fortification technique in plants grown with seleniferous manure addition in soils have been adopted in Se-replete areas, like Finland, to raise the ingestion levels of Se-food products from 25 μg/day to 110 μg/day per person (Eurola et al., 1990). This step was expected to enhance the concentration of Se in blood plasma (Varo et al., 1988; Rayman, 2008). Similarly, many other countries has adopted cost effective and zero toxic method of culturing and incubating *Saccharomycese cerevisiae* (Baker's yeast) with sodium selenite, which biologically metabolizes this inorganic form into organic forms, mainly as SeMet (Ip et al**.,** 2000; Moreno, 2004; Reyes et al., 2006). These seleniferous yeast tablets available in markets are the rich source of Se as revealed by the ICP-MS and ESI-MS Se-detectors indicating the presence of other 49 Se-species (organic and inorganic) in addition to SeMet (Preud'homme et al., 2012). Rider and his coworkers (2010) observed that the bio-absorptive capacity of Se from Se-yeast is much more than the inorganic form (selenite). Therefore, these Se-fortified products provide variable amount of both organic and inorganic sources (Lobinski et al., 2000; Dumont et al., 2006). Reilly (1996) analyzed the Se richness or poorness from these consumed food products like cereal grains (0.01-0.6 μg/g), broccoli (0.1-0.5 μg/g), Brazil nuts (0.5-0.9 μg/g) and mushrooms (0.01-1.4 μg/g fresh weight). About 70 to 90% of Se requirement from these dietary products ingested are bioavailable to carry out metabolic functions in body (Finley, 2006).

In evaluating Se intake levels in Se-rich and Se-deficient populations all over the world, different countries have set variable recommended Se-dietary allowance (RDA) values (Table 2.2). The common range of RDA value in adults is from 40 μg Se intake per day to 55 μg/day (Combs, 2001). However, the intake of 200 μg Se/day was observed to have superanutritional effects in cancer prevention (Linda et al., 2005) and 400 μg Se/day being considered as upper tolerable limit (Hurst et al., 2013), above which proves toxic to the health (Hira et al., 2003).

Table 2.2: Recommended dietary allowance (RDA) values of selenium intake in different countries to benefit human health.

Country	Se-RDA values (μg/day)	Reference
Belgium	60-70	Superior Health Council (SHC, 2009)
Europe	55	Scientific Committee in Food (SCF, 2000)
France	53.7	Scientific Committee in Food (AFSSA, 2009)
India	26-36	World Health Organization (WHO, 2004)
United kingdom	48-58	Rose et al., 2010
U.S.A.	55	US Department of Health and Human Services and US Department of Agriculture (USDA, 2015)

2.1.2 Absorption, transport and distribution of dietary selenium

The degree of Se-absorption primarily depends upon the (a) ingested chemical Se-form i.e., either organic or inorganic; and (b) the age, sex and life style of living being (Thompson, 2004). Secondly, other factors like competence with -SH groups (Waschulewski and Sunde, 1988), lipid content in dietary intake (Mutaten and Mykkanen, 1984), presence of heavy metals (Ralston et al., 2008; Reeves et al., 2007) reduce Se absorption capacity in body. Stahl et al. (2002) reported that of the total Se absorbed in body, 30% retained in liver, 30% in reflex muscles, 15% in kidney, 10% in plasma and rest in other parts like RBC's, spleen, pancreas and skeleton. The major transporter and distributor among selenoproteins is Se-plasma protein (SelP), which along with albumin travels to liver and kidney where it either undergoes Se-protein synthesis (Brandon et al., 2006;

Schumann et al., 1997; Suzuki et al., 2009) or channeled up to utilization under following categorizes:

2.1.2.1 Bioavailable fraction

This fraction accounts for Se available from food matrix for the absorption through intestinal lining (Ruby et al., 1999; Shen et al., 1997; Stahl et al., 2002). Different kind of food products like plants and meat sources have highly variable degree of bioavailability, which determines the response to Se supplementation (Meltzer et al., 1990). The most abundant bioavailable Se-species present in plants is selenomethionine (SeMet), whereas in animal meat sources, it is available as selenocysteine (SeCys) (Yang et al., 1988). In comparison, between plants and inorganic sources, SeMet-rich wheat has more Se-availability with longer retention and lower toxicity in stimulated conditions (Cubadda et al., 2010) as well as *in vivo* systems such as intestinal lumen of Se-rich trouts (Rider et al., 2009; Rider et al., 2010) and lambs (Tiwary et al., 2006), indicating better bioavailability of SeMet in contrast to inorganic Se intake (Whanger and Butler, 1988).

2.1.2.2 Bioaccessible fraction

The Se fraction absorbed through intestinal lumen and entering into circulation pathway through blood plasma, is known as bioaccessible fraction (Suzuki et al., 1998). The *in-vitro* gastrointestinal digestion studies revealed that milled and processed food has more bioaccessibility than raw food (Dhanjal et al., 2016; Reeves et al., 2007). To access the amount of cellular Se-uptake, Se-retention, transportation and the degree of utilization in metabolism of these selenoproteins (Zeng et al., 2008), Caco-2 cell model was mimicked with intestinal lumen under *ex-vivo* conditions by forming a uniform interconnected layer (Simon et al., 2007). The rate of bioaccessibility from 167 μg/g pre-digested seleniferous food matrix was more in case of Se-yeast (144±3 μg/g) followed by selenite (35±1 μg/g) and selenate (30±2 μg/g). This confirms the lower absorption and

bioaccessibility of inorganic species when compare to the organic fractions (Thiry, 2013). Seleniferous broccoli as dietary plant product, in comparison to inorganic selenite/selenate intake, provided better absorption (Zeng et al., 2008) and maximum antioxidant protection in Se-repleted mice (Finley, 1998).

2.1.2.3 Bioactive fraction

The absorbed Se, when enters the hepatic portal system through trans-selenation pathway and transforms into active selenometabolites, is known as bioactive form (Van-campen and Glann, 1999). In trans-selenation pathway, the active form of SeCys donor i.e., phosphorylated Se (Stadtman, 1996; Suzuki, 2005) bonds with dedicated t-RNA to form selenocystyl-t-RNA (Chambers et al., 1986). The product, selenocystyl-t-RNA in the presence of UGA codon (usually a stop codon) (Butler and Whanger, 1989; McConell and Hoffman, 1972) and a stem loop like structure called SECIS (selenocysteine insertion sequence), permits the recognition of UGA codon as SeCys-coding sequence (Stadtman, 1996; Whanger, 2002). It translates into 21st amino acid, selenocysteine (SeCys) (Bock et al., 1991; Suzuki, 2005). These SeCys-subunits acts as bioactive Se-form in the active center of antioxidant enzymes like glutathione peroxidase (GPx) (Latreche et al., 2012), thioredoxin reductases (TRx) and iodothyronine deiodinase (DIO). In turn, the expression of these enzymes are the marker of the bioactive Se present in the cells (Thiry et al., 2012). This fraction has various metabolic properties to conduct antioxidant, anti-inflammatory, anti-cancerous and immuno-stimulant functions (Brandon et al., 2006; Garcia et al., 2009; Schumann et al., 1997). Se-rich Brazilian nuts feeding tend to shoot up the bioactive Se content in liver cells (Chansler et al., 1986), which up regulates the GPx activity (Thompson et al., 2008).

2.1.3 Utilization and excretion

The different chemical forms of bioavailable Se have variable propensities for its utilization in human body. For an example, the bioactive Se-forms like selenite, selenate and SeCys, are the common precursor of H_2Se formation, which directly participates in Se-protein synthesis. On the other hand, SeMet passively (Stadtman, 1996) channelizes into storage form (Behne et al., 2009) in liver (Beilstein and Whanger, 1988; Schrauzer, 2000; Suzuki et al., 2006; Suzuki et al., 2008) or blood and plasma proteins (Burk et al., 2001). SeMet unspecifically imitates sulfur and get incorporated into sulfur containing proteins (Schrauzer, 2000). Thus, SeMet itself is not a selenoprotein, but considered as Se-containing protein (Alarcon and Vique, 2008; Ducros and Favier, 2004). Under Se-deficient conditions, the stored SeMet transforms into methylselenol (Suzuki et al., 2006; Zeng et al., 2008), which further dimethylate into H_2Se to take part in protein synthesis (Combs, 1988; Pedrero and Madrid, 2009; Suzuki et al., 2006).

The hyperaccumulation of Se in plants and animals may cause toxicity, therefore, the excess amount is necessary to get utilized (Suzuki et al., 2006) or excreted out (Vander Torre et al., 1991). In animals and humans, the excess Se is either urinated as trimethylselenonium ions or evaporated through breath as dimethylselenide with garlic odor (Pyrzynska, 1998; Levander, 1972; Suzuki et al., 2006). In plants, the excess form is converted into dimethylselenide ($[CH_3]_2Se$) by microbial and phytomethylation of selenite and selenate species present in soil particles (Kabata and Mukherjee, 2007; Terry et al., 2000).

2.2 Selenium Deficiency

Se is an immuno-stimulant, which in adequate concentrations, provides the maximum antioxidant properties in combination with vitamin A, C and E, β-carotene (Baraboi and Shestakova, 2004), to suppress the excess redox radicals generated in cells. Due to its deficiency in China and eastern parts of Siberia, two overt diseases, Keshan and Kaschin-Beck have been recorded (Reilly, 1996).

Keshan disease mainly occurred in children and women of childbearing age. It involved the cardiac enlargement and arrhythmia, due to coxsackie B virus prevalence along with Se and vitamin E deficiency (Yang et al., 1994; Levander and Beck, 1999; Liu et al., 2002). Kaschin-Beck disease is due to deficiency of Se and vitamin E along with iodine (Neve, 1999). It is a distortive osteoarthritis disease caused due to fulvic acid and mycotoxins present in food, resulting in dwarfism with shortened fingers and swollen joints (Reilly, 1996). The daily Se-intake by these people was less than 10 μg/day (Alarcon and Vique, 2008). Later upon Se-supplementation, the Se-replete China's population had recovered from both the diseases (Fairweather-Trait, 1997; Yang, 2006). Se supplementation in combination to vitamin E synergistically enhance the GPx activity (Thompson, 2004), which prevent necroses in liver cells of rat (Schwarz and Folts, 1957). Se deficiency had been reported to cause several health ailments like acute pancreatitis (Kuklinski et al., 1991), chronic pancreatitis (McCloy, 1998), arthritis (Peretz et al., 2009), asthma (Norton and. Hoffmann, 2012), cystic fibrosis (Kauf et al., 1994), diabetes (Kowluru et al., 2001), kwashiorkor (Ashour et al., 1999), male infertility (Rayman, 2000), muscular distrophy (Kurihara et al., 1990), osteoarthritis (Kurz et al., 2002), rheumatoid arthritis (Rayman, 2000), systemic inflammatory syndrome (Angstwurm et al., 1999).

2.3 Selenium toxicity

Dietary selenium is like a double edged sword which shows impaired performance at accidental deficiency or excess ranges i.e., from 0.1 to 7000 μg Se/day (Fordyce, 2005; Rayman, 2002; Rayman, 2008; WHO, 2004). The higher intakes in China, up to 5000 μg Se/ day (Yang et al., 1983), or lower Se-intakes of less than 50 μg Se/day in European countries (Flynn et al., 2009; Rayman, 2004; Rayman, 2005; Rayman, 2008) showed detrimental health ailments. Toxicity is significantly concentration dependent from nM to μM doses to maintain the selenoenzymatic

activities in the body. Therefore, a very thin line exists to understand the optimal intake of Se to overcome deficiency, provide essential Se and prevent toxicity. Like the deficiency resulting in health deprivation, the Se intake above upper tolerable limit of 400 μg/day can lead to selenosis (FAO/WHO, 2002). In some endemic and non-endemic areas of China, the average Se-intake values had been recorded to be >910 μg/day (Yang et al., 1989). Se toxicity in China (Oldfield, 2006) due to accidental intake of 3200 μg Se/day to 5000 μg Se/day (Combs, 2001; Letavayova et al., 2006; Whanger, 2004) divided selenosis into two categories, i.e., acute and chronic selenosis. The acute selenosis is due to long exposure of low Se concentration resulting in respiratory and gastro-intestinal problems. On the other hand, the chronic selenosis is due to extensive intake of Se above 5000 μg/day (Dhillon and Dhillon, 1991) leads to nail cracking, hair loss (Yang et al., 1983), yellowing of teeth, impaired growth rate, male sterility and poor muscle reflex action (Draize and Beath, 1935). In case of plants, chlorosis was reported in wheat grown on Se contaminated areas of Nawanshahr-Hoshiarpur districts of Punjab, India (Dhillon and Dhillon, 1991). Selenosis has also been reported in human population with intake of Se-rich diet of this region (Hira et al., 2003).

Another factor on which toxicity depends is the ingested form of Se (organic or inorganic) and its bioaccessability in the body. The half-life of inorganic Se in the body is much shorter, since in excess it is not stored and is directly excreted out (Levander et al., 1983). These inorganic forms are accepted to be more toxic and bioactive than the organic ones (Farzaneh, 2015). The excess amount of Se can lead to activate the pro-oxidant actions (Vinceti et al., 2001: Gosetti et al., 2007), thereby, resulting in non-availability of the functional state of Se (H_2Se), leading to reduced antioxidant capacity of selenoenzymes. In this condition, the oxidative stress shoots up in between cells called Se-induced toxicity (Gad and Twab, 2009). In another way, the oxidation of hydrogen

selenide (H_2Se, precursor of Se-protein synthesis) forming O^{2-} and OH^- radicals, which breaks and mutates DNA strands producing RONS (Combs and Gary, 1998). The capacity of selenite to form superoxide ion, is maximum in comparison to selenate (Mezes and Balogh, 2009), whereas, SeMet acts inert in producing free radicals and do not follow the H_2Se formation pathway. The organic forms are more bioaccessible, facilitating availability of Se as SeMet and act as efficient reservoir of Se (Behne et al., 2009). Therefore, oxidation chances of H_2Se is negligible in case of SeMet in comparison to inorganic sources (Brozmanova et al., 2010). Franke and Painter (1938) observed the decreasing toxicity order among inorganic species as selenate and selenite followed by selenide and elemental Se state.

2.4 Biological roles of selenium to benefit human health

2.4.1 Antioxidant functions

Se acts as a cofactor for various antioxidant enzymes present in humans and animals as metabollically active Se-markers (Contempre et al., 1996; Ganther, 1999; Mugesh et al., 2001). Biologically, the incorporation of Se forms into these selenoproteins exhibit the excellent antioxidant properties (Arteel and Sies, 2001; Johansson et al., 2005). The selenoenzymatic family relays upon about 30% of Se present in human body (Mezes and Balog, 2009), out of which more than half maintains the antioxidant functioning (Tapiero et al., 2003). Therefore, the dietary Se-intake has direct relationship in prevention of oxidative damages produced due to free radical generation. At least 25 selenoprotein categories were recognized in human body (Gromer et al., 2005) including glutathione peroxidase (GPx), thioredoxin reductases (TRx), deiodinases, selenoprotein H (SelH), selenoprotein I (SelI), selenoprotein K (SelK), selenoprotein M (SelM), selenoprotein N (SelN), selenoprotein P (SelP), selenoprotein R (SelR, MsrB1), selenoprotein S (SelS, Selenos), selenoprotein T (SelT), selenoprotein V (SelV), selenoprotein W (SelW) and

selenophosphate synthase (SPS2) and others (Gandhi et al., 2013; Huang et al., 2012; Behne and Kyriakopoulos, 2001) (Table 2.3).

The glutathione peroxidase family includes mainly four isoforms according to their location and function. GPx1 (cellular GPx) is the most abundant selenoenzyme presides in liver and erythrocytes (Behne and kyriakopoulos, 2001), indicates the long term storage of Se in body (Arthur, 1994). The upregulation of GPx2 has been effective in suppressing the inflammation-associated with gastrointestinal lining upon Se supplementation (Susanne et al., 2012). GPx3 (extracellular or plasma GPx) is the second most abundant selenoprotein after selenoprotein P, present in plasma. Its activity is found to influence systemic inflammatory response syndrome (SIRS) of ICU patients (William et al., 2009). GPx4 (phospholipid GPx) has been mostly found in cytosol and mitochondria, helps in reduction of fatty acid hydroperoxides that are esterified to phospholipids (Tapiero et al., 2003).

The other class of antioxidant enzyme is thioredoxin reductases (TRx) in which Se acts as an active immunomodulator in regulation of thyroid hormone biosynthesis (Allan et al., 1999; Beckett and Arthur, 2005; Kohrle, 1999). Among the three main TRx isoforms, TRx1 (cytosolic) is the most abundant. TRx2 is mitochondria related enzyme and TRx3 is specific to testis (Gromer et al., 2005). With increase in Se supplementation, the synthesis of thyroid hormone (T3) and thyroxine (T4) increases and vice-verse (Beckett et al., 1987). Another Se dependent antioxidant class is iodothyronine deiodinases, which in presence of adequate amount of Se prevents the risk of goitre (Vanderpas et al., 1993).

Table 2.3: List of selenoproteins with their specific distribution and function (Gandhi et al., 2013; Huang et al., 2012).

Selenoprotein	Localization	Significance and function	Reference
Glutathione peroxidase 1	Cytoplasm (Negro, 2008; Lei et al., 1998)	First line of defence against ingested pro-oxidants or gut microbes	Reeves and Hoffmann, 2009
		Respond to oxidative stress, inflammation	Crack et al., 2001
Glutathione peroxidase 2	Gastrointestinal (Negro, 2008; Chu et al., 1999)	Inhibiting oxidative stress by COX-2 downregulation	Banning et al., 2008
		Quench gastrointestinal inflammation and cancer development	Papp et al., 2007; Yan and Chen, 2006
		Reduce inflammation	Krehl et al.,2012
Glutathione peroxidase 3	Plasma (Negro, 2008; Lei et al., 1998)	Local extracellular antioxidant, constitute 20% of total Se in body	Koyama et al., 1999
		Supress prostate cancer	Yu et al., 2007
		Supress oxidative stress	Lee et al., 2008;
		Biomarker of systemic inflammation	Manzanares et al., 2009
		Upregulate the antioxidant capacity of PPARγ	Chung et al., 2008
		GPx-3 deficient cells promote thrombosis	Jin et al., 2011
		Cardioprotective role by reducing nitric oxide (NO) levels	Kenet et al., 1999
Glutathione peroxidase 4	Plasma membrane (Negro, 2008; Lei et al., 1998)	A phospholipid hydroperoxide in membranes	Conrad et al.,2007
		Necessary for lipooxygenase metabolism	Seiler et al., 2008; Villette et al., 2002
		Essential for male fertility	Schneider et al., 2009; Papp et al., 2007
		Sensor of oxidative stress, gives pro-apoptotic signals	Seiler et al., 2008; Ran et al., 2004
Thioredoxin reductase type I	Cytoplasm and nucleus (Calvo et al., 2013)	Regulate cell signaling, growth and proliferation	Jakupoglu et al., 2005; Rhee et al., 2005; Rundlof and Arner, 2004
		Metabolize Se-compounds like SeO_3^{2-}, SeCys, SeMet and ebselen in trans-selenation pathway.	Gromer and Gross, 2002; Zhao et al., 2002; Bjornstedt et al., 1992; Kumar et al., 1992
		Regulate NFκB activation	Sakurai et al., 2003
		Supress breast and lung cancer	Cadenas et al., 2010; Yoo et al., 2006

Thioredoxin reductase type II	Mitochondria (Choi et al., 2002)	Mitochondrial antioxidant, mutation can cause glucocorticoid deficiency Protect heart and skeletal muscles from ROS stress	Prasad et al., 2014; Nalvarte et al., 2004 Wellman et al., 2013; Stanley et al., 2011
Thioredoxin reductase type III	Testis (Vizuete et al., 2004)	Marker of spermatogenesis It is mammalian testis specific thioredoxin hormone	Jimenez et al., 2004 Vizuete et al., 2004
Deiodinase type I	Plasma membrane (Lu and Holmgren, 2008)	For systemic active thyroid levels Regulates pro-inflammatory cytokine production	Tien et al., 2007; Zavacki et al., 2005; Solis et al., 2004 Baur et al., 2000
Deiodinase type II	Endoplasmic reticulum (ER) (Lu and Holmgren, 2008)	For local active thyroid levels Deficiency can cause mental retardation in iodine deficient area Deficiency results in hearing loss and retarded cochlear development	Tu et al., 1997 Guo et al., 2004 Richard et al., 1998
Deiodinase type III	Plasma membrane (Lu and Holmgren, 2008)	Hypothyrodism, inactivate thyroid hormone	Hernandez et al., 2006; Huang et al., 2000
Selenoprotein H	Nucleus (Hatfield et al., 2014; Pillai et al., 2014; Novoselov et al., 2007)	DNA binding protein, regulates transcription Mediates antioxidant function through GPx synthesis and phase II detoxification. Reduce inflammatory response Control ER stress	Pillai et al., 2014 Panee et al., 2007 Curran et al., 2005 Gao et al., 2006
Selenoprotein I	Plasma membrane (Horibata and Hirabayashi, 2007)	Mammalian form of ethanolamine phosphotransferase I, involved in phospholipid synthesis	Horibata and Hirabayashi, 2007
Selenoprotein K	ER and plasma membrane (Verma et al., 2011; Lu et al., 2006)	Reduce the risk of prostate cancer Regulates Ca^{2+} flux Protect heart muscles by supressing ROS generation Supervise ER homeostasis	Meplan et al., 2012 Verma et al., 2011 Lu et al., 2006 Shchedrina et al., 2011; Du et al., 2010
Selenoprotein M	ER (Reeves et al., 2010)	Neuroprotective function and Ca^{2+} regulation SelM deletion leads to obesity Redox signaling to supervise peroxidase activiy	Reeves et al., 2010 Pitts et al., 2013 Triana et al., 2010
Selenoprotein N	ER (Chung et al., 2009; Petit et al., 2003)	Ca^{2+} homeostasis Redox signaling	Jurynec et al., 2008 Arbogast and Ferreiro, 2010

		Deficiency results in muscular disorders	Castets et al., 2011; Ferreiro et al., 2004; Moghadaszadeh et al., 2001
Selenoprotein 15	ER (Reeves and Hoffmann, 2009; Gromer et al., 2005)	Regulate ER stress Redox homeostasis in cancer etiology Maintain lens transparency (Anti-cataract)	Korotkov et al., 2001 Kasaikina et al., 2011 Kasaikina et al., 2011
Selenoprotein P	Endothelial cells, liver cells (Burk and Hill, 2005; Yang et al., 2000)	Hepatically drived maintain Se homeostasis in plasma Intracellular antioxidant inhibits oxidative damage Protection against oxidation of low density lipoproteins Protection against peroxy-nitrite mediated oxidation Mediates sperm maturation	Schweizer et al., 2005 Steinbrenner et al., 2006 Traulsen et al., 2009 Arteel et al., 1998 Olson et al., 2005
Selenoprotein R, MsrB1	Cytoplasm and nucleus (Negro, 2008; Kim and Gladyshev, 2004)	Function as Zn containing methionine sulfoxide reductase to control oxidative insult	Kim and Gladyshev, 2007; Kryukov et al., 2002
Selenoprotein S	ER and Plasma membrane (Jun and Sharon, 2015)	Remove the misfolded protein from ER membrane	Curran et al., 2005; Gao et al., 2004
Selenoprotein T	Golgi apparatus and perinuclear speckles (Moustafa and Antar, 2012; Sengupta et al., 2009; Grumolato et al., 2008)	Ca^{2+} mobilization and homeostasis, monitor neuro-endocrine secretion. SelT deficiency alters cell adhesion	You et al., 2014; Grumolato et al., 2008 Sengupta et al., 2009
Selenoprotein V	ER (Hatfield et al., 2014)	Testis specific expression	Hoffmann et al., 2007; Ursini et al., 1999
Selenoprotein W	Cytoplasm (Chung et al., 2009)	Glutathione dependent antioxidant Accumulates in skeletal muscles for muscle growth	Yao et al., 2013; Musiani et al., 2011; Jeong et al., 2002; Vendeland et al., 1993 Loflin et al., 2006; Gu et al., 2000
Selenophosphate synthetase	Blood plasma and nuclear membrane (Kim et al., 2010)	Precursor of Se-donor in trans-selenation pathway	Allmang et al., 2009; Papp et al., 2007; Xu et al., 2007;

2.4.2 Anti-tumorogenesis effect at superanutritional doses of selenium

Although Se is toxic in large doses, yet it is an essential micro-nutrient at superanutritional doses with reference to cancer treatment. Dose dependent Se supplementation to cancer patients depends on the degree of Se deficiency as; (a) Se deficient patients and (b) Se replete patients. The Se deficient patients can recover by optimal Se-intake, whereas the Se replete patients are dependent on superanutritional doses of SeMet and sodium selenite (Combs, 1997; El-Bayoumy, 1991; Ip, 1986; Medina and Morrison, 1988). Most of the *in-vitro* and animal studies carried out in the last 35 years demonstrate that the application of Se at supra-nutritional levels can inhibit tumorogenesis (Clark et al., 1996; Combs and Gray, 1998; Ip, 1998; Linda et al., 2005; Shamberger and Frost, 1969). To combat with cancerous effects through selenoenzymes which impart antioxidant activity, are likely to involve the production of specific anti-tumorogenic metabolites, such as methylselenol (Ip, 1998). The efficiency range to cure cancer is more in Se-methylselenocysteine (Ip et al., 1991) followed by sodium selenite and SeMet (Suzuki and Ogra, 2002). Studies have suggested that Se provided in certain forms can neutralize carcinogens, enhance the immune system, alter gene (including p53) expression, inhibit tumor cell metabolism and neo-angiogenesis (blood vessel development around tumors), and promote apoptosis (Combs and Gray, 1998; Davis et al., 2002). GPx activity in blood plasma through SelP inversely measures the degree of cancer risk (Clark et al., 1984; Ghadirian et al., 2000; Sabichi et al., 2006; Vogel and Mcpherson, 1989).

2.4.3 Antibacterial and antiviral functions

The bacterial infection from *Listeria monocytogenes* and *Citrobacter rodentium* in Se deficiency leads to weaker immunity (Smith et al., 2011; Wang et al., 2009). Se supplementation in the form of Sepp1, prevents *Trypanosoma congolense* infection (Bosschaerts et al., 2008). In viral infections too, Se has inverse relationship (Taylor, 1997) in pervention of septicaemia,

lymphoedema and viral hemorrhagic fever (Schrauzer, 1998). In Se replete HIV patients, the sepsis effect has been reported to promote AIDS (Baeten et al., 2001; Baum and Shor-Posner, 1998; Campa et al., 1999), which turns inert and harmless coxsackie virus into pathogenic form, resulting in oxidative burst among cells (Beck et al., 1998). This initiates the recurrent inflammatory pains (Tapiero et al., 2003). However, Se supplementation proves fruitful in prevention of hepatitis B viral infection (Yu et al., 1997).

2.4.4 Relationship between selenium intake and cardiovascular diseases

Se deficiency was reported in a fatal case study in a patient with coronary heart disease (Salonen et al., 1982). It was due to accumulation fatty esters and cholesterol in artery walls of heart. With Se supplementation, the phospholipid glutathione peroxidase (GPx4) activity increases to reduce these stroke diseases (Sattler et al., 1994; Tapiero et al., 2003).

2.4.5 Selenium supplementation effects on skin and hair

The beneficial effects of Se along with other antioxidants from UV-rays and chemical pollutants in environment improves the scaling effect and skin density (Heinrich et al., 2006). An inverse relationship has been observed between Se intake and keratinocyte functions resulting in erythema symptoms, hyperplastic rough epidermis and abnormal hair follicle morphogenesis, brittle hair (Sengupta et al., 2010). To control the roughness and to escape from dandruff, selenium disulfide (S_2Se) can be added to the shampoos (The American Society of Health-System Pharmacists, 2017).

2.5 Role of selenium in prevention of oxidative stress

Cellular energy (ATP) production in the aerobic organisms is conducted by mitochondrial respiratory chain through transfer of four electrons, producing the electrochemical gradient on the membrane. If in case, O_2 accepts less than four electrons, it leads to the formation of partially reduced O_2 species called reactive oxygen species (ROS) (Semchyshyn and Lushchak, 2012).

These are highly reactive compounds which tend to rip off an electron from another liaison as quickly as possible. These reactive species include hydrogen peroxide (H_2O_2), lipid hydroperoxides, superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (•OH), nitric derivatives (•NO, ONOO-) and various organic radicals (•RO) (Semchyshyn and Lushchak, 2012). At physiological concentrations, these species act as secondary messengers able to regulate apoptosis, to activate transcriptional factors and to modulate the expression of genes. But when present at high concentration (under stress, smoking, pollution, radiation, etc.), these compounds can readily oxidize the cellular material within the first line of membranous lipids, thiol groups of cell proteins and enzymes, and DNA bases. Consequences of these oxidations include the diminution of cellular exchanges, loss of some transcription signals, misfunction of certain proteins, loss of enzymatic activity, inflammation, DNA breaks, and occurrence of genetic mutations, possibly leading to cell death (aging) by apoptosis and necrosis, or to cancers (Kohen and Niska, 2002). Hydroxyl radical is extremely reactive and directly involved in DNA damage (Letavayova et al., 2006). Selenium is an essential element for antioxidant reactions in humans and animals. Biochemically, Se is a component of the enzyme glutathione peroxidase, which along with superoxide dismutase, catalase and vitamin E protects against damage to cellular components by preventing the accumulation of peroxides in the tissue.

2.5.1 Immuno-stimulant effects of dietary selenium

Dietary selenium influences the immuno-competence by enhancing the lymphocytic activity, imparting immuno-stimulant effects (Schumacher et al., 1994; Rayman, 2000). This involves the activation, proliferation and differentiation capacity of B and T-cells, natural killer cells, driving the innate and adaptive immune response. However, the Se deficiency leads to the impairment of macrophages and neutrophils (Boyne and Arthur, 1986; Spallholz et al., 1990).

2.5.2 Role of Se in inflammation and arachidonic acid pathway

Inflammation is the primary infectious-protective response of body towards external or internal stimuli like injury, physical, chemical or thermal stress, trauma or auto-antibody generated auto-immune response. To bring the timely resolution, macrophages play prime role as responders of inflammation site. They prevent the reach of protective and acute inflammation state up to destructive and chronic state. The chronic inflammation through massive RONS (reactive oxygen and nitrogen species) production prevails the most common problem as swear pain throughout the world. RONS has been recognized as important secondary messenger that affects signaling functions of variety of cell types and cell to cell communication. RONS produced by macrophages and neutrophils are essential for oxidative destruction of phagocytosed foreign pathogens to provide fully effective immunity. The antioxidant capacity of macrophages plays vital role, regulating the expression of pro-inflammatory genes (Vunta et al., 2008) with tightly controlled intracellular oxidative tone. However, the massive oxidative stress leads to propagate inflammatory signals (Reuter et al., 2010; Poli et al., 2004), which switch-on the AA pathway and MAP kinase pathway through NFκB activation, which is the key regulator of inflammation (Poli et al., 2004; Wang et al., 1998). NF-κB plays key role in the regulation of immune response to infection via pro-inflammatory signaling pathway (Lawrence et al., 2001). However, the prolonged activation of these pathways in overproduction of these pro-inflammatory cytokines leads to atherosclerosis, hypertension, cardiovascular and chronic inflammation (Bergamini et al., 2004).

Macrophage activation under oxidative stress produce two types of mediators for signaling, i.e, protein mediators and lipid mediators. The protein mediators include IL-1, IL-6 and TNF-α and lipid mediators are AA derived eicosanoids. These eicosanoids includes four additional types of

mediators, i.e., prostaglandins, prostacyclins, thromboxane, leukotrienes and lipoxins. These are the important modulators of inflammation and immune response (Ma et al., 2003). AA is the 20-carbon fatty acid chain which rearrange into multienzyme complexes called pro-/anti-inflammatory prostaglandins (PGs). Under inflammatory stimuli (LPS), COX-2 induce the AA pathway with release of key product, PGH_2, which depending upon state of inflammation further mediates different varieties of PGs like PGE_2, PGD_2, TXA_2 and PGI_2, catalyse by PG synthase enzymes namely, microsomal PGE synthase (mPGES-1), hematopoietic PGD synthase (H-PGDS), TXA_2 synthase (TXAS) and prostacyclin synthase (PGIS), respectively (Gandhi et al., 2013). These lipid mediators plays dual role in inflammation, divide PG'S into two categories, pro-inflammatory PGs (PGE_2 and TXA_2) and anti-inflammatory PGs (PGD_2 and its downstream products, i.e., Δ^{12} PGJ_2 and 15d-PGJ_2) (Figure 2.1). The resolution of acute inflammation is through shunting of pro-inflammatory PGE_2 into anti-inflammatory PGD_2 and its downstream product 15d-PGJ_2 and peroxisome proliferators activated receptor - gamma (PPARγ) (Kapoor et al., 2007). PPARγ activates M_2-specific alternate macrophages which increase the production of its markers, i.e., Arg-1, Fizz-1 and Mrc-1 (Nelson et al., 2011) to resolve inflammation (Odegaard et al., 2007). 15d-PGJ_2 inactivate the pro-inflammatory transcription factors (AP-1 and STAT3) and activate anti-inflammatory transcription factor (Nrf-2) (Surh et al., 2011; Gandhi et al., 2011; Ji et al., 2005; Itoh et al., 2004; Sawano et al., 2002) (Figure 2.2).

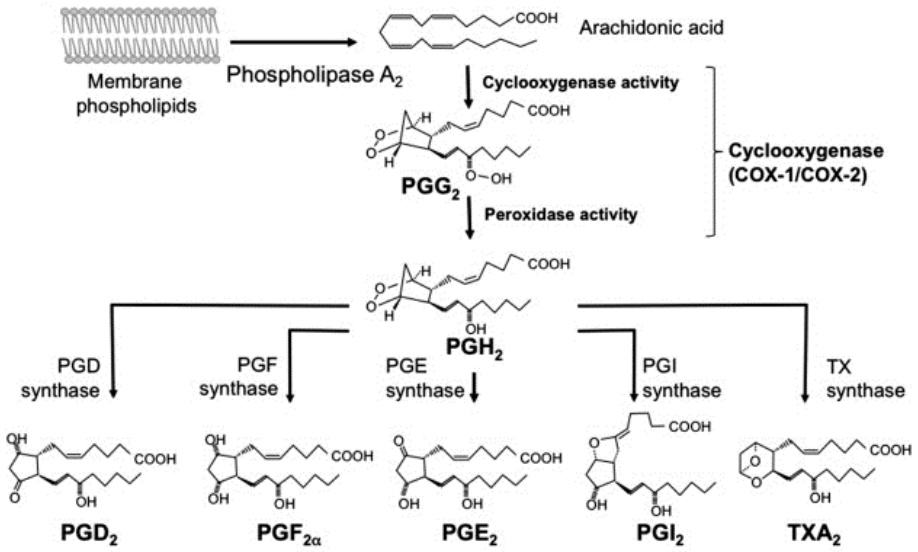


Figure 2.1: Arachidonic pathway derived pro-/anti-inflammatory prostaglandins (Kawahara et al., 2015; Smith and Murphy, 2002).

In response to external stimuli, the recruitment of leukocytes leads to the respiratory burst caused due to increased uptake of oxygen and macrophages, may express many pro-inflammatory enzymes of AA pathway including COX-2 and mPGES-1, which generate PGE_2 (Zamamiri et al., 2003). PGE_2 is the prototypical biomarker of inflammation, while PGD_2 possesses anti-inflammatory properties (Gandhi et al., 2011) and serves as the other arm of the AA pathway. COX-2 not only contributes to the onset of the inflammatory reaction through synthesis of pro-inflammatory prostaglandins, but also plays an important role in activating pathways of resolution through the preferential synthesis of PGD_2 by PGD synthase (PGDS) (Huang et al., 2012). The ability of this enzyme cascade to act on wide variety of RONS has supported the understanding of the role and importance of Se as a potential chemopreventive agent (Combs and Gray, 1998).

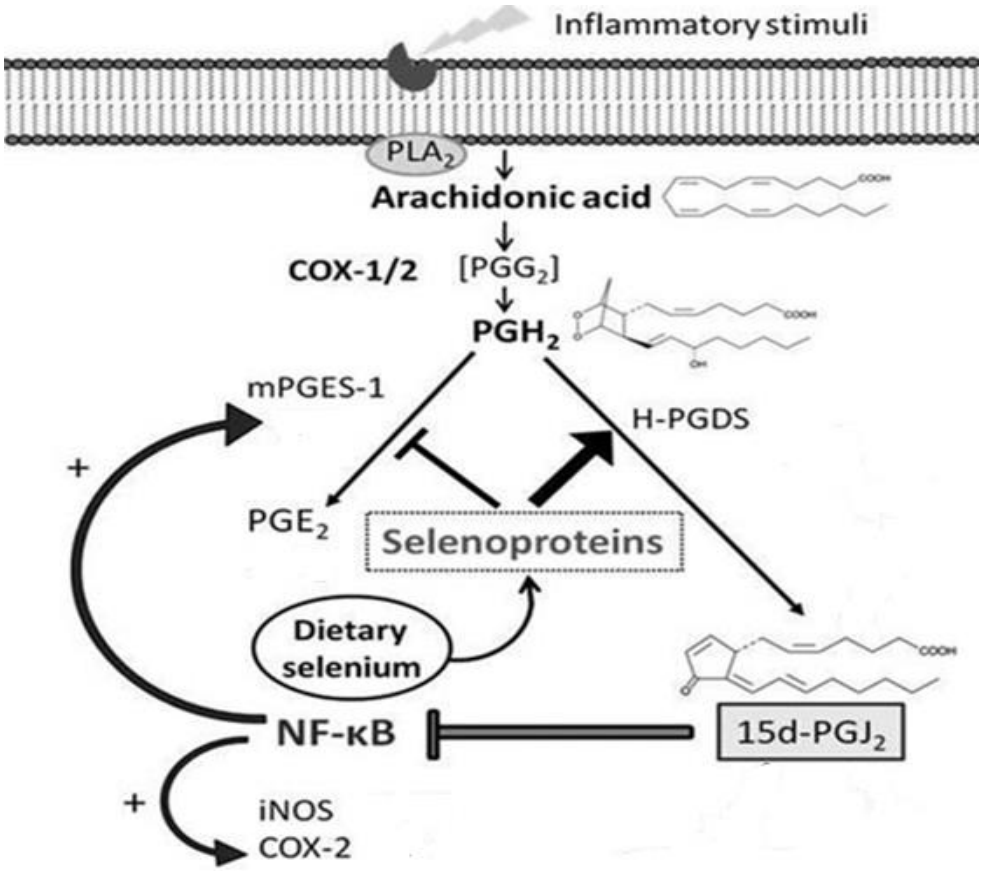


Figure 2.2: Dietary Se dependent shunting of AA pathway in suppression of NFκB, mPGES-1, iNOS and COX-2 expressions through elevated levels of anti-inflammatory prostaglandins i.e., PGD_2 and its downstream products, Δ^{12} PGJ_2 and 15d-PGJ_2 (Gandhi et al., 2013).

Se supplementation activates PPARγ, increase 15d-PGJ_2 production and modify cysteine thiols (IKK-β) for NFκB down regulation (Vunta et al., 2007). Anti-inflammatory PGs are dependent upon Se regulated GPx moieties, having H_2O_2 reducing properties through PGIS (Maddipati and Marnett, 1987). Selenoproteins such as GPx affects the activity of oxidant mediated activation of redox sensitive transcription factor NF-κB (Prabhu et al., 2002). Hattori and his co-workers (2005) had shown the importance of Se supplementation through these selenoproteins, can mitigate the production of ROS and its downstream effects like inflammatory pains. Human neutrophils stimulated with TNF-α shows increased GPx4 expression in ROS dependent manner. The increased Se levels, directly react with thiol groups on molecules to form RS-Se-SR adduct and

inhibit cell signaling events. For example, DNA binding capacity of NF-κB in cell lysates was inhibited by addition of high levels of Se (Kim and Stadtman, 1997). Higher Se status in RAW 264.7 mouse macrophages inhibited NF-κB activation induced by LPS (Youn et al., 2008). The downstream effects of NF-κB activation, i.e., expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and tumor necrosis factor- alpha (TNF-α) were also decreased with Se supplementation in rat Kupffer cells (Prabhu et al., 2002).

2.6 Other health effects

Se is essential for male fertility and motility because the selenoprotein phospholipid glutathione peroxidase (GPx4) is both an abundant antioxidant enzyme and a structural protein in mature spermatozoa (Behne et al., 1996; Maiorino and Ursini, 2002; Pfeifer et al., 2001; Ursini et al., 1999). A case study in U.S.A. on superanutritional Se intake of 226 μg/day reduces the cognitive impairment, anxiety, depression (Castano et al., 1997) and bad mood parameters as compared to the low level Se intake (32.6 μg/day) (Rayman, 2000). Se acts as potent anti-inflammatory agent in intestinal sores (Barrett et al., 2013; Krehl et al., 2012; Steinbrenner et al., 2011) with quick macrophage movement and action (Huang et al., 2012). SelP deficiency leads to movement disorders (Schweizer et al., 2004).

Gaps in knowledge and significance of the study

Selenium, in the form of selenoproteins, has a pivotal role in down-regulation of inflammation, which is a critical component that forms the underlying basis for cancer and other diseases. However, influence of bio-accessible Se from dietary sources such as Se rich cereals on inflammation has not been well understood, despite the need of identifying an alternative source of Se supplementation to selenite or selenomethionine. Significant gap exists that must be resolved in order to introduce Se rich cereals towards optimal nutrition. While there are several studies that

have examined the anti-inflammatory properties of inorganic Se as selenite, there are limited studies on selenomethionine or other naturally bio-available organic forms of Se. More specifically the ability of these accessible Se compounds to shunt pathways of arachidonic acid towards anti-inflammatory responses. Thus, the proposed study is based on the hypothesis that Se compounds bio-available in aqueous and bio-accessible fractions of cereal grains mediate the shunting of AA pathway in macrophages to negatively modulate the expression of inflammatory genes vis-a-vis enzymes.

Based on the gaps identified in the literature and the studies carried out by other research groups, following objectives were framed for the study:

1. *Extraction and quantification of selenium in various fractions of Se rich cereal grains.*
2. *Impact of Se supplementation on pro-inflammatory effects through expression of anti-inflammatory enzymes.*

Determination of gene expression profile relevant to pro- and anti-inflammatory cascade in presence of Se supplementation.

3.0 Materials and Methods

3.1 Sample collection

The post-harvested cereal grains such as wheat (*Triticum aestivum*), maize (*Zea mays*) and rice (*Oryza sativa*) were collected in November 2014 and October 2015 from Se contaminated sites near the villages of Jainpur and Barwa geographically located at 31°13’ N, 76°21’ E, in the Nawanshahr-Hoshiarpur districts of Punjab, India. Samples were collected from three different locations of each field, and composite samples were prepared and processed separately. Similarly, non-seleniferous samples (controls) were collected from agricultural fields of Patiala (30°32’ N, 76°40’E), Punjab, India. Healthy grains were manually separated from damaged ones, washed, air-dried and grounded to fine powder with an electric blender.

3.2 Quantification of selenium in various fractions

3.2.1 Extraction of Osborne fractions

The grounded flour was passed through 0.5 mm sieve to obtain homogeneous fine powder. 200 g of each samples were defatted with 300 mL hexane (twice for 2-3 h) so as to separate albumin, globulin, glutelin and prolamin subunits (Burnouf and Bietz, 1989). The samples were air-dried at room temperature to evaporate the remaining hexane present in flour and store at 4°C for further analyses.

Osborne fractions (namely, albumin, globulin, glutelin, and prolamin) were extracted from cereal grains according to the method of Ju et al. (2001) with minor modifications. The scheme was common for wheat, rice, and maize protein extracts and is given in the flow chart (Figure 3.1). Fractions were extracted, independently, from defatted sample flour based on their solubility in

distilled water (albumin), 5% NaCl (globulin), 0.1 N NaOH (glutelin), and 70% ethanol (prolamin) at 25°C. In brief, 200 g of defatted sample was stirred in 200 ml of distilled water for 4 h, at room temperature. The first fraction, albumin was separated by centrifugation of aqueous slurry at 4500 rpm, 4°C for 20 min. The supernatant containing albumin was collected and passed through 0.45 µm filter under vacuum. The residue left behind after albumin separation was re-suspended in 200 mL of distilled water containing 5% NaCl with continued stirring for 4 h, at room temperature. The supernatant containing the second fraction i.e., globulin was filtered and stored at 4°C for further processing. The left-over residue was treated with 200 ml of 0.1 N NaOH at pH 11 for 4 h, at room temperature to obtain glutelin supernatant (third fraction). For the last fraction, the residue was re-dissolved in 70% ethanol with continued stirring for 4 h, at room temperature. The extraction procedure for each fraction was repeated twice to extract the maximum amount of proteins out of slurry. For precipitation of proteins, the pH of different supernatants were adjusted to their isoelectric points (titration of aliquot and as indicated by pH electrode) by adding 5-6 drops of 1 N HCl and kept overnight at 4°C to ensure the complete aggregation. The extracts were then centrifuged at 12,000 rpm for 30 min (4°C). The supernatants were discarded and pellets were washed with acetone, cryodried, and crushed into fine powder for further analyses.

3.2.2 Crude starch extraction

The residue left after the extraction of different Osborne fractions, was repeatedly washed with 1 L of distilled water for 24 h, followed by salt washing with 2% NaCl for 24 h, alkali washing twice with 0.1 N NaOH for 48 h, and alcohol washing with 80% ethanol at 80°C in a water bath for 1 h to remove and denature the remaining protein, if any. The supernatant was discarded and the residue was air dried for overnight, at ambient temperature (37°C) to get crude starch powder (Reddy and Bhotmange, 2013). For wheat and maize, to reduce the interference of unwanted

materials such as fiber, fine cell residues, and other soluble materials, the upper white starchy layer was collected manually and resuspended in 1 L of 0.1 N NaOH for 48 h and in diethyl ether for 4 h at room temperature before the alcohol washing step.

3.3 Elemental determination and quantification of selenium

Elemental composition (K, Na, Mg, Ca, Zn, Mn and Cu) and Se levels were determined in defatted whole grain flour, starch residue, and all protein fractions of wheat, maize and rice following the procedure given by Levesque and Vendette (1971) with minor modifications (Figure 3.1). In brief, this method involved close vessel oxidation of whole grain flour, starch fraction (≈200 mg) and Osborne fractions (≈20 mg) with 5 mL of HNO_3 and $HClO_4$ (3:1) (SD Fine-Chem, India) for 30 min at 200°C, followed by reduction with 5 mL of 6 N HCl (SD FineChem, India) for 10 min at 150°C in a microwave digester (MARS 6 240/50:910905, CEM, Matthews, NC, U.S.A.). The reduced Se (Se^{+4}) was then transferred into 50 mL volumetric flask to volume made-up with 0.1 N HCl. The elemental composition was analyzed by microwave plasma atomic emission spectrometry (4100 MP-AES, Agilent Technologies). For Se quantification, 500 µL of digest was taken in a test tube along with 100 µL of 0.04 M Na_2EDTA (Himedia, India) solution in 10% hydroxylamine hydrochloride (stabilizing solution; Himedia, India) and 100 µL of 12.5 M formic acid (SD FineChem, India). The pH of solution was adjusted to 1.8 with 4.0 N NH_4OH (Himedia, India) and placed in water bath at 50°C for 10 min. After cooling, the reaction mixture was allowed to complex with 500 µL of 2,3-diamino-naphtalene (0.1% DAN in 0.1 N HCl; Himedia, India) to obtain yellow coloured piazselenol complex in 3 mL of cyclohexane layer. The colour intensity was measured with fluorescence spectrometer (LS-45, Perkin Elmer, Waltham, MA, U.S.A.) at emission spectrum of 520 nm (detection limit of 2 ng/mL) corresponding to the amount of selenite present in the cereal grains and their protein fractions. Selenium quantification in each sample was

carried out by a relative method using the emission spectrum of National Institute of Standards and Technology certified selenium ICP standard solution (SRM-1349). Every sample was analysed in triplicate and results are expressed as mean±SD.

3.4 Bioaccessibility of selenium by *In-vitro* gastrointestinal digestion

In-vitro gastrointestinal digestion (GI) was performed in triplicates according to the method of Kulkarni et al. (2007) with some modifications (Figure 3.1). The *in-vitro* enzymolysis was mimicked with human gastrointestinal digestion by incubating whole grain flour (≈500 mg) and Osborne fractions (≈20 mg) with 10 mL of gastric juice (1% w/v pepsin in 0.15 M NaCl, pH 1.8). The slurry was vigorously shaken for 1-2 min to degas the samples and the tightly sealed flasks were placed in shaker-incubator set at 37°C with gentle shaking (50 rpm) for 4 h. Further, for intestinal digestion, the pH was adjusted to 6.8 with saturated solution of $NaHCO_3$ and supplemented with 10 mL of intestinal juice (3% w/v pancreatic, 1.5% w/v α-amylase and 1% w/v bile salts in 0.15M NaCl). Flasks were again degassed and incubated at 37°C for 4 h. Samples were centrifuged at 8000 rpm for 10 min (4°C) and supernatants were filtered through 0.45 µm pore sized filters. 1.0 mL of clear digests from different fractions were subjected to quantification of bioaccessible selenium by similar method mentioned earlier (section 3.2.3). The percentage bioaccessibility (%B) of Se in GI digest was calculated using following formula:

$$\%B = 100 \times [(GI)_{Se}/(T)_{Se}]$$

Where, $(GI)_{Se}$ is the concentration of Se in gastro-intestinal digests and $(T)_{Se}$ is the total Se present in whole grain samples or Osborne fractions, respectively.

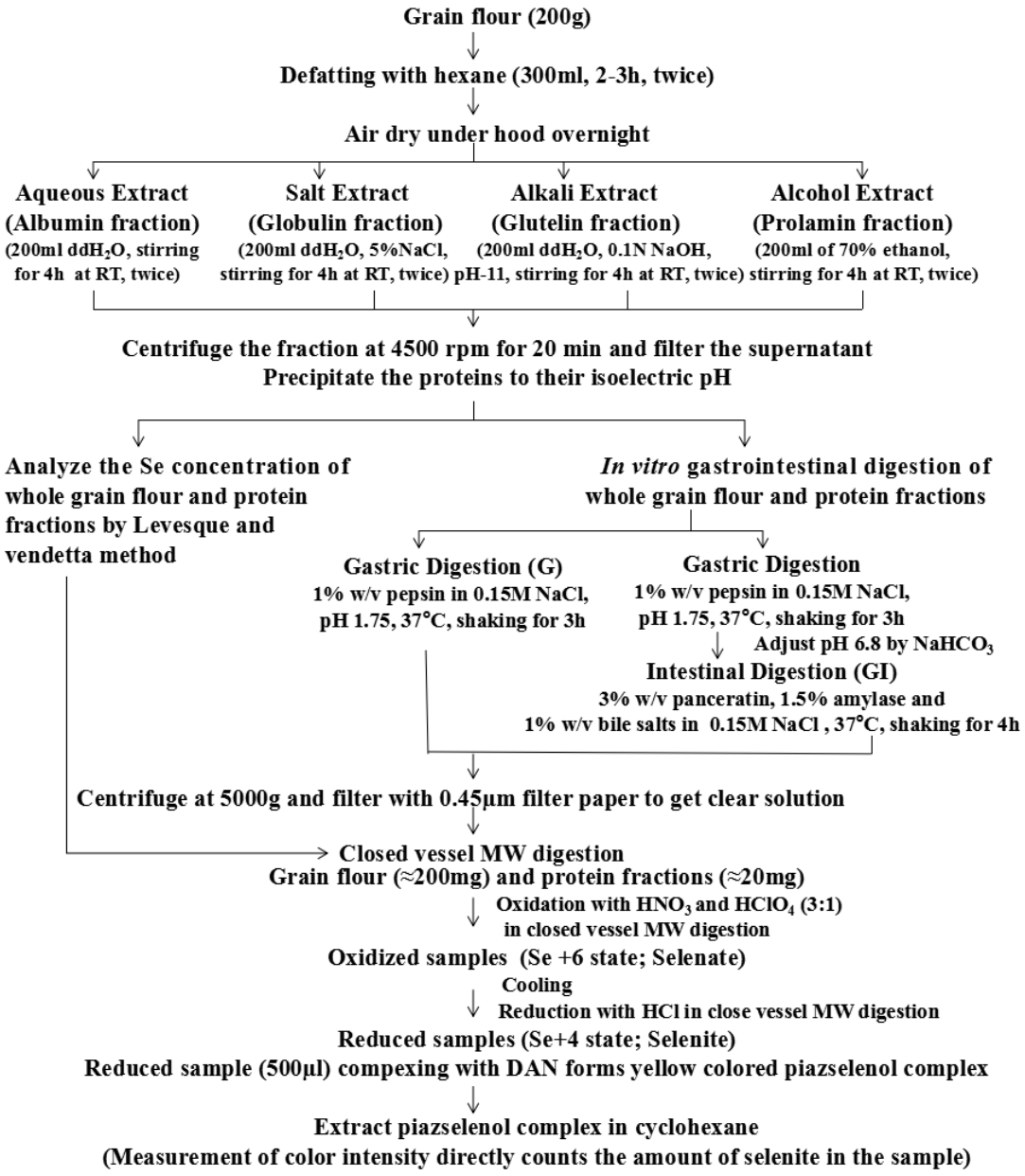


Figure 3.1: The scheme for the extraction of Osborne fractions, *in-vitro* gastrointestinal digestion and estimation of selenium (Dhanjal et al., 2016).

3.5 Anti-inflammatory studies of bioavailable selenium in cereal grains

To investigate the significant role of dietary Se in modulating pro and anti-inflammatory pathways and its bioavailability from cereal matrices, extracts were supplemented with/without stress conditions to the murine macrophage model. This section is focused on:

(i) Preparation of wheat and maize extracts (ii) Impact of Se supplementation on expression of inflammatory responses, and (iii) role of rMETase in enhancing the bioaccessability of selenium from dietary matrices. In this study, seleniferous wheat and maize grains were selected due to high Se concentration, better bioaccessibility and less starch content as compare to rice grains.

3.5.1 Extraction of water soluble selenium fraction from wheat and maize flour

5 g of seleniferous and non-seleniferous samples were dissolved in 10 mL of 1X PBS buffer (Lonza, India) and vortexed for 10 min. Samples were kept on rest for another 10 min and centrifuged at 4000 rpm for 30 min (4°C). The supernatants were transferred into ultra-centrifuge vials and re-centrifuged at 16000 rpm for 1 h (4°C). The pellets were discarded and the filterates were collected under sterile conditions by passing through 0.22 µm sterile filters twice to avoid any contamination. The Se concentration in extracts were quantified according to the procedure outlined earlier. The vials were stored at -80°C till further studies.

3.5.2 Growth conditions of RAW264.7 macrophage cell lines

A murine macrophage cell line, RAW264.7 was procured from National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Dulbecco's Modified Eagle Medium (Lonza, India) supplemented with 10% defined fetal calf serum (Himedia, India), 4.5 g/L glucose, 2 mM L-glutamine (Sigma Aldrich, U.S.A.), 1% v/v penstrep containing 100 U/mL penicillin (Sigma Aldrich, U.S.A.) and 100 µg/mL streptomycin (Sigma Aldrich, U.S.A.), 0.5% amphotericin B

solution (Sigma Aldrich, U.S.A.) at 37°C with 5% CO_2. Cells devoid of any selenium source (SeDef) were passaged and maintained with regular change in growth medium in 24 h.

3.5.3 Profiling of enzymatic expressions on Se supplementation

SeDef cells (1×10^5) were then cultured in five different Se-supplemented media (SeSup) for three days along with respective controls (Table 3.1). The growth medium was changed in every 12 h. After 72 h, cells were stimulated with 1 µg/mL bacterial endotoxin lipopolysaccharide (LPS) (Sigma Aldrich, U.S.A.). Time kinetics was set from 0 to 4 h LPS stimulation to investigate the significant change in expression profile of GPx-1 and COX-2 enzymes, with time lag studies up to 12 h for mPGES-1 and H-PGDS as their endocellular expression tend to increase after 6 h according to the literature (Gandhi et al., 2011; Shemi et al., 2000; Vunta et al., 2007). For iNOS expression and NO production, cells were incubated with LPS up to 24 h as the release of extracellular nitrates starts after 8 to 11 h (approx.) of induced stress (Prabhu et al., 2002). Cells were collected at different time intervals, washed twice with PBS (Lonza, India), scraped and centrifuged at 2000 rpm. The pellets were stored at -80°C until further processing.

The growth rate and cell viability pattern under high dose selenium (upto 500 nM) with/without LPS treatment was assessed by MTT (Himedia, India) assay in 96 well plates. It involved the mitochondrial reduction of MTT [3-(4,5-dimethylthiazolyi-2)-2,5-diphenyltetrazolium bromide] into purple coloured formazan crystals by live cells and the intensity of colour is directly proportional to the viable cells. In brief, 20 µl of MTT reagent (5 mg/mL, in PBS) was added and kept at 37°C for 4 h. On appearance of purple crystals were visible under microscope, 100 µL of dimethyl sulfoxide (DMSO; Himedia, India) was added and absorbance was measured at 570 nm.

Table 3.1: Different source of Se-supplementation with increasing three different Se concentrations (50nM, 100nM and 500nM) w.r.t. their controls. Control (C) represents cells devoid of any source of Se-supplementation. In case of (NSeW)e, (NSeM)e, (NSeW)e+rMET and (NSeM)e+rMET, 'e' represents the volume of non-seleniferous extract taken equivalent to seleniferous extracts with 500nM Se concentration.

Sr.No.	Se source in Se-supplemented cells (SeSup)	Corresponding controls in Se-deficient cells (SeDef)
1.	sodium selenite (SS)	control (C)
2.	seleniferous wheat extract (SeW)	non-seleniferous wheat extract (NSeW)
3.	seleniferous wheat extract + rMETase (SeW+rMET)	non-seleniferous wheat extract + rMETase (NSeW+rMET)
4.	seleniferous maize extract (SeM)	non-seleniferous maize extract (NSeM)
5.	seleniferous maize extract + rMETase (SeM+rMET)	non-seleniferous maize extract + rMETase (NSeM+rMET)

3.5.3.1 Preparation of cell lysates

The pellets were retro-pipetted with 100µL of CelLytic^{TM} M protein extraction reagent (Sigma Aldrich, U.S.A.) containing Pierce^{TM} protease inhibitor (Life Technologies, India) and kept for 30 min at 4°C with intermittent vortexing. CelLytic^{TM} M reagent contains dialyzable mild detergent dissolved in bicine buffer for minimal interference with cell protein. The protease inhibitor cocktail contains 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), penstatin A, aprotinin, leupeptin, bestatin and E-64 which inhibits the proteolytic degradation of serine-proteases, cysteine-proteases and other aminopeptidases present in cell lysates. The cell lysates were centrifuged at 12000 rpm for 30 min at 4°C and protein containing supernatant was collected in fresh sterile tube, stored at -20°C. For mPGES-1 analysis, the pellet containing membranous proteins were re-suspended in extraction buffer, centrifuged at 16000 rpm for 1 h at 4°C and the supernatants were collected. The protein concentration in all fractions were estimated using Bradford method (Alabi, 2014).

3.5.3.2 Protein quantification

The Bradford reagent (Sigma Aldrich, U.S.A.) contains Coomassie Brilliant Blue G-250 dye having free electrons under acidic conditions which interacts with ionzable groups present on proteins. The protein-dye complex denatures the quaternary structure exposing the hydrophobic pockets. The anionic charge on the dye forms Van der Waal interaction with these tertiary pockets of protein and stabilizes the blue form of dye having absorbance at 595nm (Reisner et al. 1975; Groth et al. 1963). To determine the concentration of protein in unknown samples, standard curve was plotted at 595nm using bovine serum albumin (BSA; Sigma Aldrich, U.S.A.) as standard protein. The microplate assay calculations for blank, standard and unknown protein is given in table 3.2.

Table 3.2: Estimated concentration and volume of standard protein (BSA, 1.0 mg/mL stock solution) taken in complexing with Bradford reagent to draw the standard curve and predict the protein concentration of unknown samples.

BSA Concentration (mg/mL)	BSA volume (stock solution-1.0 mg/mL) (µL)	Unknown sample volume (µL)	H_2O volume (µL)	Bradford reagent volume (µL)	Total volume (µL)
Blank	0	0	10	190	200
0.2	2	0	8	190	200
0.4	4	0	6	190	200
0.6	6	0	4	190	200
0.8	8	0	2	190	200
1.0	10	0	0	190	200
Unknown sample	0	10	0	190	200

3.5.3.3 Western blot analysis

A 30 µg of protein was thoroughly mixed with reaction mixture containing 2 µL of 5X denaturing dye (containing 0.15 M tris HCl (Himedia, India), 5% SDS (Sigma Aldrich, U.S.A.), 25% glycerol (Sigma Aldrich, U.S.A.), 12.5% β-mercaptoethanol (Sigma Aldrich, U.S.A.) and

0.006% bromophenol blue (Himedia, India), pH 6.8); and heated at 100°C for 5 min. Along with prestained protein ladder (PUREgene Genetix Biotech, India), samples were separated on 15% SDS-polyacrylamide gel for 1 h. In the meantime, the PVDF membrane (0.2 μm, Biorad) was incubated in methanol for 5 min and saturated with transfer buffer (1X Tris glycine containing 25 mM Tris base (Himedia, India), 190 mM glycine and 20% methanol). Simultaneously, the filter papers and cushioned pads were soaked in transfer buffer. For electrophoretic transfer, the sandwich assembly for 'wet' transfer was arranged. An electric field with constant current of 50 mA was applied for 2 h to move the proteins out of polyacrylamide gel and transblotted onto PVDF membrane. The membrane was blocked with TBST containing 10 mM Tris, pH 8.0, 150 mM NaCl (Sigma Aldrich, U.S.A.), 0.05% Tween-20 (Sigma Aldrich, U.S.A.) and 5% skim milk (Sigma Aldrich, U.S.A.) for 1h at room temperature. Skim milk prevents non-specific binding of antibody to the membrane and hence, reduce the background noise during staining. The membrane was washed with TBST for four times and cut the strips according to the molecular weight of protein to be tagged to the antibody. Anti-rabbit-GPx-1 (Abcam, Cat # ab108427); mouse anti-GAPDH (Cat # 437000) and HRP goat anti-mouse IgG (H+L) (Cat # 626520) were procured from Life Technologies; while goat anti-rabbit IgG HRP (Cat # 10004301), COX-2 polyclonal antibody (Cat # 160126), H-PGDS polyclonal antibody (Cat # 160013) and mPGES-1 polyclonal antibody (Cat # 160140) were procured from Cayman Chemical Company. The primary antibodies were probed overnight at 4°C. The blots were washed with TBST for about four times to remove the excess unbounded antibody and re-probed with an appropriate secondary antibody coupled to HRP for 1 h at room temperature. Anti-GAPDH blots were considered for normalize protein loading. The blots were re-washed with TBST to remove the excess unbound secondary antibody followed by staining with 3,3',5,5'-

tetramethylbenzidine (TMB, Sigma Aldrich, U.S.A.) to obtain signal (blue) for the presence of test-protein. The immunoreactive bands were recorded according to their molecular weight i.e., for GPx-1- 22 kDa, COX-2- 72 kDa, mPGES-1- 16 kDa, H-PGDS- 23 kDa and GAPDH- 37 kDa. These bands were analyzed densitometrically using ImageJ (Schneider et al., 2012).

3.5.4 Profiling of molecular expression of pro- and anti- inflammatory genes at pre-transcriptional levels

3.5.4.1 RNA extraction

Total RNA was extracted from SeDef and SeSup cells by TRIzol reagent (Life Technologies, India) according to the manufacturer's instructions. The technique is known as acid guanidinium thiocyanate-phenol-chloroform extraction, contains the monophasic solution of guanidine isothiocyanate and phenol. Under acidic conditions, phenol and chloroform seperates the solvent into two phases i.e., upper aqueous phase in which total RNA is dissolved and lower organic phase in which proteins are dissolved in phenol and lipids in chloroform. Whereas, guanidine isothiocyanate with lysing action, denatures the proteins, RNase and DNase enzyme activity which otherwise interrupt the pure total RNA extraction. Before starting the process of RNA extraction, all the lab-ware such as tips, tip-boxes, eppendorfs, gloves, reservoirs and millipore water were incubated in diethyl pyrocarbonate (DEPC; Himedia, India) treated water, overnight and autoclaved at 121°C for 30 min to inactivate RNase. In brief, the methodology includes addition of 500 µL of TRIzol reagent to cell pellet with retro-pipetting many times. The mixture was allowed to incubated for 5 min at room temperature and 150 µL of chloroform was added. The solution was mixed by tube-inversion for at least 15 times. The sample was then allowed to incubated for 5 min at room temperature to dissolve the proteins and lipids in organic phase. The mixture was centrifuged at 10,000 rpm for 15 min at 4°C. The upper aqueous phase containing

total RNA was carefully transferred in new eppendorf and 250 µL of isopropanol (Himedia, India) was added. The solution was mixed by inverting the tube and incubated for 10 min at room temperature for precipitation of RNA. The mixture was centrifuged at 12,000 rpm for 10 min at 4°C to spin down the RNA pellet. To inhibit any DNA contamination, RNA samples were treated with RNase-free DNase (Sigma Aldrich, U.S.A.) for 15min at room temperature. The supernatant was discarded and the pellet was washed with 70% ethanol (diluted in DEPC treated water). The sample was then centrifuged at 10,000 rpm for 5 min at 4°C. The RNA pellet was carefully tapped onto tissue paper to elute the remaining ethanol. The pellet was then dried in laminar air flow and dissolved in 100 µL of RNase free water.

The purity and yield of total RNA was determined spectrophotometrically using Nanodrop (ND-1000 V.3.7.1, Thermo Scientific, U.S.A.) on the basis of UV absorbance values at 260 and 280 nm. Pure RNA exhibits an $A_{260/280}$ ratio of 2.0 and $A_{260/230}$ ratio of 1.8. The integrity of intact RNA was checked prior to cDNA synthesis by running the gel in MOPS (Himedia, India) electrophoresis buffer (1.0 M MOPS (3-(N-morpholino) propanesulfonic acid), 1.0 M sodium acetate.$3H_2O$, 1.25 M EDTA and 37% formaldehyde). In brief, 1 µg of RNA sample was mixed with 2 µL of 5X RNA loading buffer (contains 0.025% bromophenol blue, 0.025% xylene cyanol FF, 0.025% SDS, 0.025% ethidium bromide, 20% glycerol, 95% formamide and 0.5 mM EDTA) and loaded onto 2% agarose gel. The gel was electrophoresed in 1X MOPS buffer at constant voltage of 40V to avoid overheating for 45 min. The 28S, 18S and snRNA bands were visualized under UV trans-illuminator and were quantified on GEL DOC XR system (Biorad) using Quantity One 1-D Analysis (Biorad) software program.

3.5.4.2 Semi-quantitaive reverse transcriptase PCR

3.5.4.2.1 cDNA synthesis

One microgram of total RNA was reverse-transcribed using Superscript® III First strand synthesis kit (Life Technologies, India). According to the manufacturer's protocol, 1 µg of total RNA was mixed well with 1µL of 50µM oligo$(dT)_{20}$, 1µL of 10 mM dNTP's and the volume was made to 10µL with DEPC-treated water. The primer mixture was incubated in thermal cycler at 65°C for 5 min. In the meantime, cDNA synthesis mixture (for 1 reaction) was prepared by adding 2 µL of 10X RT buffer, 4 µL of 25 mM $MgCl_2$, 2 µL of 0.1 M DTT, 1 µL of RNaseOUT^{TM} (40 U/µL) and 1 µL of SuperScript ® III RT (200 U/µL). RNaseOUT^{TM} is the recombinant RNase inhibitor was added in the reaction mixture to safeguard the target RNA degradation. In total, 10 µL of cDNA synthesis mix was added to the primer mixture, gently mixed and re-incubated at 50°C for 50 min. The reaction was terminated by heating the samples at 80°C for 5 min. 1 µL of RNaseH was then added to the tube and incubated at 37°C for 20 min to denature RNA (if present). The purity and yield of cDNA product was determined spectrophotometrically and stored at -20°C until further use.

3.5.4.2.2 Polymerization of reverse transcribed product (cDNA) for the profiling of mRNA expressions

Equal amount of cDNA (100 ng) from each sample was polymerized using Applied Biosystems Veriti thermal cycler. Primers for GPx-1, COX-2, mPGES-1, H-PGDS, iNOS, GAPDH and β-Actin were procured from Eurofins Genomics and are listed in table 3.3 with Tm values (annealing temperature). Denaturation and extension parameters for PCR amplification were similar as described for mycoplasma DNA amplification, except the specific annealing temperatures for these genes. The polymerized product was electrophoresed on 2% agarose gel and cDNA bands

were quantified on GEL DOC XR system (Biorad) using Quantity One 1-D Analysis (Biorad) software program. The intensity of bands were analyzed densitometrically using ImageJ software.

Table 3.3: Primers used for pro-/ anti-inflammatory genes in PCR amplification. One microgram of total RNA was reverse transcribed from each sample and amplified by PCR polymerization. Gene expression in SeSup and SeDef samples were then quantified densitometrically using ImageJ software program and normalized to the expression of GAPDH and β-actin.

Primer	Forward (5′ to 3′)	Reversal (5′ to 3′)	Tm value (°C)
Gpx1	ACAGTCCACCGTGTATGCCTTC	CTCTTCATTCTTGCCATTCTCCTG	66
COX-2	CCCCCACAGTCAAAGACACT	CCCCAAAGATAGCATCTGGA	65
H-PGDS	CCTGGGCAGACTTCTACTGG	AAACTGCAACACCCCTTGAG	67
mPGES	CACCTTGTAGGGTGCTGGTT	CAGCCTAATGTTCAGCGACA	66
iNOS	AATGGCAACATCAGGTCGGCCATCACT	GCTGTGTGTCACAGAAGTCTC	66
GAPDH	TGTTCCTACCCCCAATGTGT	CCCTGTTGCTGTAGCCGTAT	55
β-*actin*	TGGAATCCTGTGGCATCCATGAAAC	TAAAACGCAGCTCAGTAACAGTCCG	70

3.5.5 Nitric oxide assay

Nitric oxide (NO), released under stress into the cell free medium (as nitrite), was measured extracellularly from the culture media taken from cells cultivated and stimulated with LPS under identical conditions. In brief, 100 µL of LPS exhausted cell free media at different time intervals were spiked with 100 µL of Griess reagent (GR; Sigma Aldrich, U.S.A.) and incubated at room temperature for 15 to 20 min under dark conditions. GR is the combination of 0.2% naphthyl ethylene diamine dihydrochloride (NEDD) and 2% sulphanilamide in 5% phosphoric acid for the determination of nitrites released under stress conditions. For standard curve, $NaNO_2$ (Himedia,

India) was used from scale 0 μM to 100 μM and incubate with GR with similar experimental conditions. Extracellular nitrite excretion was measured in a plate reader at the wavelength of 548 nm.

4.0 Results and Discussion

The present study was aimed at examining the propensity of seleniferous cereal grains in modulating arachidonic acid (AA) pathway towards the suppression of LPS stimulated inflammatory responses in RAW264.7 macrophage cell lines. It includes the following activities, which have been presented in the following sections:

Section A

- Se quantification and bioaccessibility in Se-rich cereal crops with reference to non-seleniferous matrices;

Section B

- Selenium induced upregulation of anti-inflammatory responses; and

Role of rMETase in exuberating the bioaccessibility of Se from Se-rich cereal matrices.

Section A

Quantification and In-vitro bioavailability of Se from Osborne fractions of Se-rich cereal grains.

4.1 Selenium in soil

The total Se concentration in soil samples collected during different cropping seasons were analyzed by fluorescence spectrophotometer and presented as mean ± SD in table 4.1. Soil collected from seleniferous region of Nawanshahr-Hoshiarpur districts of Punjab showed 20 times higher levels of Se than soils of non-seleniferous region of Patiala, Punjab. Our earlier estimates of the Se concentration in soils of the same seleniferous region ranged from 2.7 to 6.5 µg/g, as analysed by neutron activation analysis (Sharma et al., 2009). Dhillon and Dhillon (1991) reported that soil in the said region had 4–5 times more Se than non-seleniferous regions. The concentration varied from 0.23 to 4.55 µg Se/g up to 180 cm depth of soil layers. Another group also reported this area to have significant impact of Se in irrigation water (340 µg/L) (Bajaj et al., 2011), resulting in hyperaccumulation of Se in plants and cereal grains cultivated on this soil (Dhillon and Dhillon, 2003a). In following years, Srivastava and his coworkers (2006) found a significant increase in Se levels at same site reaching up to 1.5 fold (479 µg/L) in ground water. The maximum permissible level for Se in water used for drinking is 10 µg/L and for irrigation is 20 µg/L, as recommended by United States Environmental Protection Agency (NAS-NAE, 1973). In China, a significant correlation of 0.58 ($P<0.001$) has been established between total Se and water soluble Se present in soils (Tamas et al., 2010). The soils of SeDef (selenium deficient) areas of China along with other countries like New Zealand, Denmark, Finland and the Atlantic Region of Canada contain only 0.01 to 0.6 µg Se/g (Reilly, 1996; Koivistoinen and Huttunen, 1986). Soil with Se concentration of ≤ 0.4 µg/g are generally considered SeDef (Plant et al., 2005), leading to reduced uptake of Se in diet (Rayman, 2008; Rayman, 2012), further causing numerous SeDef diseases, such as Keshan disease (Chen, 2012) and Kashin Beck disease (Jirong et al., 2012) in China. Therefore, bio-fortification of these SeDef areas was envisaged and experimented in Africa,

Australia, China, Finland, UK and other countries (Alfthan et al., 2000; Judson and Reuter, 1998; Koivistoinen and Huttunen, 1986), either through forage spraying or adding Se-rich fertilizers in soil. Such experiments have resulted in enhancing the dietary intake of Se as examplified by the foliage spraying of 0.2 to 0.5 μg sodium selenite per gram fertilizer in rice fields of Southern China providing an increased average dietary Se-intake from 6-18 μg/day to 50-100 μg/day (Hu et al., 2002).

The Se availability, its uptake by plants and utilization by livestock and human beings through food chain, ultimately depends upon the concentration and nature of Se-species present in soil. To investigate the absorptive properties of different Se-rich fertilizers, Gupta and Macheod (1994) spiked 10 g Se/hectare in SeDef field with sodium selenite, selcote and lime coated Se granules. Among these different chemical forms, sodium selenite showed better adsorption and higher uptake by plants. Biofortification of UK soil with selenate fertilizer resulted in conversion of selenate to 70% in the form of selenite and 30% to other soluble organic forms (mainly SeMet) in wheat crop (Stroud et al., 2010).

The extent of assimilation of these Se-species from soil up to the plant parts depends upon various agricultural practices like pH of soil, sulphur content present in fertilizers, redox potential of soil, aeration, water logging, rainfall, humidity etc., (Temmerman et al., 2014; Spadoni et al., 2007). The adsorption or absorption rate of Se-species is critically dependent upon pH of soil surface. At neutral pH, the selenite adsorption on soil particle predominates, whereas, shifting of pH range towards alkalinity, the solubility and absorbance rate of selenate ions increases (Goh and Lim, 2004). In contrast, under acidic conditions, the available Se complexes with iron hydroxide [$Fe(OH)_2$] molecules present in soil and thus reduces its assimilation by plant roots. Sulfur competence with Se is another factor due to similar properties which decreases its uptake by plants

(Terry et al., 2000). Dhillon and Dhillon (2000) studied the application of 0.8 ton gypsum (calcium sulfate) per hectare with significant reduction of Se accumulation by 50% in straw portions and 64% in grains of wheat and rice crops. Geering et al. (1967) observed the solubility of Se in soils were affected by redox potential created by ferric oxide-selenite complex. The presence of other ions in fertilizers like phosphate (Sors et al., 2005), chloride (Grieve et al., 2001), iron and aluminium (Jonnalagadda and Rao, 1993) forms complex with Se and further inhibits Se-uptake by plants. Under water logged conditions and poor aeration, the brown rice grains had accumulated only 0.029 μg Se/g from the soil, out of which the bioavailable form accounted for extremely low Se content up to 0.009 μg/g (Cao et al., 2001).

It can be interpreted from studies carried out since last two decades, by various research groups, that the soils of Nawanshahr-Hoshiarpur region have significantly high Se levels and therefore, it is being hypothesized that these soils can be used to produce fortified food products enriched with naturally bioavailable selenium. These matrices can further be used for formulating Se supplements according to the recommended intake values and be distributed amongst SeDef population. These cultivated Se-rich food products contain 90% of organic form of Se (dominantly as SeMet), that are relatively non-toxic and have long term retentive capacity in human body, providing slow and sustained release of bioavailable Se when required. Following section presents the data on the levels of Se in cereal grains grown in said seleniferous soils in comparison to those cultivated in non-seleniferous region.

4.2 Selenium in cereal whole grains

The concentrations of Se in whole grains of wheat grown on seleniferous area was 122.9 ± 0.6 μg/g when compared with Se level in grains (1.1 ± 0.03 μg/g) from a non-seleniferous area (Table 4.1, Figure 4.1). Se-rich wheat grains have significantly higher Se levels ($p<0.001$) with respect to

non-seleniferous wheat grains collected from non-seleniferous fields, with 112 folds increase in Se content in comparison to non-Se grains.

Table 4.1: Total Se content in dry weight of soil and cereal whole grains collected from seleniferous (Se) fields of Nawanshahr-Hoshiarpur districts of Punjab and their respective controls (NSe) grown on local fields of Patiala, Punjab. Samples were analyzed by fluorescence spectrophotometer with detection limit of 2 ng/mL. Results shown are representative of six independent analyses.

Sample	Total Selenium (µg Se/g DW)	
	Seleniferous (Se)	Non-seleniferous (NSe)
Soil	3.32±0.15	0.17±0.03
Wheat grains	122.86±0.6	1.11±0.03
Maize grains	26.45±0.2	1.29±0.63
Rice grains	19.72±0.2	1.28±0.52

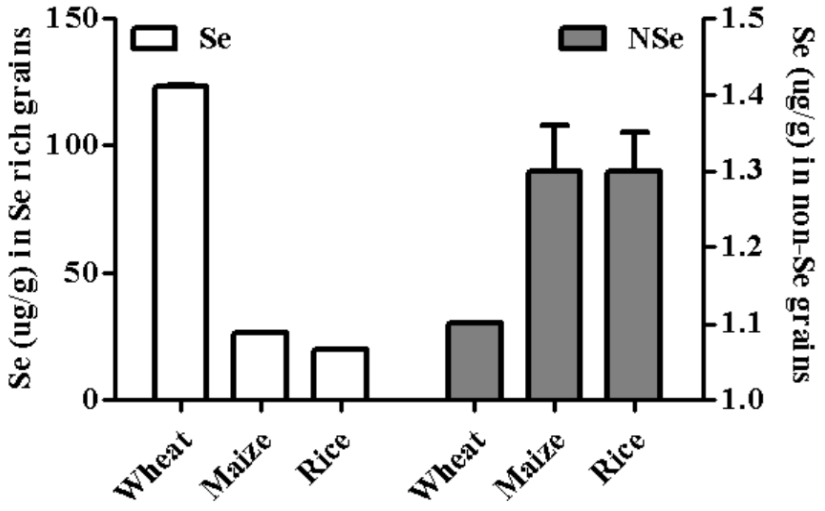


Figure 4.1: Concentration of Se in cereal grains from Se impacted (Se, scale at left) and normal soils (NSe, scale at right).

Depending upon the geographical location, globally, there is wide variation in Se content with extremely low levels of Se in wheat grains of Western Australia (0.001 µg/g) to moderately high values in South Dakota, U.S.A. (30 µg/g) (Lyons et al., 2004; White et al., 1981). Other countries like China (0.01-0.23 µg/g; Alfthan and Neve, 1996), Finland (0.01-0.015 µg/g; Koivistoinen and Varo, 1987), Eastern Europe and New Zealand (0.01-0.028 µg/g; Mihailovic et al., 1996) have sub-optimal levels of Se in wheat, wherein Se was biofortified to provide sufficient levels for normal supplementation in population. Different SeDef countries have attempted fortifying their crops by adding Se-enriched fertilizers in soil or direct forage spraying application. In Canterbury, New Zealand, the foliage spray of sodium selenate at the rate of 20 g/ha increased the Se concentration of wheat grain upto 0.5 µg/g in comparison to its control (0.03 µg/g) (Curtin et al., 2010). Even 6.45 g/ha (Tveitnes et al., 1996) or 10 g/ha (Stephen et al., 2012) sodium selenate supplementation has been reported to increase the Se concentration of wheat grain to desired level. A continuous case study of 17 years was conducted from 1982 to 1998 in United Kingdom, wherein fortification of their soils was initiated with Se fertilizers to meet the daily Se need (Adams et al., 2002). Sodium selenate application increased the Se content in wheat grains to 0.2 µg/g vis-a-vis in food products like wheat bread (6.4 to 7.1 µg Se/slice, approximately) (Hart et al., 2011). Belgian soil had low Se levels of 0.14 to 0.7 µg/g dry weight, which upon Se-fortification accumulates high Se in *Brassica* and *Allium* species (Temmerman et al., 2014). In Se-fortification process, selenate fertilizers are more efficient than selenite fertilizers (Poblaciones et al., 2014; Stroud et al., 2010). In contrast, the present study showed that the Nawanshahr-Hoshiarpur region of Punjab, India, naturally impacted with high Se soils, have Se levels in wheat grains significantly higher (123±0.6 µg/g) than those reported in other Se-contaminated countries (Aureli et al., 2012).

Se levels of rice grains collected from seleniferous site were 19.7 ± 0.2 μg/g when compared to Se content of grains (1.3 ± 0.5 μg/g) from non-Se site. This significant difference ($p<0.001$) showed 15 fold higher Se concentration in rice grains of Nawanshahr-Hoshiarpur region as compare to non-Se region (Patiala) of Punjab, India. The Se content in white polished rice samples were estimated globally to be extremely low (Table 4.2), which on average of 300 g rice consumption (per day per individual) failed to provide 70% of recommended values of Se intake (William et al., 2009). Sun and his coworkers (2010) estimated the Se distribution in rice bran was 1.94 times more than the whole grain followed by polished rice and husk. In China, two independent studies were conducted in year 2002, in which the SeDef rice grains were reported to have 0.015 - 0.046 μg Se/g. According to the China Nutrition Society, the daily consumption of 300-500 g of these SeDef rice per person provided insufficient amount of Se, i.e. 6-18 μg Se/day. Foliar spray of sodium selenite (14-18 g/ha) or sodium selenate (20 g/ha), to fortify the Se in crops significantly increased the grain Se content upto 0.18-0.042 μg/g and 0.47-0.64 μg/g, respectively. Among Se fertilizers used in these studies, selenate fertilizer proved 36% higher efficiency in increasing Se content of rice grains than selenite fertilizers (Chen et al., 2002; Hu et al., 2002). In the following years, Fang and his coworkers (2008) confirmed that the Se-fortification with 15 g/ha Se fertilizer application increased the rice grain Se content by 194% in China. The present study reports the highest Se concentration in rice grains (19.72±0.2 μg/g) in comparison to the previously reported at the same site (5-16.2 μg/g) by Dhillon and Dhillon (2009) and Sharma et al. (2009). An important observation in our results is that even non-seleniferous rice grains collected from Patiala region of Punjab, contained moderate amount of Se (1.28±0.52) in comparison to the different areas of other countries listed in table 4.2.

Maize grains collected from the same seleniferous region contained 26.5 ± 0.2 µg/g Se in comparison to non-seleniferous grains (1.3 ± 0.6 µg/g) collected from local sites of Patiala region. Se-rich grains have significant difference ($p<0.001$) with 20 fold increase in Se content when compared to the non-seleniferous grains. Maize is another important staple food consumed by population throughout the world. In Kenya, 96% of whole population consume maize as their primary staple food, in which the Se content ranges from 1.82±0.76 µg/g to 11.0±0.86 µg/g (Otieno et al., 2015). In other countries like South Africa (Courtman et al., 2012) and Malawi (Chilimba et al., 2011), 94% of maize samples contain only 0.005 µg/g Se content, which provide the dietary Se-intake of 6.7 µg Se/day per individual. This has led to several chronic health problems among SeDef areas.

Table 4.2: Global Se variation in white polished rice grains (William et al., 2009).

Country	Se concentration (µg Se/g DW)
China	0.002-1.37
Egypt	0.006-0.087
France	0.053-0.241
Ghana	0.021-0.254
India	0.035-0.371
Italy	0.032-0.158
Japan	0.026-0.109
Philippines	0.056-0.241
Spain	0.006-0.104
Thailand	0.006-0.487
U.S.A.	0.006-0.406

Even the capacity of Se-uptake among different cereal species (wheat, maize and rice) grown on similar seleniferous fields were significantly different from each other ($p<0.001$). Wheat grains

accumulate significantly high Se levels followed by maize and rice grains. Se content in Se-rich wheat grains was 4.6 fold and 6.2 fold higher than seleniferous maize and rice grains, respectively. The Se levels in the seleniferous area under study also indicated moderately higher levels when compared with global averages (Jaiswal et al., 2015).

For humans, the staple food including the cereal grains are the major source of Se supplementation (Combs, 2001). Large number of studies has been carried out by various scientists on speciation of the major forms of Se present in the cereal grains and it was found that up to 85% of total Se present is protein bound form of SeMet (Cubadda et al., 2010; Diaz et al., 2003; Guzman et al., 2009; Hart et al., 2011; Kirby et al., 2008; Olson et al., 1997; Olson and Palmer, 1976; Poblaciones et al., 2014; Sharma et al., 2009; Stadlober et al., 2001; Sun et al., 2010; Warburton et al., 2007; Whanger, 2002). Yang and his coworkers (1997) estimated the percentage of SeMet in wheat (50-81%), maize (46-82%) and rice (55-87%). Among the inorganic forms, selenate was identified as the major species present in wheat and rice, but not so significant in maize grains (Aureli et al., 2012). Selenate is more mobile up to the xylem and is less readily transformed into organic forms (Terry et al., 2000; Sager 2006), whereas selenite adsorbs on humid material present in soil, forms hydroxides and oxyhydroxides with iron, manganese, or aluminium and gets rapidly metabolized into SeMet or SeCys forms (Seby et al., 1998). Other species found are Se-methyl-selenomethionine, selenocysteine, Se-methyl-selenocysteine (Brody, 1994) and Se-containing sugars such as 2,3-dihydroxypropionyl-selenolanthionine (Aureli et al., 2012).

4.3 Elemental profile of seleniferous crops versus non-seleniferous crops

In the present study, the influence of Se levels have been observed on whole elemental profile and it is confirmed that the seleniferous crops have elevated mineral composition in comparison to the non-seleniferous crops. Potassium among macro elements and Se among micro elements were of

relatively high concentration than cereal grains grown on non-seleniferous soils. Calcium was 3.2%, 2.0% and 5.7% more in seleniferous wheat, maize and rice grains, respectively, with reference to non-seleniferous counterparts. The overall mineral composition of Se-rich wheat grains was significantly high ($p<0.001$) than maize and rice grains. As the Se trend in Se-rich wheat is followed by seleniferous maize and rice grains, the elemental profile follows the same (Table 4.3).

Table 4.3: Elemental composition (K, Na, Mg, Ca, Zn, Se, Mn and Cu) of seleniferous wheat, rice and maize grains versus non-seleniferous grains (µg Se/g) [n = 3].

Elements	Wheat		Maize		Rice	
	Se	**NSe**	**Se**	**NSe**	**Se**	**NSe**
Potassium	4871±11.4	4546±6.8	3881±7.2	3242±14.8	1545±2.1	756±2.0
Sodium	2499±3.5	483±3.2	679±1.4	227±1.9	536±2.7	187±1.8
Magnesium	1797±5.9	1215±4.7	955±8.8	747±6.9	270±6.2	231±3.8
Calcium	1319±9.8	420±5.2	220±2.9	115±0.4	301±0.9	53±0.2
Zinc	113±1.5	43±1.5	56±1.6	28±0.2	27±0.3	22±0.8
Selenium	123±0.6	1.1±0.0	27±0.2	1.3±0.6	20±0.2	1.3±0.5
Manganese	53±4.5	35±2.5	6±1.5	5±0.2	9±1.0	8±0.3
Copper	42±3.4	28±0.5	16±2.6	9±1.5	11±1.3	7±0.6

Earlier, it was reported that in whole wheat grains, the concentration of Mn, Fe, Zn, Cu and Se in µg/g were 46.0, 43.0, 35.0, 5.0 and 0.6 respectively (Miller, 1996). The buckwheat grain biomass contains 91% Zn, 87% P, 70% S, 62% Mg, 60% K, 54% Cu, 53% Mn and 35% Fe in endosperm, whereas, the husk contains 85% Ca, 84% Al, 83% Si and 76% Cl, 69% Ti and 46% Fe (Mikus et al., 2009). Maize grain have high K level (3248 µg/g) followed by P (2996 µg/g), Mg (1079 µg/g),

Na (592 µg/g), Ca (483 µg/g), Fe (48 µg/g), Zn (46 µg/g), Cu (13 µg/g) and Mn (10 µg/g) (Gu et al., 2015; Bressani et al., 1989). The polished rice have half the total Zn, two-third Fe and zero K present and after milling process, whole K, Ca and Mn content is removed (Villafane et al., 2017; Lu et al., 2013). However, Juliano (1985) observed that milled rice still retain 83% of N content, 74% Ca and 63% Na content in brown rice. Brown rice has better mineral content as compare to white polished rice (Antoine et al., 2012). In brown rice, Mg, K, Mn and P are present in outer portion with highest Mg/K ratio in rice kernals (Tomio et al., 2002). P, K, Mg high ratio is the characteristics of sticky rice (Kokot and Phuong, 1999).

The higher uptake of Se by these cereal grains seems to prevent the accumulation and toxicity of certain other elements like arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) by reducing their uptake from soil (Rogan et al., 2009). As increasing As content significantly decline the nutrient quality by limiting the uptake of other essential micronutrients (Williams et al., 2009), it is essential to understand the prevention of risk of As contamination. Carey et al. (2012) established the localization and speciation of Se and As in grains, in which Se helps in elucidating its transportation mechanism in grains. Similarly, in another heavy metal (Hg) contaminated area of Guizhou, China, 0.5 µg/mL sodium selenite application lead to least Hg accumulation in rice grains (Li et al., 2015). Se was reported to modulate (Zhao et al., 2014) and inhibit the uptake of Hg from soil (Zhang et al., 2012). Se (0.5 µg/g) is reported to decrease the metal mobility and thus, reduce the translocation of Cd and Pb by 71% and 33%, respectively (Hu et al., 2014; Lin et al., 2012). Se deficiency along with other mineral deficiency of iodine is the major reason for prevalence of goiter in Southwest zone of Shri Lanka (Fordyce et al., 2000). In contrast, Se-fortification along with Zn improves the Fe uptake and accumulation in wheat grains and hence, improving the nutrient quality of grains (D'Souza et al., 2014). Hence, Se-fortified dietary food

products also facilitate enhanced nutrient quality of essential micro and macro elements in comparison to non-Se food products, required for proper functioning and metabolism of body in addition to the supplementation of Se.

4.4 Osborne fractions and selenium content

The amount of Osborne fractions with reference to the total protein yield and Se content is listed in table 4.4. Referring to the protein yield among four protein fractions, the glutelin fraction dominated over other fractions across all the grains tested. This fraction has viscoelastic properties which allows the dough to be processed into food products like bread, pasta and noodles etc. (Shewry, 2009). Earlier it was reported that 31.3% of the total protein fraction (83.5%) was glutelin containing maximum Se content (52.3%) (Fang et al., 2010; Kamara et al., 2009) which matches with our results. Kim et al. (2013) estimated the Osborne fractions yield in rice grains in which brown rice had 7.3% glutelin, 1.9% albumin, 0.5% globulin and 0.05% prolamin, whereas milled rice had 8.4% glutelin, 0.5% albumin, 0.3% globulin and 0.1% prolamin. The previous reports from other researchers showed that albumin and globulin content was majorly present in bran part of rice grains, whereas prolamin was evenly distributed throughout the endosperm in minute quantities (Cagampang et al., 1966). Prolamin, despite being the lowest among all protein fractions, is the best storage protein with high lysine content (Doll, 1977) and our observations further confirm this aspect as indicated by significant Se levels in this fraction, across all grains (Figure 4.2). In our results, the overall Se content in prolamin and glutelin fractions was higher than in other proteins, except in case of rice. A multiple comparison test showed significant variation ($p<0.05$) in Se levels across grains but not significant amongst proteins. Nearly similar observations were also obtained in wheat samples

Table 4.4: Percent protein yield and corresponding selenium content of protein fractions.

Fraction	Wheat		Maize		Rice	
	Protein Yield (%)	**Se Conc. (µg/g)**	**Protein Yield (%)**	**Se Conc. (µg/g)**	**Protein Yield (%)**	**Se Conc. (µg/g)**
Albumin	11 ± 0.1	401 ± 1.9	20 ± 0.8	280 ± 2.6	8 ± 0.9	28 ± 1.5
Globulin	24 ± 2.6	264 ± 4.2	34 ± 0.8	192 ± 0.8	26 ± 2.2	241 ± 1.6
Glutelin	49 ± 2.1	563 ± 2.1	38 ± 1.1	359 ± 2.3	59 ± 1.8	177 ± 1.3
Prolamin	16 ± 1.1	629 ± 6.9	8 ± 0.6	339 ± 1.3	7 ± 0.5	257 ± 1.3

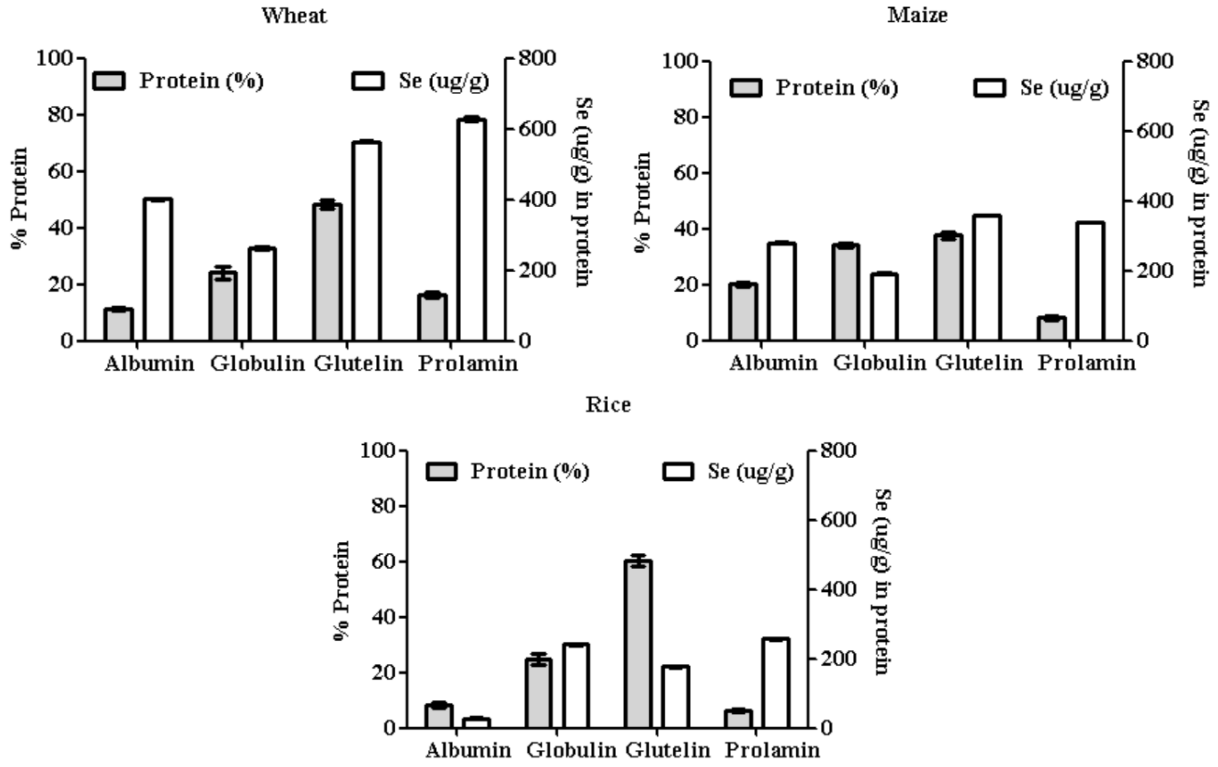


Figure 4.2: Selenium content in different protein extracts of various cereal grains, protein yield at right scale and Se content at left scale.

obtained from other locations in the seleniferous belt (Jaiswal et al., 2015). Correspondingly, Se levels in the residual starch were below detectable limits with respect to the presence of 2,3-dihydroxypropionyl-selenolanthione (*m/z* 345), hexose moiety (*m/z* 317 and *m/z* 358), and hexose pentose moiety (*m/z* 407 and *m/z* 408) as selenosugars in cereal grains (Aureli et al., 2012). In the spent residue, Se was present in traces with 2.0 ± 0.7, 0.5 ± 0.2, and 1.0 ± 0.1 µg/g concentration in wheat, maize, and rice, respectively, which was considered as percent residual loss.

Abundance of methionine and cysteine in each fraction represents nonspecific incorporation of SeMet and SeCys, respectively, into proteins (Strub et al., 2003). Aureli et al. (2012) reported that the total Se in rice is present in organic form (SeMet) dominantly in the glutelin fraction (31.3%) and only about 3% is possibly available as inorganic Se.

In turn, Se-methyl-selenocysteine and selenocystathionine are present in nonprotein fractions of Se-accumulating plants. During maturation phase of plants, the peptide subunits of these Osborne fractions vary significantly (Chrastil and Zarins, 1994), with high nutritional quality in storage proteins (Santos et al., 2013). It would, therefore, be of further interest to understand the profile of Se moieties in these Se-rich Osborne fractions. The degree of bioaccessibility, bioavailability and bioactivity of Se from different fractions will be discussed in next section on *in vitro* gastrointestinal digestion which describes the absorptive capacity of gastric and intestinal enzymatic process.

4.5 Selenium bioavailability

To access the degree of bioavailability and bioaccessibility of Se from Se-rich whole grains and their protein fractions, the present study was conducted following stimulated *in vitro* gastrointestinal digestion. The aim of this component of the study was to quantify the extent of Se absorption in *ex-vivo* conditions from cereal whole grains and their respective Osborne fractions.

G/GI method is a useful primary screening technique which gives an idea of how much of Se is bioaccessible from seleniferous dietary source, nature of food matrix and to select a considerate amount of healthy Se-intake as compare to normal Se-intake.

Different parameters like pH of gastric and intestinal juices; pepsin and pancreatic enzyme concentration; temperature; and time allowed to complete the process were mimicked with human enzymatic digestion conditions. After complete gastric and intestinal digestion, the clear extract with soluble Se content, released from whole grain flour and their respective protein fractions, were analyzed using fluorescence spectrometer. The mean ± SD percentage of bioaccessibility of Se from different samples after gastric and intestinal break down are listed in table 4.5 and table 4.6, respectively.

The bioavailability of Se in different protein fractions is directly proportional to its concentration in the fractions. Results indicate that the predigested food in stomach (G) with acidic pH partially solublizes the Se embedded in cereal matrix, which upon intestinal digestion (GI, neutral pH, pancreatin and bile salts), breaks down the Se-containing proteins.

The *in vitro* gastrointestinal digestion showed that cereal grains (Jaakkola et al., 1983) have higher Se bioavailability than from animal foodstuffs (Combs, 1988). For an example, G/GI digestion of fish and mollusk was reported to be as low as 6.7 ± 3.4% and 5.5 ± 2.4%, respectively (Pineiro et al., 2013). Whereas, from cereals (10-24%), pulses (12-29%) and green leafy vegetables (10-31%), the bioavailable fraction was significantly higher (Khanam and Platel, 2016). Se bioavailability depends upon species present in dietary food, in which SeMet is recommendable over inorganic-Se intake in a balanced diet (Thiry et al., 2012). Hakkarainen (1993) reported the Se bioavailability from wheat (83–100%), barley (78–85%), oats (41–45%),

Table 4.5: Percent bioaccessibility of Se from Osborne fractions of Se-rich cereal grains during gastric digestion (G).

Fraction	% Bioaccessibility (G) (mean ± SD)		
	Wheat	**Maize**	**Rice**
Whole grain	74.9 ± 8.0	59.1 ± 5.9	51.4 ± 9.0
Albumin	78.3 ± 0.8	78.8 ± 1.0	42.2 ± 5.0
Globulin	74.2 ± 2.3	75.1 ± 1.2	91.3 ± 2.2
Glutelin	79.4 ± 0.8	60.2 ± 1.0	70.7 ± 2.0
Prolamin	78.3 ± 0.5	44.8 ± 1.1	94.2 ± 1.0

Table 4.6: Percent bioaccessibility of Se in cereal grains and their respective protein fractions after gastric digestion followed by intestinal digestion (GI).

Fraction	% Bioaccessibility (GI) (mean ± SD)		
	Wheat	**Maize**	**Rice**
Whole grain	97.8 ± 2.1	95.2 ± 1.6	82.4 ± 4.4
Albumin	**88.3 ± 0.5**	**99.9 ± 6.5**	70.8 ± 6.8
Globulin	79.4 ± 1.5	98.4 ± 4.9	95.1 ± 1.8
Glutelin	85.3 ± 1.0	95.1 ± 1.8	81.5 ± 3.1
Prolamin	82.0 ± 1.3	80.4 ± 2.3	97.4 ± 2.4

fish (64–80%), and meat meal (22–30%). In addition, the *in vitro* G/GI digestion of plant products, such as selenized green onion and chive samples revealed that the G/GI fluids oxidize the selenite and SeMet species into selenate and SeOMet forms. The bioavailability of these oxidized forms further results in their better bioaccessibility to carry out Se dependent metabolic functions (Kapolna and Fodor, 2009). Among selenate and selenite supplementation, selenate bioavailability remains stable during G/GI digestion. However, the presence of SeMet in various Se enriched food

crops produce several minor Se-metabolites during digestion (Lavu et al., 2016; Cubadda et al., 2010). Thus, this variation in the bioavailability of major fraction (SeMet) indicate the efficacy of G/GI enzymes in facilitating complete breakdown of selenized species into simpler forms leading to their bioaccessiblity and for further utilization by human body.

The bioaccessible fraction depends upon two main factors, i.e., the amount of bioavailable Se and chemical nature of Se species present (organic or inorganic) in food matrix. In accordance to the first factor, the present data showed significant variation across the cereal grains (Table 4.4).

The food supplements mainly provide organic Se species, dominantly SeMet followed by SeCys, Se-methyl SeCys, selenite, selenate and low molecular Se-species (Lavu et al., 2012; Thiry et al., 2012). The G/GI digestion of SeMet-enriched bread containing 80% of selenomethionine showed 100 ± 3% Se-bioaccessibility (Martinez et al., 2015). Various selenocompounds were also identified during G/GI digestion in which 89 ± 3% of total Se present was SeMet and 11 ± 1% was still present in left-over behind residue as non-protein bound Se (Reyes et al., 2006). Da'Silva et al. (2013) reported the presence of SeMet and SeCys in Brazilian nuts through G/GI digestion, in which 74% of bioaccessible fraction was SeMet containing 54.8 ± 4.6 μg Se/g. Whereas, SeCys was majorly detected in urine samples, indicating that amongst the organic fractions present in nuts or food matrix, SeMet was a major bioaccessible form (Cubadda et al., 2010).

In present study, the bioaccessible percentage of Se from maize whole grains after G and GI digestion was 59.1 ± 5.9% and 95.2 ± 1.6%, respectively, which is significantly higher than reported earlier, as out of total Se concentration present in maize flour (29 μg/g), 32% and 51% was bioaccessible after G and GI digestion, respectively (Jaiswal et al., 2012). However in rice grains, the Se bioaccessibility was low. The Se in G and GI digested whole grains was 51.4 ± 9.0% and 82.4 ± 4.4% bioaccessible, respectively (Table 4.5 and 4.6). It is presumably due to: a) the

lower Se accumulation in rice grains; and b) the small proportion of Se-containing protein being still bound to the starch matrices (Fang et al., 2010). Among Osborne fractions, Se from albumin of GI digestion of wheat and maize showed higher bioaccessibility, whereas, in rice grains, Se from prolamin was higher. The order of Se availability after complete GI digestion in different protein fractions of different cereal grains were highly variable. The bioaccessibility and bioactivity of Se from water soluble fraction (presumably albumin fraction) of maize matrix was significantly higher in comparison to Se from wheat matrix, as indicated in the later section (4.7.1). It is due to the Se species present in aqueous fraction of maize grains that were observably more bioaccessible (100%) upon GI digestion when compared to the wheat grains (~88%) (Table 4.6).

About 60% of predigestion of the Se enriched food products is already completed in stomach followed by remaining 20-40% in intestine (Lavu et al., 2012). Se from cereal grains in organic forms (SeMet and SeCys) are more bioaccessible and are efficient reservoirs for long-term storage (Behne et al., 2009). In Se-rich garlic, the fractions of G/GI digestion indicated the presence of SeMet, Se-methyl SeCys and γ-glutamyl-Se-methyl SeCys in decreasing order of their abundance as bioaccessible species (Dumont et al., 2006). The HPLC-ICP MS and ES-MS quantified 12% of selenite in G/GI processed canned cod (Crews et al., 1996). Canned items had lower Se bioaccessibility than fresh raw food (Afonso et al., 2015). Other than plant sourced items, the selenized fermented milk contains 76 ± 3% of bioaccessible and 24 ± 6% of insoluble Se, in which SeCys and Se-methyl SeCys species were most abundant (Alzate et al., 2010).

Section B

Selenium dependent regulation of inflammatory responses in lipopolysaccharide-stimulated RAW264.7 murine macrophages.

This section describes the role of organically bound selenium in resolving the LPS-induced inflammatory response in RAW264.7 macrophage model, with reference to the selenite supplementation. Further, the efficacy of rMETase treatment in enhancing the accessibility of bound selenium on upregulation of GPx-1 and H-PGDS genes and corresponding down- regulation of COX-2, mPGES-1 and iNOS genes at both pre-transcriptional and post-translational levels is being demonstrated.

4.6 Cell viability

Different forms of Se supplementation viz., sodium selenite (SS), selenium-rich wheat/maize extract (SeW/SeM) in resolving LPS induced inflammation on RAW264.7 macrophages. All the sources of Se were observed to be non-toxic with 100 percent viable cells at 500 nM concentration of Se, as indicated by MTT assay.

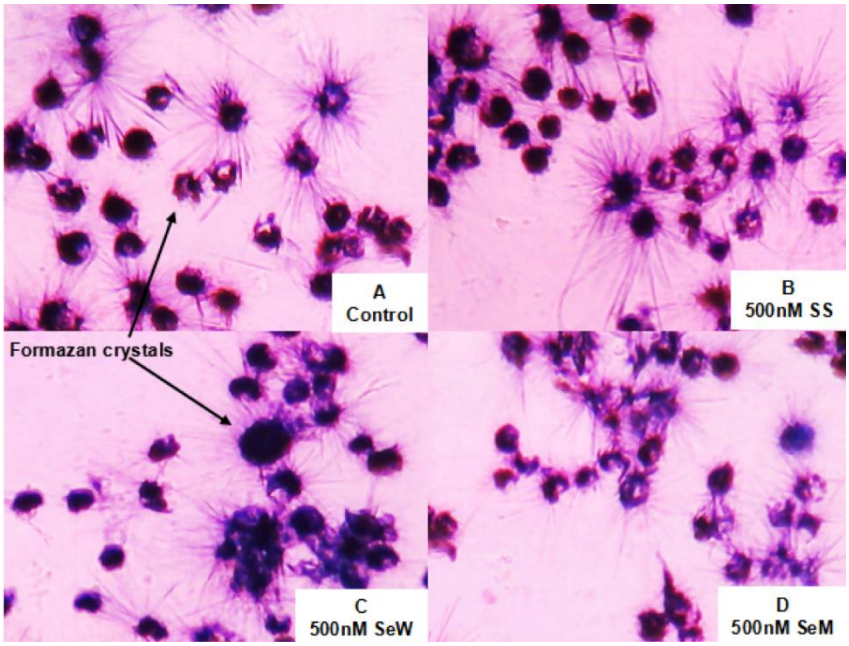


Figure 4.3: Microscopic image (at 400X) showing formazan crystals formed in live cells of RAW264.7 macrophages, i.e., control (A) and pretreated with 500 nM sodium selenite (B); 500 nM seleniferous wheat extract (C); 500 nM seleniferous maize extract (D).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is the well-known water soluble, yellow coloured tetrazolium salt used for colorimetric assay. It gets reduced by NADPH dependent dehydrogenase enzyme into purple coloured formazan crystals (Figure 4.3), only in metabolically active cells. These crystals can be solubilized in DMSO and quantified spectrophotometrically. Cell viability is directly proportional to the extent of MTT reduction and intensity of purple colour production.

During oxidative stress, the free radicals generated by oxidation of low density lipoproteins initiate the cytotoxic and apoptotic effects and results in cell death. Sodium selenite, at 100 µM, was reported to result in generation of RONS and apoptotic condition (Song et al., 2009). In contrast, the lower concentration of sodium selenite supplementation from 50 nM to 500 nM did not affect the cell cycle and had no variation in morphological structure (Vunta et al., 2008; Zeng and Botnen, 2007). Thus, it suggested that Se supplementation up to 500 nM provides anti-toxic and anti-apoptotic effect along with protection from organic peroxides.

4.7 GPx as a marker of selenium supplementation

GPx is the Se containing enzyme, in which the selenol group of selenocysteine is present at the catalytic site of the enzyme (Roy et al., 2005). The increase in Se levels accounts for the up regulation of gene expression of this antioxidant selenoenzyme, GPx (Vunta et al., 2008; MacPherson, 1997; Reilly, 1996). For studies at cellular and organismal level, Se-status in plasma (SelP) is a useful biomarker of GPx activity and GPx saturation is considered to be pre-requisite for efficient antioxidant functioning (Duffield et al., 1999; Thomson et al., 1993).

In present study, GPx-1 expression was found negligible in RAW264.7 macrophages that were devoid of Se supplementation (control). However, the expression increased up to 9.4 fold with 1500 nM sodium selenite supplementation. At 50 nM of Se supplementation, GPx-1 expression

reached at saturation point with 7.7 fold increase, beyond which the expression levels stabilized (Figure 4.4). Earlier reports showed that GPx levels saturated at 100 nM sodium selenite with 10 fold increase in GPx-1 expression in RAW264.7 cells (Vunta et al., 2008). The present study further confirmed the expression profile of GPx-1 under similar conditions.

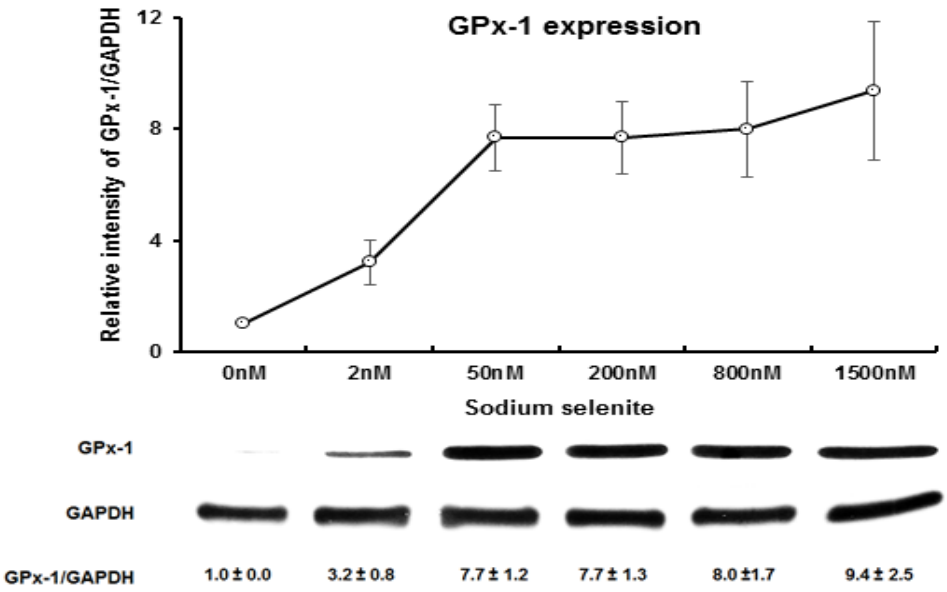


Figure 4.4: GPx-1 expression profile with exogenous sodium selenite supplementation in RAW264.7 cells. Densitometric values normalized to GAPDH are shown below each panel with mean ± SD of triplicate samples.

Se is an antioxidant that modulates RONS, inflammation and other immune responses (Hoffmann and Berry, 2008). The role of selenoproteins have already been well-documented in regulation of intracellular redox signaling through mitigation of RONS induced oxidative stress (Papp et al., 2007). Being one of the major selenoprotein, i.e., GPx, maintains the whole mammalian antioxidant network in prevention of lipid peroxidation with reduction of phospholipid H_2O_2 in membranes, mitigating inflammation, atherogenesis, neurodegeneration and resistance to γ-

radiation (Conrad et al., 2007; Yant et al., 2003). Depending upon the state of inflammation, it has pivotal role in regulation of macrophage markers from classically activated M1 markers (inflammatory precursors) towards alternatively activated M2 markers (inflammatory suppressor). M1 macrophage markers (TNF-β and IL-1β), tend to produce RONS in SeDef-GPx knockout mice, whereas, M2 markers (Arg-1, Fizz1 and Mrc-1) attenuates RONS with Se supplementation maintaining GPx activity. Therefore, Se supplementation mediates PPARγ dependent macrophage mode switch on from M1 to M2 type, resolving inflammatory process and wound healing (Nelson et al., 2011).

Dietary intake of supranutritional doses of Se increases the GPx activity, up to 16-18 times, within hours (Sun et al., 1998). This increased activity gives significant protection against inflammation, apoptosis and carcinogenesis (Krehl et al., 2012). Se supplementation can increase the GPx-1 and GPx-2 expression by 13 and 5 fold, respectively, preventing the hydroperoxide damage in $CaCO_2$ cell line and balancing the oxidative challenge (Wingler et al., 2000). These two isoforms are also inflammatory markers of inflammatory bowel disease (IBD) (Te-Velde et al., 2008), that prevent the inflammatory response of intestinal mucosa (Esworthy et al., 2001; Tham et al., 1998). Immunohistochemistry revealed that in the presence of Se, GPx-2 increases the expression of its isoform GPx-1 in crypt bases of ileum and colon where it is typically localised (Florian et al., 2010).

Pivotal role of Se- dependent GPx-1 against RONS is known to mitigate inflammation and bolster immunity that involves down-regulation of COX-2 and mPGES-1, while upregulating H-PGDS to effect a process called “eicosanoid class switching mechanism” (Yui et al., 2015; Duntas, 2009; Vunta et al., 2007). However, in SeDef conditions, there is increased ROS generation that is closely associated with increased inflammatory genes and augmentation of oxidative stress, which further

exacerbates the modulation of pro-inflammatory cytokines via activation of transcription factor NFκB (Cai et al., 2015). Prolonged NFκB activation results in neuro-inflammation followed by neurotoxicity, as it is ubiquitously expressed in neurons (Shih et al., 2015). GPx-1 deficiency under atherogeneses, increased the adhesion molecule expression by augmenting NFκB activation, promoting the pro-inflammatory state (Lubos et al., 2011). Therefore, increased GPx-1 expression upon Se supplementation is necessary to keep up the antioxidant activity and down regulation of pro-inflammatory eicosanoids including NFκB deactivation. In present study, the increase in GPx-1 expression is directly proportional to the concentration of Se supplementation and it further increases upon LPS stimulation, which proves that under oxidative stress, cellular GPx-1 protects the cells from systemic manifestation of reactive oxygen species.

Keeping in view, the concentration of total Se present in Se-rich cereal grain matrices, further studies on resolution of inflammation by organic/inorganic Se was carried out with Se concentrations ranging from 50-500 nm.

The expression profile of Se-dependent pro-/anti-inflammatory genes were measured using RAW264.7 macrophage model. Cells were grown in various conditions as SeDef (Control, NSeW, NSeW+rMET, NSeM and NSeM+rMET) and Se supplemented media (SS, SeW and SeW+rMET, SeM and SeM+rMET) with different Se concentrations (50 nM, 100 nM and 500 nM). The anti-inflammatory enzyme expression of GPx-1 was increased with increasing sodium selenite concentration (500 nM > 100 nM > 50 nM > 0 nM, 0h) of SeSup cells with 3.5 fold higher expression as compared to SeDef cells (Figure 4.5 A). With oxidative stress, induced due to LPS stimulation, control RAW264.7 cells that were devoid of Se, also showed mild GPx-1 expression after 4 h (Figure 4.5 A) which clearly indicates the self defense mechanism against the ROS generation. However, upon LPS stimulation followed by incubation of up to 4 h, Se

supplementation increased GPx-1 levels to a significant extent with increasing concentration (500 nM > 100 nM > 50nM) with respect to control i.e., from 2.4 fold at 50 nM to 9.0 fold at 500 nM (Figure 4.5 A).

4.7.1 rMETase facilitated bio-availability of selenium from cereal matrices

The figure 4.5 shows both western blots and mRNA profiles of GPx-1/GAPDH expressions, with reference to role of rMETase on facilitation of Se from Se-rich cereal matrices in comparison to the sodium selenite supplementation. rMETase is the recombinant L-methionine γ-lyase or methioninase, a pyridoxal 5'-phosphate containing enzyme. It catalyzes the α- and γ-elimination of methionine, sulfur containing amino acids. Likewise, it is able to cleave C-Se and C-O bonds of selenium and oxygen containing amino acids (Fukumoto et al., 2012; Nakayama et al., 1984) and making them optically more active and bioavailable in functioning (Tanaka et al, 1985).

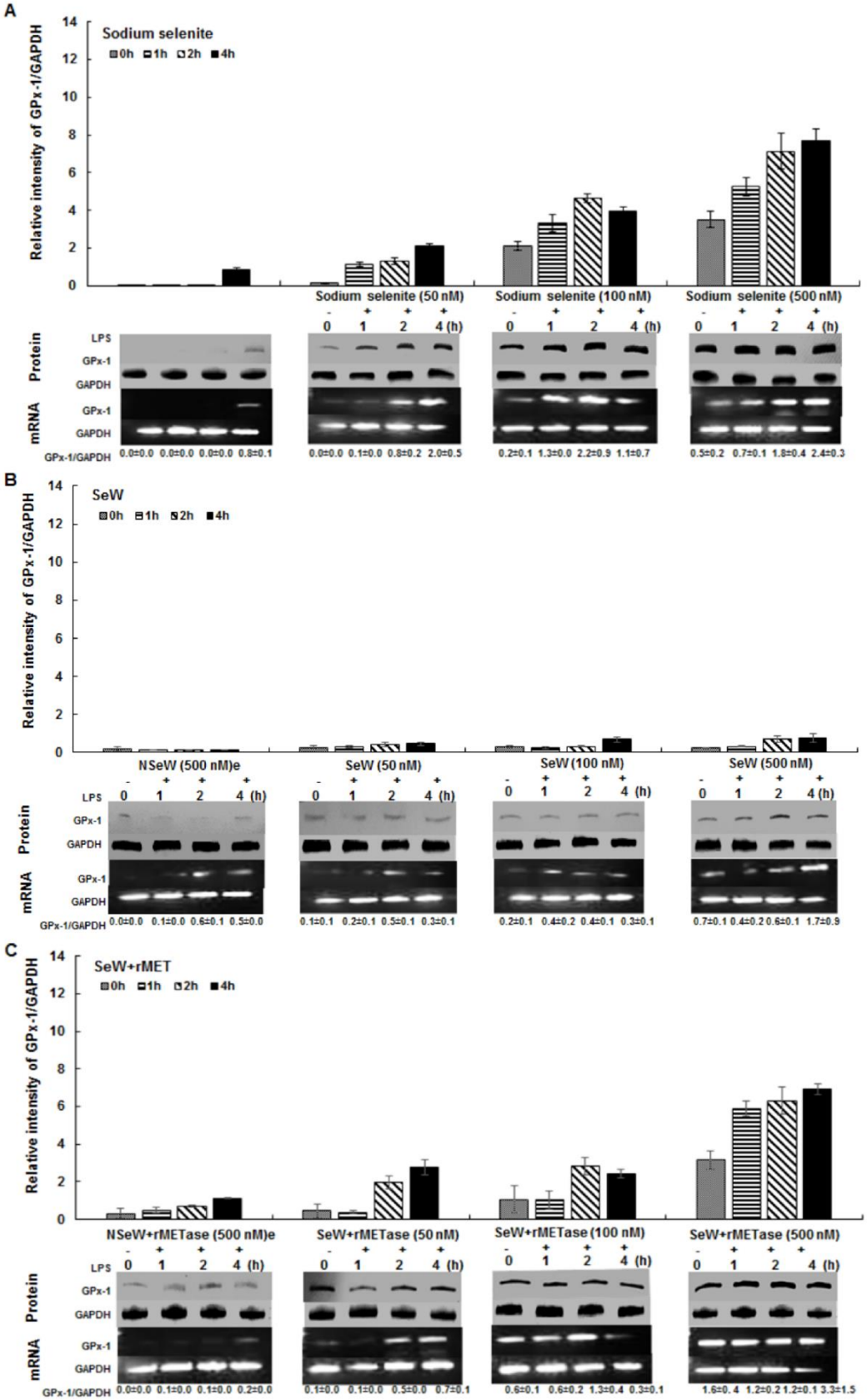
Se-supplementation in the form of seleniferous wheat extract (SeW) marginally elevated the overall GPx-1 expression when compared to non-seleniferous wheat extract (NSeW). Even at maximum concentration of SeW tested (500 nM), RAW264.7 cells showed 1.1 fold increase in GPx-1 expression at 4 h of LPS stimulation with respect to control (C) (Figure 4.5 B). The SS and SeW+rMET incubated cells showed 9.0 and 8.0 fold higher GPx-1 expression, respectively, over SeW supplemented cells at similar concentration and time. This clearly demonstrates that Se bound to the wheat matrix was not completely bio-available and accessible to the cells. Hence, to facilitate the accessibility of Se and assess its efficacy on GPx-1 expression, cells were incubated with rMETase (Gandhi et al., 2011) at 0.2 units/mL along with SeW extract. The observations clearly indicated enhanced GPx-1 expression trending similar to SS-stimulated cells, as a function of the Se released from matrices. Our results also showed the significant difference with rMETase application in comparison to controls with facilitation of free Se from wheat matrices, impacting

the elevated trend of anti-inflammatory enzymes and down-regulation of pro-inflammatory enzymes.

In LPS unstimulated (0h) SeW+rMET supplemented cells, the GPx-1 expression enhanced up to 3.2 fold with increase in Se concentration (500 nM > 100 nM > 50 nM), in comparison to NSeW+rMET incubated cells (Figure 4.5 C). Under similar conditions, the supplementation of SeW extract showed poor GPx-1 response of up to 1.1 fold (Figure 4.5 B). At 4 h of LPS stimulation, further GPx-1 expression increased to 6.2 fold with respect to NSeW+rMET (Figure 4.5 C).

Similarly, with Se-supplementation in the form of seleniferous maize extract (SeM), GPx-1 was 1.5 to 2.3 fold higher than wheat matrix (SeW, 0.2 to 0.3 fold) at 50 to 500 nM supplementation (Figure 4.5 B, D). However, with rMETase treatment and 500 nM SeM+rMET supplementation (without LPS stimulation, 0h), the GPx-1 expression was up to 8.6 fold in comparison to SeM (2.3 fold). Upon LPS stimulation of 4h, 500nM SeM+rMET showed 14.0 fold increase in GPx-1 expression as compare to SeM treated cells (5.0 fold) (Figure 4.5 D, E).

From these observations, it was evident that, although wheat grains accumulate higher Se levels than maize grains, the accessibility and activity of Se from water soluble fraction of maize extract was significantly higher than that of wheat extract when supplemented with RAW264.7 macrophages. As albumin is highly water soluble with binding sites on its surface that adsorb positively or negatively charged molecules (Se in this case), it is expected to effectively deliver the non-covalently bound Se molecules to various parts of the body (Bronze-Uhle et al., 2017).


A
Sodium selenite
0h 1h 2h 4h
Relative intensity of GPx-1/GAPDH
Sodium selenite (50 nM)
Sodium selenite (100 nM)
Sodium selenite (500 nM)
LPS
Protein
GPx-1
GAPDH
mRNA
GPx-1
GAPDH
GPx-1/GAPDH
0.0±0.0 0.0±0.0 0.0±0.0 0.8±0.1
0.0±0.0 0.1±0.0 0.8±0.2 2.0±0.5
0.2±0.1 1.3±0.0 2.2±0.9 1.1±0.7
0.5±0.2 0.7±0.1 1.8±0.4 2.4±0.3
B
SeW
0h 1h 2h 4h
Relative intensity of GPx-1/GAPDH
NSeW (500 nM)e
SeW (50 nM)
SeW (100 nM)
SeW (500 nM)
LPS
Protein
GPx-1
GAPDH
mRNA
GPx-1
GAPDH
GPx-1/GAPDH
0.0±0.0 0.1±0.0 0.6±0.1 0.5±0.0
0.1±0.1 0.2±0.1 0.5±0.1 0.3±0.1
0.2±0.1 0.4±0.2 0.4±0.1 0.3±0.1
0.7±0.1 0.4±0.2 0.6±0.1 1.7±0.9
C
SeW+rMET
0h 1h 2h 4h
Relative intensity of GPx-1/GAPDH
NSeW+rMETase (500 nM)e
SeW+rMETase (50 nM)
SeW+rMETase (100 nM)
SeW+rMETase (500 nM)
LPS
Protein
GPx-1
GAPDH
mRNA
GPx-1
GAPDH
GPx-1/GAPDH
0.0±0.0 0.1±0.0 0.1±0.0 0.2±0.0
0.1±0.0 0.1±0.0 0.5±0.0 0.7±0.1
0.6±0.1 0.6±0.2 1.3±0.4 0.3±0.1
1.6±0.4 1.2±0.2 1.2±0.1 3.3±1.5

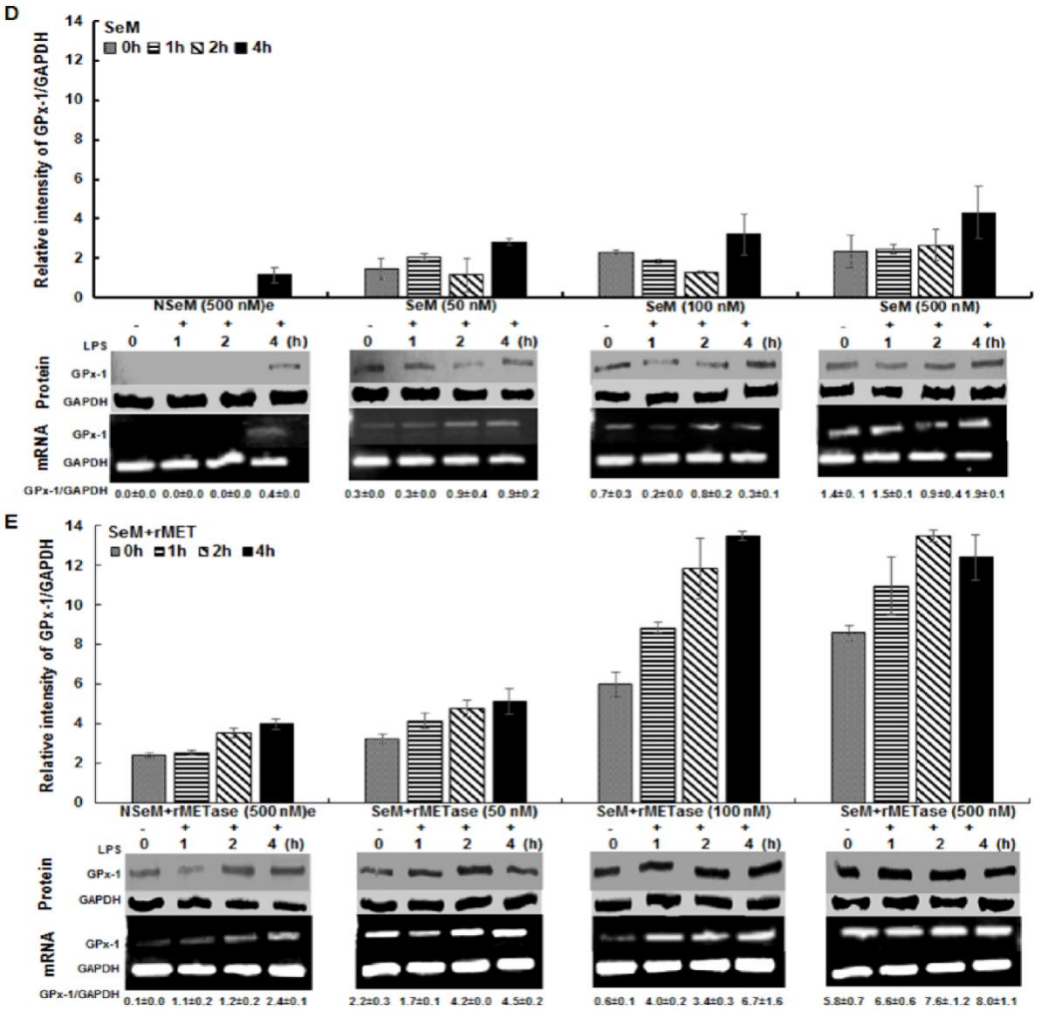


Figure 4.5: Western blots and mRNA profiles of GPx-1 expression in RAW264.7 macrophages [n=3]. Cells were treated with 50nM, 100nM and 500nM Se-concentration in different forms: (A) Sodium selenite (SS), (B) Seleniferous wheat extract (SeW), (C) Seleniferous wheat extract in presence of rMETase incubation (SeW+rMET) (D) Seleniferous maize extract (SeM) and (E) Seleniferous maize extract in presence of rMETase incubation (SeM+rMET) with respective to their controls, for 72 h followed by inflammation induced with 1 μg/mL LPS for 4 h. Densitometric values normalized to GAPDH and mean±SD and values are given below each panel of mRNA expression.

Additionally, maize albumin containing feruloylated oligo-/polysaccharides, exhibit enhanced antioxidant activity in comparison to wheat extract (Yang et al., 2014). Even the non-seleniferous maize grains had notable GPx-1 expressions in comparison to non-seleniferous wheat grains

with/without rMETase treatment (Figure 4.5 B, C, D and E), which proves that the bioaccessibility and bioactivity of even low levels of Se in these controls significantly differs in different cereal crops due to the nature and properties of these matrices.

Collectively, our data confirms the role of rMETase in facilitating the release of bio-available Se from Se-rich maize and wheat grain (SeM+rMET and SeW+rMET) and its antioxidant expression in comparison to the SeM and SeW supplementation, respectively. The accessibility and activity of Se from SeM and SeM+rMET was significantly higher than SeW and SeW+rMET.

About 72-85% of selenocompounds present in these Se-rich cereal grains account to selenomethionine (SeMet) (Cubadda et al., 2010), which is the major form consumed by humans (Finley, 2006). It can thus be hypothesized that Se-rich natural dietary matrices facilitate sustained and slow release of Se through enzymatic cleavage of SeMet to be metabolized to bioactive forms that increase plasma Se levels with better anti-oxidant and anti-inflammatory capacity (Cao et al., 2014). SeMet supplementation in minute concentration up to 3 μg/g abrogates the allergies caused due to these inflammatory responses under Se deficiency (Sakazaki et al., 2014). The present study confirms that although the overall trend of cellular GPx expression increases with increase in Se supplementation through Se-rich cereal grains, the release of SeMet from these matrices was less accessible in comparison to the enzyme treatment (rMETase). Similarly, the enzymatic processes in biological systems can also facilitate sustained release of Se from bound organic dietary matrices during supplementation.

4.8 COX-2 as a marker of inflammation

The cardinal signs of inflammation, i.e., dolor (pain), calor (heat), rubor (redness), tumor (swelling) and functio laesa (loss of function), switch on the expression of main three inflammatory signaling indicators, a) inflammatory cyclooxygenase mediators (COXs), b) oxidant generating

enzymes (iNOS), c) arachidonic acid or p38 MAPK signaling pathway that leads to NFκB activation (Ohshima et al., 2005; Prabhu et al., 2002). The COXs mediators upon receiving the inflammatory signal, initiate the arachidonic acid pathway to express the prostaglandin H synthase which further catalyzes synthesis of PGH_2. Depending upon the state of inflammation, i.e, acute or chronic, PGH_2 starts stimulating substances such as eicosanoids (Wallace, 2002). There are four principle bioactive eicosanoids including PGD_2, PGE_2, PGI_2 and PGF2α, which are ubiquitously produced as lipid mediators to maintain the homeostatic condition of body. These help in cell proliferation, permeability, blood clotting, tissue repair and inflammation. Therefore, the regulation and synthesis of these eicosanoids finally depend upon the release of synthase enzymes like COXs, which through cyclooxygenase pathway start releasing inflammatory signals (Smith et al., 2000).

COXs have two isoforms, i.e., COX-1 and COX-2. COX-1 is the housekeeping enzyme with cytoprotective properties. It is constitutively expressed throughout the gastrointestinal mucosa so as to maintain the homeostatic condition (Ricciotti and FitzGerald, 2011). In contrast, COX-2 is an inducible isoform, expressed under oxidative insult, in addition to malfunctioning of hormones, etc. It is one of the nine genes of arachidonic acid metabolism (annexin-I, annexin-II, S100A8, S100A10, S100P, GPx-3, phosphotidyl choline transfer protein, aldo-keto reductase family 1 and COX-2) that up regulates in sepsis-condition of oesophageal squamous cell carcinoma (Zhi et al., 2003). It preferentially couples with PGE_2, PGI_2 and $PGF_2\alpha$ isoforms that respond to inflammatory expressions. COX-2 and oxidative stress is directly linked to each other (Pathak et al., 2005). To combat the initial phase of acute inflammation, COX-1 expresses in the target tissue, however, COX-2 appears to be dominant during adverse inflammatory effects and in present study, these adverse effects were induced with 1 μg/mL bacterial endotoxin lipopolysaccharide in RAW264.7

macrophages to reflect the COX-2 expression. The intensity of the expression increased up to 9.2 fold with increase in duration of LPS stimulus from 0 to 24 h (Figure 4.6).

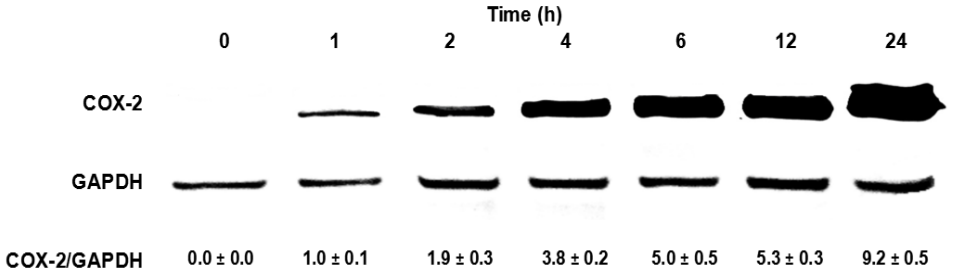
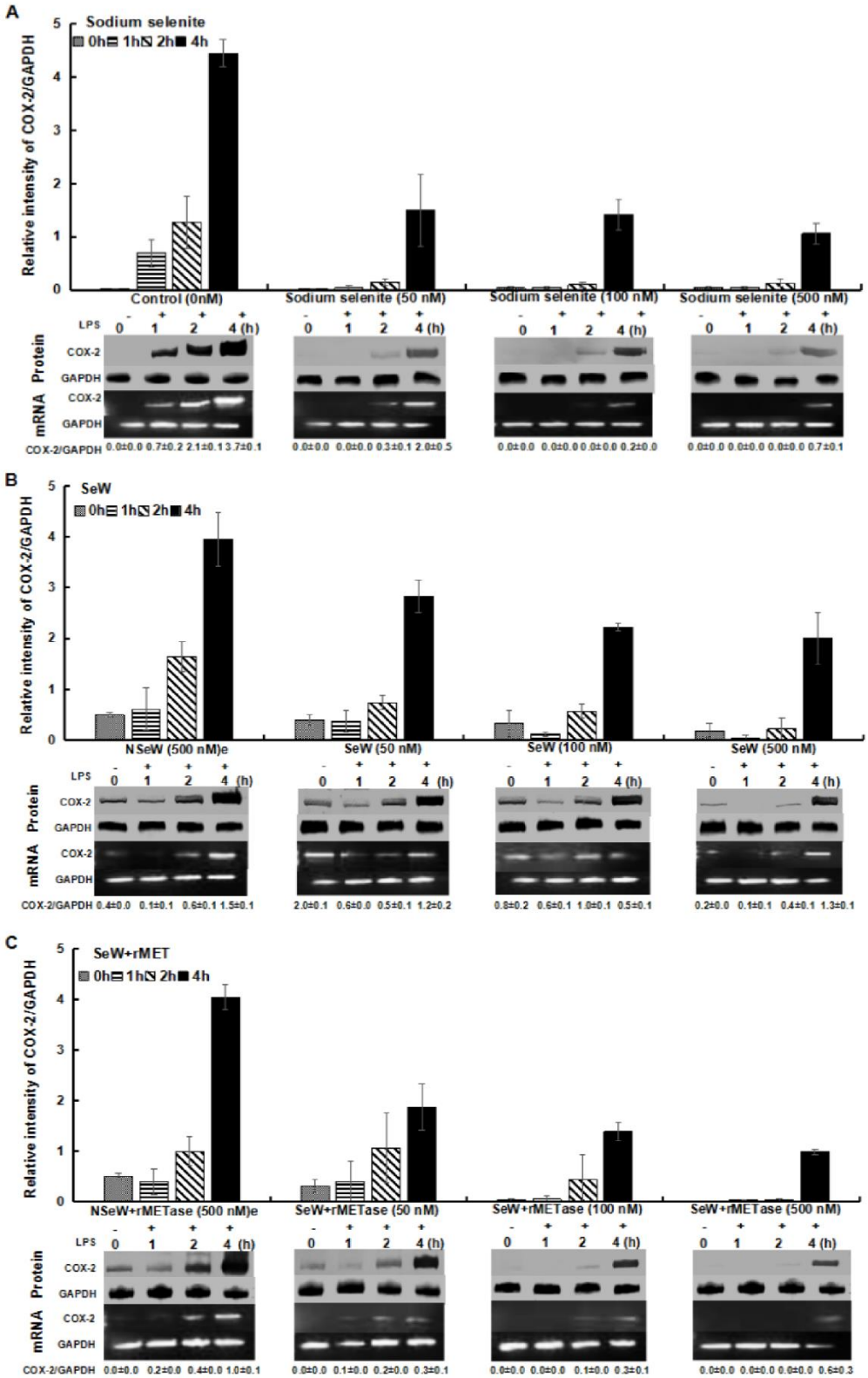


Figure 4.6: COX-2 expression in RAW264.7 cells with exogenous stimulation of 1 µg/mL LPS up to 24 h. Densitometric values normalized to GAPDH are shown below each panel with mean ± SD of triplicate samples.

However, Se supplementation down-regulated the expression of COX-2 (Figure 4.7). With LPS stimulation, the COX-2 levels increased by 4.5 fold during first 4 h in SeDef cells in comparison to SeSup cells (Figure 4.7 A). Se supplementation in the form of SeW+rMET showed nearly similar trend to SS-incubated cells with weaker expression of COX-2 after 4 h of LPS stimulation in comparison to SeW treated cells (Figure 4.7 A, B, C).

Upon SS supplementation, the COX-2 expression was down-regulated by 3.0 to 4.3 folds in 4 h with 50 nM and 500 nM Se, respectively (Figure 4.7 A). Among SeW and SeW+rMET treated cells, COX-2 levels decreased from 1.4 to 2.0 fold and 2.2 to 4.0 fold with increasing dietary Se levels (500 nM >100 nM >50 nM) as compared to the NSeW and NSeW+rMET treated cells, respectively **(**Figure 4.7 B, C). The observations further confirmed the role of rMETase in release of bound Se and enhancing its availability in down-regulating pro-inflammatory COX-2.


A
Sodium selenite
0h 1h 2h 4h
Relative intensity of COX-2/GAPDH
Control (0nM)
Sodium selenite (50 nM)
Sodium selenite (100 nM)
Sodium selenite (500 nM)
LPS
0 1 2 4 (h)
Protein
COX-2
GAPDH
mRNA
COX-2
GAPDH
COX-2/GAPDH 0.0±0.0 0.7±0.2 2.1±0.1 3.7±0.1
0.0±0.0 0.0±0.0 0.3±0.1 2.0±0.5
0.0±0.0 0.0±0.0 0.0±0.0 0.2±0.0
0.0±0.0 0.0±0.0 0.0±0.0 0.7±0.1
B
SeW
0h 1h 2h 4h
Relative intensity of COX-2/GAPDH
NSeW (500 nM)e
SeW (50 nM)
SeW (100 nM)
SeW (500 nM)
LPS
0 1 2 4 (h)
Protein
COX-2
GAPDH
mRNA
COX-2
GAPDH
COX-2/GAPDH 0.4±0.0 0.1±0.1 0.6±0.1 1.5±0.1
2.0±0.1 0.6±0.0 0.5±0.1 1.2±0.2
0.8±0.2 0.6±0.1 1.0±0.1 0.5±0.1
0.2±0.0 0.1±0.1 0.4±0.1 1.3±0.1
C
SeW+rMET
0h 1h 2h 4h
Relative intensity of COX-2/GAPDH
NSeW+rMETase (500 nM)e
SeW+rMETase (50 nM)
SeW+rMETase (100 nM)
SeW+rMETase (500 nM)
LPS
0 1 2 4 (h)
Protein
COX-2
GAPDH
mRNA
COX-2
GAPDH
COX-2/GAPDH 0.0±0.0 0.2±0.0 0.4±0.0 1.0±0.1
0.0±0.0 0.1±0.0 0.2±0.0 0.3±0.1
0.0±0.0 0.0±0.0 0.1±0.0 0.3±0.1
0.0±0.0 0.0±0.0 0.0±0.0 0.6±0.3

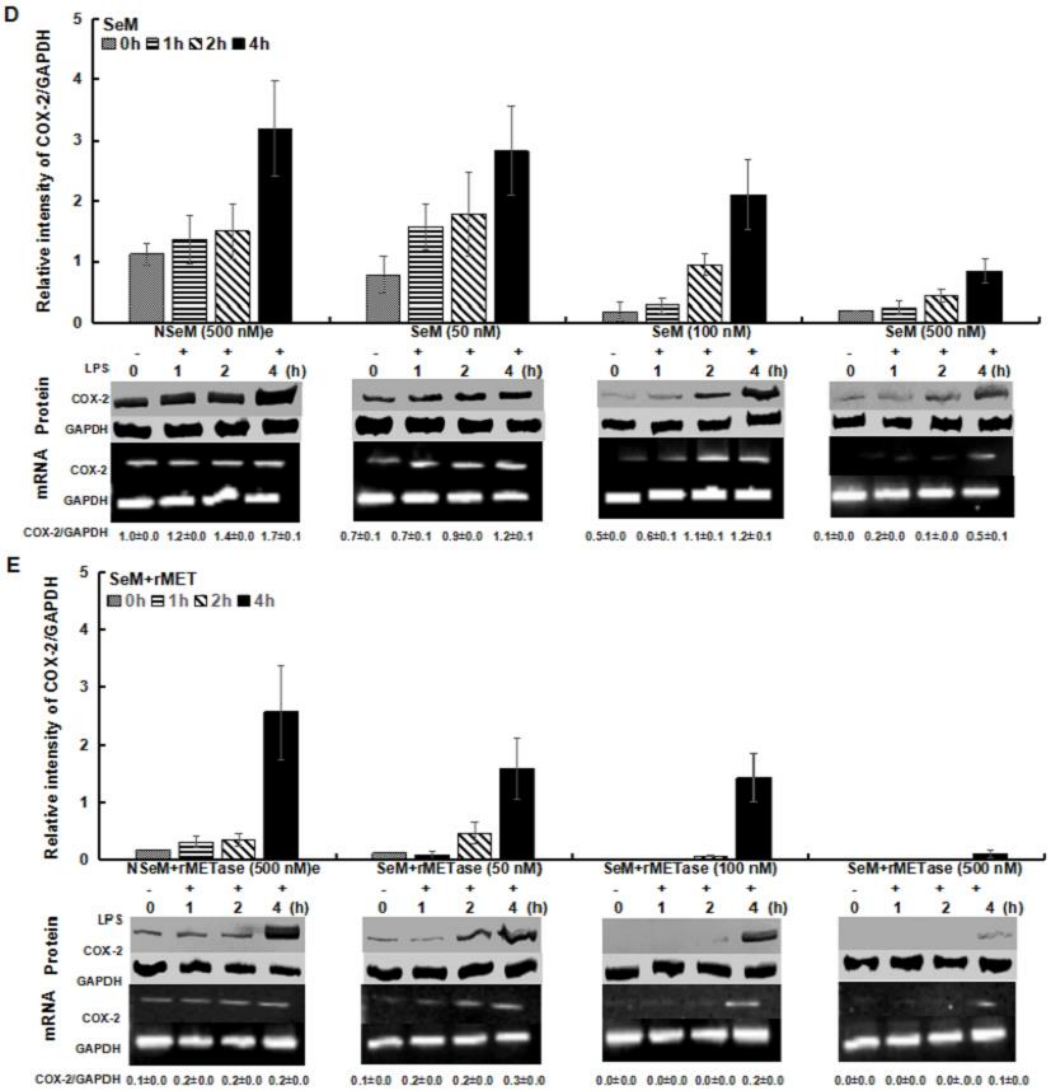


Figure 4.7: Western blots and mRNA profiles of COX-2 expression in RAW264.7 macrophages [n=3]. Cells were treated with 50nM, 100nM and 500nM Se-concentration in different forms: (A) Sodium selenite (SS), (B) Seleniferous wheat extract (SeW), (C) Seleniferous wheat extract in presence of rMETase incubation (SeW+rMET) (D) Seleniferous maize extract (SeM) and (E) Seleniferous maize extract in presence of rMETase incubation (SeM+rMET) with respective to their controls, for 72 h followed by 1 μg/mL LPS for framed stimulation over 4 h. Densitometric values normalized to GAPDH and mean±SD and values are given below each panel of mRNA expression.

The maize extracts also exhibited notable down regulation of COX-2 similar to Se-rich wheat and sodium selenite treated samples. Among SeM and SeM+rMET treated cells, COX-2 levels

decreased from 1.1 to 3.8 fold and 1.6 to 24.8 fold with increasing dietary Se levels from 50 to 500 nM in comparison to NSeM and NSeM+rMET treated cells, respectively **(**Figure 4.7 D, E). The results showed that at 500 nM, COX-2 down regulation was 12.4, 6.2 and 5.8 fold higher through maize supplementation (SeM+rMET) when compared to SeW, SeW+rMET and SS treated cells, respectively (Figure 4.7).

Thus, the bioactivity of Se from maize was observably higher than that of wheat matrix. The non-seleniferous counterparts down regulated COX-2 up to 1.1 (NSeW), 1.1 (NSeW+rMET), 1.4 (NSeM) and 1.7 (NSeM+rMET) folds only, which explains the marginal activity of constituents other than Se.

4.9. Downregulation of mPGES-1 expression by Se supplementation in LPS stimulated RAW264.7 cells

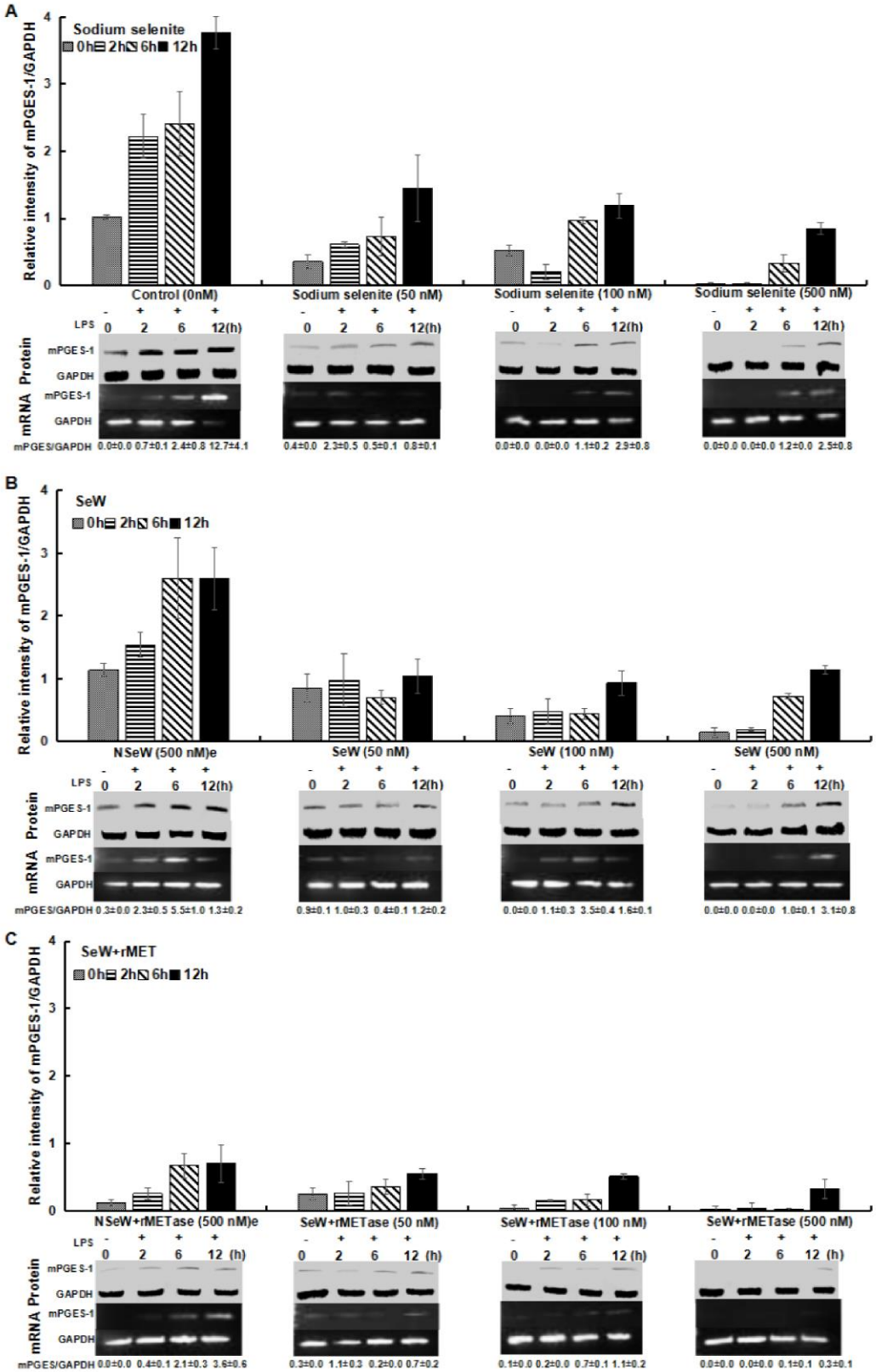
mPGES-1 is the glutathione dependent enzyme which induce the production of PGE_2 (Chen et al., 2015). It has three isoforms including mPGES-1, mPGES-2 and cPGES (cytosolic) present in fibroblasts and epithelial cells, among which only isoform-1 parallels the inducible COX-2 production and is critical for PGE_2 expression under excessive RONS production with acute and chronic inflammatory signals (Baragatti et al., 2008; Gudis et al., 2005). Various studies have demonstrated the role of PGE_2 in cancer cell proliferation and tumor development due to activation of NFκB, p38 MAP-kinase and JNK pathways in myocytes and fibroblasts to induce high inflammation (Cahlin et al., 2008; Giannico et al., 2005; Radmark and Samuelsson, 2010).

The effect of Se on mPGES-1 expression, a downstream marker of COX-2 activity, was therefore examined. RAW264.7 cells were grown in SeDef and SeSup DMEM media with/without 1 μg/mL LPS stimulation from 0 to 12 h. In all SeDef and SeSup cells, an overall increasing trend in mPGES-1 expression was seen with respect to time (Figure 4.8). Under normal cell conditions

(without LPS stimulation, 0 h), 500 nM sodium selenite completely down-regulated the mPGES-1 expression as shown in figure 4.8 A. In contrast, upon LPS stimulation vis-a-vis generation of RONS, in SeDef conditions (control 0 nM), the mPGES-1 levels increased by 3.8 fold at 12 h in comparison to the SeSup cells (Figure 4.8 A). However, Se supplementation in the form of sodium selenite with LPS stimulation of 12 h, decreased the mPGES-1 expression from 2.5 fold at 50 nM to 4.5 fold at 500 nM. This accounts to 62 to 78 % suppression with respect to control (Figure 4.8 A). Increase in SS supplementation from 25 to 250 nM has already been reported to mitigate inflammation with reduced mPGES-1 expression in RAW264.7 macrophages (Gandhi et al., 2011). However, similar trends in mPGES-1 expression, with supplementation of Se from dietary matrices, has not been reported earlier. Thus, it was noted that with seleniferous wheat and maize grains showed significant down regulation of mPGES-1 similar to that of SS treatment, when Se was made accessible through rMETase treatment (Figure 4.8).

At 12 h of LPS stimulation and 500 nM SeW+rMET supplementation, mPGES-1 expression significantly decreased up to 12 fold in comparison to the SeW cells (3.3 fold) (Figure 4.8 B, C). Similar to the decreased COX-2 expression, a significant down regulation of mPGES-1 expression was also noticed in cells supplemented with seleniferous maize extracts when compared to wheat extracts (Figure 4.8 D, E). The 500 nM Se as SeM+rMET suppressed the mPGES-1 upto 21 fold in comparison to SeW+rMET (12 fold) treated cells (Figure 4.8 C, E).

The results showed the damping of mPGES-1 with Se supplementation in early hours (4 h) of LPS stimulation, which further modulates over a 12 h period (Davis et al., 2002; Eskew et al., 1989). During resolution of inflammation, Se supplementation shifts AA metabolism from PGE_2 towards PGD_2 and its down-regulated product 15d-PGJ_2 through inactivation of NF-κB (Davis et


A
Sodium selenite
0h 2h 6h 12h
Relative intensity of mPGES-1/GAPDH
Control (0nM)
Sodium selenite (50 nM)
Sodium selenite (100 nM)
Sodium selenite (500 nM)
LPS
0 2 6 12(h)
Protein
mPGES-1
GAPDH
mRNA
mPGES-1
GAPDH
mPGES/GAPDH 0.0±0.0 0.7±0.1 2.4±0.8 12.7±4.1
0.4±0.0 2.3±0.5 0.5±0.1 0.8±0.1
0.0±0.0 0.0±0.0 1.1±0.2 2.9±0.8
0.0±0.0 0.0±0.0 1.2±0.0 2.5±0.8
B
SeW
0h 2h 6h 12h
Relative intensity of mPGES-1/GAPDH
NSeW (500 nM)e
SeW (50 nM)
SeW (100 nM)
SeW (500 nM)
LPS
0 2 6 12(h)
Protein
mPGES-1
GAPDH
mRNA
mPGES-1
GAPDH
mPGES/GAPDH 0.3±0.0 2.3±0.5 5.5±1.0 1.3±0.2
0.9±0.1 1.0±0.3 0.4±0.1 1.2±0.2
0.0±0.0 1.1±0.3 3.5±0.4 1.6±0.1
0.0±0.0 0.0±0.0 1.0±0.1 3.1±0.8
C
SeW+rMET
0h 2h 6h 12h
Relative intensity of mPGES-1/GAPDH
NSeW+rMETase (500 nM)e
SeW+rMETase (50 nM)
SeW+rMETase (100 nM)
SeW+rMETase (500 nM)
LPS
0 2 6 12 (h)
Protein
mPGES-1
GAPDH
mRNA
mPGES-1
GAPDH
mPGES/GAPDH 0.0±0.0 0.4±0.1 2.1±0.3 3.6±0.6
0.3±0.0 1.1±0.3 0.2±0.0 0.7±0.2
0.1±0.0 0.2±0.0 0.7±0.1 1.1±0.2
0.0±0.0 0.0±0.0 0.1±0.1 0.3±0.1

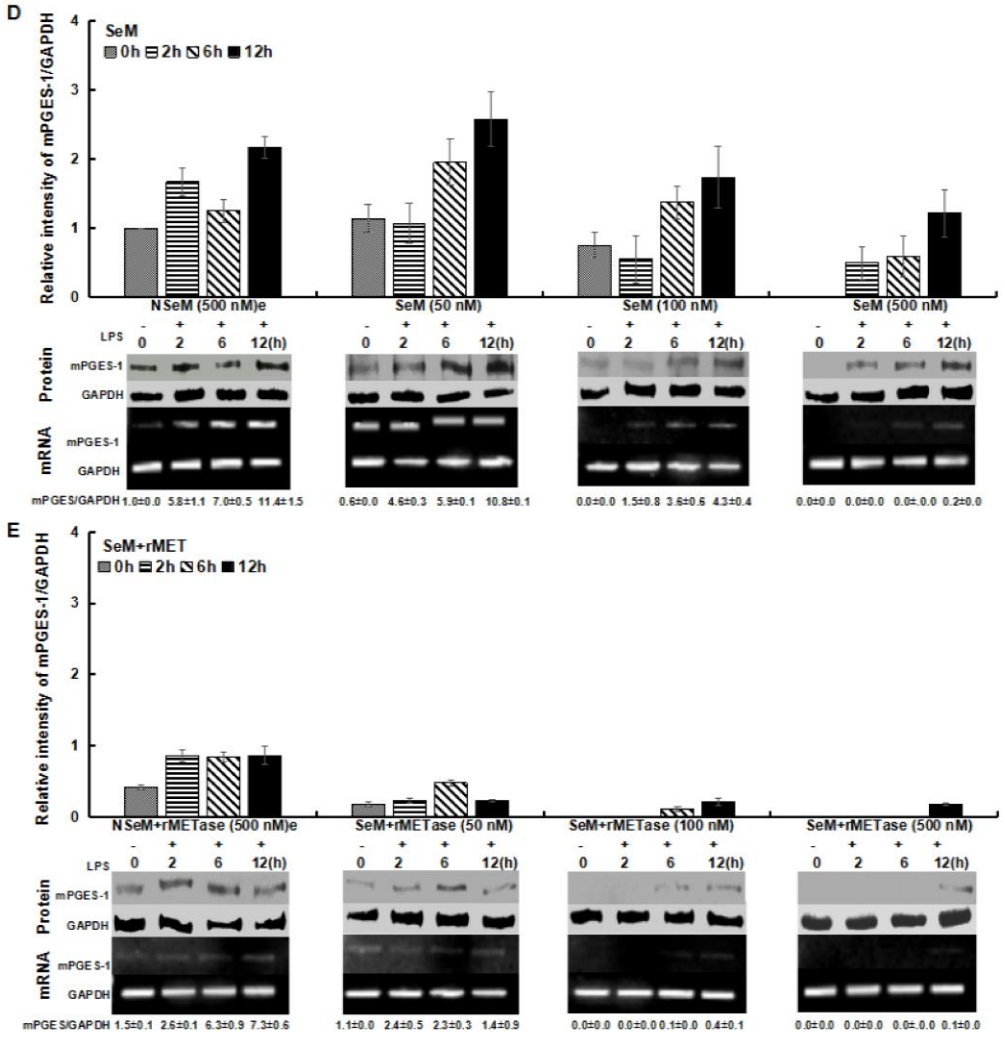


Figure 4.8: Western blots and mRNA profiles of mPGES-1 expression in RAW264.7 macrophages [n=3]. Cells were treated with 50nM, 100nM and 500nM Se-concentration in different forms: (A) Sodium selenite (SS), (B) Seleniferous wheat extract (SeW), (C) Seleniferous wheat extract in presence of rMETase incubation (SeW+rMET) (D) Seleniferous maize extract (SeM) and (E) Seleniferous maize extract in presence of rMETase incubation (SeM+rMET) with respective to their controls, for 72 h followed by 1 μg/mL LPS stimulation for 4 h. Densitometric values normalized to GAPDH and mean±SD and values are given below each panel of mRNA expression.

al., 2002; Gandhi et al., 2011; Kaushal et al, 2014; Nelson et al., 2011; Vunta et al., 2007). Further, 15d-PGJ_2 covalently modifies key cysteine thiols in I Kappa B Kinase (IKK)-β (IKK2), which

regulates the activation of NF-κB in LPS-treated macrophages (Cai et al., 2015; Gandhi et al., 2011).

In resolution of acute inflammation, COX-2 enzyme modulates its downstream enzyme, mPGES-1 and thus, helps in the activation of other anti-inflammatory PG enzymes such as H-PGDS, which produces the precursor of anti-inflammatory cyclopentenone prostaglandins, Δ^{12}-PGJ_2 and 15d-PGJ_2. With a delayed time lag of expression, levels of COX-2 and mPGES-1 correlate with each other and NF-κB transcription (Kirkbya et al., 2016) with an increase at the protein level under Se deficiency upon LPS stimulation (Davis et al., 2002). COX-1 and COX-2 deficiency reduce the PGE_2 level up to 25% and 75%, respectively (Langenbach et al., 1999). However, under enhanced levels of COX-2, COX-1 switches on its housekeeping balance towards generation of anti-inflammatory PGs, such as PGD_2 and 15d PGJ_2 with low levels of PGE_2 (Vunta et al., 2007; Morteau et al., 2000) and PGI_2 (Weaver et al., 2001), resolving the process through PPARγ activation (Bell-Parikh et al., 2003). PPARγ induce 15 hydroxyprostaglandin dehydrogenase, which plays vital role in degradation of COX-2 and PGE_2 (Hirsch and Lippman, 2005). Such a functional coupling of COX-2 and mPGES-1 versus COX-1 and H-PGDS, through PPARγ activation is known to drive the PG metabolism towards either pro-inflammatory or anti-inflammatory pathways under differential Se status (Vunta et al., 2007; Gandhi et al., 2011).

The over-expression of COX-2 and mPGES-1 due to Se deficiency is thought to be one of the key underlying basis of inflammation that is seen in inflammatory bowel disease (IBD) (Kudva et al., 2015; Reifen et al., 2015), AIDS, (Ipp et al., 2014), asthma (Daham et al., 2014), brain tumour (Eberstal et al., 2014), breast cancer (Jana et al., 2014), colorectal cancer (Roelofs et al., 2014), diabetic cardiac heterotrophy (Dhanya et al., 2014; Li et al., 2010), gynaecological cancer (Adnan and Rouba, 2005), head and neck cancer (Park et al., 2003; Lin et al., 2002; Gallo et al., 2001),

lung cancer (Sandler and Dubinett, 2004), pancreatic cancer (Hill et al., 2012), prostate cancer (Fenner, 2016), urinary bladder cancer (Tabriz et al., 2013) and UV rays induced skin cancer (Zhu et al., 2015).

Collectively, the up-regulation of these COX-2and mPGES-1 inductive inflammatory enzymes are due to increased production of RONS, thiobarbituric acid reactive substances (TBARS), iNOS, TNFα, IL-β, IL-6 mRNA expressions and low levels of GSH and antioxidant enzyme production (Zhu et al., 2015). However, Se supplementation bolster the antioxidant level through up-regulated selenoenzymes like GPx, which combat both COX-2 activity and COX-2 dependent PGE_2 expression (Banning et al., 2008). Se down regulates the acylation of histone H4 at K12 and K16 position of COX-2, NFκB and TNFα promoters for epigenetic regulation of inflammatory gene expression (Narayan et al., 2015), which curtails the process of angiogenesis, deter invasion and bolster immune surveillance (Wallace, 2002). Numerous studies had explored the application of sodium selenite upon suppression of LPS stimulated COX-2, TNFα and NFκB activation along with κB degradation, ERK, JNK and P38 phosphorylation (Morin et al., 2015; Zhang et al., 2014; Gandhi et al., 2011; Vunta et al., 2007; Davis et al., 2002; Prabhu et al., 2002). However, there are limited reports available at the application of SeMet and other forms of Se present in Se-rich cereals and plant dietary source in resolving these inflammatory mediators. Few researchers have reported the direct application of commercially available SeMet upon the anti-inflammatory therapy to inhibit the overproduction of COX-2, TNFα, iNOS, NO, and PGE_2 expression (Shen et al., 2015; Ying et al., 2013; Gandhi et al., 2011; Baines et al., 2002). SeMet supress the activation of NFκB activation by blocking IκBα proteins and translocation of P50 subunits (Shen et al., 2015; Gandhi et al., 2011).

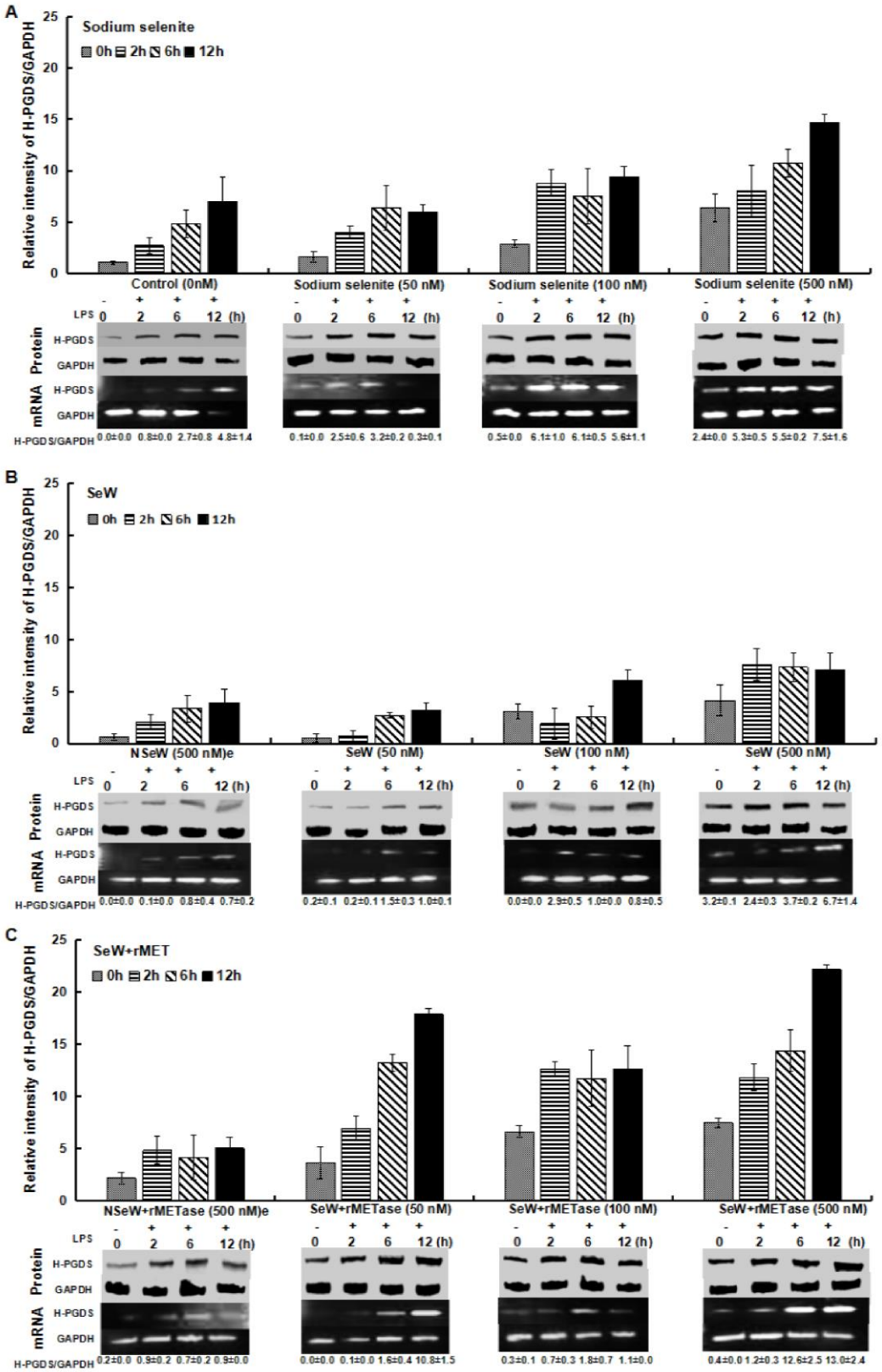
4.10. Expression of hematopoietic prostaglandin-D synthase as a function of Se supplementation

H-PGDS catalyzes the synthesis of anti-inflammatory D and J series of prostanoids (Joo and Sadikot, 2012; Kanaoka et al., 2000; Ricciotti and Fitzgerald, 2011) from its precursor PGH_2 (Urade and Hayaishi, 2011). It has two isoforms, H-PGDS (hematopoietic) and L-PGDS (lipocalin), with different chromosomal sequences, tertiary structure, cellular localization and tissue distribution (Helliwell et al., 2004; Urade and Hayaishi, 2000). H-PGDS is the glutathione-dependent enzyme (Kanaoka and Urade, 2003; Meyer and Thomas, 1995; Ujihara et al., 1988), characterized as a member of sigma class of glutathione gene family (Kanaoka et al., 1997). The localization of H-type is cytosolic in antigen presenting cells, mast cells, dendritic cells and langerhans (Mahmud et al., 1997) in response to inflammatory allergic signals in various tissues and diseases (Kanaoka and Urade, 2003; Rajakariar et al., 2007; Redensek et al., 2011; Sarashinaa et al., 2014; Satoh et al., 2006; Scher and Pillinger, 2017; Tanaka et al., 2000; Urade et al., 1989). In contrast, L-type PGDS is a glutathione-independent enzyme, present in cerebrospinal fluid (Hoffmann et al., 1993; Watanabe et al., 1994) responsible for PGD_2 synthesis in brain (Narumiya et al., 1982; Ogorochi et al., 1984), heart (Osanai and Okumura, 2011; Tokudome et al., 2009), kidneys (Donadio, 2010) and lungs (Ragolia et al., 2010). It has tissue based several key regulatory roles (Herlong and Scott, 2006) such as sleep-wake cycle (Hayaishi and Urade, 2002; Jordan et al., 2004; Pinzar et al., 2000; Urade and Hayaishi, 2011), labor pain during gestational period (Helliwell et al., 2006), male reproductive organs (Tokugawa et al., 1998), decline obesity (Tanaka et al., 2009) and inhibit lung cancer (Ando et al., 2003; Murata et al., 2013), arthritic joint destruction (Shan et al., 2004), suppress spinal inflammation (Grill et al., 2008) and nasal polyp formation (Hyo et al., 2007). PGD_2 further generates the downstream non-enzymatic metabolites

such as Δ^{12}-PGJ_2 and 15 deoxy $\Delta^{12\ 14}$ PGJ_2, which activates PPARγ receptors (Herlong and Scott, 2006; Jowsey et al., 2003) to inhibit the pro-inflammatory transcription factors such as NFκB (McAdam et al., 2000; Ricote et al., 1998; Simmons et al., 2004). Therefore, collectively the H-PGDS gene expression accounts for innate immune response with resolution of chronic allergic inflammation through PGD_2 and its downstream mediators production (Gilroy et al., 2004; Satoh et al., 2006; Serhan et al., 2007).

In present study, the culturing of RAW264.7 cells in LPS stimulated SeDef and SeSup cells showed enhanced H-PGDS expression. Under Se deficient environment and LPS stimulation of 0 to 12 h, a 7.0 fold difference was observed in H-PGDS expression (Figure 4.9 A). This increase in expression of H-PGDS against the LPS generated RONS clearly indicates the self defense mechanism of macrophages. Upon Se supplementation, the expression of H-PGDS increased with concentration of Se (500 nM > 100 nM > 50 nM) either in form of SeW+rMET or SS as compared to the corresponding controls with respect to time from 0 to 12 h (Figure 4.9 A, C). Under normal conditions (without LPS stimulation, 0 h), the Se supplementation in the form of SS increased the H-PGDS levels from 1.5 fold at 50 nM to 5.9 fold at 500 nM. Under similar experimental conditions, a prominent hike in H-PGDS levels were observed in SeW+rMET and SeM+rMET treated cells, from 3.4 to 6.9 fold and 5.6 to 16.5 fold in comparison to SeW (0.6 to 3.8 fold) and SeM (2.3 to 5.4 fold) treated cells.

These observations suggested that the natural source of Se supplementation up-regulates the H-PGDS expression to significantly high levels compared to inorganic SS supplementation, with maize extract showing relatively better bioactivity at 500 nM. With LPS stimulation of 12 h, 500 nM of SeW+rMET and SeM+rMET showed 3.2 and 3.3 fold increase in expression levels when compared to the SS (2.1 fold), SeW (1.0 fold) and SeM (1.0 fold) treated cells (Figure 4.9).


A
Sodium selenite
0h 2h 6h 12h
Relative intensity of H-PGDS/GAPDH
Control (0nM)
Sodium selenite (50 nM)
Sodium selenite (100 nM)
Sodium selenite (500 nM)
LPS
0 2 6 12 (h)
Protein
H-PGDS
GAPDH
mRNA
H-PGDS
GAPDH
H-PGDS/GAPDH
0.0±0.0 0.8±0.0 2.7±0.8 4.8±1.4
0.1±0.0 2.5±0.6 3.2±0.2 0.3±0.1
0.5±0.0 6.1±1.0 6.1±0.5 5.6±1.1
2.4±0.0 5.3±0.5 5.5±0.2 7.5±1.6
B
SeW
0h 2h 6h 12h
Relative intensity of H-PGDS/GAPDH
NSeW (500 nM)e
SeW (50 nM)
SeW (100 nM)
SeW (500 nM)
LPS
0 2 6 12 (h)
Protein
H-PGDS
GAPDH
mRNA
H-PGDS
GAPDH
H-PGDS/GAPDH
0.0±0.0 0.1±0.0 0.8±0.4 0.7±0.2
0.2±0.1 0.2±0.1 1.5±0.3 1.0±0.1
0.0±0.0 2.9±0.5 1.0±0.0 0.8±0.5
3.2±0.1 2.4±0.3 3.7±0.2 6.7±1.4
C
SeW+rMET
0h 2h 6h 12h
Relative intensity of H-PGDS/GAPDH
NSeW+rMETase (500 nM)e
SeW+rMETase (50 nM)
SeW+rMETase (100 nM)
SeW+rMETase (500 nM)
LPS
0 2 6 12 (h)
Protein
H-PGDS
GAPDH
mRNA
H-PGDS
GAPDH
H-PGDS/GAPDH
0.2±0.0 0.9±0.2 0.7±0.2 0.9±0.0
0.0±0.0 0.1±0.0 1.6±0.4 10.8±1.5
0.3±0.1 0.7±0.3 1.8±0.7 1.1±0.0
0.4±0.0 1.2±0.3 12.6±2.5 13.0±2.4

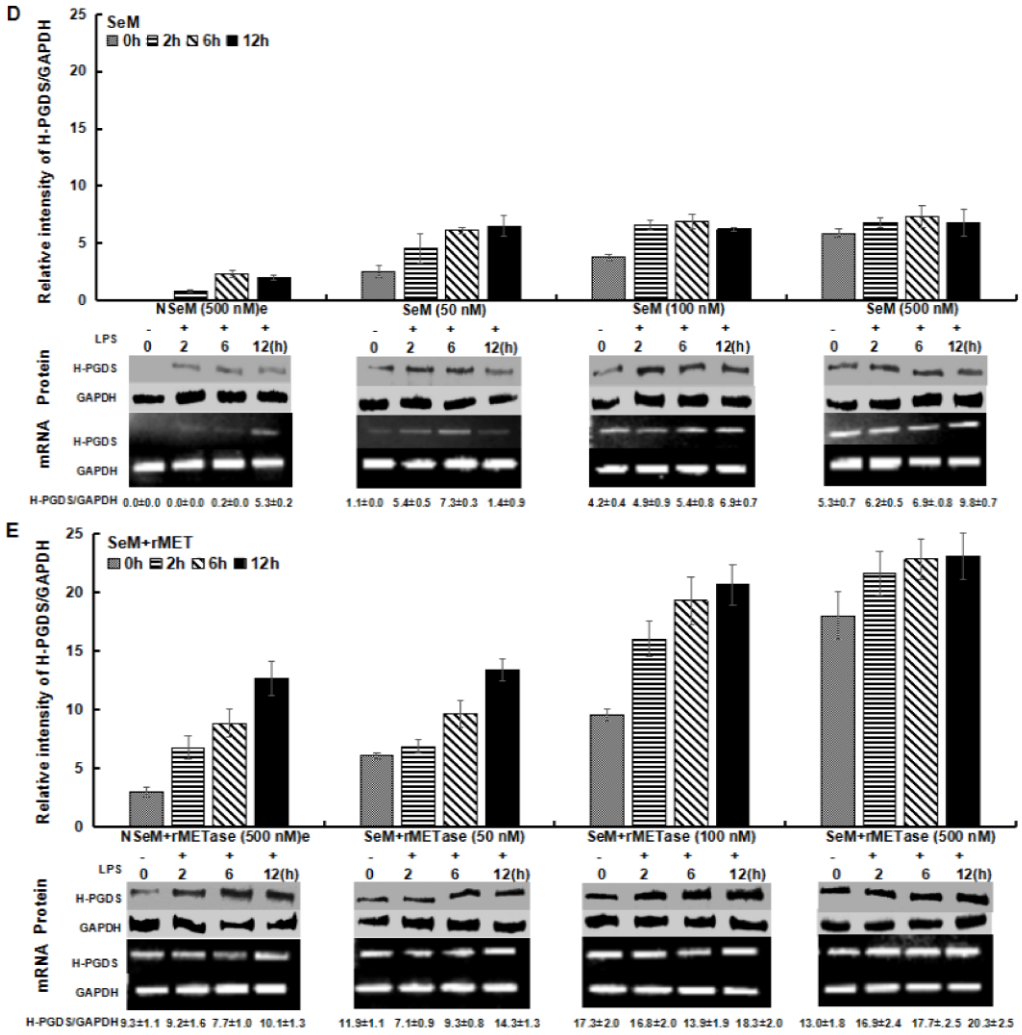


Figure 4.9: Western blots and mRNA profiles of H-PGDS expression in RAW264.7 macrophages [n=3]. Cells were treated with 50nM, 100nM and 500nM Se-concentration in different forms: (A) Sodium selenite (SS), (B) Seleniferous wheat extract (SeW), (C) Seleniferous wheat extract in presence of rMETase incubation (SeW+rMET) (D) Seleniferous maize extract (SeM) and (E) Seleniferous maize extract in presence of rMETase incubation (SeM+rMET) with respective to their controls, for 72 h followed by stimulation with 1 µg/mL LPS over 4 h. Densitometric values normalized to GAPDH and mean±SD and values are given below each panel of mRNA expression.

Similar results were observed in earlier studies by Gandhi et al. (2011) with SS stimulation of macrophages wherein enhanced levels of H-PGDS enzyme catalyzed PGH_2 to PGD_2 conversion

for the down-regulation of inflammatory processes. This PGD_2 product metabolizes into anti-inflammatory 15d-PGJ_2 eicosanoids by a non-enzymatic reactions (Ishii, 2015; Shan et al., 2004), which acts as an endogenous ligand for PPARγ receptor, a transcription factor that inhibits the expression of NF-kB regulated genes (Scher and Pillinger, 2005; Tyagi et al., 2011). During resolution phase of inflammation in wound repair, the PGD_2 and the downstream mediators such as 15-Deoxy-$\Delta^{12,14}$-PGJ_2 and PPARγ deter the overproduction of inducible COX-2 and mPGES-1 expression (Banning et al., 2008; Grill et al., 2006; Kapoor et al., 2007).

4.11. Inducible nitric oxide synthase (iNOS) and nitric oxide (NO) production

iNOS catalyzes the production of nitric oxide (NO), when L-arginine oxidises into citrulline (Knowles and Honcada, 1994). L-arginine is the co-factor for overproduction of NO and iNOS levels (Aktan, 2003). NOS have three isoforms including two constitutive (neuronal, NOS1 [nNOS] and endothelial, NOS3 [eNOS]) and one inducible (NOS 2, [iNOS]) type. nNOS, iNOS and eNOS are located at chromosome number 12, 17 and 7, respectively (Knowles and Honcada, 1994). nNOS helps in cell communication by producing NO in nervous tissues and skeletal muscles (Forstermann and Sessa, 2012). eNOS generates NO in endothelium of blood vessels for vasodilation (Manicam et al., 2017). In present study, the NO level of SeSup supernatants were significantly lower as compared to the SeDef supernatants (Figure 4.10).

The time kinetics from 0 to 24 h was carried out, in which the release of nitric ions gradually increased after 6h of LPS stimulation, in all cases, although there was no significant different ($p>0.05$) in the trend of NO release through time. However, Se supplementation revoked the extracellular release of these ions. At 500 nM Se supplementation, RAW264.7 macrophages released 1.6, 1.4, 2.0, 1.6 and 2.4 fold less NO in SS, SeW, SeW+rMET, SeM and SeM+rMET

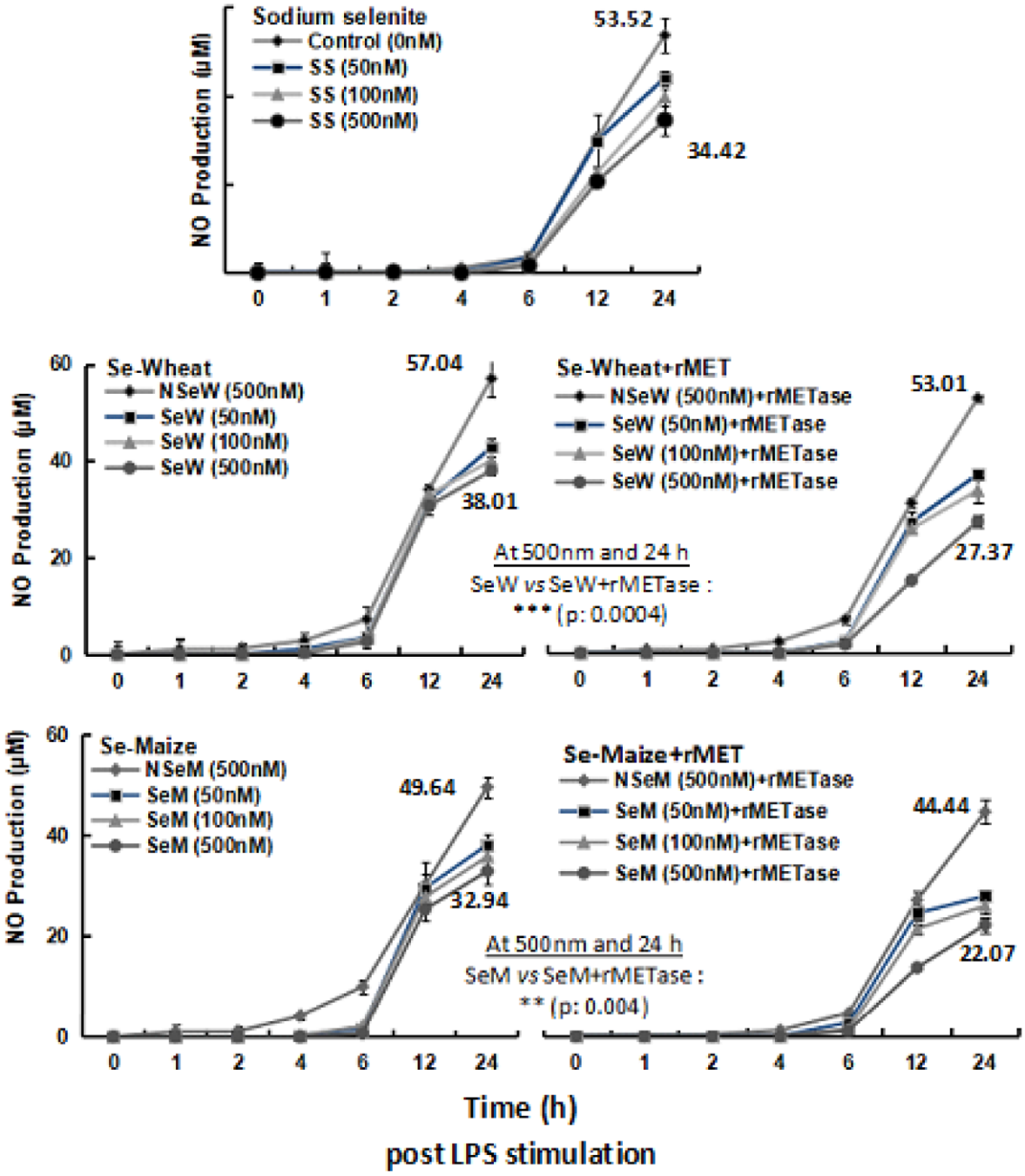


Figure 4.10: NO [n=3] in cell-free supernatant (CFS) of SeSup and SeDef RAW264.7 macrophages. Nitrite levels were measured in CFS with 1µg/mL post LPS stimulation in culture media using Griess reagent. Values indicate the mean NO levels at 24h and at concentration of 500nm with reference to their respective controls.

supplemented media in 24 h of LPS stimulation with respect to controls, respectively (Figure 4.10). At 24h (of LPS stimulation followed by 500 nM Se supplementation), the NO levels in Se supplemented samples, without rMETase, were significantly different with reference to their respective controls [NSeW vs SeW – p: 0.001; NSeM vs SeM – p: 0.001]. In the cells that were pre-treated with rMETase, the release of NO significantly decreased, when compared to those that were not pre-treated [SeW vs SeW+rMETase – p: 0.0004; SeM vs SeM+rMETase – p: 0.004]. With reference to the comparison with the sodium selenite (SS) treatment, Se rich wheat extract, with rMETase pre-treatment, showed relatively less significant difference (SS vs SeW+rMETase – p: 0.02) than Se rich maize extract with rMETase (SS vs SeM+rMETase – p: 0.003).

The observations clearly indicate that upon LPS stimulation, Se deficiency results in exogenous NO production (Hoffmann, 2007), which is part of inflammatory markers along with other cytokines like TNF-α, IL-1β, and IL-6 in LPS-induced J774.1 mouse macrophages (Safir et al., 2003). The presence of NO (measured as nitrite) in cell free supernatants directly counts to the level of iNOS expression and peroxynitrite production in cytosol (Possel et al., 2000). Therefore, to evaluate the effect of Se supplementation upon down-regulation of intracellular iNOS production, RAW264.7 cells were grown in SeSup and SeDef conditions and molecular expressions of iNOS gene were studied at pre-transcriptional levels. Upon LPS stimulation, iNOS expression was increased in all treatments. However, in SeDef (i.e., C, NSeW, NSeW+rMET, NSeM and NSeM+rMET treated cells), the mRNA expression was significantly higher than in SeSup cells (Figure 4.11). With increasing concentration of Se from 50 to 500 nM, iNOS expression was effectively inhibited within 4 h in all SeSup cells. The rMETase treated grain extracts (SeW+rMET and SeM+rMET) showed comparatively higher inhibition of iNOS expression (10.5 fold) as compared to the SeW (3.5 fold), SeM (4.2 fold) and SS (3.0 fold)

stimulated cells with respect to control (C, 24 h). In SeDef cells, devoid of LPS stimulation, marginal expression of iNOS could be seen in NSeW (0.8 fold), NSeW+rMET (0.2 fold) NSeM (0.3 fold) and NSeM+rMET (0.2 fold) as compared to the control (C) (Figure 4.11). It is known that LPS and IFNγ induction up-regulates the iNOS expression to augment inflammatory responses in human U937 macrophages (Grkovich et al., 2006). Our results corroborate with the previous report on the ability of SS supplementation to reduce iNOS expression by 3 fold as compared to SeDef, LPS unstimulated RAW 264.7 macrophages (Prabhu et al., 2002).

Similar results of NO quenching along with down-regulation of iNOS transcription were seen with the role of *Aster yomena* (Kim et al., 2017), *Eupartoium makinoi* (Ahn et al., 2015), *Siegesbeckia glabrescens* (Lee et al., 2011), Platycodon grandiflorum saponins (Ahn et al., 2005), *Andrographis paniculata* (Chiou et al., 2000), lansai C and D (Taechowisan et al., 2010), *Caesalpinia sappan*, *Curcuma longa*, *Daphne genkwa* and *Morus alba* (Hong et al., 2002), which further decreases COX-2 and PGE_2 mRNA levels in LPS induced macrophages.

iNOS, gained recognition in year 1992 as an important biological mediator (Nussler and Billiar, 1993), which stimulates the immune defense system against pathogens through inflammatory mediated cellular responses, producing large quantity of O_2^- and NO from the reductase domain (Xia et al., 1998), via stimulation of IL-1, TNFα and other pro-inflammatory cytokines (Green et al., 1994). The overproduced NO reacts with other free radicals such as O_2^- ions to form peroxynitrite leading to the oxidative burst of macrophages and thus, results in lipid peroxidation, cytotoxicity and vascular damage (Aktan, 2003; Buttery et al., 1996; Mungrue et al., 2002). In hemorrhage case, iNOS is expressed in nearly every organ (Taylor and Geller, 2000).

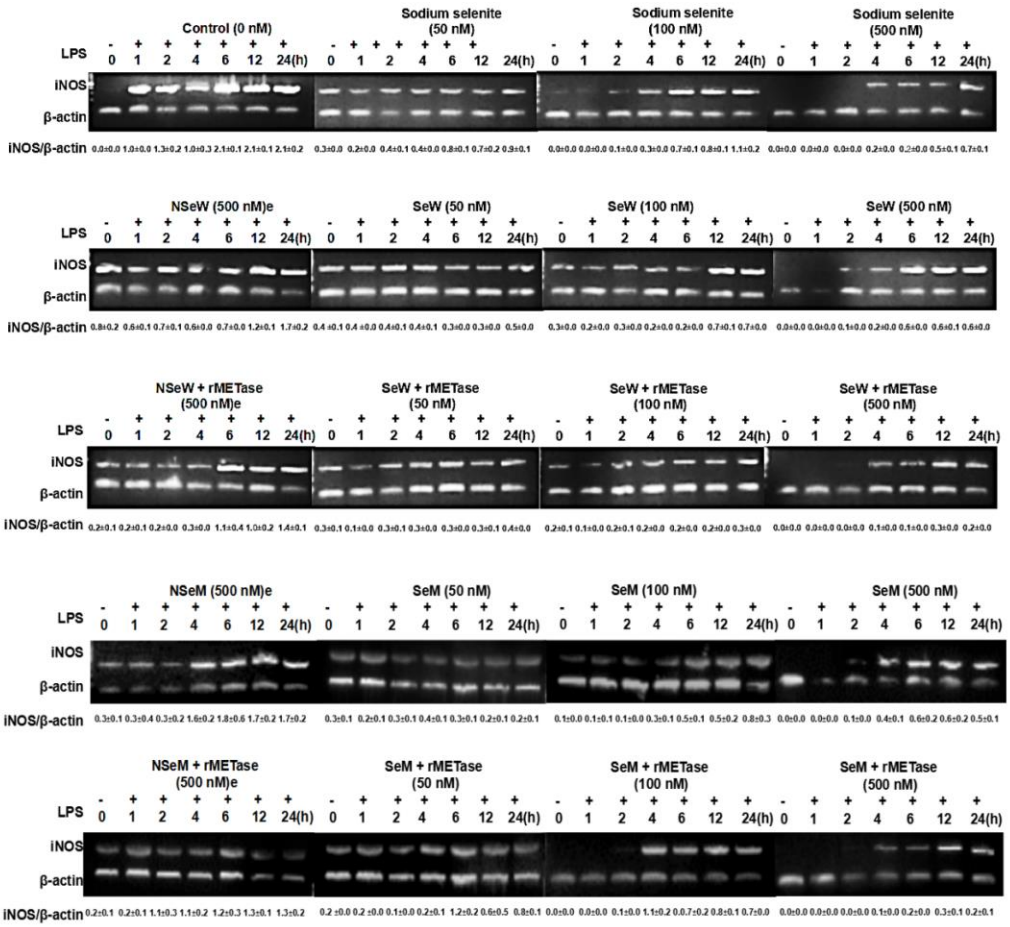


Figure 4.11: Relative intensities of iNOS/β-actin mRNA levels. Densitometry of iNOS and β-actin bands as quantified by ImageJ software program and representative of n=3 is shown.

iNOS expressed for longer periods with constant NO production has pathological actions such as inflammation, liver cirrhosis, systemic sclerosis and diabetes (Lechner et al., 2005; Yamamoto et al., 1998). In inflammatory arthritides, the NO and iNOS production from CD14+ synoviocytes, chondrocytes and endothelial cells persist in synovium and cartilage of patients with rheumatoid arthritis (Sakurai et al., 1995). In contrast, nNOS and eNOS have short pulsative NO synthesis with physiological roles such as blood pressure regulation, wound repair and host defense mechanism (Kroncke et al., 1997). During cutaneous wound repair, NO helps in collagen synthesis

and accumulation at the site of injury (Thornton et al., 1998). This explains the dual function of NOS, either with antibacterial activity in cytostatic condition or RONS production in cytotoxic condition (Xia and Zweier, 1997) and thus entitled as "double-edged biological sword" (Karpuzoglu and Ahmed, 2006). Increased levels of iNOS leads to numerous inflammation related health ailments (Kroncke et al., 1998), such as cerebral ischemia (Iadecola et al., 1997), neurological diseases (Koprowski et al., 1993), prostate cancer (Aaltoma et al., 2001), rheumatiod arthritis (Grabowski et al., 1997), osteoarthritis (Pelletier et al., 1998), tuberculosis (Nicholson et al., 1996) and esophageal adenocarcinomas (Wilson et al., 1998).

During inflammation, iNOS and COX-2 are the two up-regulated inducible enzymes present in cells (Vane et al., 1994), in which iNOS enhance the catalytic activity of COX-2, further which switch on the interaction of inflammatory systems to generate eicosanoids, depending upon the state of inflammation, i.e., acute or chronic (Kim et al., 2005; Klimp et al., 2001). Therefore, iNOS-COX-2 bond disruption is required to inhibit the release of NO which further deters the activation of COX-2. Suh et al. (1998) reported the application of synthetic oleananes (3,12-dioxoolean-1-en-28-oic acid and 3,11-dioxoolean-1,12-dien-28-oic acid) to inhibits the iNOS-COX-2 formation. Other research groups including Chen et al. (2001), Liang et al. (1999), Mediavilla et al. (2007) and Raso et al. (2001) reported the anti-inflammatory affects of flavonoids and polyphenolic compounds such as apigenin, naringenin, galangin, quercetin, morin, silymarin, kaempferol, baicalin, baicalein and wogonin in down-regulation of iNOS activity along with PGE_2 and COX-2 gene expression. Prabhu et al. (2002), Vunta et al., (2007), Davis et al. (2002), and Gandhi et al. (2011) reported the application of Se in the form of sodium selenite in down regulation of iNOS along with PGE_2 gene expression and up-regulation of PGD_2 and 15 deoxy $\Delta^{12,14}$ PGJ_2 products. The downstream product of anti-inflammatory PGs such as 15 deoxy $\Delta^{12,14}$ PGJ_2 is more potent

than its precursor (PGD_2) to down-regulate the iNOS promotor activity, mRNA and protein levels (Petrova et al., 1999). However, the dietary Se supplementation in the form of organic cereal source has not been reported till present date and the present study showed Se supplementation in the form of seleniferous wheat and maize extracts with significant decreased extracellular NO production and iNOS expression at pre-transcriptional levels in comparison to the inorganic sodium selenite supplementation. The results conclude that dietary Se supplementation revoke the generation of free radicals such as reactive oxygen and nitrogen species (RONS) in better way, which if in excess, stimulates the overproduction of pro-inflammatory enzymes such as iNOS, mPGES-1 and COX-2, that regulate the inflammatory pathways such as arachidonic acid or mitogen activated protein kinase (MAPKs).

Thus, this section describes the effect of Se supplementation through seleniferous cereal grains upon profiling of molecular and enzymatic expressions of anti-inflammatory (GPx-1 and H-PGDS) and pro-inflammatory enzymes (COX-2, mPGES-1 and iNOS) at both pre-transcriptional and post-translational levels through mRNA and protein expressions, respectively. The results showed the significant difference in role of naturally Se-enriched cereal grains (± rMETase treatment) in up-/down-regulation of anti-/pro-inflammatory genes in comparison to inorganic sodium selenite supplementation. However, the degree of Se-accumulation, bioaccessibility and bioactivity were different in different Se-crops grown on similar Se-contaminated field. Despite of highest Se levels in wheat crop, maize grains showed relatively higher bioaccessibility and bioactivity in shunting of arachidonic acid pathway from pro- to anti-inflammatory responses, in LPS stimulated murine macrophages.

Conclusions

- *The Se levels in soil and grains were significantly higher ($p<0.001$) with their respective non-Se controls. Se-uptake capacity among different cereal crops (wheat, maize and rice) grown on similar seleniferous fields were significantly different from each other ($p<0.001$).*
- *Se levels influence the whole elemental profile with elevated mineral composition in comparison to the non-seleniferous crops.*
- *Se levels among four protein fractions, the glutelin and prolamin fractions were dominated over other grains tested. The bioavailability of Se in different protein fractions is directly proportional to its concentration in the fractions.*
- *Se as an antioxidant is directly proportional to GPx-1 and H-PGDS expression and have inverse relationship with COX-2, mPGES-1 and iNOS profiles.*
- *Among dietary Se supplementation through seleniferous wheat and maize extracts, maize extracts confirm better accessibility of Se, although wheat grains accumulate higher Se levels than maize grains.*
- *The role of rMETase in facilitation of free Se from cereal matrices to suppress inflammation has showed significant difference in comparison to SeW and SeM supplementation alone.*

References

1. Aaltoma S.H., Lipponen P.K. and Kosma V.M. **2001**. Inducible nitric oxide synthase (iNOS) expression and its prognostic value in prostate cancer. *Anticancer Research*, **21:** 3101-3106.
2. Abdel-Hafez S.H. **2008**. Selenium containing heterocycles: synthesis, anti-inflammatory, analgesic and anti-microbial activities of some new 4-cyanopyridazine-3(2H)selenone derivatives. *European Journal of Medicinal Chemistry*, **43:** 1971-1977.
3. Abdel-Hafez S.H. **2010**. Synthesis of novel selenium-containing sulfa drugs and their antibacterial activities. *Bioorganicheskaia Khimiia*, **36:** 403-409.
4. Adams M.L., Lombi E., Zhao F.J. and McGrath S.P. **2002**. Evidence of low selenium concentrations in UK bread-making wheat grain. *Science of Food and Agriculture*, **82:** 1160-1165.
5. Adnan M. and Rouba A.F. **2005**. COX-2: a protein with an active role in gynecological cancers. *Current Opinion in Obstetrics & Gynecology*, **17:** 49-53.
6. Afonso C., Costa S., Cardoso C., Oliveira R., Lourenço H.M., Viula A., Batista I., Coelho I. and Nunes M.L. **2015**. Benefits and risks associated with consumption of raw, cooked, and canned tuna (*Thunnus* spp.) based on the bioaccessibility of selenium and methylmercury. *Environmental Research*, **143:**130-137.
7. AFSSA. **2009**. Avis relatif a levaluation des teneurs en vitamines et mineraux des denrées enrichies et des complements alimentaires : synthese.
8. Ahn K.S., Noh E.J., Zhao H.L., Jung S.H., Kang S.S. and Kim Y.S. **2005**. Inhibition of inducible nitric oxide synthase and cyclooxygenase II by Platycodon grandiflorum saponins via suppression of nuclear factor-κB activation in RAW 264.7 cells. *Life Sciences*, **76:** 2315-2328.
9. Ahn S.I., Lim S.J., Gu G.J., Kim J.S., Paek J.H., Kim S., Lim S.S. and Youn, H. S. **2015**. *Eupartoium makinoi* suppresses lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase-2 expression. *Food and Agriculture Immunology*, **26:** 496-503.
10. Aktan F. **2003**. iNOS-mediated nitric oxide production and its regulation. *Life Sciences*, **75:** 639-653.
11. Alabi G. **2014**. Bradford's law and its application. *International Library Review*, **11:** 151-158.

12. Alarcon M.N. and Martinez M.C. **2000**. Essentiality of selenium in human body: relationship with different diseases. *The Science of the Total Environment*, **249:** 347-371.
13. Alarcon M.N. and Vique C.C. **2008**. Selenium in food and the human body: a review. *Science of the total environment*, **400:** 115-141.
14. Alfthan G. and Neve J. **1996.** Selenium intakes and plasma selenium levels in various populations. In Natural Antioxidants and Food Quality in Atherosclerosis and Cancer Prevention. *Cambridge: Royal Society of Chemistry*, pp. 161–167.
15. Alfthan G.1., Xu G.L., Tan W.H., Aro A., Wu J., Yang Y.X., Liang W.S., Xue W.L. and Kong L.H. **2000**. Selenium supplementation of children in a selenium-deficient area in China: blood selenium levels and glutathione peroxidase activities. *Biological Trace Element Research*, **73:** 113-125.
16. Allan C.B., Lacourciere G.M. and Stadtman T.C. **1999**. Responsiveness of selenoproteins to dietary selenium. *Annual Review of Nutrition*, **19:** 1-16.
17. Allmang C., Wurth L. and Krol A. **2009**. The selenium to selenoprotein pathway in eukaryotes: More molecular partners than anticipated. *Biochimica et Biophysica Acta (BBA)-General Subjects*, **1790:** 1415-1423.
18. Alzate A., Conde M.C.P., Gutierrez A.M., Camara C. **2010**. Selenium-enriched fermented milk: A suitable dairy product to improve selenium intake in humans. *International Dairy Journal*, **20:** 761-769.
19. Ammar E. and Couri D. **1981**. Acute toxicity of sodium selenite and selenomethionine in mice after ICV or IV administration. *Neurotoxicology*, **2:** 383-386.
20. Amoako P., Uden P. and Tyson J. **2009**. Speciation of selenium dietary supplements; formation of S-(methylseleno) cysteine and other selenium compounds. *Analytica Chimica Acta*, **652:** 315-323.
21. Ando M., Murakami Y., Kojima F., Endo H., Kitasato H., Hashimoto A., Kobayashi H., Majima M., Inoue M., Kondo H., Kawai S. and Hayashi I. **2003**. Retrovirally introduced prostaglandin D2 synthase suppresses lung injury induced by bleomycin. *American Journal of Respiratory Cell and Molecular Biology*, **28:** 582-591.
22. Andren A.W., Klein D.H. and Talmi Y. **1975**. Selenium in coal-fired steam plant emissions. *Environmental Science and Technology*, **9:** 856-858.

23. Angstwurm M. W. A., Schottdorf J., Schopophil J. and Gaertner R. **1999**. Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome. *Critical Care Medicine,* **27:** 1807-1813.
24. Antoine J.M.R., Hoo Fung L.A., Grant C.N., Dennis H.T. and Lalor G.C. **2012**. Dietary intake of minerals and trace elements in rice on the Jamaican market. *Journal of Food Composition and Analysis,* **26:** 111–121.
25. Arbogast S. and Ferreiro A. **2010**. Selenoproteins and Protection against Oxidative Stress: Selenoprotein N as a Novel Player at the Crossroads of Redox Signaling and Calcium Homeostasis. *Antioxidants and Redox Signaling,* **12:** 893-904.
26. Arora S. P., Kaur P., Khirwar S. S., Chopra R. C., Ludri R. S. **1975**. Selenium levels in fodders and its relationship with Degnala Disease. *Indian Journal of Dairy Sciences,* **28:** 249-253.
27. Arteel G.E., Mostert V., Oubrahim H., Briviba K., Abel J. and Sies H. **1998**. Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. *Biological Chemistry,* **379:** 1201-1205.
28. Arteel G. and Sies H. **2001**. The biochemistry of selenium and the glutathione system. *Environmental Toxicology and Pharmacology,* **10:** 153-158.
29. Arthur J.R. **1994**. New metabolic role of selenium. *Proceedings of the Nutrition Society,* **53:** 615-624.
30. Ashour M.N., Salem S.I., El-Gadban H.M., Elwa N.M. and Basu T.K. **1999**. Antioxidant status in children with protein energy malnutrition (PEM) living in Cairo, Egypt. *European Journal of Clinical Nutrition,* **53:** 669-673.
31. Aureli F., Ouerdane L., Bierla K., Szpunar J., Prakash N.T. and Cubadda F. **2012**. Identification of selenosugars and other low-molecular weight selenium metabolites in high-selenium cereal crops. *Metallomics,* **4:** 968-978.
32. Avissar N., Kerl E.A., Baker S.S. and Cohen H.J. **1994**. Extracellular glutathione peroxidase mRNA and protein in human cell lines. *Archives of Biochemistry and Biophysics,* **309:** 239-246.
33. Baeten J.M, Mostad S.B., Hughes M.P., Overbaugh J., Bankson D.D., Mandaliya K., Ndinya-Achola J.O., Bwayo J.J. and Kreiss J.K. **2001**. Selenium deficiency is associated with shedding of HIV-1-infected cells in female genital tract. *Journal of Acquired Immune Deficiency Syndrome,* **26:** 360-364.

34. Baines A., Taylor-Parker M., Goulet A.C., Renaud C., Gerner E.W. and Nelson M.A. **2002**. Selenomethionine inhibits growth and suppresses cyclooxygenase-2 (COX-2) protein expression in human colon cancer cell lines. *Cancer Biology & Therapy*, **1:** 370-374.
35. Bajaj M., Eiche E., Neumann T., Winter J., Gallert C. **2011**. Hazardous concentrations of selenium in soil and groundwater in North-West India. *Journal of Hazardous Materials*, **189:** 640-646.
36. Banning A., Florian S., Deubel S., Thalmann S., Muller-Schmehl K., Jacobasch G. and Brigelius-Flohe R. **2008**. GPx2 Counteracts PGE_2 Production by Dampening COX-2 and mPGES-1 Expression in Human Colon Cancer Cells. *Antioxidants and Redox Signaling*, **10:** 1491-1500.
37. Banning A., Kipp A., Schmitmeier S., Lowinger M., Florian S., Krehl S., Thalmann S., Thierbach R., Steinberg P. and Flohe R.B. **2008**. Glutathione peroxidase 2 inhibits cyclooxygenase-2–mediated migration and invasion of HT-29 adenocarcinoma cells but supports their growth as tumors in nude mice. *Cancer Research*, **68:** 9746-9753.
38. Baraboi V.A. and Shestakova E.N. **2004**. Selenium: the biological role and antioxidant activity. *Ukrainian Biochemical Journal*, **76:** 23-32.
39. Baragatti B., Sodini D., Uematsu S. and Coceani F. **2008**. Role of microsomal prostaglandin E synthase-1 (mPGES1)-derived PGE2 in patency of the ductus arteriosus in the mouse. *Pediatric Research*, **64:** 523-527.
40. Barrett C.W., Ning W., Chen X., Smith J.J., Washington M.K., Hill K.E., Coburn L.A., Peek R.M. Chaturvedi R., Wilson K.T., Burk R.F. and Williams C.S. **2013**. Tumor suppressor function of the plasma glutathione peroxidase Gpx3 in colitis-associated carcinoma. *Cancer Research*, **73:** 1245-1255.
41. Baum M.K. and Shor-Posner G. **1998**. Micronutrient status in relationship to mortality in HIV-1 disease. *Nutrition Reviews*, **56:** 135-139.
42. Baur A., Bauer K., Jarry H. and Kohrle J. **2000**. Effects of proinflammatory cytokines on anterior pituitary 5'-deiodinase type I and type II. *Journal of Endocrinology*, **167:** 505-515.
43. Beck M., Esworthy R., Ho Y. and Chu F. **1998**. Glutathione peroxidase protects mice from viral-induced myocarditis. *Faseb Journal*, **12:** 1143- 1149.
44. Beckett G.J., Beddows S.E. and Morrice P.C. **1987**. Inhibition of hepatic de-iodination of thyroxine is caused by selenium deficiency in rats. *Biochemical Journal*, **248:** 443-447.

45. Beckett G.J. and Arthur J.R. **2005**. Selenium and endocrine systems. *Journal of Endocrinology*, **184:** 455-465.
46. Behne D., Weiler H. and Kyriakopoulos A. **1996**. Effects of selenium deficiency on testicular morphology and function in rats. *Journal of Reproduction and Fertility*, **106:** 291-297.
47. Behne D. and Kyriakopoulos A. **2001**. Mammalian selenium-containing proteins. *Annual Reviews of Nutrition*, **21:** 453-473.
48. Behne D., Alber D. and Kyriakopoulos A. **2009**. Effects of long-term selenium yeast supplementation on selenium status studied in the rat. *Journal of Trace Elements in Medicine and Biology*, **23:** 258-264.
49. Beilstein M. and Whanger P. **1988**. Glutathione peroxidase activity and chemical forms of selenium in tissues of rats given selenite or selenomethionine. *Journal of Inorganic Biochemistry*, **33:** 31-46.
50. Bell-Parikh L.C., Ide T., Lawson J.A., McNamara P., Reilly M. and FitzGerald G.A. **2003**. Biosynthesis of 15-deoxy-delta12,14-PGJ2 and the ligation of PPAR gamma. *Journal of Clinical Investigation*, **112:** 945–955.
51. Bergamini C.M. Gambetti S., Dondi A. and Cervellati C. **2004**. Oxygen, Reactive Oxygen Species and Tissue Damage. *Current Pharmaceutical Design*, **10:** 1611-1626.
52. Birringer M., Pilawa S., and Flohe L. **2002**. Trends in selenium biochemistry. *Natural Product Research*, **19:** 693-718.
53. Bjornstedt M., Kumar S. and Holmgren A. **1992**. Selenodiglutathione Is a Highly Efficient Oxidant of Reduced Thioredoxin and a Substrate for Mammalian Thioredoxin Reductase. *The Journal of Biological Chemistry*, **267:** 8030-8034.
54. Bock A., Forchhammer K., Heider J., Leinfelder W., Sawers G., Veprek B. and Zinoni F. **1991**. Selenocysteine: the 21st amino acid. *Molecular Microbiology*, **5:** 515-520.
55. Boldrin P.F., Faquin V., Ramos S.J., Boldrin K.V.F., William F., Avila F.W. and Guilherme L.R.G. **2013**. Soil and foliar application of selenium in rice biofortification. *Journal of Food Composition and Analysis*, **31:** 238-244.
56. Bosschaerts T., Guilliams M., Noel W., Herin M., Burk R.F., Hill K.E., Brys L., Raes G., Ghassabeh G.H., De Baetselier P. and Beschin A. **2008**. Alternatively activated myeloid cells limit pathogenicity associated with African Tryoanosomiasis through the IL-10 inducible gene selenoprotein P. *Journal of Immunology*, **180:** 6168-6175.

57. Boyne R. and Arthur J.R. **1986**. The response of selenium deficient mice to Candida albicans infection. *The Journal of Nutrition*, **116:** 816-822.
58. Brandon E., Oomen A., Rompelberg C., Versantvoort C., van Engelen J. and Sips A. **2006**. Consumer product in vitro digestion model: Bioaccessibility of contaminants and its application in risk assessment. *Regulatory Toxicology and Pharmacology*, **44:** 161-171.
59. Brenneisen P., Steinbrenner H. and Sies H. **2005**. Selenium, oxidative stress, and health aspects. *Molecular Aspects of Medicine*, **26:** 256-267.
60. Bressani R., Breuner M. and Ortiz M.A. **1989**. Contenidodefibraacidoy neutro-detergente y de minerales menores en maíz y su tortilla. *Archivos Latinoamericanos de Nutricion*, **39:** 382-391.
61. Brigelius F.R. and Kipp A. **2009**. Glutathione peroxidases in different stages of carcinogenesis. *Biochimicia et Biophysica Acta*, **1790:** 1555-1568.
62. Brody, T. **1994**. Nutritional Biochemistry. Academic Press, Inc. New York.
63. Bronze-Uhle E.S., Costa B.C., Ximenes V.F. and Lisboa-Filho P.N. **2017**. Synthetic nanoparticles of bovine serum albumin with entrapped salicylic acid. *Nanotechnology Science and Applications*, **10:** 11-21.
64. Brown T. A. and Shrift A. **1982**. Selenium: toxicity and tolerance in higher plants. *Biological Reviews*, **57:** 59-84.
65. Brozmanova J., Manikoa, D., Vlckova V. and Chovanec M. **2010**. Selenium: A double-edged sword for defense and offence in cancer. *Archives of Toxicology*, **84:** 919-938.
66. Burk R.F., Hill K.E. and Motley A.K. **2001**. Plasma selenium in specific and non-specific forms. *Biofactors*, **14:** 107-114.
67. Burk R.F. and Hill K.E. **2005**. Selenoprotein P: An extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annual Review of Nutrition*, **25:** 215-235.
68. Burk R.F., Olson G.E., Winfrey V.P., Hill K.E. and Yin D. **2011**. Glutathione peroxidase-3 produced by the kidney binds to a population of basement membranes in the gastrointestinal tract and in other tissues. *American journal of physiology. Gastrointestinal and liver*, **301:** 32-38.
69. Burnouf T. and Bietz J.A. **1989**. Rapid purification of wheat glutenin for reverse-phase high-performance liquid chromatography: comparison of dimethyl sulfoxide with traditional solvents. *Cereal Chemistry*, **66:** 121-127.

70. Butler J. A. and Whanger P.D. **1989**. Influence of dietary methionine on the metabolism of selenomethionine in rat. *Journal of Nutrition*, **119:** 1001-1009.
71. Buttery L.D., Springall D.R., Chester A.H., Evans T.J., Standfield E.N., Parums D.V., Yacoub M.H. and Polak J.M. **1996**. Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, **75:** 77-85.
72. Cabanero A.I., Madrid Y. and Camara C. **2006**. Selenium Long-Term Administration and Its Effect on Mercury Toxicity. *Journal of Agriculture and Food Chemistry*, **54:** 4461-4468.
73. Cadenas C., Franckenstein D., Schmidt M., Gehrmann M., Hermes M., Geppert B., Schormann W., Maccoux L.J., Schug M., Schumann A., Wilhelm C., Freis E., Ickstadt K., Rahnenfuhrer J., Baumbach J.I., Sickmann A. and Hengstler J.G. **2010**. Role of thioredoxin reductase 1 and thioredoxin interacting protein in prognosis of breast cancer. *Breast Cancer Research*, **12:** 1-15.
74. Cagampang G.B., Cruz L.J., Espiritu S.G., Santiago R.G. Juliano B.O. **1966**. Studies on the extraction and composition of rice proteins. *Cereal Chemistry*, **43:** 145-155.
75. Cahlin C., Lonnroth C., Arvidsson A., Nordgren S. and Lundholm K. **2008**. Growth associated proteins in tumor cells and stroma related to disease progression of colon cancer accounting for tumor tissue PGE2 content. *International Journal of Oncology*, **32:** 909-918.
76. Cai Y., Sukhova G.K., Wong H.K., Xu A., Tergaonkar V., and Vanhoutte P.M. **2015**. Rap1 induces cytokine production in pro-inflammatory macrophages through NFκB signaling and is highly expressed in human atherosclerotic lesions. *Cell Cycle*, **14:** 3580-3592.
77. Calatayud M., Devesa V., Virseda J.R., Barbera R., Montoro R., Velez D. **2012**. Mercury and selenium in fish and shellfish: Occurrence, bioaccessibility and uptake by Caco-2 cells. *Food and Chemical Toxicology*, **50:** 2696-2702.
78. Calvo I.A., Boronat S., Domenech A., Santamarina S.G., Ayte J. and Hidalgo E. **2013**. Dissection of a Redox Relay: H_2O_2-Dependent Activation of the Transcription Factor Pap1 through the Peroxidatic Tpx1-Thioredoxin Cycle. *Cell Reports*, **5:** 1413-1424.
79. Campa A., Shor-Posner G. and Indacochea. **1999**. Mortality risk in selenium-deficient HIV-positive children. *Journal of Acquired Immune Deficiency Syndrome*, **20:** 508-513.
80. Cao Z.H., Wang X.C., Yao D.H., Zhang X.L. and Wong M.H. **2001**. Selenium geochemistry of paddy soils in Yangtze River Delta. *Environmental International*, **26:** 335-339.

81. Cao S., Durrani F.A., Toth K. and Rustum Y.M. **2014**. Se-methylselenocysteine offers selective protection against toxicity and potentiates the antitumour activity of anticancer drugs in preclinical animal models. *British Journal of Cancer*, **110:** 1733-1743.
82. Carey A.M., Lombi E., Donner E., de Jonge M.D., Punshon T., Jackson B.P., Guerinot M.L., Price A.H. and Meharg A.A. **2012**. A review of recent developments in the speciation and location of arsenic and selenium in rice grain. *Analytical and Bioanalytical Chemistry*, **402:** 3275-3286.
83. Castano A., Ayala A., Rodriguez-Gomez J.A., Herrera A.J., Cano J. and Machado A. **1997**. Low selenium diet increases the dopamine turnover in prefrontal cortex of the rat. *Neurochemistry International*, **30:** 549-555.
84. Castets P., Bertrand A.T., Beuvin M., Ferry A., Grand F.L., Castets M., Chazot G., Rederstorff M., Krol A., Lescure A., Romero N.B., Guicheney P. and Allamand V. **2011**. Satellite cell loss and impaired muscle regeneration in selenoprotein N deficiency. *Human Molecular Genetics*, **20:** 694-704.
85. Chambers I., Framoton J., Goldfarb P., Affara N., McBain W. and Harrison P.R. **1986**. The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. *European Molecular Biology Organization Journal*, **5:** 1221-1227.
86. Chansler M.W., Mutanen M., Morris V.C. and Levander O.A. **1986**. Nutritional bioavailability to rats of selenium in Brazil nuts and mushrooms. *Nutrition Research*, **6:** 1419-1428.
87. Charter R.A., Tabatabai M.A. and Schafer J.W. **1995**. Arsenic, molybdenum, selenium and tungsten contents of fertilizers and phosphate rocks. *Communication in Soil Science and Plant Analysis*, **26:** 3051-3062.
88. Chen Y.C., Shen S.C., Chen L.G., Flee T.J. and Yang L.L. **2001**. Wogonin, baicalin, and baicalein inhibition of inducible nitric oxide synthase and cyclooxygenase-2 gene expressions induced by nitric oxide synthase inhibitors and lipopolysaccharide. *Biochemical Pharmacology*, **61:** 1417-1427.
89. Chen L., Yang F., Xu J., Hu Y., Hu Q., Zhang Y. and Pan G. **2002**. Determination of Selenium Concentration of Rice in China and Effect of Fertilization of Selenite and Selenate on Selenium Content of Rice. *Journal of Agricultural and Food Chemistry*, **50:** 5128-5130.

90. Chen Y.J., Chen Y.Y., Wu C.T., Yu C.C. and Liao H.F. **2010**. Prolamin, a rice protein, augments anti-leukaemia immune response. *Journal of Cereal Science*, **51:** 189-197.
91. Chen J. **2012**. An original discovery: selenium deficiency and Keshan disease (an endemic heart disease). *Asia Pacific Journal of Clinical Nutrition*, **21:** 320-326.
92. Chen Y., Liu H., Xu S., Wang T. and Li W. **2015**. Targeting microsomal prostaglandin E2 synthase-1 (mPGES-1): the development of inhibitors as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs). *Medicinal Chemical Communications*, **6:** 2081-2123.
93. Chilimba A.D.C., Young S.D., Black C.R., Rogerson K.B., Ander E.L., Watts M.J., Lammel J. and Broadley M.R. **2011**. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. *Scientific Reports*, **72:** 1-9.
94. Chiou W.F., Chen C.F. and Lin J.J. **2000**. Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide. *British Journal of Pharmacology*, **129:** 1553-1560.
95. Choi J.H., Kim T.N., Kim S., Baek S.H., Kim J.H., Lee S.R. and Kim J.R. **2002**. Overexpression of mitochondrial thioredoxin reductase and peroxiredoxin III in hepatocellular carcinomas. *Anticancer Research*, **22:** 3331-3335.
96. Chrastil J and Zarins Z. **1994**. Changes in peptide subunit composition of albumins, globulins, prolamins, and oryzenin in maturing rice grains. *Journal of Agricultural and Food Chemistry*, **42:** 2152-2155.
97. Christophersen O.A., Lyons G., Haug A. and Steinnes E. **1995**. Selenium. In: Alloway, BJ (ed.), *Heavy Metals in Soils*, Glasgow: Blackwell, pp. 429-463.
98. Chu S.C., Konczalik J.M., Wu H.P., Banks T.C. and Moss J. **1998**. Analysis of the Cytokine-Stimulated Human Inducible Nitric Oxide Synthase (iNOS) Gene: Characterization of Differences between Human and Mouse iNOS Promoters. *Biochemical and Biophysical Research Communications*, **248:** 871-878.
99. Chu F.F., Esworthy R.S., Lee L. and Wilczynski S. **1999**. Retinoic Acid Induces Gpx2 Gene Expression in MCF-7 Human Breast Cancer Cells. *The Journal of Nutrition*, **129:** 1846-1854.
100. Chu F.F., Esworthy R.S. and Doroshow J.H. **2004**. Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. *Free Radical Biology and Medicine*, **36:** 1481-1495.

101. Chung S.S., Kim M., Youn B.S., Lee N.S., Park J.W., Lee I.K., Lee Y.S., Kim J.B., Cho Y.M., Lee H.K. and Park K.S. **2008**. Glutathione Peroxidase 3 Mediates the Antioxidant Effect of Peroxisome Proliferator-Activated Receptor γ in Human Skeletal Muscle Cells. *Molecular and Cellular Biology*, **29:** 20-30.
102. Chung Y.W., Jeong D., Noh O.J., Park Y.H., Kang S.I., Lee M.G., Lee T.H., Yim M.B. and Kim I.Y. **2009**. Antioxidative role of selenoprotein W in oxidant-induced mouse embryonic neuronal cell death. *Molecules and Cells*, **27:** 609-613.
103. Clark L.C., Graham G.F., Crounse R.G., Grimson R., Hulka B. and Shy C.M. **1984**. Plasma selenium and skin neoplasms: a case control study. *Nutrition and Cancer*, **6:** 13-21.
104. Clark L.C., Combs G.F., Turnbull B.W., Slate E.H., Chalker D.K., Chow J., Davis L.S., Glover R.A., Graham G.F., Gross E.G., Krongrad A., Lesher J.L. Park H.K., Sanders B.B., Smith C.L. and Taylor J.R. **1996**. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA*, **276:** 1957-63.
105. Combs, G.F. **1988**. Selenium in Foods. Advances in Food Research (Chichester, C.O. and Schweiger, B.S., editors). San Diego, Academic press, pp. 85-113.
106. Combs, G.F. **1997**. Selenium and cancer. In: Garewal HS, (ed.), *Antioxidants and Disease Prevention*, New York: CRC Press, pp. 97-113.
107. Combs G.F. and Gray W.P. **1998**. Chemopreventive agents: Selenium. *Pharmacology and Therapeutics*, **79:** 179-192.
108. Combs, G.F., Clark, L.C., and Turnbull, B. W**. 2001**. An analysis of cancer prevention by selenium. *Biofactors*, **14:**153-159.
109. Combs G.F. **2001**. Selenium in global food systems. *British Journal of Nutrition*, **85:** 517-547.
110. Conrad M., Schneider M., Seiler A. and Bornkamm G.W. **2007**. Physiological role of phospholipid hydroperoxide glutathione peroxidase in mammals. *Biological Chemistry*, **388:** 1019-1025.
111. Contempre B, Le Moine O., Dumont J.E., Denef J.F. and Many M.C. **1996**. Selenium deficiency and thyroid fibrosis. A key role for macrophages and TGF-α. *Methods in Enzymology*, **124:** 7-15.

112. Courtman C., Ryssen J.B.J.V. and Oelofse A. **2012**. Selenium concentration of maize grain in South Africa and possible factors influencing the concentration. *South African Journal of Animal Science*, **42:** 454-458.
113. Crack P.J., Taylor J.M., Flentjar N.J., Haan J.D., Hertzog P., Iannello R.C. and Kola I. **2001**. Increased infarct size and exacerbated apoptosis in the glutathione peroxidase-1 (Gpx-1) knockout mouse brain in response to ischemia/reperfusion injury. *Journal of Neurochemistry*, **78:** 1389-1399.
114. Crews H.M., Clarke P.A., Lewis D.J., Owen L.M., Strutt P.R. and Izquierdo A. **1996**. Investigation of selenium speciation in *in vitro* gastrointestinal extracts of cooked cod by high-performance liquid chromatography–inductively coupled plasma mass spectrometry and electrospray mass spectrometry. *Journal of Analytical Atomic Spectrometry*, **11:** 1177-1182.
115. Cubadda F., Aureli F., Ciardullo S., D'Amato M., Raggi A., Acharya R., Reddy R.A.V., and Nagaraja T.P. **2010**. Changes in Selenium Speciation Associated with Increasing Tissue Concentrations of Selenium in Wheat Grain. *Journal of Agricultural and Food Chemistry*, **58:** 2295–2301.
116. Curran J.E., Jowett J.B.M, Elliott K.S., Gao Y., Gluschenko K., Wang J., Azim D.M.A., Cai G., Mahaney M.C., Comuzzie A.G., Dyer T.D., Walder K.R., Zimmet P., MacCluer J.W., Collier G.R., Kissebah A.H. and Blangero J. **2005**. Genetic variation in selenoprotein S influences inflammatory response. *Nature Genetics*, **37:** 1234-1241.
117. Curtin D., Hanson R., Lindley T.N. and Butler R.C. **2010**. Selenium concentration in wheat (*Triticum aestivum*) grain as influenced by method, rate, and timing of sodium selenate application. *New Zealand Journal of Crop and Horticultural Science*, **34:** 329-339.
118. Daham K., James A., Balgoma D., Kupczyk M., Billing B., Lindeberg A., Henriksson E., FitzGerald G.A., Wheelock C.E., Dahlen S.E. and Dahlen B. **2014**. Effects of selective COX-2 inhibition on allergen-induced bronchoconstriction and airway inflammation in asthma. *The Journal of Allergy and Clinical Immunology*, **134:** 306-313.
119. Da'Silva E.G., Mataveli L.R.V. and Arruda M.A.Z. **2013**. Speciation analysis of selenium in plankton, Brazil nut and human urine samples by HPLC–ICP-MS. *Talanta*, **110:** 53-57.
120. Davis F.Z. , Lu Y., Thompson J.T, Prabhu S.K., Reddy P.V., Sordillo L.M. and Reddy C.C. **2002**. Nuclear factor-κB mediates over-expression of cyclooxygenase-2 during activation of

RAW 264.7 macrophages in selenium deficiency. *Free Radical Biology and Medicine*, **32:** 890-897.

121. Davis C.D., Zeng H. and Finley J.W. **2002**. Selenium enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice. *Journal of Nutrition*, **132:** 307-309.
122. Dedina J. and Tsalev D.L. **1995**. Hydride generationatomic absorption spectrometry. Chemical Analysis: *A Series of Monographs on Analytical Chemistry and Its Applications*, **6:** 1-544.
123. Desai D., Madhunapantula S.V., Gowdahalli K., Sharma A., Chandagaludoreswamy R., El-Bayoumy K., Robertson G.P. and Amin S. 2010. Synthesis and Characterization of a Novel iNOS/Akt Inhibitor *Se, Se′*-1,4-phenylenebis(1,2-ethanediyl)bisisoselenourea (PBISe) – against colon cancer. Biorganic and Medicinal Chemistry Letters, **20:** 2038-2043.
124. Desai D., Kaushal N., Gandhi U.H., Arner R.J., D'Souza C., Chen G., Vunta H., El-Bayoumy K., Amin S. and Prabhu S.K. **2010**. Synthesis and evaluation of the anti-inflammatory properties of selenium-derivatives of celecoxib. *Chemico-Biological Interactions*, **188:** 446-56.
125. Dey S., Stafford R., Deb Roy M. K., Bhattacharjee C. R., Khathing D. T., Bhattacharjee P. C., Dkhar P. S. **1999**. Metal toxicity and trace element deficiency in some wild animal species from north-east India, as revealed by cellular, bio-inorganic and behavioural studies. *Current Science*, **77:** 276-280.
126. Dhanjal N.I., Sharma S., and Nagaraja T.P. **2016**. Quantification and *in vitro* bioaccessibility of selenium from Osborne fractions of selenium rich cereal grains. *Cereal Chemistry*, **93:** 339-343.
127. Dhanya B.L., Swathy R.P. and Indira M. **2014**. Selenium Downregulates Oxidative Stress-Induced Activation of Leukotriene Pathway in Experimental Rats with Diabetic Cardiac Hypertrophy. *Biological Trace Element Research*, **161:** 107-115.
128. Dhillon K. S. and Dhillon S. K. **1991**. Selenium toxicity in soil, plants and animals in some parts of Punjab. *International Journal of Environmental Studies*, **37:** 15-24.
129. Dhillon K. S., Bawa S. S., Dhillon S. K. **1992**. Selenium toxicity in some plants and soils of Punjab. *Journal of Indian Society of Soil Science*, **40:** 132-136.

130. Dhillon K. S., Dhillon S. K. **1997**. Distribution of seleniferous soils in north-west India and associated toxicity problems in the soil–plant–animal–human continuum. *Land Contamination and Reclamation*, **5:** 313-322.
131. Dhillon K. S. and Dhillon S. K. **2000**. Selenium accumulation by sequentially grown wheat and rice as influenced by gypsum application in a seleniferous soil. *Plant and Soil*, **227:** 243–248.
132. Dhillon K. S., Dhillon S. K. **2003a**. Distribution and management of seleniferous soils. *Advances in Agronomy*, **79:** 119-184.
133. Dhillon K. S., Dhillon S. K. **2003**. Quality of underground water and its contribution towards selenium enrichment of the soil-plant system for a seleniferous region of northwest India. *Journal of Hydrology*, **272:** 120-130.
134. Dhillon S.K. and Dhillon K.S. **2009**. Phytoremediation of selenium-contaminated soils: the efficiency of different cropping systems. *Soil Use and Management*, **25:** 441-453.
135. Dhingra S. and Bansal M. P. **2006**. Attenuation of LDL receptor gene expression by selenium deficiency during hypercholesterolemia. *Molecular and Cellular Biochemistry*, **282:** 75–82.
136. Diaz J.P., Navarro M., Lopez H. and Lopez M.C. **1996**. Selenium (IV) and (VI) levels in potable, irrigation and waste waters from an industrial zone in southeastern Spain. *Science of the Total Environment*, **186:** 231-236.
137. Diaz H.V., Hinojosa R.L., Marchante-Gayon J.M., Fernandez S.M.L. and Sanz-Medel A. **2003**. Total determination and quantitative speciation analysis of selenium in yeast and wheat flour by isotope dilution analysis ICP-MS. *Journal of Analytical Atomic Spectrometry*, **18:** 1243–1247.
138. Diaz H.V., Fernandez S.M. and Sanz M.A. **2006**. An attempt to differentiate HPLC-ICP-MS selenium speciation in natural and selenised Agaricus mushrooms using different species extraction procedures. *Analytical and Bioanalytical Chemistry*, **384:** 902-907.
139. Diwakar B.T., Finch E.R., Liao C., Shay A.E. and Prabhu S.K. **2016**. The Role of Selenoproteins in Resolution of Inflammation. *Selenium*, **42:** 499-510.
140. Doll H. **1977**. Storage Proteins in Cereals. *Genetic Diversity in Plants*, **8:** 337-347.

141. Donadio C. **2010**. Serum and urinary markers of early impairment of GFR in chronic kidney disease patients: diagnostic accuracy of urinary β-trace protein. *American Journal of Physiology*, **299:** 1407-1423.
142. Doran J.W. and Alexander M. **1977**. Microbial formation of volatile Se compounds in soil. *Soil Science Society of America Journal*, **40:** 687-690.
143. Draize J.H. and Beath O.A. **1935**. Observation on pathology of blood staggers and alkali disease. *Journal of American Veterinary Medical Association,* **39:** 753-763.
144. D'Souza A.P., Sieberg R., Li H., Cahill H.R., Zhao D., Jorge T.C.A., Tanowitz H.B. and Jelicks L.A. **2010**. The role of selenium in intestinal motility and morphology in a murine model of *Typanosoma cruzi* infection. *Parasitology Research,* **106:** 1293-1298.
145. D'Souza G.A., Hart J.J., Carvalho J.G., Rutzke M.A., Albrecht J.C., Guilherme L.R.G., Kochian L.V. and Li L. **2014**. Genotypic variation of zinc and selenium concentration in grains of Brazilian wheat lines. *Plant Science,* **224:** 27-35.
146. Ducros V. and Favier A. **2004**. Metabolisme du selenium. *EMC-Endocrinologie,* **1:** 19-28.
147. Du S., Zhou J., Jia Y.and Huang K. **2010**. SelK is a novel ER stress-regulated protein and protects HepG2 cells from ER stress agent-induced apoptosis. *Archives of Biochemistry and Biophysics*, **502:** 137-143.
148. Duffield A.J., Thomson C.D., Hill K.E. and Williams S. **1999**. An estimation of selenium requirements for New Zealanders. *American Journal of Clinical Nutrition*, **70:** 896–903.
149. Dumont E., Vanhaecke F. and Cornelis R. **2006**. Selenium speciation from food source to metabolites: a critical review. *Analytical and Bioanalytical Chemistry*, **385:** 1304-1323.
150. Dumont E., Ogra Y., Vanhaecke F., Suzuki K.T. and Cornelis R. **2006**. Liquid chromatography–mass spectrometry (LC–MS): a powerful combination for selenium speciation in garlic (*Allium sativum*). *Analytical and Bioanalytical Chemistry,* **384:** 1196-1206.
151. Duntas L.H. **2009**. Selenium and inflammation: underlying anti-inflammatory mechanisms. *Hormone and Metabolic Research,* **41:** 443-447.
152. Eberstal S., Sanden E., Fritzell S., Darabi A., Visse E. and Siesjo P. **2014**. Intratumoral COX-2 inhibition enhances GM-CSF immunotherapy against established mouse GL261 brain tumors. *International Journal of Cancer*, **134:** 2748-2753.

153. El-Bayoumy K. **1991**. The role of selenium in cancer pervention. In: deVita, VT, Hellman S, Rosenberg SS (eds.), *Practice of Oncology* (4th Ed.). Philadelphia: Lippincott, pp. 1-15.
154. El-Bayoumy K., Upadhyaya P., Chae Y.H., Sohn O.S., Rao C.V., Fiala E. and Reddy B.S. **1995**. Chemoprevention of cancer by organoselenium compounds. *Journal of Cellular Biochemistry Supplement*, **22:** 92-100.
155. Eskew M.L., Zarkower A., Scheuchenzuber W.J., Burgess J.R., Scholz R.W., Hildenbrandt G. and Reddy CC. **1989**. Effects of inadequate vitamin E and/or selenium nutrition on the release of arachidonic acid metabolites in rat alveolar macrophages. *Prostaglandins*, **38:** 79-89.
156. Esworthy R.S., Aranda R., Martín M.G., Doroshow J.H., Binder S.W. and Chu F.F. **2001**. Mice with combined disruption of Gpx1 and Gpx2 genes have colitis. *American Journal of Physiology, Gastrointestinal and Liver Physiology*, **281:** 848-855.
157. Eurola M.H., Ekholm P.I., Ylinen M., Koivistoinen P.E. and Varo P.T. **1990**. Effects of selenium fertilization on the selenium content of cereal grains, flour, and bread produced in Finland. *Cereal Chemistry*, **67:** 334- 337.
158. Fairweather-Tait S. 1997. Bioavailability of selenium. *European Journal of Clinical Nutrition*, 51: 20-23.
159. Fairweather-Tait S. J., Bao Y., Broadley M. R., Collings R., Ford D., Hesketh J. E. and Hurst R. **2011**. Selenium in Human Health and Disease. *Antioxidants and Redox Signaling*, **14:** 1337-1383.
160. Fang Y., Wang L., Xin Z., Zhao L., An X. and Hu Q. **2008**. Effect of Foliar Application of Zinc, Selenium, and Iron Fertilizers on Nutrients Concentration and Yield of Rice Grain in China. *Journal of Agricultural and Food Chemistry*, **56:** 2079-2084.
161. Fang Y., Catron B., Zhang Y., Zhao L., Caruso J. and Hu Q. **2010**. Distribution and in Vitro Availability of Selenium in Selenium-Containing Storage Protein from Selenium-Enriched Rice Utilizing Optimized Extraction. *Journal of Agricultural and Food Chemistry*, **58:** 9731-9738.
162. FAO/WHO. **2004**. Vitamin and Mineral Requirements in Human Nutrition, 2nd Edition. World Health Organization, Geneva, Switzerland.

163. FAO/WHO. **2002**. Human vitamin and mineral requirements. Report of a joint FAO/WHO consultation, Bangkok, Thailand. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.
164. Farzaneh G. **2015**. The toxicity of different selenium forms and compounds – Review. *Agrartudomanyi Kozlemenyek*, **64:** 33-38.
165. Fenner A. **2016**. Prostate cancer: A COX-2 far? *Nature Reviews Urology*, **13:** 496.
166. Feng G.J., Goodridge H.S., Harnett M.M., Wei X.Q., Nikolaev A.V., Higson A.P. and Liew F.Y. **1999**. Extracellular Signal-Related Kinase (ERK) and p38 Mitogen-Activated Protein (MAP) Kinases Differentially Regulate the Lipopolysaccharide-Mediated Induction of Inducible Nitric Oxide Synthase and IL-12 in Macrophages: *Leishmania* Phosphoglycans Subvert Macrophage IL-12 Production by Targeting ERK MAP Kinase. *The Journal of Immunology*, **163:** 6403-6412.
167. Ferreiro A., Roy S.Q., Pichereau C., Moghadaszadeh B., Goemans N., Bonnemann C., Jungbluth H., Straub V. and Villanova M. **2002**. Mutations of the Selenoprotein N Gene, Which Is Implicated in Rigid Spine Muscular Dystrophy, Cause the Classical Phenotype of Multiminicore Disease: Reassessing the Nosology of Early-Onset Myopathies. *American Society of Human Genetics*, **71:** 739–749.
168. Ferreiro A., Groote C.C.D., Marks J.J., Goemans N., Schreiber G., Hanefeld F., Fardeau M., Martin J.J., Goebel H.H., Richard P., Guicheney P. and Bonnemann C.G. **2004**. Desmin-related myopathy with mallory body–like inclusions is caused by mutations of the selenoprotein N gene. *Annals of Neurology*, **55:** 676-686.
169. Finley J.W. **1998**. The absorption and tissue distribution of selenium from high-selenium broccoli are different from selenium from sodium selenite, sodium selenate, and selenomethionine as determined in selenium-deficient rats. *Journal of Agricultural Food Chemistry*, **46:** 3702–3707.
170. Finley J.W. and Davis C.D. **2001**. Selenium (Se) from high-selenium broccoli is utilized differently than selenite, selenate and selenomethionine, but is more effective in inhibiting colon carcinogenesis. *Biofactors*, **14:** 191-196.
171. Finley J.W. **2006**. Bioavailability of selenium from foods. *Nutrition Reviews*, **64:** 146–151.
172. Fleming G.A. **1980**. Essential micronutrients II: Iodine and selenium. In: Davis BE (ed.), *Applied Soils Trace Elements* (1st Ed.), New York: John Wiley & Sons, pp. 199-234.

173. Florian S., Krehl S., Loewinger M., Kipp A., Banning A., Esworthy S., Chu F.F. and Brigelius-Flohe R. **2010**. Loss of GPx2 increases apoptosis, mitosis, and GPx1 expression in the intestine of mice. *Free Radical Biology and Medicine*, **49:** 1694-1702.

174. Flynn A., Hirnoven T., Mensisk G.B., Ocke M.C., Serra-Majem L., Stos K., Szponar L., Tetenus I., Turrini A., Fletcher R. and Wildemann T. **2009**. Intake of selected nutrients from foods, from fortification and from supplements in various European countries. *Journal of Food and Nutrition* Research, **53:** 1-51.

175. Fordyce F.M., Johnson C.C., Navaratna U.R.B., Appleton J.D., Dissanayake C.B. **2000**. Selenium and iodine in soil, rice and drinking water in relation to endemic goitre in Sri Lanka. *Science of The Total Environment*, **263:** 127-141.

176. Fordyce F. **2005**. Selenium deficiency and toxicity in the environment. In: Selinus O. (ed.). Essentials of Medical Geology(1st Ed.), Uppsala: Elsevier, pp. 373-415.

177. Forstermann U. and Sessa W.C. **2012**. Nitric oxide synthases: regulation and function. *European Heart Journal*, **33:** 829-837.

178. Fox T., Van den H.E., Atherton C., Dainty J., Lewis D., Langford N., Crews H., Luten J., Lorentzen M., Sieling F., van Aken-Schneyder P., Hoek M., Kotterman M., Van Dael P. and Fairweather-Tait S. **2004**. Bioavailability of selenium from fish, yeast and selenate: a comparative study in humans using stable isotopes. *European Journal of Clinical Nutrition*, **58:** 343-349.

179. Franke and Painter E. **1938**. A study on toxicity and selenium content of seleniferous diets: with statistical consideration. *Cereal Chemistry*, **15:** 1-24.

180. Fukumoto M., Kudou D., Murano S., Shiba T., Sato D., Tamura T., Harada S. and Inagaki K. **2012**. The Role of Amino Acid Residues in the Active Site of L-Methionine -lyase from Pseudomonas putida. *Bioscience, Biotechnology and Biochemistry*, **76:** 1275-1284.

181. Gad M. and Twab A.E.S. **2009**. Selenium toxicosis assessement (in vivo and in vitro) and the protective role of vitamin B12 in male quail (Coturnix Coturnix). *Environmental Toxicology and Pharmacology*, **27:** 7- 16.

182. Gallo O., Franchi A., Magnelli L., Sardi L., Vannacci A., Boddit V.,Chiarugi V. and Masini E. **2001**. Cyclooxygenase-2 Pathway Correlates with VEGF Expression in Head and Neck Cancer. Implications for Tumor Angiogenesis and Metastasis. *Neoplasia*, **3:** 53-61.

183. Gandhi U.H., Kaushal N., Ravindra K.C., Hegde S., Nelson S.M., Narayan, V., Vunta H., Pulson R.F. and Prabhu K.S. **2011**. Selenoprotein-dependent up-regulation of hematopoietic prostaglandin d_2 synthase in macrophages is mediated through the activation of peroxisome proliferator-activated receptor (PPARγ). *The Journal of Biological Chemistry*, **286:** 27471–27482.
184. Gandhi U.H., Nagaraja T.P. and Prabhu S.K. **2013**. Selenoproteins and their Role in Oxidative Stress and Inflammation. *Current Chemical Biology*, **7:** 65-73.
185. Gandhi U.H., Kaushal N., Hegde S., Finch E.R., Kudva A.K., Kennett M.J., Jordan C.T., Paulson R.F. and Prabhu S.K. **2014**. Selenium suppresses leukemia through the action of endogenous eicosanoids. *Cancer Research*, **74:** 3890-3901.
186. Gao Y., Feng H.C., Walder K., Bolton K., Sunderland T., Bishara N., Quick M., Kantham L. and Collier G.R. **2004**. Regulation of the selenoprotein SelS by glucose deprivation and endoplasmic reticulum stress - SelS is a novel glucose-regulated protein. *FEBS Letters*, **563:** 185-190.
187. Gao Y. , Hannan N.R.F., Wanyonyi S., Konstantopolous N., Pagnon J., Feng H.C., Jowett J.B.M., Kim K.H., Walder K. and Collier G.R. **2006**. Activation of the selenoprotein SEPS1 gene expression by pro-inflammatory cytokines in HepG2 cells. *Cytokine*, **33:** 246-251.
188. Garcia A.M., Garcia C and Fernandez R.J. **2009**. Cannabinoids and Parkinson's disease. *CNS Neurol Disord Drug Targets*, **8:** 432-439.
189. Gavett S.H., Madison S.L., Chulada P.C., Scarborough P.E., Qu W., Boyle J.E., Tiano H.F., Lee C.A., Langenbach R., Roggli V.L. and Zeldin D.C. **1999**. Allergic lung responses are increased in prostaglandin H synthase-deficient mice. *Journal of Clinical Investigation*, **104:** 721–732.
190. Geering H.R., Cary E.E., Jones L.H.P. and Allaway W.H. **1967**. Solubility and Redox Criteria for the Possible Forms of Selenium in Soils. *Soil Science Society of America Journal*, **32:** 35-40.
191. Ghadirian P., Maisonneuve P., Perret C., Kennedy G., Boyle P., Krewski D. and Lacroix A.A. **2000**. Case-control study of toenail selenium and cancer of the breast, colon, and prostate. *Cancer Detection and Prevention*, **24:** 305-313.

192. Ghosh A., Sarkar S., Pramanik A.K., Chaudhary P.S. and Ghosh S. **1993**. Selenium toxicosis in grazing buffaloes and its relationship with soil and plant of West Bengal. *Indian Journal of Animal Sciences*, **63:** 557-560.

193. Giannico G, Mendez M. and LaPointe M.C. **2005**. Regulation of the membrane-localized prostaglandin E synthases mPGES-1 and mPGES-2 in cardiac myocytes and fibroblasts. *American Journal of Physiology - Heart and Circulatory Physiology*, **288:** 165-174.

194. Gilroy D.W., Lawrence T., Perretti M. and Rossi A.G. **2004**. Inflammatory resolution: new opportunities for drug discovery. *Nature Reviews Drug Discovery*, **3:** 401–416.

195. Goh K. and Lim T. **2004**. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. *Chemosphere*, **55:** 849-859.

196. Gosetti F., Frascarolo P., Polati S., Medana C., Gianotti V., Palma P., Aigotti R., Baiocchi C. and Gennaro M. **2007**. Speciation of selenium in diet supplements by HPLC-MS/MS methods. *Food Chemistry*, **105:** 1738- 1747.

197. Grabowski P.S., Wright P.K., Van 't Hof R.J., Helfrich M.H., Ohshima H. and Ralston S.H. **1997**. Immunolocalization of inducible nitric oxide synthase in synovium and cartilage in rheumatoid arthritis and osteoarthritis. *Rheumatology*, **36:** 651–655.

198. Green S.J., Scheller L.F., Marletta M.A., Seguin M.C., Klotz F.W., Slayter M., Nelson B.J. and Nacy C.A. **1994**. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunology Letters*, **43:** 87-94.

199. Grieve C.M., Poss J.A., Suarez D.L. and Dierig D.A. **2001**. Lesquerella growth and selenium uptake affected by saline irrigation water composition. *Industrial Crops and Products*, **13:** 57-65.

200. Grill M., Peskar B.A., Schuligoi R. and Amann R. **2006**. Systemic inflammation induces COX-2 mediated prostaglandin D2 biosynthesis in mice spinal cord. *Neuropharmacology*, **50:** 165-173.

201. Grill M., Heinemann A., Hoefler G., Peskar B.A., Schuligoi R. **2008**. Effect of endotoxin treatment on the expression and localization of spinal cyclooxygenase, prostaglandin synthases, and PGD2 receptors. *Journal of Neurochemistry*, **104:** 1345-1357.

202. Grkovich A., Johnson C.A., Buczynski M.W. and Dennis E.A. **2006**. Lipopolysaccharide-induced cyclooxygenase-2 expression in human U937 macrophages is phosphatidic acid phosphohydrolase-1-dependent. *The Journal of Biological Chemistry*, **281:** 32978-32987.

203. Gromer S. and Gross J.H. **2002**. Methylseleninate is a substrate rather than an inhibitor of mammalian thioredoxin reductase. Implications for the antitumor effects of selenium. *The Journal of Biological Chemistry*, **277:** 9701-9706.

204. Gromer S., Eubel B., Lee B. and Jacob J. **2005**. Human selenoproteins at a glance. *Cellular and Molecular Life Sciences*, **62:** 2414-2437.

205. Groth F.S., Webster R.G., and Datyner A. **1963**. Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. *Biochimicia et Biophysica Acta*, **71:** 377-391.

206. Grumolato L., Ghzili H., Hadjadje M.M., Gasman S., Lesage J., Tanguy Y., Galas L., Ali D.A., Leprince J., Guerineau N.C., Elkahloun A.G., Fournier A., Vieau D., Vaudry H. and Anouar Y. **2008**. Selenoprotein T is a PACAP-regulated gene involved in intracellular Ca^{2+}mobilization and neuroendocrine secretion. *The Official Journal of the Federation of American Societies for Experimental Biology*, **22:** 1756-1768.

207. Gu Q.P., Sun Y., Ream L.W. and Whanger P.D. **2000**. Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. *Molecular and Cellular Biochemistry*, **204:** 49-56.

208. Gu R., Chen F., Liu B., Wang X., Liu J., Li P., Pan Q., Pace J., Soomro A.A., Lubberstedt T., Mi G. and Yuan L. **2015**. Comprehensive phenotypic analysis and quantitative trait locus identification for grain mineral concentration, content, and yield in maize (*Zea mays* L.). *Theoretical and Applied Genetics*. **128:** 1777-1789.

209. Gudis K., Tatsuguchi A., Wada K., Futagami S., Nagata K., Hiratsuka T., Shinji Y., Miyake K., Tsukui T., Fukuda Y. and Sakamoto C. **2005**. Microsomal prostaglandin E synthase (mPGES)-1, mPGES-2 and cytosolic PGES expression in human gastritis and gastric ulcer tissue. *Laboratory Investigations*, **85:** 225-236.

210. Guo T.W., Zhang F.C., Yang M.S., Gao X.C., Bian L., Duan S.W., Zheng Z.J., Gao J.J., Wang H., Li R.L., Feng G.Y., Clair D.S. and He L. **2004**. Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. *Journal of Medical Genetics*, **41:** 585-590.

211. Gupta U.C. and MacLeod J.A. **1994**. Effect of various sources of selenium fertilization on the selenium concentration of feed crops. *Canadian Journal of Soil Science*, **74:** 285-290.
212. Gupta U.C. and Gupta S.C. **2008**. Selenium in soils and crops, its deficiencies in livestock and humans: Implications for management. *Communications in Soil Science and Plant Analysis*, **31:** 1791-1807.
213. Guzman M.J., Reyes L., Rahman G. and Kingston H. **2009**. Simultaneous extraction of arsenic and selenium species from rice products by microwave-assisted enzymatic extraction and analysis by Ion Chromatography-Inductively Coupled Plasma-Mass Spectrometry. *Journal of Agricultural Food and Chemistry*, **57:** 3005-3013.
214. Hakkarainen J. **1993**. Bioavailability of selenium. *Journal of Agricultural Science*, **11:** 21-35.
215. Hart D.J., Fairweather-Tait S.J., Broadley M.R., Dickinson S.J., Foot I., Knott P., McGrath S.P., Mowat H., Norman K., Scott P.R., Stroud J.L., Tucker M., White P.J., Zhao F.J. and Hurst R. **2011**. Selenium concentration and speciation in biofortified flour and bread: Retention of selenium during grain biofortification, processing and production of Se-enriched food. *Food Chemistry*, **126:** 1771-1778.
216. Hartikainen H. **2005**. Biogeochemistry of selenium and its impact on food chain quality and human health. *Journal of Trace Elements in Medicine and Biology*, **18:** 309-318.
217. Hatfield D.L., Tsuji P.A., Carlson B.A. and Gladyshev V.N. **2014**. Selenium and selenocysteine: roles in cancer, health, and development. *Trends in Biochemical Sciences*, **39:** 112-120.
218. Hattori H., Imai H., Furuhama K., Sato O. and Nakagawa Y. **2005**. Induction of phospholipid hydroperoxide glutathione peroxidase in human polymorphonuclear neutrophils and HL60 cells stimulated with TNF-alpha. *Biochemical and Biophysical Research Communications*, **337:** 464–473.
219. Hawkesford M.J. and Zhao F.J. **2007**. Strategies for increasing the selenium content of wheat. *Journal of Cereal Science*, **46:** 282-292.
220. Hayaishi O. and Urade Y. **2002**. Prostaglandin D2 in sleep-wake regulation: recent progress and perspectives. *Neuroscientist*, **8:** 12–15.
221. Heinrich U., Tronnier H., Stahl W., Bejot M. and Maurette J. **2006**. Antioxidant supplements improve parameters related to skin structure in humans. *Skin Pharmacology and Physiology*, **19:** 224-231.

222. Hefferon K.L. **2015**. Nutritionally Enhanced Food Crops; Progress and Perspectives. *International Journal of Molecular Sciences*, **16:** 3895-3914.

223. Helliwell R.J., Adams L.F. and Mitchell M.D. **2004**. Prostaglandin synthases: recent developments and a novel hypothesis. *Prostaglandins Leukotrienes and Essential Fatty Acids*, **70:** 101–113.

224. Helliwell R.J., Keelan J.A., Marvin K.W., Adams L., Chang M.C., Anand A., Sato T.A., O'Carroll S., Chaiworapongsa T., Romero R.J., and Mitchell M.D. **2006**. Gestational age-dependent up-regulation of prostaglandin D synthase (PGDS) and production of PGDS-derived antiinflammatory prostaglandins in human placenta. *The Journal of Clinical Endocrinology and Metabolism*, **91:** 597-606.

225. Herlong J.L. and Scott T.R. **2006**. Positioning prostanoids of the D and J series in the immunopathogenic scheme. *Immunology Letters*, **102:** 121-131.

226. Hernandez A., Martinez M.E., Fiering S., Galton V.A. and Germain D.S. **2006**. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *The Journal of Clinical Investigation*, **116:** 476-484.

227. Hill R., Li Y., Tran L.M., Dry S., Calvopina J.H., Garcia A., Kim C., Wang Y., Donahue T.R., Herschman H.R. and Wu H. **2012**. Cell intrinsic role of COX-2 in pancreatic cancer development. *Molecular Cancer Therapeutics*, **11:** 2127-2137.

228. Hira C.K., Partal K. and Dhillon K.S. **2003**. Dietary selenium intake by men and women in high and low selenium areas of Punjab. *Public Health and Nutrition*, **7:** 39-43.

229. Hirsch F.R. and Lippman S.M. **2005**. Advances in the Biology of Lung Cancer Chemoprevention. *Journal of Clinical Oncology*, **23:** 3186-3197.

230. Hoffmann A., Conradt H.S., Gross G., Nimtz M., Lottspeich F. and Wurster U. **1993**. Purification and chemical characterization of β-trace protein from human cerebrospinal fluid: its identification as prostaglandin D synthase. *Journal of Neurochemistry*, **61:** 451–456.

231. Hoffmann P.R. **2007**. Mechanisms by which selenium influences immune responses. *Archivum Immunologiae et Therapia Experimentalis (Warsz)*, **55:** 289-297.

232. Hoffmann P.R., Hoge S.C., Li P.A., Hoffmann F.W., Hashimoto A.C. and Berry M.J. **2007**. The selenoproteome exhibits widely varying, tissue-specific dependence on selenoprotein P for selenium supply. *Nucleic Acid Research*, **35:** 3963-3973.

233. Hoffmann P.R. and Berry M.J. **2008**. The influence of selenium on immune responses. *Molecular Nutrition and Food Research*, **52:** 1273-1280.
234. Hong C.H., Hur S.K., Oh O.J., Kim S.S., Nam K.A. and Lee S.K. **2002**. Evaluation of natural products on inhibition of inducible cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) in cultured mouse macrophage cells. *Journal of Ethnopharmacology*, **83:** 153-159.
235. Horibata Y. and Hirabayashi Y. **2007**. Identification and characterization of human ethanolaminephosphotransferase1. *Journal of Lipid Research*, **48:** 503-508.
236. Hu Q., Chen L., Xu J., Zhang Y. and Pan G. **2002**. Determination of selenium concentration in rice and the effect of foliar application of Se-enriched fertilizer or sodium selenite on the selenium content of rice. *Science of Food and Agriculture*, **82:** 869-872.
237. Hu Y., Norton G.J., Duan G., Huang Y. and Liu Y. **2014**. Effect of selenium fertilization on the accumulation of cadmium and lead in rice plants. *Plant and Soil*, **384:** 131-140.
238. Huang S.A., Tu H.M., Harney J.W., Venihaki M., Butte A.J., Kozakewich H.P.W., Steven J., Fishman, M.D., and Larsen P.R. **2000**. Severe Hypothyroidism Caused by Type 3 Iodothyronine Deiodinase in Infantile Hemangiomas. *The New England Journal of Medicine*, **343:** 185-189.
239. Huang Z., Rose A.H. and Hoffmann P.R. **2012**. The role of selenium in inflammation and immunity: From molecular mechanism to therapeutic oppurtunities. *Antioxidants and Redox Signaling*, **16:** 705–743.
240. Huerta D.V., Fernandez S.M.L. and Sanz-Medel A. **2006**. An attempt to differentiate HPLC-ICP-MS selenium speciation in natural and selenised Agaricus mushroom using different species extraction procedures. *Analytical and Bioanalytical Chemistry*, **384:** 902-907.
241. Hurst R., Armah C.N., Dainty J.R., Hart D.J., Teucher B., Goldson A.J., Broadley M.R., Motley A.K. and Fairweather-Tait S.J. **2010**. Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. *The American Journal of Clinical Nutrition*, **91:** 923-931.
242. Hurst R., Collings R., Harvey L.J., King M., Hooper L., Bouwman J., Gurinovic M. and Fairweather-Tait S.J. **2013**. EURRECA-Estimating Selenium Requirements for Deriving Dietary Reference Values. *Critical Reviews in Food Science and Nutrition*, **53:** 1077-1096.

243. Hyo S., Kawata R., Kadoyama K., Eguchi N., Kubota T., Takenaka H. and Urade Y. **2007**. Expression of prostaglandin D2 synthase in activated eosinophils in nasal polyps. *Archives of Otolaryngology – Head & Neck Surgery*, **133:** 693-700.
244. Iadecola C., Zhang F., Xu S., Casey R. and Ross M.E. **1995**. Inducible Nitric Oxide Synthase Gene Expression in Brain following Cerebral Ischemia. *Journal of Cerebral Blood Flow and Metabolism*, **15:** 378-384.
245. Iadecola C., Zhang F., Casey R., Nagayama M. and Ross M.E. **1997**. Delayed Reduction of Ischemic Brain Injury and Neurological Deficits in Mice Lacking the Inducible Nitric Oxide Synthase Gene. *The Journal of Neuroscience*, **17:** 9157-9164.
246. IMFNB (Food and nutrition board, Institute of medicine-national academy of sciences dietary reference intakes: Recommended intakes for individuals for vitamin C, vitamin E, selenium and carotenoids. Bookshelf ID: NBK225466.
247. Institute of Medicine, Food and Nutrition Board, USA. **2000**. Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. The National Academies Press, Washington DC.
248. Ip, C. **1986**. The chemopreventive role of selenium in carcinogenesis. *Journal of the American College of Toxicology***, 5:** 7-20.
249. Ip C., Hayes C., Budnick R.M. and Ganther H.E. **1991**. Chemical form of selenium, critical metabolites, and cancer prevention. *Cancer Research*, **51:** 595-600.
250. Ip, C. **1998**. Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition***, 128:** 1845-1854.
251. Ip C., Birringer M., Block E., Kotrebai M., Tyson J., Uden P. and Lisk D. **2000**. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *Journal of Agricultural Food and Chemistry*, **48:** 2062-2070.
252. Ipp H., Zemlin A.E., Erasmus R.T. and Glashoff R.H. **2014**. Role of inflammation in HIV-1 disease progression and prognosis. *Critical Reviews in Clinical Laboratory Sciences*, **51:** 98-111.
253. Ishii T. **2015**. Selenium suppresses leukemia through the action of endogenous eicosanoids. Cancer Research. *Free Radical Biology and Medicine*, **88:** 189-198.

254. Itoh K., Mochizuki M., Ishii Y. and Yamamoto M. **2004**. Transcription Factor Nrf2 Regulates Inflammation by Mediating the Effect of 15-Deoxy-Δ12, 14-Prostaglandin J2. *Molecular and Cellular Biology*, **24:** 36-45.

255. Jaakkola K., Tummavuori J., Pirinen A., Kurkela P., Tolonen M. and Arstila A.U. **1983**. Selenium levels in whole blood of Finnish volunteers before and during organic and inorganic selenium supplementation. *Scandinavian Journal of Clinical and Laboratory Investigation*, **43:** 473-476.

256. Jaiswal S.K., Prakash R., Acharya R., Nathaniel T.N., Reddy A.V.R. and Prakash N.T. **2012a**. Bioaccessibility of selenium from Se-rich food grains of the seleniferous region of Punjab, India as analyzed by instrumental neutron activation analysis. *CyTA – Journal of Food*, **10:** 160-164.

257. Jaiswal S. K., Prakash R., Acharya R., Reddy A. V. R. and Tejo Prakash N. **2012b**. Selenium content in seed, oil and oil cake of Se hyperaccumulated *Brassica juncea* (Indian mustard) cultivated in a seleniferous region of India. *Food Chemistry*, **134:** 401-404.

258. Jaiswal S.K., Prakash R. and Nagaraja T.P. **2015**. Selenium in storage proteins of wheat cultivated on selenium impacted soils of Punjab, India. *Acta Alimentaria*, **44:** 235-241.

259. Jakupoglu C., Przemeck G.K., Schneider M., Moreno S.G., Mayr N., Hatzopoulos A.K., de Angelis M.H., Wurst W., Bornkamm G.W., Brielmeier M. and Conrad M. **2005**. Cytoplasmic thioredoxin reductase is essential for embryogenesis but dispensable for cardiac development. *Molecular and Cellular Biology*, **25:** 1980-1988.

260. Jana D., Sarkar D.K., Ganguly S., Saha S., Sa G., Manna A.K., Banerjee A. and Mandal S. **2014**. Role of cyclooxygenase 2 (COX-2) in prognosis of breast cancer. *Indian Journal of Surgical Oncology*, **5:** 59–65.

261. Jeong D.W., Kim T.S., Chung Y.W., Lee B.J. and Kim I.Y. **2002**. Selenoprotein W is a glutathione-dependent antioxidant in vivo. *FEBS Letters*, **517:** 225-228.

262. Ji J.D., Kim H.J., Rho Y.H., Choi S.J., Lee Y.H., Cheon H.J., Sohn J. and Song G.G. **2005**. Inhibition of IL-10-induced STAT3 activation by 15-deoxy-Δ12, 14-prostaglandin J_2. *Rheumatology*, **44:** 983-988.

263. Jimenez A., Zu W., Rawe V.Y., Huikko M.P., Flickinger C.J., Sutovsky P., Gustafsson J.A., Oko R. and Vizuete A.M. **2004**. Spermatocyte/Spermatid-specific Thioredoxin-3, a Novel

Golgi Apparatus-associated Thioredoxin, Is a Specific Marker of Aberrant Spermatogenesis. *Journal of Biological Chemistry*, **279:** 34971-34982.

264. Jin R.C., Mahoney C.E., Anderson L.C., Ottaviano F., Croce K., Leopold J.A., Zhang Y.Y., Tang S.S., Handy D.E. and Loscalzo J. **2011**. Glutathione Peroxidase-3 Deficiency Promotes Platelet-Dependent Thrombosis In Vivo. *Circulation*, **123:** 1963-1973.
265. Jirong Y., Huiyun P., Zhongzhe Y., Birong D., Weimin L., Ming Y. and Yi S. **2012**. Sodium selenite for treatment of Kashin-Beck disease in children: a systematic review of randomised controlled trials. *Osteoarthritis Cartilage*, **20:** 605-13.
266. Johann M.R., Antoine J.M.R., Fung L.A.H., Grant C.N., Dennis H.T. and Lalor G.C. **2012**. Dietary intake of minerals and trace elements in rice on the Jamaican market. *Journal of Food Composition and Analysis*, **26:** 111-121.
267. Johansson L., Gafvelin, G., and Arner E. **2005**. Selenocysteine in proteins -properties and biotechnological use. *Biochimica et Biophysica Acta*, **1726**: 1-13.
268. Jonnalagadda S.B. and Rao P.V. **1993**. Toxicity, bioavailability and metal speciation. *Comparative Biochemistry and Physiology*, **106:** 585-595.
269. Joo M. and Sadikot R.T. **2012**. PGD Synthase and PGD2 in Immune Resposne. *Mediators of Inflammation*, **2012:** 1-6.
270. Jordan W., Tumani H., Cohrs S., Eggert S., Rodenbeck A., Brunner E., Rüther E. and Hajak G. **2004**. Prostaglandin D synthase (β-trace) in healthy human sleep," *Sleep*, **27:** 867–874.
271. Jowsey I.R., Murdock P.R., Moore G.B., Murphy G.J., Smith S.A., Hayes J.D. **2003**. Prostaglandin D2 synthase enzymes and PPARgamma are co-expressed in mouse 3T3-L1 adipocytes and human tissues. *Prostaglandins and Other Lipid Mediators*, **70:** 267-284.
272. Judson G.J. and Reuter D.J. **1998**. Selenium. Soil Analysis: An Interpretation Manual, South Australia Research & Development Institute (SARDI), Urrbrae, South Australia, pp. 325-329.
273. Ju Z.Y., Hettiarachchy N.S. and Rath N. **2001**. Extraction, denaturation and hydrophobic properties of rice flour proteins. *Journal of Food Science*, **66:** 229-232.
274. Juliano B.O. **1985**. Rice bran. American Association of Cereal Chemists: St. Paul, MN, *Rice Chemistry and Technology*, pp. 289-348.
275. Jun L. and Sharon R. **2015**. Membrane-Bound Selenoproteins. *Antioxidants and Redox signaling*, **23:** 795-813.

276. Jurynec M.J., Xia R., Mackrill J.J., Gunther D., Crawford T., Flanigan K.M., Abramson J.J., Howard M.T. and Grunwald D.J. **2008**. Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle. *Proceedings of the National Academy of Sciences U.S.A.*, **105:** 12485–12490.
277. Kabata P.A. and Mukherjee A.B. **2007**. Trace Elements from Soil to Human. *Springer-Verlag Berlin Heidelberg*, pp. 1-23.
278. Kamara M.T., Ming Z.H. and Kexue Z. **2009**. Extraction, Characterization and Nutritional Properties of Two Varieties of Defatted Foxtail Millet Flour (*Setaria italica* L.) grown in China. *Asian Journal of Biochemistry,* **4:** 88-98.
279. Kaminski M., Kiessling M., Suss D., Krammer P.H. and Gulow K. **2007**. Novel role for mitochondria: protein kinase Ctheta-dependent oxidative signaling organelles in activation-induced T-cell death. *Molecular and Cell Biology*, **27:** 3625–3639.
280. Kanaoka Y., Ago H., Inagaki E., Nanayama T., Miyano M., Kikuno R., Fujii Y., Eguchi N., Toh H., Urade Y. and Hayaishi O. **1997**. Cloning and crystal structure of hematopoietic prostaglandin D synthase. *Cell*, **90:** 1085–1095.
281. Kanaoka Y., Fujimori K., Kikuno R., Sakaguchi Y., Urade Y. and Hayaishi O. **2000**. Structure and chromosomal localization of human and mouse genes for hematopoietic prostaglandin D synthase. Conservation of the ancestral genomic structure of sigma-class glutathione S-transferase. *European Journal of Biochemistry,* **267:** 3315-3322.
282. Kanaoka Y. and Urade Y. **2003**. Hematopoietic prostaglandin D synthase. *Prostaglandins Leukotrienes and Essential Fatty Acids*, **69:** 163–167.
283. Kapolna E. and Fodor P. **2009**. Bioavailability of selenium from selenium-enriched green onions (*Allium fistulosum*) and chives (*Allium schoenoprasum*) after '*in vitro*' gastrointestinal digestion. *International Journal of Food Sciences and Nutrition*, **58:** 282-296.
284. Kapoor M., Clarkson A.N., Sutherland B.A. and Appleton I. **2005**. The role of antioxidants in models of inflammation: emphasis on L-arginine and arachidonic acid metabolism. *Inflammopharmacology*, **12:** 505–519.
285. Kapoor M., Koima F., Yang L. and Crofford L.J. **2007**. Sequential induction of pro- and anti-inflammatory prostaglandins and peroxisome proliferators-activated receptor-gamma during normal wound healing: a time course study. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, **76:** 103-112.

286. Karpuzoglu E. and Ahmed S.A. **2006**. Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: Implications for immunity, autoimmune diseases, and apoptosis. *Nitric Oxide*, **15:** 177-186.

287. Kasaikina M.V., Fomenko D.E., Labunskyy V.M., Lachke S.A., Qiu W., Moncaster J.A., Zhang J., Wojnarowicz M.W., Natarajan S.K., Malinouski M., Schweizer U., Tsuji P.A., Carlson B.A., Maas R.L., Lou M.F., Goldstein L.E., Hatfield D.L. and Gladyshev V.N. **2011**. Roles of the 15-kDa Selenoprotein (Sep15) in Redox Homeostasis and Cataract Development Revealed by the Analysis of Sep 15 Knockout Mice. *Journal of Biological Chemistry*, **286:** 33203-33212.

288. Kauf E., Dawczynski H., Jahreis G., Janitsky E. and Winnefeld K. **1994**. Sodium selenite therapy and thyroid-hormone status in cystic fibrosis and congenital hypothyroidism. *Biological Trace Element Research*. **40:** 247-253.

289. Kaushal N., Gandhi U.H., Nelson S.M., Narayan V. and Prabhu S.K. **2011**. Selenium and inflammation. *Selenium*, **3:** 443-456.

290. Kaushal N., Narayan V., Patterson A.D. and Prabhu S.K. **2012**. Enhanced Metabolic Inactivation of PGE2 by Selenium in Macrophages is Mediated by the Up regulation of 15-hydroxy-prostaglandin dehydrogenase. *FASEB Journal*, **26:** 253-255.

291. Kaushal N., Kudva A.K., Patterson A.D., Chiaro C., Kennett M.J., Desai D., Amin S., Carlson B.A., Cantorna M.T. and Prabhu K.S. **2014**. Crucial role of macrophage selenoproteins in experimental colitis. *The Journal of Immunology*, **193:** 3683-3692.

292. Kawahara K., Hohjoh H., Inazumi T., Tsuchiya S. and Sugimoto Y. **2015**. Prostaglandin E_2-induced inflammation: Relevance of prostaglandin E receptors. *Biochimica et Biophysica Acta (BBA)-General Subjects*, **1851:** 414-421.

293. Kelly S., Baxter M., Chapman S., Rhodes C., Dennis J. and Brereton P. **2002**. The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. *European Food Research and Technology*, **214:** 72-78.

294. Kelly E., Greene C.M., Carroll T.P., McElvaney N.G. and O'Neill S.J. **2009**. Selenoprotein S/SEPS1 Modifies Endoplasmic Reticulum Stress in Z Variant α1-Antitrypsin Deficiency. *Journal of Biological Chemistry*, **284:** 16891-16897.

295. Kenet G., Freedman J., Shenkman B., Regina E., Simoni F.B., Holzman F., Vavva F., Brand N., Michelson A., Trolliet M., Loscalzo J. and Inbal A. **1999**. Plasma glutathione peroxidase

deficiency and platelet insensitivity to nitric oxide in children with familial stroke. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19:** 2017-2023.

296. Khanam A. and Platel K. **2016**. Bioaccessibility of selenium, selenomethionine and selenocysteine from foods and influence of heat processing on the same. *Food Chemistry*, **194:** 1293-1299.
297. Kim Y.M., Lee B.S., Yi K.Y. and Paik S.G. **1997**. Upstream NF-κB site is required for the maximal expression of mouse inducible nitric oxide synthase gene in interferon-γ plus lipopolysaccharide-induced RAW 264.7 macrophages. *Biochemical and Biophysical Research Communications*, **236:** 655-660.
298. Kim I.Y. and Stadtman T.C. **1997**. Inhibition of NF-kappaB DNA binding and nitric oxide induction in human T cells and lung adenocarcinoma cells by selenite treatment. *Proceedings of the National Academy of Sciences USA*, **94:** 12904–12907.
299. Kim H.Y. and Gladyshev V.N. **2004**. Methionine Sulfoxide Reduction in Mammals: Characterization of Methionine-*R*-Sulfoxide Reductases. *Molecular Biology of the Cell*, **15:** 1055-1064.
300. Kim S.F., Huri D.A. and Snyder S.H. **2005**. Inducible nitric oxide synthase binds, s-nitrosylates, and activates cyclooxygenase-2. *Science*, **310:** 1966-1970.
301. Kim H.Y. and Gladyshev V.N. **2007**. Methionine sulfoxide reductases: selenoprotein forms and roles in antioxidant protein repair in mammals. *Biochemical Journal*, **407:** 321-329.
302. Kim J.Y., Lee K.H., Shim M.S., Shin H., Xu X.M., Carlson B.A., Hatfield D.L. and Lee B.J. **2010**. Human selenophosphate synthetase 1 has five splice variants with unique interactions, subcellular localizations and expression patterns. *Biochemical and Biophysical Research Communications*, **397:** 53-58.
303. Kim J.W., Kim B.C., Lee J.H., Lee D.R., Rehman S. and Yun S.J. **2013**. Protein content and composition of waxy rice grains. *Pakistan Journal of Botany*, **45:** 151-156.
304. Kim J.S., Kim A.Y., Shin H.M., Ahn S.I., Shim H.J., Nam K.W., Hwang K.A. and Youn H.S. **2017**. *Aster yomena* suppresses LPS-induced cyclooxygenase-2 and inducible nitric oxide synthase expression. *Food and Agriculture Immunology*, **28:** 202-210.
305. Kirby J.K., Lyons G.H. and Karkkainen M.P. **2008**. Selenium speciation and bioavailability in biofortified products using species-unspecific isotope dilution and reverse phase ion

pairing-inductively coupled plasma-mass spectrometry. *Journal of Agriculture and Food Chemistry*, **56:** 1772–1779.

306. Kirkby N.S., Chan M.V., Zaiss A.K., Vaz E.G., Jiao J., Berglund L.M., Verdu E.F., Shala A.B., Wallace J.L., Herschman H.R., Gomez M.F. and Mitchella, J. A. **2016**. Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-κB and NFAT transcriptional pathways. *Proceedings of the National Academy of Sciences*, **113:** 434-439.
307. Klimp A.H., Hollema H., Kempinga C., van der Zee G.J., de Vries E.G.E. and Daemen T. **2001**. Expression of Cyclooxygenase-2 and Inducible Nitric Oxide Synthase in Human Ovarian Tumors and Tumor-associated Macrophages. *Cancer Research*, **61:** 7305-7309.
308. Knowles R.G. and Moncada S. **1994**. Nitric oxide synthases in mammals. *Biochemical Journal*, **298:** 249-258.
309. Kohen R. and Nyska A. **2002**. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicologic Pathology Journal*, **30:** 620-650.
310. Kohrle J. **1999**. The trace element selenium and the thyroid gland. *Biochimie*, **81:** 527-533.
311. Koivistoinen P. and Huttunen J.K. **1986**. Selenium in food and nutrition in Finland. An overview on research and action. *Annals of clinical research*. **18:** 13-17.
312. Koivistoinen P. and Varo P. **1987.** Selenium in Finnish food. In: Combs GF, Spallholz JE, Levander OA, Oldfield JE, editors. Selenium in biology and medicine. Part B. New York: Van Nostrand Reinhold Company, pp. 645–651.
313. Kokot S. and Phuong T.D. **1999**. Elemental content of Vietnamese rice Part 2. Multivariate data analysis. *Analyst*, **124:** 561-569.
314. Koprowski H., Zheng Y.M., Heber-Katz E., Fraser N., Rorke L., Fu Z.F., Hanlon C. and Dietzschold B. **1993**. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proceedings of the National Academy of Sciences of the United States of America*, **90:** 3024-3027.
315. Korotkov K.V., Kumaraswamy E., Zhou Y., Hatfield D.L. and Gladyshev V.N. **2001**. Association between the 15-kDa Selenoprotein and UDP-glucose:Glycoprotein Glucosyltransferase in the Endoplasmic Reticulum of Mammalian Cells. *Journal of Biological Chemistry*, **276:** 15330-15336.

316. Kowluru R.A., Tang J. and Kern T. S. **2001**. Abnormalities of retinal metabolism in diabetes and experimental galactosemia.VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes*, **50:** 1938-1942.
317. Koyama H., Omura K., Ejima A., Kasanuma Y., Watanabe C. and Satoh H. **1999**. Separation of Selenium-Containing Proteins in Human and Mouse Plasma Using Tandem High-Performance Liquid Chromatography Columns Coupled with Inductively Coupled Plasma-Mass Spectrometry. *Analytical Biochemistry*, **267:** 84-91.
318. Krehl S., Loewinger M., Florian S., Kipp A.P., Banning A., Wessjohann L.A., Brauer M.N., Iori R., Esworthy R.S., Chu F.F. and Flohe R.B. **2012**. Glutathione peroxidase-2 and selenium decreased inflammation and tumors in a mouse model of inflammation-associated carcinogenesis whereas sulforaphane effects differed with selenium supply. *Carcinogenesis*, **33:** 620-628.
319. Krishnan K., Campbell S., Rahman A.F., Whaley S. and Stone W.L. **2003**. Cancer chemoprevention drug targets. *Current Drug Targets*, **4:** 45-54.
320. Kroncke K.D., Fehsel K. and Kolb-Bachofen V. **1997**. Nitric oxide: cytotoxicity versus cytoprotection. How, why, when, where? *Nitric Oxide*, **1:** 107-120.
321. Kroncke K.D., Fehsel K. and Kolb-Bachofen V. **1998**. Inducible nitric oxide synthase in human diseases. *Clinical and Experimental Immunology*, **113:** 147-156.
322. Kryukov G.V., Kumar R.A., Koc A., Sun Z. and Gladyshev V.N. **2002**. Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. *Proceedings of National Academy of Sciences U.S.A.*, **99:** 4245-4250.
323. Kudva A.K., Shay A.E. and Prabhu S.K. **2015**. Selenium and inflammatory bowel disease. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, **309:** 71-77.
324. Kuklinski B., Buchner M., Schweder R. and Nagel R. **1991**. Acute pancreatitis--a free radical disease. Decrease in fatality with sodium selenite (Na2SeO3) therapy. *Zeitschrift fur die gesamte innere Medizin und ihre Grenzgebiete*, **46:** 145-149.
325. Kulkarni S.D., Acharya R., Rajurkar N.S. and Reddy A.V.R. **2007**. Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum L.*) by in vitro digestion method. *Food Chemistry*, **103:** 681-688.

326. Kumar S., Bjornstedt M. and Holmgren A. **1992**. Selenite is a substrate for calf thymus thioredoxin reductase and thioredoxin and elicits a large non-stoichiometric oxidation of NADPH in the presence of oxygen. *European Journal of Biochemistry*, **207:** 435-439.
327. Kumaraswamy E., Malykh A., Korotkov K.V., Kozyavkin S., Hu Y., Kwon S.Y., Moustafa M.E., Carlson B.A., Berry M.J., Lee B.J., Hatfield D.L., Diamond A.M. and Gladyshev V.N. **2000**. Structure-Expression Relationships of the 15-kDa Selenoprotein Gene, possible role of the protein in cancer etiology. *Journal of Biological Chemistry*, **275:** 35540-35547.
328. Kurihara T., Kishi M., Saito N., Komoto M., Hidaka T. and Kinoshita M. **1990**. Electrical myotonia and cataract in X-linked muscular dystrophy (mdx) mouse. *Journal of the Neurological Sciences*, **99:** 83-92.
329. Kurz B., Jost B. and Schunke M. **2002**. Dietary vitamins and selenium diminish the development of mechanically induced osteoarthritis and increase the expression of antioxidative enzymes in the knee joint of STR/1N mice. *Osteoarthritis and Cartilage*, **10:** 119-126.
330. Labunskyy V.M., Hatfield D.L. and Gladyshev V.N. **2014**. Selenoproteins: Molecular pathways and physiological roles. *Physiological Reviews*, **94:** 739-777.
331. Lakin H.W. **1972**. Selenium accumulation in soils and its absorption by plants and animals. *Geological Society of America Bulletin*, **83:** 181-189.
332. Lands W., Lee R. and Smith W. **1971**. Factors regulating the biosynthesis of various prostaglandin. *Annals of the New York Academy of Sciences*, **180:** 107-122.
333. Langenbach R., Loftin C., Lee C. and Tiano H. **1999**. Cyclooxygenase knockout mice-Models for elucidating isoform-specific functions. *Biochemical Pharmacology*, **58:** 1237–1246.
334. Latreche, L., Duhieu S., Hamici Z.T., Jean O.J. and Chavatte L. **2012**. The differential expression of glutathione peroxidase 1 and 4 depends on the nature of the SECIS element. *RNA Biology*, **9:** 681-690.
335. Lavu R.V.S., Laing G.D., De Wiele T.V., Pratti V.L., Willekens K., Vandecasteele B. and Tack F. **2012**. Fertilizing Soil with Selenium Fertilizers: Impact on Concentration, Speciation, and Bioaccessibility of Selenium in Leek (*Allium ampeloprasum*). *Journal of Agricultural and Food Chemistry*, **60:** 10930-10935.

336. Lavu R.V.S., Wiele T.V.D., Pratti V.L., Tack F. and Laing G.D. **2016**. Selenium bioaccessibility in stomach, small intestine and colon: Comparison between pure Se compounds, Se-enriched food crops and food supplements. *Food Chemistry*, **197:** 382-387.
337. Lawrence T., Gilroy D.W., Colville-Nash P.R. and Willoughby D.A. **2001**. Possible new role of NFkB in the resolution of inflammation. *Nature Medicine*, **7:** 1291-1297.
338. Lechner M., Lirk P. and Rieder J. **2005**. Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. *Seminars in Cancer Biology*, **15:** 277-289.
339. Lee Y.S., Kim A.Y., Choi J.W., Kim M., Yasue S., Son H.J., Masuzaki H., Park K.S. and Kim J.B. **2008**. Dysregulation of Adipose Glutathione Peroxidase 3 in Obesity Contributes to Local and Systemic Oxidative Stress. *Molecular Endocrinology*, **22:** 2176-2189.
340. Lee J.H., Kang B.S., Hwang K.H. and Kim G.H. **2011**. Evaluation for anti-inflammatory effects of *Siegesbeckia glabrescens* extract in vitro. *Food and Agriculture Immunology*, **22:** 145-160.
341. Lei X.G., Dann H.M., Ross D.A., Cheng W.H., Combs G.F. and Roneker K.R. **1998**. Dietary Selenium Supplementation Is Required to Support Full Expression of Three Selenium-Dependent Glutathione Peroxidases in Various Tissues of Weanling Pigs. *The Journal of Nutrition*, **128:** 130-135.
342. Letavayova L., Vlckova V. and Brozmanova J. **2006**. Selenium: From cancer prevention to DNA damage. *Toxicology*, **227:** 1-14.
343. Levander O.A. **1972**. Metabolic interrelationships and adaptations in selenium toxicity. *Annals New York Academy of Sciences*, **192:** 181-192.
344. Levander O.A., Alfthan G., Arvilommi H., Gref C., Huttunen J., Kataja M., Koivistoinen P. and Pikkarainen J. **1983**. Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *The American Journal of Clinical Nutrition*, **37:** 887-897.
345. Levander O.A. and Beck M.A. **1999**. Selenium and viral virulence. *British Medical Bulletin*, **55:** 528-533.
346. Levesque M. and Vendette E.D. **1971**. Selenium determination in soil and plant materials. *Canadian Journal of Soil Science*, **51:** 85-93.

347. Li Y.B., Han J.Y. Jiang W. and Wang J. **2010**. Selenium inhibits high glucose-induced cyclooxygenase-2 and P-selectin expression in vascular endothelial cells. *Molecular Biology Reports*, **38:** 2301-2306.

348. Li Y.F., Zhao J., Li Y., Li H., Zhang J., Li B., Gao Y., Chen C., Luo M., Huang R. and Li J. **2015**. The concentration of selenium matters: a field study on mercury accumulation in rice by selenite treatment in qingzhen, Guizhou, China. *Plant and Soil*, **391:** 195-205.

349. Liang Y.C., Huang Y.T., Tsai S.H., Shiau S.Y.L., Chen C.F. and Lin J.K. **1999**. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis*, **20:** 1945-1952.

350. Lin D.T., Subbaramaiah K., Shah J.P., Dannenberg A.J. and Boyle J.O. **2002**. Cyclooxygenase-2: A novel molecular target for the prevention and treatment of head and neck cancer. *Journal of The Sciences and Specialties of The Head and Neck*, **24:** 792-799.

351. Lin L., Zhou W., Dai H., Cao F., Zhang G. and Wu F. **2012**. Selenium reduces cadmium uptake and mitigates cadmium toxicity in rice. *Journal of Hazardous Material*, **235:** 343-351.

352. Linda B., Ulla-Britta T., Servet E., Christina N., Arrigo C., Erik H.L., Mikael B. and Lennart C.E. **2005**. Selenium prevents tumor development in a rat model for chemical carcinogenesis. *Carcinogenesis*, **26:** 125-131.

353. Lintschinger J., Fuchs N., Moser J., Kuehnelt D. and Goessler W. **2000**. Selenium-Enriched Sprouts. A Raw Material for Fortified Cereal-Based Diets. *Journal of Agricultural and Food Chemistry*, **48:** 5362-5368.

354. Liu Y., Chiba M., Inaba Y. and Kondo M. **2002**. Keshan disease. A review from the aspect of history and etiology. *Japanese Journal of Hygiene*, **56:** 641-648.

355. Liu K., Chen F., Zhao Y., Gu Z. and Yang H. **2011**. Selenium accumulation in protein fractions during germination of Se-enriched brown rice and molecular weights distribution of Se-containing proteins. *Food Chemistry*, **127:** 1526-1531.

356. Loflin J., Lopez N., Whanger P.D. and Kioussi C. **2006**. Selenoprotein W during development and oxidative stress. *Journal of Inorganic Biochemistry*, **100:** 1679-1684.

357. Lu C., Qiu F., Zhou H., Peng Y., Hao W., Xu J., Yuan J., Wang S., Qiang B., Xu C. and Peng X. **2006**. Identification and characterization of selenoprotein K: An antioxidant in cardiomyocytes. *FEBS Letters*, **580:** 5189-5197.

358. Lu J. and Holmgren A. **2008**. Selenoproteins. *Journal of Biological Chemistry*, **284:** 723-727.

359. Lu L., Tian S., Liao H., Zhang J., Yang X., Labavitch J.M., Chen W. **2013**. Analysis of Metal Element Distributions in Rice (*Oryza sativa* L.) Seeds and Relocation during Germination Based on X-Ray Fluorescence Imaging of Zn, Fe, K, Ca, and Mn. *PLOS ONE*, **8:** 1-9.
360. Lubos E., Kelly N.J., Oldebeken S.R., Leopold J.A., Zhang Y.Y., Loscalzo J. and Handy D.E. **2011**. Glutathione Peroxidase-1 (GPx-1) deficiency augments pro-inflammatory cytokine-induced redox signaling and human endothelial cell activation. *Journal of Biological chemistry*, **286:** 35407-35417.
361. Lyons G.H., Yusuf Genc Y., Stangoulis C.R., Palmer L.T. and Graham R.D. **2004**. Selenium distribution in wheat grain, and the effect of postharvest processing on wheat selenium content. *Biological Trace Element Research*, **103:** 155-168.
362. Lyons G., Monasterio I.O., Stangoulis J. and Graham R. **2005**. Selenium concentration in wheat grain: Is there sufficient genotypic variation to use in breeding? *Plant and Soil*, **269:** 369-380.
363. Ma J., Chen T., Mandelin J., Ceponis A., Miller N.E., Hukkanen M., Ma G.F., and Konttinen Y.T. **2003**. Regulation of macrophage activation. *Cellular and Molecular Life Sciences*, **60:** 2334-2346.
364. MacPherson A., Barclay M.N.I., Scott R. and Yates R.W.S. **1997**. Loss of Canadian wheat lowers selenium intake and status of the Scottish population. In: Fischer PWF, L'Abbe MR, Cockell KA, Gibson RS, eds. Trace elements in man and animals 9: proceedings of the ninth international symposium on trace elements in man and animals. *Ottawa: NRC Research Press*, 203-205.
365. Maddipati K.R. and Marnett L.J. **1987**. Characterization of the major hydroperoxide-reducing activity of human plasma. Purification and properties of a selenium-dependent glutathione peroxidase. *The Journal of Biological Chemistry*, **262:** 17398-17403.
366. Mahmud I., Ueda N., Yamaguchi H., Yamashita R., Yamamoto S., Kanaoka Y., Urade Y. and Hayaishi O. **1997**. Prostaglandin D synthase in human megakaryoblastic cells. *Journal of Biological Chemistry*, **272:** 28263–28266.
367. Maiorino M. and Ursini F. **2002**. Oxidative stress, spermatogenesis and fertility. *Biological Chemistry*, **383:** 591-597.

368. Manicam C., Ginter N., Li H., Xia N., Goloborodko E., Zadeh J.K., Musayeva A., Pfeiffer N. and Gericke A. **2017**. Compensatory vasodilator mechanisms in the ophthalmic artery of endothelial nitric oxide synthase gene knockout mice. *Scientific Reports*, **7:** 1-12.

369. Mann J.R., Backlund M.G. and Dubois R.N. **2005**. Mechanisms of Disease: inflammatory mediators and cancer prevention. *Nature Clinical Practice: Oncology*, **2:** 202-210.

370. Manzanares W., Biestro A., Galusso F., Torre M.H., Manay N., Pittini G., Facchin G. and Hardy G. **2009**. Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill? *Intensive Care Medicine*, **35:** 882-889.

371. Marian E. **1984**. Introduction on environmental chemistry and global cycle of Cr, Ni, Co, Be, As, Cd and Se and their derivatives. *Toxicological and Environmental Chemistry*, **8**: 1-9.

372. Martinez M.S., Corona T.P., Camara C. and Madrid Y. **2015**. Preparation and Characterization of a Laboratory Scale Selenomethionine-Enriched Bread. Selenium Bioaccessibility. *Journal of Agricultural and Food Chemistry*, **63:** 120-127.

373. Masscheleyn P.H., Delaune R.D. and Patrick W.H. **1991**. Biogeochemical behaviour of selenium in anoxic soils and sediments equilibrium thermodynamics approach. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering*, **26:** 555-573.

374. Mattmiller S.A., Carlson B.A. and Sordillo L.M. **2013**. Regulation of inflammation by selenium and selenoproteins: impact on eicosanoid biosynthesis. *Journal of Nutritional Science*, **2:** 1-13.

375. Mayland H.F., James J.G., Panter K.E. and Sonderegger J.L. **1989**. Selenium in seleniferous environments. In: Jacob J.W. (ed.), *Selenium in Agriculture and the Environment*, SSSA Publication 23, Madison: SSSA, pp. 15-50.

376. McAdam B.F., Mardini I.A., Habib A., Burke A., Lawson J.A., Kapoor S. and FitzGerald G.A. **2000**. Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. *Journal of Clinical Investigation*, **105:** 1473-1482.

377. McCloy R. **1998**.Chronic pancreatitis at Manchester, UK. Focus on antioxidant therapy. *Digestion*, **59:** 36-48.

378. McConnell K.P. and Hoffman J.L. **1972**. Methionine-selenomethionine parallels in rat liver polypeptide chain synthesis. *Federation of European Biochemical Societies Journal*, **24:** 60-62.
379. McNeal J.M., and Balistrieri L.S. **1989**. Geochemistry and occurrence of selenium—An overview, in Jacobs, L.W., ed., Selenium in agriculture and the environment. *Soil Science Society of America*, **23:** 1–13.
380. Mediavilla V.G., Crespo I., Collado P.S., Esteller A., Campos S.S., Tunon M.J. and Gallego J.G. **2007**. The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *European Journal of Pharmacology*, **557:** 221-229.
381. Medina D. and Morrison D.G. **1988**. Current ideas on selenium as a chemopreventive agent. *Pathology and Immunopathology Research*, **7:** 187-199.
382. Meltzer H., Norheim G., Bibow K., Myhre K. and Holm H. **1990**. The form of selenium determines the response to supplementation in a selenium replete population. *European Journal of Clinical Nutrition*, **44:** 435-446.
383. Meltzer H. M., Bibow K., Paulsen I. T., Mundal H. H., Norheim G., Holm H. **1993**. Different bioavailability in humans of wheat and fish, selenium as measured by blood platelet response to increased dietary Se. *Biological Trace Element Research*, **36:** 229-241.
384. Meplan C., Rohrmann S., Steinbrecher A., Schomburg L., Jansen E., Linseisen J., Hesketh J. **2012**. Polymorphisms in Thioredoxin Reductase and Selenoprotein K Genes and Selenium Status Modulate Risk of Prostate Cancer. *PLoS ONE*, **7:** 1-10.
385. Meyer D.J. and Thomas M. **1995**. Characterisation of rat spleen prostaglandin H D-isomerase as a sigma-class GSH transferase. *Biochemical Journal*, **311:** 739-742.
386. Mezes M. and Balogh K. **2009**. Prooxidant mechanisms of selenium toxicity - a review. *Acta Biologica Szegediensis*, **53:** 15-18.
387. Mihailovic M., Lindberg P. and Jovanovic I. **1996.** Selenium content in foodstuffs in Serbia. *Acta Veterinaria Belgrade*, **46:** 343–348.
388. Mikus K.V., Pelicon P., Vavpetic P., Kreft I. and Regvar M. **2009**. Elemental analysis of edible grains by micro-PIXE: Common buckwheat case study. Nuclear Instruments and

Methods in Physics Research Section B: Beam *Interactions with Materials and Atoms*, **267:** 2884-2889.

389. Miller D.D. **1996**. Minerals, *Food Chemistry*, New York, Marcel Dekker, Inc. pp. 617- 650.
390. Moghadaszadeh B., Petit N., Jaillard C., Brockington M., Roy S.Q., Merlini L., Romero N., Estournet B., Desguerre I., Chaigne D, Muntoni F., Topaloglu H. and Guicheney P. **2001**. Mutations in SEPN1 cause congenital muscular dystrophy with spinal rigidity and restrictive respiratory syndrome. *Nature Genetics*, **29:** 17-18.
391. Mohri I., Aritake K., TaniguchI H., Sato Y., Kamauchi S., Nagata N., Maruyama T., Taniike M. Urade Y. **2009**. Inhibition of prostaglandin D synthase suppresses muscular necrosis. *The American Journal of Pathology*, **174:** 1735-1744.
392. Moore K.L., Schroder M., Lombi E., Zhao F.J., McGrath S.P., Hawkesford M.J., Shewry P.R. and Grovenor C.R.M. **2009**. NanoSIMS analysis of arsenic and selenium in cereal grain. *New Phytologist*, **185:** 434-445.
393. Moreno P., Quijano M., Gutierrez A., Perez-Conde M. and Camara C. **2004**. Study of selenium species distribution in biological tissues by size exclusion and ion exchange chromatagraphy inductively coupled plasma-mass spectrometry. *Analytica Chimica Acta*, **524:** 315-327.
394. Morgan D.M.L. **1998**. Tetrazolium (MTT) Assay for Cellular Viability and Activity. *Methods in Molecular Biology*, **79:** 179-184.
395. Morin C., Blier P.U. and Fortin S. **2015**. Eicosapentaenoic acid and docosapentaenoic acid monoglycerides are more potent than docosahexaenoic acid monoglyceride to resolve inflammation in a rheumatoid arthritis model. *Arthritis Research and Therapy*, **17:** 1-12.
396. Morteau O., Morham S.G., Sellon R., Dieleman L.A., Langenbach R., Smithies O. and Sartor R.B. **2000**. Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. *Journal of Clinical Investigation*, **105:** 469–478.
397. Motghare K.S., Bhutey A., Murrhar B.B., Gupta M., Meshram A.W. and Balsubramanium Y. **2001**. Lipid peroxidation and glutathione peroxidase in ischemic heart disease. *Indian Journal of Clinical Biochemistry*, **16:** 213-215.
398. Moustafa M.E. and Antar H.A. **2012**. A bioinformatics approach to characterize mammalian selenoprotein T. *Biochemical Genetics*, **50:** 736-747.

399. Mugesh G., du Mont W., Sies H. **2001**.Chemistry of Biologically Important Synthetic Organoselenium Compounds. *Chemical Review*, **101:** 2125-2180.
400. Mungrue I.N., Husain M. and Stewart D.J. **2002**. The role of NOS in heart failure: lessons from murine genetic models. *Heart Failure Reviews*, **7:** 407–22.
401. Murata T., Aritake K., Tsubosaka Y., Maruyama T., Nakagawa T., Hori M., Hirai H., Nakamura M., Narumiya S., Urade Y. and Ozaki H. **2013**. Anti-inflammatory role of PGD2 in acute lung inflammation and therapeutic application of its signal enhancement. *Proceedings of the National Academy of Sciences*, **110:** 5205-5210.
402. Murawaki Y., Tsuchiya H., Kanbe T., Harada K., Yashima K., Nozaka K., Tanida O., Kohno M., Mukoyama T., Nishimuki E., Kojo H., Matsura T., Takahashi K., Osaki M., Ito H., Yodoi J., Murawaki Y. and Shiota G. **2008**. Aberrant expression of selenoproteins in the progression of colorectal cancer. *Cancer Letters*, **259:** 218-230.
403. Musiani F., Ciurli S. and Dikiy A. **2011**. Interaction of Selenoprotein W with 14-3-3 Proteins: A Computational Approach. *Journal of Proteome*, **10:** 968-976.
404. Mutanen M., and Mykkanen H. **1984**. Effect of dietary fat on plasma glutathione peroxidase levels and intestinal absorption of 76Se-labeled sodium selenite in chicks. *The Journal of Nutrition*, **114:** 829-834.
405. Nakayama T, Esaki N., Lee W.J., Tanaka I., Tanaka H. and Soda K. **1984**. Purification and Properties of L-Methionine y-Lyase from Aeromonas sp. *Agricultural and Biological Chemistry*, **48:** 2367-2369.
406. Nalvarte I., Damdimopoulos A.E. and Spyrou G. **2004**. Human mitochondrial thioredoxin reductase reduces cytochrome c and confers resistance to complex III inhibition. *Free Radical Biology and Medicine*, **36:** 1270-1278.
407. Narayan V., Ravindra K.C., Liao C., Kaushal N., Carlson B.A. and Prabhu S.K. **2015**. Epigenetic regulation of inflammatory gene expression in macrophages by selenium. *The Journal of Nutritional Biochemistry*, **26:** 138-145.
408. Narumiya S., Ogorochi T., Nakao K. and Hayaishi O. **1982**. Prostaglandin D2 in rat brain, spinal cord and pituitary: basal level and regional distribution. *Life Sciences*, **31:** 2093-2103.
409. National Academy of Sciences–National Academy of Engineering. **1973**. Water quality criteria: a report of the committee on water quality criteria. US Environmental Protection Agency, pp. 232–353.

410. Navarro A.M. and Lopez M.M. **2000**. Essentiality of selenium in the human body: relationship with different diseases. *The Science of Total Environment*, **249:** 347-371.
411. Nawaz F., Ashraf M.Y., Ahmad R., Waraich E.A., Shabbir R.N. and Bukhar M.A. **2015**. Supplemental selenium improves wheat grain yield and quality through alterations in biochemical processes under normal and water deficit conditions. *Food Chemistry*, **175:** 350-357.
412. Neal R.H. and Sposito G. **1989**. Selenate adsorption on alluvial soils. *Soil Science Society of America Journal*, **53:** 70-74.
413. Needleman P., Turk J., Jakschik B.A., Morrison A.R. and Lefkowith J.B. **1986**. Arachidonic acid metabolism. *Annual Reviews of Biochemistry*, **55:** 69-102.
414. Negro R. **2008**. Selenium and thyroid autoimmunity. *Biologics Targets and Therapy*, **2:** 265-273.
415. Nelson S.M., Lei X. and Prabhu S.K. **2011**. Selenium levels affect the IL-4-induced expression of alternative activation markers in murine macrophages. *The Journal of Nutrition*, **141:** 1754-1761.
416. Neve J. **1999**. Combined selenium and iodine deficiency in Kashin-Beck osteoarthropathy. In: *STDA Bulletin*, Grimbergen: *Selenium-Tellurium Development Association*, pp. 1-3.
417. Nicholson S., Almeida M.G.B., Lapa e Silva J.R., Nathan C., Xie Q.W., Mumford R., Weidner J.R., Calaycay J., Geng J., Boechat N., Linhares C., Rom W. and Ho J.L. **1996**. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *The Journal of Experimental Medicine*, **183:** 2293-2302.
418. Ng L., Goodyear R.J., Woods C.A., Schneider M.J., Diamond E., Richardson G.P., Kelley M.W., Germain D.L.S., Galton V.A. and Forrest D. **2004**. Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. *Proceedings of the National Academy of Sciences U.S.A.*, **101:** 3474–3479.
419. Norton R.L. and. Hoffmann P.R. **2012**. Selenium and asthma. *Molecular Aspects of Medicine*, **33:** 98-106.
420. Novoselov S.V., Kryukov G.V., Xu X.M., Carlson B.A., Hatfield D.L. and Gladyshev V.N. **2007**. Selenoprotein H Is a Nucleolar Thioredoxin-like Protein with a Unique Expression Pattern. *Journal of Biological Chemistry*, **282:** 11960-11968.

421. Nussler A.K. and Billiar T.R. **1993**. Inflammation, immunoregulation, and inducible nitric oxide synthase. *Journal of Leukocyte Biology*, **54:** 171-178.

422. Odegaard J.I., Ricardo-Gonzalez R.R., Goforth M.H., Morel C.R., Subramanian V., Mukundan L., Red Eagle A., Vats D., Brombacher F., Ferrante A.W., and Chawla A. **2007**. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature*, **447:** 1116-20.

423. Ogorochi T., Narumiya S., Mizuno N., Yamashita K., Miyazaki H. and Hayaishi O. **1984**. Regional distribution of prostaglandins D2, E2, and F2α and related enzymes in postmortem human brain. *Journal of Neurochemistry*, **43:** 71–82.

424. Ogra Y., Ishiwata K., Encinar J.R., Lobinski R. and Suzuki K.T. **2004**. Speciation of selenium in selenium-enriched shiitake mushroom. Lentinula edodes, *Analytical and Bioanalytical Chemistry*, **379:** 861-866.

425. Oldfield J.E. **2006**. Selenium:A historical prospective. In: Hatfield, DL, Berry MJ, Gladyshev VN (eds.), *Selenium: Its Molecular Biology and Role in Human Health* (2nd Ed.), New York: Springer, pp. 1-6.

426. Olson O.E., Novacek E.J., Whitehead E.I. and Palmer I.C. **1970**. Investigations on selenium in wheat. *Phytochemistry*, **9:** 1181-1188.

427. Olson O.E. and Palmer I.S. **1976**. Selenoamino acids in tissues of rats administered inorganic selenium. *Metabolism*, **25:** 299-306.

428. Olson O.E. **1997**. The Human Animal: Personal Identity Without Psychology. New York: Oxford University Press, pp. 1-200.

429. Olson G.E., Winfrey V.P., Nagdas S.K., Hill K.E. and Burk R.F. **2005**. Selenoprotein P Is Required for Mouse Sperm Development. *Biology of Reproduction*, **73:** 2001-2011.

430. Ohshima H., Tazawa H., Sylla B.S. and Sawa T. **2005**. Prevention of human cancer by modulation of chronic inflammatory processes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **591:** 110-122.

431. Osanai T. and Okumura K. **2011**. Lipocalin-type PGD synthase as a novel biomarker for coronary vasospasm. *Circulation Journal*, **75:** 784-785.

432. Otieno S.B., Jayne T.S. and Muyanga M. **2015**. The Effects of Soil Chemical Characteristics on Accumulation of Native Selenium by Zea mays Grains in Maize Belt in Kenya.

International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, **9:** 1154-1158.

433. Panee J., Stoytcheva Z.R., Liu W. and Berry M.J. **2007**. Selenoprotein H is a Redox-sensing High Mobility Group Family DNA-binding Protein That Up-regulates Genes Involved in Glutathione Synthesis and Phase II Detoxification. *Journal of Biological Chemistry*, **282:** 23759-23765.
434. Papp L.V., Lu J., Holmgren A. and Khanna K.K. **2007**. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health. *Antioxidants and Redox signaling*, **9:** 775-806.
435. Park S.W., Lee S.G., Song S.H., Heo D.S., Park B.J., Lee D.W., Kim K.H. and Sung M.W. **2003**. The effect of nitric oxide on cyclooxygenase-2 (COX-2) overexpression in head and neck cancer cell lines. *Cancer Cell Biology*, **107:** 729-738.
436. Pathak S.K., Sharma R.A., Steward W.P., Mellon J.K., Griffiths T.R.L. and Gescher A.J. **2005**. Oxidative stress and cyclooxygenase activity in prostate carcinogenesis: targets for chemo preventive strategies. *European Journal of Cancer*, **41:** 61-70.
437. Pedrero Z. and Madrid Y. **2009**. Novel approaches for selenium speciation in foodstuffs and biological specimens: a review. *Analytica Chimica Acta*, **634:** 135-152.
438. Pelletier J.P., Jovanovic D., Fernandes J.C., Manning P., Connor J.R., Currie M.G., Di Battista J.A. and Pelletier J.M. **1998**. Reduced progression of experimental osteoarthritis in vivo by selective inhibition of inducible nitric oxide synthase. *Arthritis and Rheumatology*, **41:** 1275-1286.
439. Peretz A, Siderova V. and Neve J. **2009**. Selenium supplementation in rheumatoid arthritis investigated in a double blind, placebo-controlled trial. *Scandinavian Journal of Rheumatology*, **30:** 208-212.
440. Petit N., Lescure A., Rederstorff M., Krol A., Moghadaszadeh B., Wewer U.M and Guicheney P. **2003**. Selenoprotein N: an endoplasmic reticulum glycoprotein with an early developmental expression pattern. *Human Molecular Genetics*, **12:** 1045-1053.
441. Petrova T.V., Akama K.T. and Eldik L.J.V. **1999**. Cyclopentenone prostaglandins suppress activation of microglia: Down-regulation of inducible nitric-oxide synthase by 15-deoxy-Δ12,14-prostaglandin J2. *Proceedings of the National Academy of Sciences USA*, **96:** 4668-4673.

442. Pfeifer H., Condar M., Roethlein D., Kyriakopoulos A., Brielmeier M., Bornkamm G. and Behne D. **2001**. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. *Federation of American Societies for Experimental Biology Journal*, **15:** 1236-1238.
443. Piedra C.J., Clemente M.J., Devesa V. and Velez D. **2016.** Influence of Physiological Gastrointestinal Parameters on the Bioaccessibility of Mercury and Selenium from Swordfish. *Journal of Agricultural and Food Chemistry*, **64:** 690-698.
444. Pillai R., Lock J.H.U. and Bellinger F.P. **2014**. Selenium and selenoprotein function in brain disorders. *IUBMB Life*, **66:** 229-239.
445. Pineiro J.M., Pineiro A.M., Hortas V.R., Gonzolez R.D., Rodriguez E.A., Mahia P.L. and Lorenzo S.M. **2013**. *In vitro* bioavailability of total selenium and selenium species from seafood. *Food Chemistry*, **139:** 872-877.
446. Pinzar E., Kanaoka Y., Inui T., Eguchi N., Urade Y. and Hayaishi O. **2000**. Prostaglandin D synthase gene is involved in the regulation of non-rapid eye movement sleep. *Proceedings of the National Academy of Sciences, U.S.A.*, **97:** 4903-4907.
447. Pitts M.W., Reeves M.A., Hashimoto A.C., Ogawa A., Kremer P., Seale L.A. and Berry M.J. **2013**. Deletion of Selenoprotein M Leads to Obesity without Cognitive Deficits. *Journal of Biological Chemistry*, **288:** 26121-26134.
448. Plant J.A., Kinniburgh D.G., Smedley P.L., Fordyce F.M. and Klinck B.A. **2005**. Arsenic and selenium. *Environmental Geochemistry*, **9:** 17-66.
449. Poblaciones M.J., Rodrigo S., Santamaría O., Chen Y. and McGrath S.P. **2014**. Agronomic selenium biofortification in Triticum durum under Mediterranean conditions: From grain to cooked pasta. *Food Chemistry*, **146:** 378-384.
450. Poli G., Leonarduzzi G., Biasi F. and Chiarpotto E. **2004**. Oxidative Stress and Cell signaling. *Current Medical Chemistry*, **11:** 1163-1182.
451. Possel H., Noack H., Putzke J., Wolf G. and Sies H. **2000**. Selective upregulation of inducible nitric oxide synthase (iNOS) by lipopolysaccharide (LPS) and cytokines in microglia: In vitro and in vivo studies. *GLIA*, **32:** 51-59.
452. Prabhu S.K., Zamamiri-Davis F., Stewart J.B., Thompson J.T. Sordillo L.R. and Reddy C.C. **2002**. Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW

264.7 macrophages: Role of nuclear factor kappa- B in up-regulation. *Biochemical Journal*, **366:** 203-209.

453. Prasad R., Chan L.F., Hughes C.R., Kaski J.P., Kowalczyk J.C., Savage M.O., Peters C.J., Nathwani N., Clark A.J.L., Storr H.L. and Metherell L.A. **2014**. Thioredoxin Reductase 2 (*TXNRD2*) Mutation Associated With Familial Glucocorticoid Deficiency (FGD). *The Journal of Clinical Endocrinology and Metabolism*, **99:** 1556-1563.
454. Preud'homme H., Far J., Gil-Casal S. and Lobinski R. **2012**. Large-scale identification of selenium metabolites by online size-exclusion-reversed phase liquid chromatography with combined inductively coupled plasma (ICP-MS) and electrospray ionization linear trap-Orbitrap mass spectrometry (ESI-MSn). *Metallomics*, **4:** 422-432.
455. Pyrzynska K. **1998**. Speciation of selenium compounds. *Analytical Sciences*. **14:** 479-483.
456. Radmark O. and Samuelsson B. **2010**. Microsomal prostaglandin E synthase-1 and 5-lipoxygenase: potential drug targets in cancer. *Journal of Internal Medicine*, **268:** 5-14.
457. Ragolia L., Palaia T., Hall C.E., Klein J. and Buyuk A. **2010**. Diminished lipocalin-type prostaglandin D2synthase expression in human lung tumors. *Lung Cancer*, **70:** 103–109.
458. Rajakariar R., Hilliard M., Lawrence T., Trivedi S., Colville-Nash P. Bellingan G., Fitzgerald D., Yaqoob M.M. and Gilroy D.W. **2007**. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyΔ12−14 PGJ2. *Proceedings of the National Academy of Sciences of the United States of America*, **104:** 20979–20984.
459. Ralston N., Ralston C., Blackwell J. and Raymond L. **2008**. Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology*, **29:** 802-811.
460. Ran Q., Liang H., Gu M., Qi W., Walter C.A., Roberts L.J., Herman B., Richardson A. and Remmen H.V. **2004**. Transgenic Mice Overexpressing Glutathione Peroxidase 4 Are Protected against Oxidative Stress-induced Apoptosis. *Journal of Biological Chemistry*, **279:** 55137-55146.
461. Raso G.M., Meli R., Carlo G.D., Pacilio M. and Carlo R.D. **2001**. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. *Life Sciences*, **68:** 921-931.
462. Rayman M.P. **2000**. The importance of selenium to human health. *Lancet*, **356:** 233-241.

463. Rayman M.P. **2002**. The argument for increasing selenium intake. *Proceedings of the Nutrition Society*, **61:** 203–215.
464. Rayman M.P. **2004**. The use of high-selenium yeast to raise selenium status: how does it measure up. *British Journal of Nutrition*, **92:** 557-573.
465. Rayman M.P. **2005**. Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proceedings of the Nutrition Society*, **64:** 527-542.
466. Rayman M.P. **2008**. Food-chain selenium and human health: spotlight on speciation. *British Journal of Nutrition*, **100:** 238-253.
467. Rayman M.P. **2008**. Food-chain selenium and human health: emphasis on intake. *British Journal of Nutrition*, **100:** 254-268.
468. Rayman M.P. **2012**. Selenium and human health. *Lancet*, **379:** 1256-1268.
469. Ravikovitch, S. and Margolin, M. **1957**. Selenium in soils and plants. *Ktavim*, **7:** 41-52.
470. Reddy D.K. and Bhotmange M.G. **2013**. Isolation of starch from rice (*Oryza sativa* L.) and its morphological study using scanning electron microscopy. *International Journal of Agriculture Food and Science Technology*, **4:** 859-866.
471. Redensek A., Rathore K.I., Berard J.L., Vales R.L., Swayne L.A., Bennett S.A.L., Mohri I., Taniike M., Urade Y. and David S. **2011**. Expression and detrimental role of hematopoietic prostaglandin D synthase in spinal cord contusion injury. *GLIA*, **59:** 603-614.
472. Reeves P., Gregoire B., Garvin D., Hareland G., Lindlauf J., Johnson L. and Finley J. **2007**. Determination of selenium bioavailability from wheat mill fractions in rats by using the slope-ratio assay and a modified Torula yeast-based diet. *Journal of Agricultural Food and Chemistry*, **55:** 516-522.
473. Reeves M.A. and Hoffmann P.R. **2009**. The human selenoproteome: recent insights into functions and regulation. *Cellular and Molecular Life Sciences*, **66:** 2457-2478.
474. Reeves M.A., Bellinger F.P. and Berry M.J. **2010**. The Neuroprotective Functions of Selenoprotein M and its Role in Cytosolic Calcium Regulation. *Antioxidants and Redox signaling*, **12:** 809-818.
475. Reifen R., Karlinsky A., Stark A.H., Berkovich Z. and Nyska A. **2015**. α-Linolenic acid (ALA) is an anti-inflammatory agent in inflammatory bowel disease. *The Journal of Nutritional Biochemistry*, **26:** 1632-1640.

476. Reiko Tanaka R., Miwa Y., Mou K., Tomikawa M., Eguchi N., Urade Y., Takahashi-Yanaga F., Morimoto S., Wake N. and Sasaguri T. **2008**. Knockout of the l-pgds gene aggravates obesity and atherosclerosis in mice. *Biochemical and Biophysical Research Communications*, **378:** 851-856.

477. Reilly C. **1996**. Selenium in Food and Health. *London: Blackie Academic and Professional*, pp. 1-338.

478. Reisner A.H., Nemes P., and Bucholtz C. **1975**. The use of Coomassie Brilliant Blue G-250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. *Analytical Biochemistry*, **64:** 509-516.

479. Rengel Z., Batten G.D. and Crowley D.E. **1999**. Agronomic approaches for improving the micronutrient density in edible portions of field crops. *Field Corp Research*, **60:** 27-40.

480. Reuter S., Gupta S.C., Chaturvedi M.M. and Aggarwal B.B. **2010**. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine*, **49:** 1603-1616.

481. Reyes L.H., Encinar J.R., Gayon M.M., Alonso J.I.G. and Medel A.S. **2006**. Selenium bioaccessibility assessment in selenized yeast after "in vitro" gastrointestinal digestion using two-dimensional chromatography and mass spectrometry. *Journal of Chromatography A*, **1110:** 108-116.

482. Rhee S.G., Chae H.Z. and Kim K. **2005**. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radical Biology and Medicine*, **38:** 1543-1552.

483. Ricciotti E. and FitzGerald G.A.. **2011**. Prostaglandins and Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **31:** 986-1000.

484. Richard K., Hume R., Kaptein E., Sanders J.P., van Toor H., De Herder W.W., den Hollander J.C., Krenning E.P. and Visser T.J. **1998**. Ontogeny of iodothyronine deiodinases in human liver. *The Journal of Clinical Endocrinology and Metabolism*, **83:** 2868–2874.

485. Ricote M., Li A.C., Willson T.M., Kelly C.J. and Glass C.K. **1998**. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature*, **391:** 79-82.

486. Rider S., Davies S., Jha A., Fisher A., Knight J. and Sweetman J. **2009**. Supranutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout

(Oncorhynchus mykiss): implications on selenium status and health responses. *Aquaculture*, **295:** 282-291.

487. Rider S., Davies S., Jha A., Clough R. and Sweetman J. **2010**. Bioavailability of co-supplemented organic and inorganic zinc and selenium sources in a white fishmeal-based rainbouw trout (Oncorhynchus mykiss) diet. *Journal of Animal Physiology and Animal Nutrition*, **94:** 99-110.
488. Rodriguez M.M.J., Rivero C.V. and Ballesta J.R. **2005**. Selenium distribution in topsoils and plants of semi-arid mediterranian environments. *Environmental Geochemistry and Health*, **27:** 513-519.
489. Roelofs H.M.J., MorscheR.H.M., Heumen B.W.H., Nagengast F.M. and Peters W.H.M. **2014**. Over-expression of COX-2 mRNA in colorectal cancer. *BMC Gastroenterology*, **14:** 1-6.
490. Rogan N., Serafimovski T., Dolenec M., Tasev G. and Dolenec T. **2009**. Heavy metal contamination of paddy soils and rice (*Oryza sativa* L.) from Kocani Field (Macedonia). *Environmental Geochemistry and Health*, **31:** 439-451.
491. Rose M., Baxter M., Brereton N. and Baskaran C. **2010**. Dietary exposure to metals and other elements in the 2006 total diet study and some trends over the last 30 years. *Food Additives and Contaminants*, **27:** 1380-1404.
492. Rosenfeld I., and Beath O.A. **1964**. Selenium: Geobotany, Biochemistry, Toxicity and Nutrition. New York: Academic Press, pp.411.
493. Roy G., Sarma B.K., Phadnis P.P. and Mugesh G. **2005**. Selenium-containing enzymes in mammals: Chemical perspectives. *Journal of Chemical Sciences*, **117:** 287-303.
494. Ruby M., Schoof R., Brattin W., Post G., Harnois M., Mosby D., Casteel S., Bert W., Carpenter M., Edwards D., Cragin D. and Chappell W. **1999**. Advances in evaluating the bioavailability of inorganics in soil for use in human health risk assessment. *Environmental Science and Technology*, **33:** 3697-3705.
495. Rundlof A.K. and Arner E.S.J. **2004**. Regulation of the Mammalian Selenoprotein Thioredoxin Reductase 1 in Relation to Cellular Phenotype, Growth, and Signaling Events. *Antioxidants and Redox signaling*, **6:** 41-52.
496. Sabichi A.L., Lee J.J., Taylor R.J., Thompson I.M., Miles B.J., Tangen C.M., Minasian L.M., Pisters L.L., Caton J.R., Basler J.W., Lerner S.P., Menter D.G., Marshall

J.R., Crawford E.D. and Lippman S.M. **2006**. Selenium accumulation in prostate tissue during a randomized, controlled short-term trial of *l*-selenomethionine: a southwest oncology group study. *Clinical Cancer Research*, **12:** 2178-2184.

497. Safir N., Wendel A., Saile R. and Chabraoui L. **2003**. The effect of selenium on immune functions of J774.1 cells. *Clinical Chemistry and Laboratory Medicine*, **41:** 1005-1011.
498. Sager M. **2006**. Selenium in agriculture, food and nutrition. *Pure and Applied Chemistry*, **78:** 111-133.
499. Sakazaki F., Arakawa T., Shimizu R., Ogino H., Okuno T. and Ueno H. **2014**. Allergies are aggravated by mild selenium deficiency and abrogated by supplementation with selenomethionine. *Food and Agricultural Immunology*, **25:** 477-485.
500. Sakurai H., Kohsaka H., Liu M.F., Higashiyama H., Hirata Y., Kanno K., Saito I. and Miyasaka N. **1995**. Nitric oxide production and inducible nitric oxide synthase expression in inflammatory arthritides. *The Journal of Clinical Investigation*, **96:** 2357-2363.
501. Sakurai A., Yuasa K., Shoji Y., Himeno S., Tsujimoto M., Kunimoto M., Imura N. and Hara S. **2003**. Overexpression of thioredoxin reductase 1 regulates NF-κB activation. *Journal of Cellular Physiology*, **198:** 22-30.
502. Salonen J., Alfthan G., Huttunen J., Pikkarainen J. and Puska P. **1982**. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *The Lancet*, **2:** 175-179.
503. Sandler A.B. and Dubinett S.M. **2004**. COX-2 inhibition and lung cancer. *Seminars in Oncology*, **31:** 45-52.
504. Santos K.F.N., Silveira R.D.D., Didonet C.C.G. and Brondani C. **2013**. Storage protein profile and amino acid content in wild rice *Oryza glumaepatula*. *Pesquisa Agropecuaria Brasileira*, **48:** 66-72.
505. Sarashina H., Tsubosaka Y., Omori K., Aritake K., Nakagawa T., Hori M., Hirai H., Nakamura M., Narumiya S., Urade Y., Ozaki H. and Murata T. **2014**. Opposing Immunomodulatory Roles of Prostaglandin D2 during the Progression of Skin Inflammation. *The Journal of Immunology*, **192:** 459-465.
506. Satoh T., Moroi R., Aritake K., Urade Y., Kanai Y., Sumi K., Yokozeki H., Hirai H., Nagata K., Hara T., Utsuyama M., Hirokawa K., Sugamura K., Nishioka K. and Nakamura M. **2006**.

Prostaglandin D2 Plays an Essential Role in Chronic Allergic Inflammation of the Skin via CRTH2 Receptor. *The Journal of Immunology*, **177:** 2621-2629.

507. Sattler W., Maiorino M. and Stocker R. **1994**. Reduction of HDL- and LDLAssociated Cholesterylester and Phospholipid Hydroperoxides by Phospholipid Hydroperoxide Glutathione Peroxidase and Ebselen (PZ- 51). *Archives of Biochemistry and Biophysics*, **309:** 214-221.
508. Sawano H., Haneda M., Sugimoto T., Inoki K., Koya D. and Kikkawa R. **2002**. 15-Deoxy-Delta12, 14-prostaglandin J2 inhibits IL-1beta-induced cyclooxygenase-2 expression in mesangial cells. *Kidney International*, **61:** 1957-1967.
509. Scher J.U. and Pillinger M.H. **2005**. 15d-PGJ2: The anti-inflammatory prostaglandin? *Clinical Immunology*, **114:** 100-109.
510. Scher J.U. and Pillinger M.H. **2017**. The Anti-Inflammatory Effects of Prostaglandins. *Journal of Investigative Medicine*, **57:** 703-708.
511. Schneider M., Forster H., Boersma A., Seiler A., Wehnes H., Sinowatz F., Neumuller C., Deutsch M.J., Walch A., Angelis M.H., Wurst W., Ursini F., Roveri A., Maleszewski M., Maiorino M. and Conrad M. **2009**. Mitochondrial glutathione peroxidase 4 disruption causes male infertility. *Federation of American Societies for Experimental Biology*, **23:** 3233-3242.
512. Schneider C.A., Rasband W.S. and Eliceiri K.W. **2012**. "NIH Image to ImageJ: 25 years of image analysis". *Nature Methods*, **9:** 671-675.
513. Schrauzer G.N. **1998**. Selenium: present and future applications in medicine. In: *STDA Sixth International Symposium on Uses of Selenium and Tellurium*, Scottsdale, USA. Grimbergen, Belgium: Selenium Tellurium Development Association, pp. 37-40.
514. Schrauzer G.N. **2000**. Selenomethionine: a review of its biological significance, metabolism and toxicity. *The Journal of Nutrition*, **130:** 1653-1656.
515. Schumacher K.L., Roy M., Wishe H.I., Cohen M.W. and Stotzky G. **1994**. Supplementation with selenium and human immune cell functions. *Biological Trace Element Research*, **41:** 115-127.
516. Schumann K., Classen H., Hages M., Prinz-Langenohl R., Pietrzik K. and Biesalki H. **1997**. Bioavailability of oral vitamins, minerals, and trace elements in perspective. *Arzneim.-Forsch./Drug Research*, **47:** 369-380.

517. Schwarz K., and Folts S.M. **1957**. Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society*, **79**: 3292-3293.
518. Schweizer U., Brauer A.U., Kohrle J., Nitsch R. Savaskan N.E. **2004**. Selenium and brain function: a poorly recognized liaison. *Brain Research Reviews*, **45:** 164-178.
519. Schweizer U., Streckfu F., Pelt P., Carlson B.A., Hatfield D.H., Kohrle J. and Schomburg L. **2005**. Hepatically derived selenoprotein P is a key factor for kidney but not for brain selenium supply. *Biochemical Journal*, **386:** 221-226.
520. Seby F., Potin-Gautier M., Giffaut E. and Donard O. **1998**. Assessing the speciation and the biogeochemical processes affecting the mobility of selenium from a geological repository of radioactive wastesto the biosphere. *Analysis*, **26:** 193-198.
521. Seiler A., Schneider M., Forster H., Roth S., Wirth E.K., Culmsee C., Plesnila N., Kremmer E., Radmark O., Wurst W., Bornkamm G.W., Schweizer U. and Conrad M. **2008**. Glutathione Peroxidase 4 Senses and Translates Oxidative Stress into 12/15-Lipoxygenase Dependent- and AIF-Mediated Cell Death. *Cell Metabolism*, **8:** 237-248.
522. Semchyshyn H. and Lushchak V. **2012**. Interplay netween oxidative and carbonyl stresses: Molecular mechanisms, biological effects and therapeutic strategies of protection. In. Oxidative stress: Molecular mechanisms and biological effects, Lushchak, V. and Semchyshyn, H. Eds., Intech Publishers, pp. 1-33.
523. Sengupta A., Carlson B.A., Labunskyy V.M., Gladyshev V.N. and Hatfield D.L. **2009**. Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells. *Biochemistry and Cell Biology*, **87:** 953-961.
524. Sengupta A., Lichti U., Carlson B., Ryscavage A., Gladyshev V., Yuspa S. and Hatfield D. **2010**. Selenoproteins are essential for proper keratinocyte function and skin development. *PLoS ONE*, **5:** 12249.
525. Serhan C.N., Brain S.D., Buckley C.D. Gilroy D.W., Haslett C., O'Neill L.A., Perretti M., Rossi A.G. and Wallace J.L. **2007**. Resolution of inflammation: state of the art, definitions and terms. *The FASEB Journal*, **21:** 325–332.
526. SCF (Scientific Committee of Food). **2000**. Opinion of the scientific committee on food on the tolerable upper intake level of selenium.
527. Shamberger R. and Frost D. 1969. Possible protective effect of selenium against human cancer. Canadian Medical Association Journal, **100:** 682.

528. Shan Z.Z., Hongo K.M., Dai S.M., Nakamura H., Kato T. and Nishioka K. **2004**. A potential role of 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 for induction of human articular chondrocyte apoptosis in arthritis. *Journal of Biological Chemistry*, **279:** 37939-37950.
529. Sharma S. and Singh R. **1983**. Selenium distribution in soils of bio-clamatic zones of Haryana, India. *Journal of the Indian Society of Soil Science*, **24**: 62-67.
530. Sharma N., Prakash R., Srivastava A., Sadana U. S., Achary R., Prakash T. and Reddy A. V. R. **2009**. Profile of selenium in soil and crops in seleniferous area of Punjab, India by neutron activation analysis. *Journal of Radioanalytical and Nuclear Chemistry*, **281:** 59-62.
531. SHC (Superior Health Council). **2009**. Recommandations nutritionnelles pour la Belgique, n°8309.
532. Shchedrina V.A., Everley R.A., Zhang Y., Gygi S.P., Hatfield D.L. and Gladyshev V.N. **2011**. Selenoprotein K Binds Multiprotein Complexes and Is Involved in the Regulation of Endoplasmic Reticulum Homeostasis. *Journal of Biological Chemistry*, **286:** 42937-42948.
533. Shemi D., Azab A.N. and Kaplanski J. **2000**. Time-dependent effect of LPS on PGE2 and TNF-alpha production by rat glial brain culture: influence of COX and cytokine inhibitors. *Journal of Endotoxin Research*, **6:** 377-81.
534. Shen L., Van Dyck K., Luten J. and Deelstra H. **1997**. Diffusibility of selenate, selenite, seleno-methionine and seleno-cystine during simulated gastrointestinal digestion. *Biological Trace Element Research*, **58:** 55-63.
535. Shen Y., Yang S., Shi Z., Lin T., Zhu H., Bi F., Liu A., Ying X., Liu H., Yu K. and Yan S. **2015**. SeMet Mediates Anti-inflammation in LPS-Induced U937 Cells Targeting NF-κB Signaling Pathway. *Inflammation*, **38:** 736-744.
536. Shewry P.R. **2009**. Wheat. *Journal of Experimental Botany*, **60:** 1537–1553.
537. Shih R.H., Wang C.Y. and Yang C.M. **2015**. NF-kappaB Signaling Pathways in Neurological Inflammation: A Mini Review. *Frontiers in Molecular Neuroscience*, **8:** 1-8.
538. Simon A.P., Turck N., Sidhoum J.M., Gradwohl G. and Kedinger M. **2007**. In vitro models of intestinal epithelial cell differentiation. *Cell Biology and Toxicology*, **23:** 241-256.
539. Simmons D.L., Botting R.M. and Hla T. **2004**. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacological Reviews*, **56:** 387–437.
540. Smith W.L., DeWitt D.L. and Garavito R.M. **2000**. Cyclooxygenases: Structural, cellular, and molecular biology. *The Annual Review of Biochemistry*, **69:** 145–182.

541. Smith W.L. and Murphy R.C. **2002**. The eicosanoids: cyclooxygenase, lipoxygenase, and epoxygenase pathways. *Biochemistry of Lipids, Lipoproteins and Membranes*, **13:** 341-371.

542. Smith A., Cheung L. and Botero S. **2011**. Long-term selenium deficiency increases the pathogenecity of *Citrobacter rodentium* infection in mice. *Biological Trace Element Research*, **144:** 965-982.

543. Spadoni M., Voltaggio M., Carcea M., Coni E., Raggi A. and Cubadda, F. **2007**. Bioaccessible selenium in Italian agricultural soils: Comparison of the biogeochemical approach with a regression model based on geochemical and pedoclimatic variables. *Science of the Total Environment*, **376:** 160-177.

544. Spallholz J.E., Boylan L.M. and Larsen H.S. **1990**. Advances in understanding selenium's role in the immune system. *Annals of the New York Academy of Sciences*, **587:** 123-139.

545. Solis S.J.C., Villalobos P., Orozco A. and Valverde R.C. **2004**. Comparative kinetic characterization of rat thyroid iodotyrosine dehalogenase and iodothyronine deiodinase type 1. *Journal of Endocrinology*, **181:** 385-392.

546. Song H., Hur I., Park H., Nam J., Park G.B., Kong K.H., Hwang Y.M., Kim Y.S., Cho D.H., Lee W.J. and Hur D.Y. **2009**. Selenium Inhibits Metastasis of Murine Melanoma Cells through the Induction of Cell Cycle Arrest and Cell Death. *Immune Network*, **9:** 236-242.

547. Sors T.G., Ellis D.R. and Salt D.E. **2005**. Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research*, **86:** 373-389.

548. Srivastava A., Tejo Prakash N., Bhatnagar S., Kumar A., Gupta S., Sadana U. S., and Spallholz J. E. **2006**. Selenium toxicity in water, soil, and crops in Nawanshahr region of India. In: Proceedings of the International Conference on Selenium in Biology and Medicine, Wisconsin, USA, pp. 120.

549. Stadlober M., Sager M. and Irgolic K. **2001**. Effects of selenate supplemented fertilisation on the selenium level of cereals - identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chemistry*, **73:** 357-366.

550. Stadtman T. **1996**. Selenocysteine. *Annual Review of Biochemistry*, **65:** 83-100.

551. Stahl W., Berg H., Arthur J., Bast A., Dainty J., Faulks R., Gartner C., Haenen G., Hollman P., Holst B., Kelly F., Polidori M., Rice-Evans C., Southon S., Vliet T., Ribes J., Williamson G. and Astley S. **2002**. Bioavailability and metabolism. *Molecular Aspects of Medicine*, **23:** 39-100.

552. Stanley B.A., Sivakumaran V., Shi S., McDonald I., Lloyd D., Watson W.H., Aon M.A. and Paolocci N. **2011**. Thioredoxin Reductase-2 Is Essential for Keeping Low Levels of H_2O_2 Emission from Isolated Heart Mitochondria. *Journal of Biological Chemistry*, **286:** 33669-33677.

553. Stephen R.C., Saville D.J. and Watkinson J.H. **2012**. The effects of sodium selenate applications on growth and selenium concentration in wheat. *New Zealand Journal of Crop and Horticultural Science*, **17:** 229-237.

554. Steinbrenner H., Alili L., Bilgic E., Sies H. and Brenneisen P. **2006**. Involvement of selenoprotein P in protection of human astrocytes from oxidative damage. *Free Radical Biology and Medicine*, **40:**1513–1523.

555. Steinbrenner H., Steinbrenner H., Bilgic E., Steinbrenner H., Bilgic E., Alili L., Sies H. and Brenneisen P. **2009**. Selenoprotein P protects endothelial cells from oxidative damage by stimulation of glutathione peroxidase expression and activity. *Free Radical Research*, **40:** 936-943.

556. Steinbrenner H., Speckmann B., Pinto A. and Sies H. **2011**. High selenium intake and increased diabetes risk: experimental evidence for interplay between selenium and carbohydrate metabolism. *Journal of Clinical Biochemistry and Nutrition*, **48:** 40-45.

557. Stroud J.L., Broadley M.R., Foot I., Fairweather-Tait S.J., Hart D.J., Hurst R., Knott P., Mowat H., Norman K., Scott P., Tucker M., White P.J, McGrath S.P. and Zhao F.J. **2010**. Soil factors affecting selenium concentration in wheat grain and the fate and speciation of Se fertilizers applied to soil. *Plant and Soil*, **332:** 19-30.

558. Strub M.P., Hoh F., Sanchez J.F., Strub J.M., Bock A., Aumelas A. and Dumas C. **2003**. Selenomethionine and selenocysteine double labeling strategy for crystallographic phasing. *Structure*, **11:** 1359-1367.

559. Suh N., Honda T., Finlay H.J., Barchowsky A., Williams C., Benoit N.E., Xie Q., Nathan C., Gribble G.W. and Sporn M.B. **1998**. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Research*, **58:** 717-723.

560. Sun Y., Ha P.C., Butler J.A., Ou B.R., Yeh J.Y. and Whange P. **1998**. Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of the rat. *The Journal of Nutritional Biochemistry*, **9:** 23-27.

561. Sun G.X., Liu X., Williams P.N. and Zhu Y.G. **2010**. Distribution and Translocation of Selenium from Soil to Grain and Its Speciation in Paddy Rice (*Oryza sativa* L.). *Environmental Science and Technology*, **44:** 6706-6711.

562. Surh Y.J., Na H.K., Park J.M., Lee H.N., Kim W., Yoon I.S. and Kim D.D. **2011**. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2, an electrophilic lipid mediator of anti-inflammatory and pro-resolving signaling. *Biochemical Pharmacology*, **82:** 1335-1351.

563. Susanne K., Maria L., Simone F., Anna P.K., Antje B., Ludger A.W., Martin N.B., Renato I., Robert S.E., Fong F.C. and Regina B.F. **2012**. Glutathione peroxidase-2 and selenium decreased inflammation and tumors in a mouse model of inflammation-associated carcinogenesis whereas sulforaphane effects differed with selenium supply. *Carcinogenesis*, **33:** 620–628.

564. Suzuki T. **1965**. A geochemical study of selenium in volcanic exhalation and sulphur deposits. II. On the behavior of selenium and sulphur in volcanic exhalation and sulphur deposits. *Bulletin of Chemical Society of Japan*, **38:** 1940-1946.

565. Suzuki K.T., Sasakura C. and Yoneda S. **1998**. Binding sites for the (HgSe) complex on selenoprotein P bioavailability. *Biochimica et Biophysica Acta*, **1429:** 102-11.

566. Suzuki K. T. and Ogra Y. **2002**. Metabolic pathway for selenium in the body: Speciation by HPLC-ICP MS with use of enriched Se. *Food Additives and Contaminants*, **19:** 974-983.

567. Suzuki K. **2005**. Metabolomics of selenium: Se metabolites based on speciation studies. *Journal of Health Science*, **51:** 107-114.

568. Suzuki K., Kurasaki K., Ogawa S. and Suzuki N. **2006**. Metabolic transformation of methylseleninic acid through key selenium intermediate selenide. *Toxicology and Applied Pharmacology*, **215:** 189- 197.

569. Suzuki K., Tsuji Y., Ohta Y. and Suzuki N. **2008**. Preferential organ distribution of methylselenol source Se-methylselenocysteine relative to methylseleninic acid. *Toxicology and Applied Pharmacology*, **227:** 76- 83.

570. Suzuki Y., Hashiura Y., Matsumura K., Matsukawa T., Shinohara A. and Furuta N. **2009**. Dynamic pathways of selenium metabolism and excretion in mice under different selenium nutritional stresses. *Metallomics*, **2:** 126-132.

571. Swaine, D.J. **1955**. The trace element content of soils. *Commonwealth Bureau of Soil Science. Technical Communication* no. 48. HMSO, London.

572. Tabriz H.M., Olfati G., Ahmadi S.A. and Yusefnia S. **2013**. Cyclooxygenase-2 expression in urinary bladder transitional cell carcinoma and its association with clinicopathological characteristics. *Asian Pacific Journal of Cancer Prevention*, **14:** 4539-4543.
573. Taechowisan T., Wanbanjob A., Tuntiwachwuttikul P. and Liuin J. **2010**. Anti-inflammatory effects of lansai C and D cause inhibition of STAT-1 and NF-κB activations in LPS-induced RAW 264.7 cells. *Food and Agriculture Immunology*, **21:** 57-64.
574. Tamas M., Mandoki Z. and Csapo J. **2010**. The role of selenium content of wheat in the human nutrition. A literature review. *Acta Universitatis Sapientiae, Alimentaria*, **3:** 5-34.
575. Tan J., Zhu W., Wang W., Li R., Hou S., Wang D. and Yang L. **2002**. Selenium in soil and endemic diseases in China. *Science of The Total Environment*, **284:** 227-235.
576. Tanaka H., Esaki N. and Soda K. **1985**. A versatile bacterial enzyme: L-methionine y-lyase. *Enzyme and Microbial Technology*, **7:** 530-537.
577. Tanaka K., Ogawa K., Sugamura K., Nakamura M., Takano S. and Nagata M. **2000**. Differential production of prostaglandin D2 by human helper T cell subsets. *Journal of Immunology*, **164:** 2277–2280.
578. Tanaka R., Miwa Y., Mou K., Tomikawa M., Eguchi N., Urade Y., Takahashi-Yanaga F., Morimoto S., Wake N. and Sasaguri T. **2009**. Knockout of the l-pgds gene aggravates obesity and atherosclerosis in mice. *Biochemical and Biophysical Research Communications*, **378:** 851–856.
579. Tamari Y., Ogawa H., Fukumoto Y., Tsuji H. and Kusaka Y. **1990**. Selenium content and its oxidation-state in igneous rocks, rock-forming minerals, and a reservoir sediment. *Bulletin of the Chemical Society of Japan*, **63:** 2631-2638.
580. Tapiero H., Townsend D. and Tew K. **2003**. The antioxidant role of selenium and seleno-compounds. *Biomedicine and Pharmacotherapy*, **57:** 134-144.
581. Taylor E.W. **1997**. Selenium and viral diseases: Facts and hypotheses. *Journal of Orthomolecular Medicine*, **12:** 227-239.
582. Taylor B.S. and Geller D.A. **2000**. Molecular regulation of the human inducible nitric oxide synthase (iNOS) gene. *Shock*, **13:** 413-424.
583. Temmerman L.D., Waegeneers N., Thiry C., Laing G.D., Tack F. and Ruttens A. **2014**. Selenium content of Belgian cultivated soils and its uptake by field crops and vegetables. *Science of The Total Environment*, **468:** 77-82.

584. Terry N., Zayed A.M., de Souza M.P. and Tarun A.S. **2000**. Selenium in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **51:** 401-432.

585. Te-Velde A.A., Pronk I., de Kort F. and Stokkers P.C.F. **2008**. Glutathione peroxidase 2 and aquaporin 8 as new markers for colonic inflammation in experimental colitis and inflammatory bowel diseases: an important role for H_2O_2? *Gastroenterology and Hepatology*, **20:** 555-560.

586. Tham D.M., Whitin J.C., Kim K.K., Zhu S.X. and Cohen H.J. **1998**. Expression of extracellular glutathione peroxidase in human and mouse gastrointestinal tract. *The American Journal of Physiology*, **275:** 1463-1471.

587. The American Society of Health-System Pharmacists. "Selenium Sulfide". Retrieved 8 January **2017**.

588. Thiry C., Ruttens A., Temmerman L.D., Schneider Y.J. and Pussemier L. **2012**. Current knowledge in species-related bioavailability of selenium in food. *Food Chemistry*, **130:** 767-784.

589. Thiry C., Schneider Y.J., Pussemier L., Temmerman L.D. and Ruttens A. **2013**. Selenium Bioaccessibility and Bioavailability in Se-Enriched Food Supplements. *Biological Trace Element Research*, **152:** 152-160.

590. Thompson C. **2004**. Assessment of requirements for selenium and adequacy of selenium status: a review. *European Journal of Clinical Nutrition*, **58:** 391-402.

591. Thompson C., Chisholm A., McLachlan S. and Campbell J. **2008**. Brazil nuts: an effective way to improve selenium status. *American Journal of Clinical Nutrition*, **87:** 379-384.

592. Thomson C.D., Robinson M.F., Butler J.A. and Whanger P.D. **1993**. Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (EC 1.11 1.9) in blood components of New Zealand women. *British Journal of Nutrition*, **69:** 577-588.

593. Thomson C.D. **2004**. Assessment of requirements for selenium and adequacy of selenium status: a review. *European Journal of Clinical Nutrition*, **58:** 391-402.

594. Thornton F.J., Schaffer M.R., Witte M.B., Moldawer L.L., MacKay S.L.D., Abouhamze A.B.S., Tannahill C.L. and Barbul A. **1998**. Enhanced collagen accumulation following direct transfection of the inducible nitric oxide synthase gene in cutaneous wounds. *Biochemical and Biophysical Research Communications*, **246:** 654-659.

595. Tien E.S., Matsui K., Moore R. and Negishi M. **2007**. The Nuclear Receptor Constitutively Active/Androstane Receptor Regulates Type 1 Deiodinase and Thyroid Hormone Activity in the Regenerating Mouse Liver. *Journal of Pharmacology and Experimental Therapeutics*, **320:** 307-313.

596. Tiwary A., Stegelmeier B., Panter K., James L. and Hall J. **2006**. Comparative toxicosis of sodium selenite and selenomethionine in lambs. *Journal of Veterinary Diagnostic Investigation*, **18:** 61-70.

597. Tokudome S., Sano M., Shinmura K., Matsuhashi T., Morizane S., Moriyama H., Tamaki K., Hayashida K., Nakanishi H., Yoshikawa N., Shimizu N., Endo J., Katayama T., Murata M., Yuasa S., Kaneda R., Tomita K., Eguchi N., Urade Y., Asano K., Utsunomiya Y., Suzuki T., Taguchi R., Tanaka H. and Fukuda K. **2009**. Glucocorticoid protects rodent hearts from ischemia/reperfusion injury by activating lipocalin-type prostaglandin D synthase-derived PGD2biosynthesis. *Journal of Clinical Investigation*, **119:** 1477-1488.

598. Tokugawa Y., Kunishige I., Kubota Y., Shimoya K., Nobunaga T., Kimura T., Saji F., Murata Y., Eguchi N., Oda H., Urade Y. and Hayaishi O. **1998**. Lipocalin-type prostaglandin D synthase in human male reproductive organs and seminal plasma. *Biology of Reproduction,* **58:** 600-607.

599. Tomio I., Tamaki M., Arai E. and Horino T. **2002**. Distribution of Amylose, Nitrogen, and minerals in Rice Kernels with Various Characters. *Journal of Agricultural and Food Chemistry*, **50:** 5326–5332.

600. Toyokuni S. **1999**. Reactive oxygen species-induced molecular damage and its application in pathology. *Pathology International*, **49:** 91–102.

601. Traulsen H., Steinbrenner H., Buchczyk D.P., Klotz L.O. and Sies H. **2009**. Selenoprotein P Protects Low-density Lipoprotein Against Oxidation. *Free Radical Research*, **38:** 123-128.

602. Triana A.G., Jimenez S.J., Uriarte A.B.P., Zavala A.L., Aguilar G.G., Mundo R.R.S., Soto E.M.V. **2010**. Expression and silencing of Selenoprotein M (SelM) from the white shrimp Litopenaeus vannamei: Effect on peroxidase activity and hydrogen peroxide concentration in gills and hepatopancreas. Comparative Biochemistry and Physiology Part A: *Molecular and Integrative Physiology*, **155:** 200–204.

603. Tsoyi K.,Park H.B., Kim Y.M., Chung J.I., Shin S.C., Lee W.S., Seo H.G.,Lee J.H., Chang K.C. and Kim H.J. **2008**. Anthocyanins from black soybean seed coats inhibit UVB-Induced

inflammatory cylooxygenase-2 gene expression and PGE2 production through regulation of the nuclear factor-KB and phosphatidylinositol 3-kinase/Akt pathway. *Journal of Agricultural and Food Chemistry*, **56:** 8969-8974.

604. Tu H.M., Kim S.W., Salvatore D., Bartha T., Legradi G., Larsen P.R., Lechan R.M. **1997**. Regional Distribution of Type 2 Thyroxine Deiodinase Messenger Ribonucleic Acid in Rat Hypothalamus and Pituitary and Its Regulation by Thyroid Hormone. *Endocrinology*, **138:** 3359-3368.
605. Tveitnes S., Singh B.R. and Ruud L. **1996**. Selenium concentration in spring wheat as influenced by basal application and top dressing of selenium-enriched fertilizers. *Fertilizer Research*, **45:** 163-167.
606. Tyagi S., Gupta P., Saini A.S., Kaushal C. and Sharma S. **2011**. The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. *Journal of Advanced Pharmaceutical Technology and Research*, **2:** 236-240.
607. Ujihara M., Tsuchida S., Satoh K., Sato K. and Urade Y. **1988**. Biochemical and immunological demonstration of prostaglandin D2, E2 and F2α formation from prostaglandin H2 by various rat glutathione S-transferase isozymes. *Archives of Biochemistry and Biophysics*, **264:** 428–437.
608. Urade Y., Ujihara M., Horiguchi Y., Ikai K. and Hayaishi O. **1989**. The major source of endogenous prostaglandin D2 production is likely antigen presenting cells : localisation of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic and Kupffer cells in various tissues. *Journal of Immunology*, **143:** 2982–2989.
609. Urade Y. and Hayaishi O. **2000**. Prostaglandin D synthase: structure and function. *Vitamins and Hormones*, **58:** 89–120.
610. Urade Y. and Hayaishi O. **2011**. Prostaglandin D2 and sleep/wake regulation. *Sleep Medicine Reviews*, **15:** 411–418.
611. Ursini F., Heim S., Kiess M., Maiorino M., Roveri A., Wissing J. and Flohe L. **1999**. Dual function of the selenoprotein PHGPx during sperm maturation. *Science*, **285:** 1393–1396.
612. Van Campen D. R. and Glahn R. P. **1999**. Micronutrient bioavailability techniques: Accuracy, problems and limitations. *Field Crops Research*, **60:** 93-113.
613. Van Metre D.C. and Callan R.J. **2001**. Selenium and vitamin E. *The Veterinary Clinics of North America: Food Animal Practice*, **17:** 373-402.

614. Vander Torre H.W., Van Dokkum W.T.M., Schaafsma G., Wedel M. and Ockhuizen. **1991**. Effect of various levels of selenium in wheat and meat on blood Se status indices and on Se balance in Dutch men. *British Journal of Nutrition*, **65:** 69-80.

615. Vanderpas J.B., Contempre B., Duale N.L., Deckx H., Bebe N., Longombe A.O., Thilly C.H., Diplock A.T. and Dumont J.E. **1993**. Selenium deficiency mitigates hypothyroxinemia in iodine deficient subjects. *The American Journal of Clinical Nutrition*, **57:** 271-275.

616. Vane J.R., Mitchell J.A., Appleton I., Tomlinson A., BaileyD.B., Croxtall J. and Willoughby D.A. **1994**. Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. *Proceedings of the National Academy of Sciences of the United State of America*, **91:** 2046-2050.

617. Varo P., Alfthan G., Ekholm P., Aro A., Koivistoinen P. **1988**. Selenium intake and serum selenium in Finland: effects of soil fertilization with selenium. *American Journal of Clinical Nutrition*, **48:** 324-329.

618. Vega L., Sosab M.R., Garcia-Montalvoa E.A., Razoa L.M. and Elizondo G. **2007.** Non-optimal levels of dietary selenomethionine alter splenocyte response and modify oxidative stress markers in female mice. *Food and Chemical Toxicology*, **45:** 1147-1153.

619. Vendeland S.C., Beilstein M.A., Chen C.L., Jensen O.N., Barofsky E. and Whanger P.D. **1993**. Purification and properties of selenoprotein W from rat muscle. *Journal of Biological Chemistry*, **268:** 17103-17107.

620. Verma S, Hoffmann F.W., Kumar M., Huang Z., Roe K., Wu E.N., Hashimoto A.S. and Hoffmann P.R. **2011**. Selenoprotein K Knockout Mice Exhibit Deficient Calcium Flux in Immune Cells and Impaired Immune Responses. *The Journal of Immunology*, **186:** 2127-2137.

621. Vignais P.V. **2002**. The superoxide-generating NADPH oxidase: structural aspects and activation mechanism. *Cellular and Molecular Life Sciences*, **59:** 1428–1459.

622. Villafane R., Hidalgo M., Piccoli A., Marchevsky E., Pellerano R. **2017**. Non-essential element concentrations in brown grain rice: Assessment by advanced data mining techniques. *Environmental Science and Pollution Research*, pp. 1-6.

623. Villette S., Kyle J.A.M., Brown K.M., Pickard K., Milne J.S., Nicol F., Arthur J.R. and Hesketh J.E. **2002**. A Novel Single Nucleotide Polymorphism in the 3′ Untranslated Region of Human Glutathione Peroxidase 4 Influences Lipoxygenase Metabolism. *Blood Cells, Molecules, and Diseases*, **29:** 174-178.

624. Vinceti M., Wei E.T., Malagoli C., Bergomi M. and Vivoli G. **2001**. Adverse health effects of selenium in humans. *Reviews on Environmental Health*, **16:** 233-251.

625. Vizuete A.M., Sadek C.M., Jimenez, A., Krause W.J., Sutovsky P. and Oko R. **2004**. The Mammalian Testis-Specific Thioredoxin System. *Antioxidants and Redox signaling*, **6:** 25-40.

626. Vogel V.G. and McPherson R.S. **1989**. Dietary epidemiology of colon cancer. *Hematology Oncology Clinics of North America*, **3:** 35-63.

627. Vunta H., Davis F., Palempalli U.D., Bhat D., Arner R.J., Thompson J.T., Peterson D.G., Reddy C.C. and Prabhu S.K. **2007**. The anti-inflammatory effects of selenium are mediated through 15-deoxy-Delta12, 14-prostaglandin J2 in macrophages. *The Journal of Biological Chemistry*, **282:** 17964-17973.

628. Vunta H., Belda B.J., Arner R.J., Reddy C.C., Vanden-Heuvel J.P. and Prabhu S.K. **2008**. Selenium attenuates pro-inflammatory gene expression in macrophages. *Molecular Nutrition and Food Research*, **52:** 1316-1323.

629. Wallace J.M. **2002**. Nutritional and botanical modulation of the inflammatory cascade-eicosanoids, cyclooxygenases, and lipoxygenases- as an adjunct in cancer therapy. *Integrative Cancer Therapies*, **1:** 7-37.

630. Wang X., Martindale J.L., Liu Y. and Holbrook N.J. **1998**. The cellular response to oxidative stress: influences of mitogen-activated protein kinase signaling pathways on cell survival. *Biochemical Journal*, **333:** 291-300.

631. Wang C., Wang H., Luo J., Hu Y., Wei L., Duan M. and He H. **2009**. Selenium deficiency impairs host innate immune response and induces susceptibility to *Listeria monocytogenes* infection. *BMC Immunology*, **10:** 55.

632. Wang Y.D., Wang X. and Wong Y.S. **2013**. Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. *Food Chemistry*, **141:** 2385-2393.

633. Wang J., Wang Z., Mao H., Zhao H. and Huang D. **2013**. Increasing Se concentration in maize grain with soil- or foliar-applied selenite on the Loess Plateau in China. *Feild Crops Research*, **150:** 83-90.

634. Warburton E., Goenaga A. and Infante H. **2007**. Methane mixed plasma-improved sensitivity of inductively coupled plasma mass spectrometry detection for selenium speciation analysis of wheat-based food. *Journal of Analytical Atomic Spectrometry*, **22:** 370-376.

635. Waschulewski I.H. and Sunde R.A. **1988**. Effect of dietary methionine on tissue selenium and glutathione peroxidase activity in rats given selenomethionine. *British Journal of Nutrition*, **60:** 57-68.

636. Watanabe K., Urade Y., Mäder M., Murphy C. and Hayaishi O. **1994**. Identification of β-trace as prostaglandin D synthase. *Biochemical and Biophysical Research Communications,* **203:** 1110–1116.

637. Weaver J.A., Maddox J.F., Cao Y.Z., Mullarky I.K. and.Sordillo L.M. **2001**. Increased 15-HPETE production decreases prostacyclin synthase activity during oxidant stress in aortic endothelial cells. *Free Radical Biology and Medicine,* **30:** 299-308.

638. Weinberg J.B., Misukonis M.A., Shami P.J., Mason S.N., Sauls D.L., Dittman W.A., Wood E.R., Smith G.K., McDonald B. and Bachus K.E. **1995**. Human mononuclear phagocyte inducible nitric oxide synthase (iNOS): analysis of iNOS mRNA, iNOS protein, biopterin, and nitric oxide production by blood monocytes and peritoneal macrophages. *Blood,* **86:** 1184-1195.

639. Weiss H.V., Koide M and Goldberg E.D. **1971**. Selenium and S in Greenland ice sheet: Relation to fossil fuel combustion. *Science,* **172:** 261-263.

640. Wellman K.H.F., Mattox T.A., Thayne K., Katunga L.A., Favor J.D.L., Neufer P.D., Hickner R.C., Wingard C.J. and Anderson E.J. **2013**. Novel role for thioredoxin reductase-2 in mitochondrial redox adaptations to obesogenic diet and exercise in heart and skeletal muscle. *The Journal of Physiology*, **591:** 3471-3486.

641. Whanger P. and Butler J. **1988**. Effects of various dietary levels of selenium as selenite or selenomethionine on tissue selenium levels and glutathione peroxidase activity in rats. *The Journal of Nutrition*, **118:** 846-852.

642. Whanger P.D. **2002**. Selenocompounds in plants and animals and their biological significance. *The Journal of American College of Nutrition*, **21:** 223-232.

643. Whanger P.D. **2004**. Selenium and its relationship to cancer: an update dagger. *British Journal of Nutrition*, 91: 11-28.

644. Whanger P.D. **2009**. Selenoprotein expression and function—Selenoprotein W. *Biochimica et Biophysica Acta (BBA) – General Subjects,* **1790:** 1448-1452.

645. WHO, Food and Agriculture Organisation, International Atomic Energy Agency expert group. **1996**. Trace elements in human nutrition and health. Geneva: WHO.

646. World Health Organization (WHO). **2004**. Vitamin and mineral requirements in human nutrition. In: *Report of a Joint FAO-WHO Expert Consultation*, Bangkok, Thailand. Switzerland: WHO, pp. 194-216.

647. White C.L., Robson A.D. and Fisher H.M. **1981**. Variation in nitrogen, sulphur, selenium, cobalt, manganese, copper and zinc contents of grain from wheat and two lupin species grown in a range of Mediterranean environment. *Australian Journal of Agricultural Research*, **32:** 47–59.

648. White P.J., Bowen H.C., Parmaguru P., Fritz M., Spracklen W., Spiby R.E., Meacham M.C., Mead A., Harriman M., Trueman L.J., Smith B.M., Thomas B. and Broadley M.R. **2004**. Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana. Journal of Experimental Botany*, **55:** 1927-1937.

649. White P.J. and Broadley M.R. **2005**. Biofortifying crops with essential mineral elements. *Trends in Plant Science*, **10:** 586-593.

650. Wilber C.G. **1993**. Toxicology of selenium: A review. *Clinical Toxicology*, **17:** 171-230.

651. William M., Alberto B., Federico G., Maria H.T., Nelly M., Gustavo P., Gianella F. and Gil H. **2009**. Serum selenium and glutathione peroxidase-3 activity: biomarkers of systemic inflammation in the critically ill? *Intensive Care Medicine*, **35:** 882–889.

652. Williams P.N., Lombi E., Sun G.X., Scheckel K., Zhu Y.G, Feng X., Zhu J., Carey A.M., Adomako E., Lawgali Y., Deacon C. and Meharg A.A. **2009**. Selenium Characterization in the Global Rice Supply Chain. *Environmental Science and Technology*, **43:** 6024-6030.

653. Williams P.N., Islam S., Islam R., Jahiruddin M., Adomako E., Soliaman A.R.M., Rahman G.K.M.M., Lu Y., Deacon C., Zhu Y.G. and Meharg A.A. **2009**. Arsenic Limits Trace Mineral Nutrition (Selenium, Zinc, and Nickel) in Bangladesh Rice Grain. *Environmental Science and Technology*, **43:** 8430-8436.

654. Wilson K.T., Fu S., Ramanujam K.S. and Meltzer S.J. **1998**. Increased expression of inducible nitric oxide synthase and cyclooxygenase-2 in barrett's esophagus and associated adenocarcinomas. *Cancer Research*, **58:** 2929-2934.

655. Wingler K., Muller C., Schmehl K., Florian S. and Brigelius F.R. **2000**. Gastrointestinal glutathione peroxidase prevents transport of lipid hydroperoxides in CaCo-2 cells. *Gastroenterology*, **119:** 420-430.

656. Wrobel K., Kannamkumarath S., Caruso J., Wysocka I., Bulska E., Swiatek J. and Wierzbicka M. **2004**. HPLC-ICP-MS speciation of selenium in enriched onion leaves - a potential dietary source of Semethylselenocysteine. *Food Chemistry*, **86:** 617-623.

657. Xia Y. and Zweier J.L. **1997**. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. *Proceedings of the National Academy of Sciences of the United States of America*, **94:** 6954-6958.

658. Xia Y., Roman L.J., Masters B.S.S. and Zweier J.L. **1998**. Inducible nitric-oxide synthase generates superoxide from the reductase domain. *Journal of Biological Chemistry*, **273:** 22635-22639.

659. Xu X.M., Carlson B.A., Irons R., Mix H., Zhong N., Gladyshev V.N. and Hatfield D.L. **2007**. Selenophosphate synthetase 2 is essential for selenoprotein biosynthesis. *Biochemical Journal*, **404:** 115-120.

660. Yamamoto T., Katayama I. and Nishioka K. **1998**. Nitric oxide production and inducible nitric oxide synthase expression in systemic sclerosis. *The Journal of Rheumatology*, **25:** 314-317.

661. Yan W. and Chen X. **2006**. GPX2, a Direct Target of p63, Inhibits Oxidative Stress-induced Apoptosis in a p53-dependent Manner. *Journal of Biological Chemistry*, **281:** 7856-7862.

662. Yang G., Wang S., Zhou R. and Sun S. **1983**. Endemic Selenium Intoxication of Humans in China. *American Journal of Clinical Nutrition*, **37:** 872-881.

663. Yang G.Q., Zhu L.Z., Liu L.Z., Gu L.Z., Qian P.C., Huang J.H. and Lu M.D. **1987**. Human selenium requirements in China. *Selenium in Biology and Medicine*, pp. 589–607.

664. Yang G., Ge K., Chen J., and Chen X. **1988**. Selenium-related endemic diseases and the daily selenium requirement of humans. *World Review of Nutrition and Dietetic*, **55**: 98-152.

665. Yang G., Yin S., Zhou R., Gu L., Yan B., Liu Y., Liu Y. **1989**. Studies of safe maximal daily selenium intake in a seleniferous area in China. II. Relation between Se intake and the manifestation of clinical signs and certain biochemical alterations in blood and urine. *Journal of Trace Elements and Electrolytes in Health and Disease*, **3:** 123–130.

666. Yang F.Y., Lin Z.H., Xing J.R., Li S.G., Yang J., San S. and Wu L. **1994**. Selenium deficiency is a necessary but not sufficient factor required for the pathogenesis of Keshan disease. *Journal of Clinical Biochemistry and Nutrition*, **16:** 101-110.

667. Yang X., Tian Y., Ha P. and Gu L. **1997**. Determination of selenomethionine content in grain and human blood. *Wei Sheng Yan Jiu*, **26:** 113-116.

668. Yang X., Hill K.E., Maguire M.J. and Burk R.F. **2000**. Synthesis and secretion of selenoprotein P by cultured rat astrocytes. *Biochimica et Biophysica Acta (BBA) General Subjects*, **1474:** 390-396.

669. Yang F. **2006**. Keshan disease and mitochondrial cardiomyopathy. *Science in China Series C: Life Sciences*, **49:** 513-518.

670. Yang J., Maldonado-Gomez M.X., R.W. and Rose D.J. **2014**. Production and *in Vitro* fermentation of soluble, non-digestible, feruloylated oligo- and polysaccharides from maize and wheat brans. *Journal of Agricultural and Food Chemistry*, **62:** 159-166.

671. Yang X., Yao H., Chen Y., Sun L., Li Y., Ma X., Duan S., Li X., Xiang R., Han J. and Duan Y. **2015**. Inhibition of Glutathione Production Induces Macrophage CD36 Expression and Enhances Cellular-oxidized Low Density Lipoprotein (oxLDL) Uptake. *Journal of Biological Chemistry*, **290:** 21788-21799.

672. Yant L.J., Ran Q., Rao L., Van R.H., Shibatani T., Belter J.G., Motta L., Richardson A. and Prolla T.A. **2003**. The selenoprotein GPX4 is essential for mouse development and protects from radiation and oxidative damage insults. *Free Radical Biology and Medicine*, **34:** 496-502.

673. Yao H.D., Wu Q., Zhang Z.W., Li S., Wang X.L., Lei X.G. and Xu S.W. **2013**. Selenoprotein W serves as an antioxidant in chicken myoblasts. *Biochimica et Biophysica Acta (BBA) – General Subjects*, **1830:** 3112-3120.

674. Yi J.S., Holbrook B.C., Michalek R.D., Laniewski N.G. and Grayson J.M. **2006**. Electron transport complex I is required for CD8 + T cell function. *The Journal of Immunology*, **177:** 852–862.

675. Ying X., Chen X., Cheng S., Zhao Z., Guo X., Chen H., Hong J., Peng L.and Xu H. **2013**. SeMet Inhibits IL-1[beta]-Induced Rheumatoid Fibroblast-Like Synoviocytes Proliferation and the Production of Inflammatory Mediators. *Biological Trace Element Research*, **153:** 437-445.

676. Yoo M.H., Xu X.M., Carlson B.A., Gladyshev V.N. and Hatfield D.L. **2006**. Thioredoxin Reductase 1 Deficiency Reverses Tumor Phenotype and Tumorigenicity of Lung Carcinoma Cells. *Journal of Biological Chemistry*, **281:** 13005-13008.

677. Yoo M.H., Gu X., Xu X.M., Kim J.Y., Carlson B.A., Patterson A.D., Cai H., Gladyshev V.N. and Hatfield D.L. **2010**. Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage and in Alzheimer's disease. *Antioxidants and Redox Signaling*. **12:** 819-827.

678. You L., Liu C., Yang Z.J., Li M. and Li S. **2014**. Prediction of Selenoprotein T Structure and Its Response to Selenium Deficiency in Chicken Immune Organs. *Biological Trace Element Research*, **160:** 222–231.

679. Youn H.S., Lim H.J., Choi Y.J., Lee J.Y., Lee M.Y. and Ryu J.H. **2008**. Selenium suppresses the activation of transcription factor NF-kappa B and IRF3 induced by TLR3 or TLR4 agonists. *International Immunopharmacology*, **8:** 495–501.

680. Yu S., Zhu Y. and Li W. **1997**. Protective role of Selenium against hepatitis B virus and primary liver cancer in Qidong. *Biological Trace Element Research*, **56:** 117-124.

681. Yu Y.P., Yu G., Tseng G., Cieply K., Nelson J., Defrances M., Zarnegar R., Michalopoulos G. and Luo G.H. **2007**. Glutathione Peroxidase 3, Deleted or Methylated in Prostate Cancer, Suppresses Prostate Cancer Growth and Metastasis. *Cancer Research*, **67:** 8043-8050.

682. Yui K., Imataka G., Nakamura H., Ohara N. and Naito Y. **2015**. Eicosanoids derived from arachidonic acid and their family prostaglandins and cyclooxygenase in psychiatric disorders. *Current Neuropharmacology*, **13:** 776-785.

683. Zamamiri D.F., Lu Y., Thompson J.T., Prabhu S.K., Reddy P.V., Sordillo L.M. and Reddy C.C. **2003**. Nuclear factor-kappaB mediates over-expression of cyclooxygenase-2 during activation of RAW 264.7 macrophages in selenium deficiency. *Free Radical Biology and Medicine*, **32:** 890–897.

684. Zavacki A.M., Ying H., Christoffolete M.A., Aerts G., So E., Harney J.W., Cheng S.Y., Larsen P.R. and Bianco A.C. **2005**. Type 1 Iodothyronine Deiodinase Is a Sensitive Marker of Peripheral Thyroid Status in the Mouse. *Endocrinology*, **146:** 1568-1575.

685. Zeng H and Botnen J.H. **2007**. Selenium is critical for cancer-signaling gene expression but not cell proliferation in human colon Caco-2 cells. *Biofactors*, **31:** 155-164.

686. Zeng H., Botnen J.H. and Johnson L. **2008**. A selenium-deficient Caco-2 cell model for assessing differential incorporation of chemical or food selenium into glutathione peroxidase. *Biological Trace Element Research*, **123:** 98-108.

687. Zhang L., Shi W. and Wang X. **2006**. Difference in selenite absorption between high- and low-selenium rice cultivars and its mechanism. *Plant and Soil*, **282:** 183-193.

688. Zhang H., Luo Y., Zhang W., He Y., Dai S., Zhang R., Huang Y., Bernatchez P., Giordano F. J., Shadel G., Sessa W. C. and Min W. **2007**. Endothelial-specific expression of mitochondrial thioredoxin improves endothelial cell function and reduces atherosclerotic lesions. *American Journal of Pathology*, **170:** 1108-1120.

689. Zhang H., Feng X., Zhu J., Sapkota A., Meng B., Yao H., Qin H. and Larssen T. **2012**. Selenium in Soil Inhibits Mercury Uptake and Translocation in Rice (*Oryza sativa* L.). *Environmental Science and Technology*, **46:** 10040-10046.

690. Zhang W., Zhang R., Wang T., Jiang H., Mengyao Guo M., Zhou E., Sun Y., Yang Z., Xu S., Cao Y. and Zhang N. **2014**. Selenium Inhibits LPS-Induced Pro-inflammatory Gene Expression by Modulating MAPK and NF-κB Signaling Pathways in Mouse Mammary Epithelial Cells in Primary Culture. *Inflammation*, **37:** 478-485.

691. Zhang M., Tang S., Huang X., Zhang F., Pang Y., Huang Q. and Yi Q. **2014**. Selenium uptake, dynamic changes in selenium content and its influence on photosynthesis and chlorophyll fluorescence in rice (Oryza sativa L.). *Environmental and Experimental Botany*, **107:** 39-45.

692. Zhao R., Masayasu H. and Holmgren A. **2002**. Ebselen: a substrate for human thioredoxin reductase strongly stimulating its hydroperoxide reductase activity and a superfast thioredoxin oxidant. *Proceedings of the National Academy of Sciences U.S.A.*, **99:** 8579-8584.

693. Zhao F.J. and McGrath S.P. **2009**. Biofortification and phytoremediation. *Current Opinion in Plant Biology*, **12:** 373-380.

694. Zhao F.J., Su Y.H., Dunham S.J., Rakszeg M., Bedo Z., McGrath S.B. and Shewry P.R. **2009**. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *Journal of Cereal Science*, **49:** 290-295.

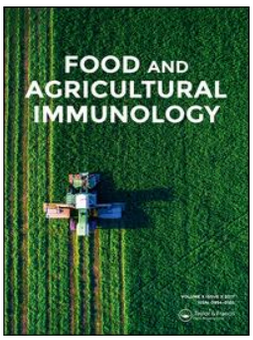
695. Zhao J., Li Y., Li Y., Gao Y., Li B., Hu Y., Zhao Y. and Chai Z. **2014**. Selenium modulates mercury uptake and distribution in rice (Oryza sativa L.), in correlation with mercury species and exposure level. *Metallomics*, **6:** 1951-1957.

696. Zhi H., Zhang J., Hu G., Lu J., Wang X., Zhou C., Wu M. and Liu Z. **2003**. The deregulation of arachidonic acid metabolism-related genes in human esophageal squamous cell carcinoma. *Cancer Cell Biology*, **106:** 327-333.
697. Zhu X., Jiang M., Song E., Jiang X. and Song Y. **2015**. Selenium deficiency sensitizes the skin for UVB-induced oxidative damage and inflammation which involved the activation of p38 MAPK signaling. *Food and Chemical Toxicology*, **75:** 139-145.
698. Zoller W.H., Gladney E.S. and Duce R.A. **1974**. Atmospheric concentrations and sources of trace metals apt the South Pole. *Science*, **183:** 198-200.

Publications

- **Dhanjal N.I.**, Sharma S. and Tejo Prakash, N., **2016**. Quantification and in vitro bioaccessibility of selenium from osborne fractions of selenium-rich cereal grains. *Cereal Chemistry*, **93:** 339-343.

- **Dhanjal N.I.**, Sharma S., Prabhu K.S. and Tejo Prakash, N., **2017**. Selenium supplementation through Se-rich dietary matrices can upregulate the anti-inflammatory responses in lipopolysaccharide-stimulated murine macrophages. *Food and Agricultural Immunology*, **28:** 1374-1392.



Food and Agricultural Immunology

ISSN: 0954-0105 (Print) 1465-3443 (Online) Journal homepage: http://www.tandfonline.com/loi/cfai20

Selenium supplementation through Se-rich dietary matrices can upregulate the anti-inflammatory responses in lipopolysaccharide-stimulated murine macrophages

Noorpreet Inder kaur Dhanjal, Siddharth Sharma, K. Sandeep Prabhu & N. Tejo Prakash

To cite this article: Noorpreet Inder kaur Dhanjal, Siddharth Sharma, K. Sandeep Prabhu & N. Tejo Prakash (2017) Selenium supplementation through Se-rich dietary matrices can upregulate the anti-inflammatory responses in lipopolysaccharide-stimulated murine macrophages, Food and Agricultural Immunology, 28:6, 1374-1392, DOI: 10.1080/09540105.2017.1343805

To link to this article: https://doi.org/10.1080/09540105.2017.1343805

Published online: 19 Jul 2017.

FOOD AND AGRICULTURAL IMMUNOLOGY, 2017
VOL. 28, NO. 6, 1374–1392
https://doi.org/10.1080/09540105.2017.1343805

Taylor & Francis
Taylor & Francis Group

Selenium supplementation through Se-rich dietary matrices can upregulate the anti-inflammatory responses in lipopolysaccharide-stimulated murine macrophages

Noorpreet Inder kaur Dhanjal[a], Siddharth Sharma[a], K. Sandeep Prabhu[b] and N. Tejo Prakash [c]

[a]Department of Biotechnology, Thapar University, Patiala, India; [b]Department of Veterinary Science and The Center for Molecular Toxicology and Carcinogenesis, The Pennsylvania State University, University Park, PA, USA; [c]School of Energy and Environment, Thapar University, Patiala, India

ABSTRACT

The accessibility of selenium from naturally enriched sources such as cereals crops can potentially be used as selenium supplements to support nutritional requirements. Dietary selenium supplementation, as Se-rich wheat extracts, on RAW264.7 macrophage cells enhanced the antioxidant capacity via augmentation of cellular selenoprotein glutathione peroxidase 1 (GPx-1) expression in the absence or presence of lipopolysaccharide (LPS) treatment. Cells were supplemented with Se in the form of sodium selenite (SS), seleniferous wheat extract (SeW) and seleniferous wheat extract with rMETase treatment (SeW + rMET) at three different concentrations. Cells supplemented with SS and SeW + rMET showed increase in GPx-1 expression as compared to SeW-treated cells. SeW + rMET, further, downregulated the LPS-induced expression of cyclooxygenase-2, microsomal PGE synthase-1 and inducible nitric oxide synthase w.r.t. Se-deficient cells, while the expression of haematopoietic PGD synthase was upregulated. This demonstrates SeSup effectively modulates the expression of inflammatory responses, indicating the potential benefits of dietary selenium supplementation.

ARTICLE HISTORY
Received 5 May 2017
Accepted 14 June 2017

KEYWORDS
Seleniferous wheat; glutathione peroxidase-1; cyclooxygenase-2; prostaglandin metabolism; reactive oxygen species; rMETase

Introduction

Macrophages are the prime responders to diverse stimuli for cell-to-cell communication. Activation of these innate immune cells is usually accompanied by oxidative burst, as seen by increased reactive oxygen and nitrogen species (RONS), which affects the pathogen. In addition, macrophage activation is also associated with the release of various mediators, including arachidonic acid (AA)-derived prostaglandins such as PGE_2, TXA_2 and PGD_2, and its metabolite 15d-PGJ_2 (Ricciotti & FitzGerald, 2011). However, any disruption in RONS encountering enzymes will lead to massive oxidation by cell populations in inadequate mitigation of pathogens. This represents a potential toxic insult, resulting in DNA damage and inactivation of enzymes, leading to recurrent infections and persistent

CONTACT N. Tejo Prakash ntejoprakash@thapar.edu School of Energy and Environment, Thapar University, Patiala147004, India

inflammation. The basic reactions involved in oxidative burst are through chemically reactive secondary messenger molecules such as $NO^{\bullet}$, $O_2^{\bullet}$ and H_2O_2. These superoxide radicals may be rapidly reduced to H_2O_2 by SOD (Conrado, D'Angelantonio, Torreggiani, Pecci, & Fontana, 2014), and further to H_2O by antioxidant/anti-inflammatory enzymes like selenoproteins such as glutathione peroxidase and selenium-independent enzymes such as H-PGDS, which belongs to the sigma-class of glutathione S-transferase with non-selenium-dependent peroxidase activity (Higgins & Hayes, 2011).

The biological effects of dietary Se is through its incorporation into selenoproteins that influence various cells and their functions such as activation, migration, proliferation capacity and signalling strength like cytokine production and phagocytosis (Huang, Rose, & Hoffmann, 2012). The ex-vivo supplementation of Se boosts up the innate/adaptive immune responses, which further directly/indirectly regulate the cellular redox tone by inhibiting the expression of pro-inflammatory genes and, hence, reducing the inflammatory response (Rayman, 2012).

Dietary Se supplementation is proved to elevate the transcription of anti-inflammatory enzymes (GPx and H-PGDS) and downregulate the transcription of pro-inflammatory inducible enzymes (COX-2 and iNOS), pro-inflammatory cytokines (IL-1, IL-2, IL-6 and TNF-α) and pro-inflammatory chemokines (IL-8, MIP-1α, MCP1, RANTES and eotaxin) (Gandhi, Nagaraja, & Prabhu, 2013). Se can potentially help in switching the AA pathway from pro-inflammatory expression to anti-inflammatory responses and reduce the risk of cancer (Lu et al., 2016), arthritis (Onal, Naziroglu, Colak, Bulut, & Flores-Arce, 2011) and other cardiovascular diseases (Bukkens et al., 1990). In the recent past, our group has reported the significant hyperaccumulation of Se in staple crops ranging from 20 to 123 μg/g in wheat, maize and rice matrices (Dhanjal, Sharma, & Nagaraja, 2016). In Se-rich wheat (Cubadda et al., 2010) and rice (Premarathna et al., 2012), 90% of Se content is in the form of seleniomethionine (SeMet). The *in vitro* gastrointestinal digestion studies show a better accessibility of Se in terms of SeMet during intestinal digestion (Dhanjal et al., 2016; Cubadda et al., 2010). These Se-rich matrices provide an efficient reservoir for long-term shortage (Behne, Alber, & Kyriakopoulos, 2009), facilitating the availability of Se through catabolism of SeMet during the natural protein turn-over (Zeng, Botnen, & Jhonson, 2008). However, the ability of these Se-rich foods to mitigate expression of inflammatory genes while upregulating those proteins that are key in resolution of inflammation has not been demonstrated. In this study, we demonstrate the influence of bioavailable dietary Se in resolving the inflammatory responses through upregulation of expression of anti-inflammatory enzymes such as GPx-1 and H-PGDS, while downregulating pro-inflammatory enzymes (COX-2, mPGES-1 and iNOS) in SeDef and SeSup RAW264.7 macrophage cells.

Experimental

Extraction and quantification of selenium in wheat extract

The extraction of Se from wheat matrices (SeW and NSeW) was performed in duplicate with 1X PBS buffer. The samples were vortexed for 10 min and centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was centrifuged at 16,000 rpm for 1 h at 4°C. The filtrate was collected under sterile conditions and passed twice through 0.22 μm sterile filters.

The extracts were kept at −80°C until quantification was done. Se in wheat extract was oxidized in acid using microwave digestion (MARS 6 240/50:910905, CEM-NC) with nitric acid and perchloric acid (SD FineChem, India) followed by reduction with HCl (SD FineChem, India). The reduced Se (Se^{+4}) was then allowed to complex with 2,3-diamino-naphtalene (Himedia, India) to obtain yellow coloured piazselenol complex in cyclohexane. The colour intensity was measured with a fluorescence spectrometer (Perkin Elmer LS-45) at emission spectrum of 520 nm corresponding to the amount of selenite present in the wheat extracts (SeW/NSeW). The emission spectrum of NIST-certified Selenium ICP standard solution (SRM-1349) was used for Se quantification (Dhanjal et al., 2016).

Cell culture and stimulation

RAW264.7 murine macrophage cell line was cultured in DMEM (Lonza) containing 10% defined FCS (Himedia), 4.5 g/L glucose, 2 mM L-Glutamine, 1 % v/v penstrep containing 100 U/mL of penicillin and 100 µg/mL streptomycin (Sigma), 0.5% amphotericin B solution (Sigma) at 37°C with 5% CO_2 devoid of any Se source (SeDef). SeDef cells (1×10^5) were then cultured in three different Se-supplemented media (SeSup) for three days along with respective controls (Table 1). After 72 h, cells were stimulated with lipopolysaccharide (LPS) (Sigma) at 1 µg/mL. Cells were collected at different time intervals, washed twice with PBS (Lonza), scraped and centrifuged at 2000 rpm. Both SeDef and SeSup cells had similar growth rate and cell viability pattern.

Preparation of cell lysates

The pellets were re-suspended in 100 µL of CelLytic™ M (Sigma) protein extraction reagent containing Pierce™ protease inhibitor (Thermo Scientific Life Sciences) and kept for 30 min at 4°C with intermittent vortexing. The cell lysate was centrifuged at 12,000 rpm for 30 min at 4°C and protein containing supernatant was collected. For mPGES-1 analysis, the pellet containing membranous proteins was re-suspended in extraction buffer and centrifuged at 16,000 rpm for 1 h at 4°C. Supernatant was collected and the protein concentration was estimated using the Bradford method (Alabi, 1979).

Western blot analysis

Along with prestained protein ladder (PUREgene Genetix Biotech), 30 µg of protein was separated on 15% SDS-polyacrylamide gel (Sigma) and transblotted onto the PVDF membrane (0.2 µm, Biorad). The membrane was blocked with TBST containing 10 mM Tris,

Table 1. Different sources of Se supplementation with increasing three different Se concentrations (50, 100 and 500 nM) w.r.t. their controls. Control (C) represents cells devoid of any source of Se supplementation. In case of (NSeW)e and (NSeW)e + rMET, "e" represents the volume of non-seleniferous wheat extract taken equivalent to seleniferous wheat extract with 500 nM Se concentration.

Se source in Se-supplemented cells (SeSup)	Corresponding controls in Se-deficient cells (SeDef)
Sodium selenite (SS)	Control (C)
Seleniferous wheat extract (SeW)	Non-seleniferous wheat extract (NSeW)e
Seleniferous wheat extract + rMETase (SeW + rMET)	Non-seleniferous wheat extract + rMETase ((NSeW)e + rMET)

pH 8.0 (Himedia), 150 mM NaCl (Sigma), 0.05% Tween-20 (Sigma) and 5% skim milk (Sigma) for 1 h at room temperature. The membrane was probed with primary antibody followed by an appropriate secondary antibody coupled to HRP. Anti-GPx-1 was procured from Abcam (Cat # ab108427); mouse anti-GAPDH (Cat # 437000) and HRP goat anti-mouse IgG (H + L) (Cat # 626520) were procured from Life Technologies; while goat anti-rabbit IgG HRP (Cat # 10004301), COX-2 (mouse) polyclonal antibody (Cat # 160126), H-PGDS polyclonal antibody (Cat # 160013) and mPGES-1 polyclonal antibody (Cat # 160140) were procured from Cayman Chemical Company. Anti-GAPDH was re-probed with blots for normalized protein loading. The immunoreactive bands were analysed densitometrically using the ImageJ software program (Schneider, Rasband, & Eliceiri, 2012), developed at the National Institute of Health (NIH).

Semi-quantitative reverse transcriptase PCR

Total RNA was extracted from SeDef and SeSup cells by TRIzol reagent (Life Technologies) according to the manufacturer's instructions and quantified on the basis of UV absorbance values at 260 and 280 nm. To reduce any DNA contamination, RNA samples were treated with RNase-free DNase (Promega) for 15 min at 20–25°C. One microgram of total RNA was reverse-transcribed using Superscript® III First strand synthesis (Invitrogen). Equal amounts of DNA (100 ng) from each sample were polymerized using Applied Biosystems Veriti thermal cycler. Primers for GPx-1, COX-2, mPGES-1, H-PGDS, iNOS, GAPDH and β-Actin, as listed in Table 2, were used for PCR amplification and the product was analysed on 2% agarose gel (3B Black Bio Biotech India Ltd.). DNA bands were quantified on a GEL DOC XR system (Biorad) using Quantity One 1-D Analysis (Biorad) software program and the intensity of bands were analysed densitometrically using ImageJ software program (Schneider et al., 2012), developed by the National Institutes of Health.

Nitrite assay

Nitric oxide (NO) release into cell-free medium was measured extracellularly from the culture media (as nitrite) taken from cells cultivated and stimulated with LPS under identical conditions. For standard curve, $NaNO_2$ was used from scale 0 to 100 μM and incubated with Griess reagent (Sigma) for 15–20 min under dark conditions. Extracellular nitrite excretion was measured in a plate reader at 548 nm.

Table 2. Primers used for pro-/anti-inflammatory genes in PCR amplification. One microgram of total RNA was reverse transcribed from each sample and amplified by PCR polymerization. Gene expression in SeSup and SeDef samples were then quantified densitometrically using ImageJ software program and normalized to the expression of GAPDH.

Primer	Forward (5′ to 3′)	Reversal (5′ to 3′)
mouse Gpx1	ACAGTCCACCGTGTATGCCTTC	CTCTTCATTCTTGCCATTCTCCTG
COX-2	CCCCCACAGTCAAAGACACT	CCCCAAAGATAGCATCTGGA
H-PGDS	CCTGGGCAGACTTCTACTGG	AAACTGCAACACCCCTTGAG
mPGES-1	CACCTTGTAGGGTGCTGGTT	CAGCCTAATGTTCAGCGACA
iNOS	AATGGCAACATCAGGTCGGCCATCACT	GCTGTGTGTCACAGAAGTCTC
GAPDH	TGTTCCTACCCCCAATGTGT	CCCTGTTGCTGTAGCCGTAT
Mouse β-*actin*	TGGAATCCTGTGGCATCCATGAAAC	TAAAACGCAGCTCAGTAACAGTCCG

Results and discussion

Quantification of selenium wheat extract

The Se concentration of seleniferous wheat grain extract measured by fluorescence spectrometer was 7.1 ± 0.2 µg/ml. Earlier reports from our group showed the hyperaccumulation of Se in different cereal grains and their protein fractions from seleniferous belt of Punjab, India. The whole wheat grains accumulate up to 122.9 ± 0.6 µg/g Se than maize (26.5 ± 0.2 µg/g) and rice (19.7 ± 0.2 µg/g). In the *in vitro* gastrointestinal digestion studies, the intestinal accessibility of Se describes its potential bioavailability through dietary supplementation to the SeDef population (Dhanjal et al., 2016).

Dietary selenium transforms the expression profile of pro-inflammatory genes (COX-2, mPGES-1) into the anti-inflammatory genes (GPx-1, H-PGDS)

The expression profile of Se-dependent pro-/anti-inflammatory genes were measured in RAW264.7 cells. Cells were grown in various conditions as SeDef (Control, NSeW and NSeW + rMET) and Se-supplemented media (SS, SeW and SeW + rMET) with different Se concentrations (50, 100 and 500 nM). As GPx is a well-known marker for the Se status, the anti-inflammatory enzyme expression of Se-GPx-1 increased with increasing sodium selenite concentration (500 nM > 100 nM > 50 nM > 0 nM, 0 h) of SeSup cells with 3.5-fold higher expression as compared to SeDef cells (Figure 1(A)). With oxidative stress upon LPS stimulation, control RAW264.7 cells that were devoid of Se also showed mild GPx-1 expression after 4 h (Figure 1(A)) which clearly indicates the self-defense mechanism against the ROS generation. However, upon LPS stimulation up to 4 h, Se supplementation increased GPx-1 levels to a significant extent with increasing concentration (500 nM > 100 nM > 50 nM) w.r.t. control, i.e. from 2.4-fold at 50 nM to 9.0-fold at 500 nM. Increased expression of selenoproteins, including GPx1, is known to mitigate inflammation and bolster immunity that involves downregulation of COX-2 and mPGES-1, while upregulating H-PGDS to effect a process called "eicosanoid class switching mechanism" in Se-stimulated RAW264.7 cells (Yui, Imataka, Nakamura, Ohara, & Naito, 2015; Duntas, 2009; Vunta et al., 2007). Higher Se supplementation up to 2 µM in the form of sodium selenite is also reported to inhibit NFκB-dependent transcription (Nettore et al., 2017; Meplan et al., 2016; Youn et al., 2008; Maehira, Mivagi, & Eguchi, 2003). Under Se-deficient conditions, there is increased ROS generation that is closely associated with increased inflammatory genes, which results in augmentation of oxidative stress in cells. Thus, imbalances in oxidative stress exacerbate the modulation of repressor activator protein 1 (RAP1), which induces pro-inflammatory cytokines via activation of transcription factor NFκB (Cai et al., 2015). Prolonged NFκB activation results in neuro-inflammation followed by neuro-toxicity, as it is ubiquitously expressed in neurons (Shih, Wang, & Yang, 2015).

rMETase increases the bioavailability of selenium from cereal matrices

rMETase is the recombinant L-methionine γ-lyase or methioninase, a pyridoxal 5'-phosphate containing enzyme. It catalyses the α- and γ-elimination of methionine, sulphur-containing amino acids. Likewise, it is able to cleave C-Se and C-O bonds of

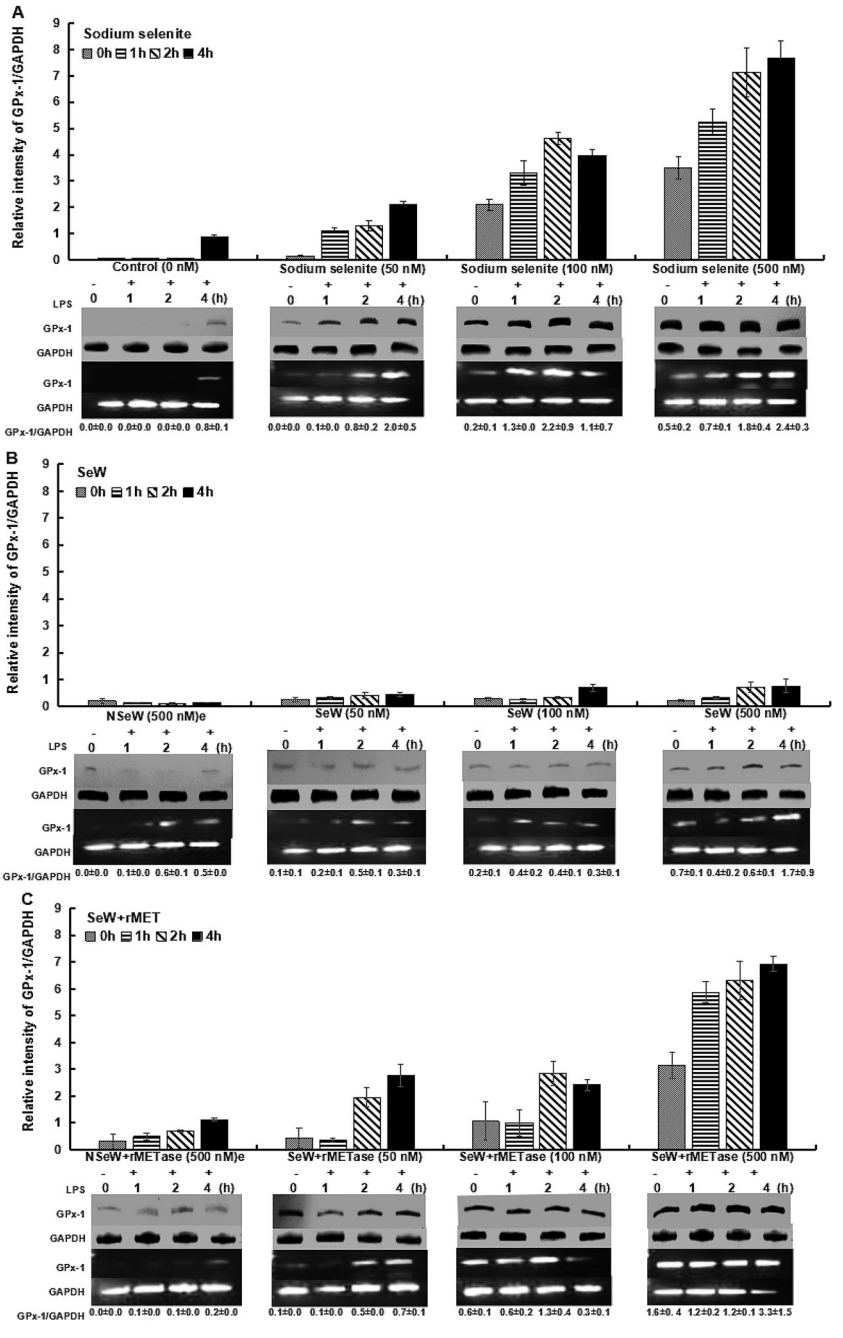


Figure 1. GPx-1 expression in RAW264.7 macrophages. Cells were treated with 50, 100 and 500 nM Se concentrations in different forms: (A) Sodium selenite (SS), (B) Seleniferous wheat extract (SeW) and (C) Seleniferous wheat extract in the presence of rMETase incubation (SeW + rMET) with respect to their controls, for 72 h and then inflamed with 1 μg/mL LPS for framed time interval up to 4 h. Densitometric values normalized to GAPDH are graphed with mean ± S.D. for protein expression and the values indicated below each panel of mRNA expression.

selenium- and oxygen-containing amino acids (Fukumoto et al., 2012; Nakayama et al., 1984) and making them optically more active and bioavailable in functioning (Tanaka, Esaki, & Soda, 1985). Our results also showed the significant difference with rMETase application in comparison to controls demonstrating the facilitation of free Se from wheat matrices, and impacting the elevated trend of anti-inflammatory enzymes vis-à-vis downregulation of pro-inflammatory enzymes.

A marginally elevated trend of overall GPx-1 expression was observed when cells were incubated with ex-vivo dietary Se-supplementation in the form of seleniferous wheat extract (SeW) when compared to non-seleniferous wheat extract (NSeW). Even at highest concentration of SeW extract (500 nM), RAW264.7 cells showed 1.1-fold increase in GPx-1 expression at 4 h of LPS stimulation w.r.t. control (C) (Figure 1 (B)). The SS and SeW + rMET-incubated cells showed 9.0- and 8.0-fold higher GPx-1 expression, respectively, than SeW-supplemented cells at a similar concentration and time. This clearly demonstrates that Se bound to the wheat matrix was not completely bio-available and accessible to the cells. Hence, to facilitate the accessibility of Se and assess its efficacy, cells were incubated with rMETase (Gandhi et al., 2011) at 0.2 units/mL media along with SeW extract. The observations clearly indicated enhanced GPx-1 expression trending similar to SS-stimulated cells. In LPS-unstimulated (0 h) SeW + rMET-supplemented cells, the GPx-1 expression increased with increase in SeW + rMET extract concentration (500 nM > 100 nM > 50 nM) up to 3.2-fold in comparison to NSeW + rMET-incubated cells (Figure 1(C)). Under similar conditions, the supplementation of SeW extract showed poor GPx-1 response of up to 1.1-fold (Figure 1(B)). At 4 h of LPS stimulation, SeW + rMET supplementation (50 to 500 nM) w.r.t. NSeW + rMET enhanced the GPx-1 level from 2.5-fold to 6.2-fold, respectively (Figure 1(C)).

Collectively, our data confirm the role of rMETase in facilitating the release of bio-available Se from Se-rich wheat grain (SeW + rMET) and its antioxidant expression in comparison to the SeW supplementation. Previous reports from our group showed that selenomethionine (SeMet) accounts for 72–85% of selenocompounds present in Se-rich wheat grains (Cubadda et al., 2010) and is the major form consumed by humans (Finley, 2006). It can be, thus, hypothesized that Se-rich natural dietary matrices facilitate sustained and slow release of Se through enzymatic cleavage of SeMet to be metabolized to bioactive forms that increase plasma Se levels with better antioxidant and anti-inflammatory capacity (Cao, Guo, Zhang, Dong, & Gong, 2014). SeMet supplementation in minute concentration of up to 3 μg/g abrogate the allergies caused due to these inflammatory responses under Se deficiency (Sakazaki et al., 2014).

Dietary selenium supplementation downregulates the COX-2 expression in comparison with SeDef macrophages

In contrast to GPx1, Se supplementation downregulated the expression of a well-known pro-inflammatory gene, COX-2. COX-2 is a marker of inflammation and is activated by diverse stimuli, including LPS. COX-2 levels were increased by 4.5-fold during the first 4 h in SeDef cells as compared to the SeSup group (Figure 2(A)). However, SeW with

rMETase treatment (SeW + rMET) showed a nearly similar trend to SS-incubated cells with moderately weaker expression of COX-2 after 4 h of LPS stimulation (Figure 2(C)).

Upon SS supplementation, the COX-2 expression was downregulated within 2–4 h (Figure 2(A)) and reduced by 3.0- to 4.3-fold in 4 h with 50 and 500 nM Se, respectively. Among SeW- and SeW + rMET-treated cells, COX-2 levels decreased from 1.4- to 2.0-fold and 2.2- to 4.0-fold with increasing dietary Se levels (500 nM > 100 nM > 50 nM) as compared to the NSeW- and NSeW + rMET-treated cells, respectively (Figure 2(B,C)). Further at 500 nM, SeW + rMET, COX-2 expression significantly decreased by 4 h as compared to SeW-treated cells (Figure 2(C)).

These results suggested that SeW extract with bio-available Se modulates the COX-2 expression leading to the downregulation of its downstream product mPGES-1 (Figure 3 (C)) and thus helps in the activation of other anti-inflammatory prostaglandin enzymes such as H-PGDS, which produces the precursor of anti-inflammatory cyclopentenone prostaglandins, D^{12}-PGJ_2 and 15d-PGJ_2. With a delayed time lag of expression, levels of COX-2 and mPGES-1 correlate with each other and NF-κB transcription (Kirkbya et al., 2016) with an increase at the protein level under Se deficiency upon LPS stimulation (Davis et al., 2002). Such a functional coupling of COX-2 and mPGES-1 versus COX-1 and H-PGDS is known to drive the PG metabolism towards either pro-inflammatory or anti-inflammatory pathways under differential Se status (Gandhi et al., 2011; Vunta et al., 2007). The overexpression of COX-2 and mPGES-1 due to Se deficiency is thought to be one of the key underlying basis of inflammation that is seen in breast cancer (Jana et al., 2014), AIDS, (Ipp, Zemlin, Erasmus, & Glashoff, 2014) and asthma (Daham et al., 2014).

mPGES-1 expression is downregulated by dietary supplementation in LPS-stimulated RAW264.7 cells

To demonstrate the effect of Se on mPGES-1 expression as a downstream marker of COX-2 activity, cells were grown in SeDef and SeSup RAW264.7 cells with/without 1 μg/mL LPS stimulation from 0 to 12 h. Based on the literature (Davis et al., 2002; Vunta et al., 2007; Gandhi et al., 2011), a time course experiment was set up to check the expression of COX-2 and mPGES-1. In SeDef LPS-stimulated cells (control 0 nM), the mPGES-1 level was increased by 3.8-fold from 0 to 12 h as compared to the SeSup cells (Figure 3(A)). However, an increasing trend in mPGES-1 expression over time was seen in all SeSup cells. In contrast to control (C, 12 h) SeW + rMET incubation of RAW264.7 cells clearly indicated a significant decreased mPGES-1 expression up to 12-fold at higher concentration (500 nM) as compared to the SeW cells (3.3-fold) (Figure 3(B, C)).

Interestingly, in LPS-stimulated NSeW + rMET-supplemented cells, rMETase co-treatment decreased mPGES-1 expression up to 5.9-fold over 12 h (Figure 3(C)). This result highlights the role of rMETase in promoting the bioavailability of Se to plausibly skew the AA pathway. During resolution of inflammation, Se supplementation shifts AA metabolism from PGE_2 towards PGD_2 and its downregulated product 15d-PGJ_2 through inactivation of NF-κB (Gandhi et al., 2011; Kaushal et al., 2014; Nelson, Lei, & Prabhu, 2011; Vunta et al., 2007; Davis et al., 2002). By virtue of being a Michael electrophile, 15d-PGJ_2 covalently modifies key cysteine thiols in I Kappa B Kinase (IKK)-β (IKK2), which regulates the activation of NF-κB in LPS-treated macrophages (Cai et al., 2015; Gandhi et al., 2011).

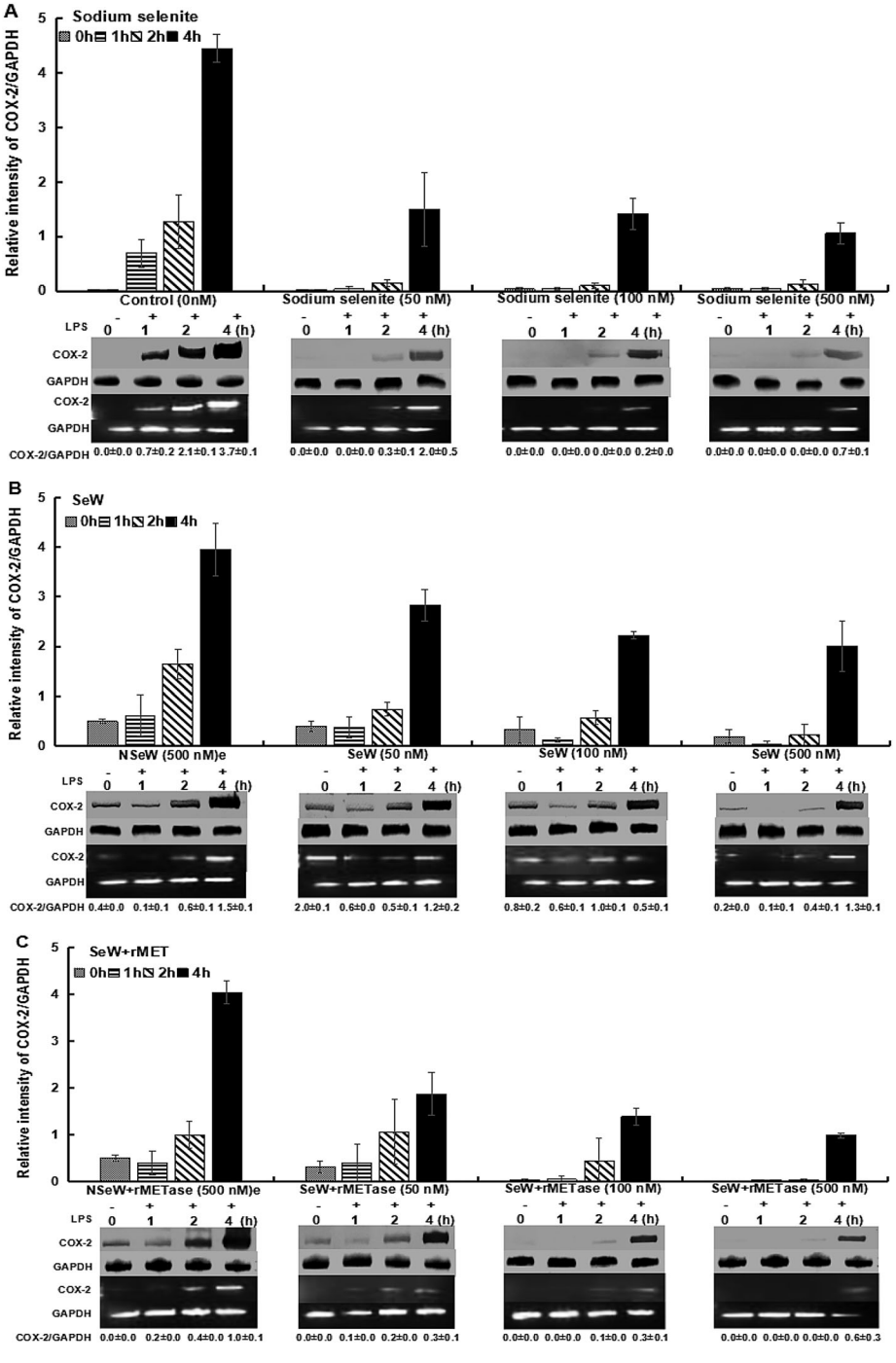


Figure 2. LPS-induced expression of COX-2 in RAW264.7 macrophages with same experimental conditions as for GPx-1. The graph shows the inverse relationship with GPx-1 upregulation with increased selenium concentration up to 4 h of LPS incubation. $n = 3$ is shown.

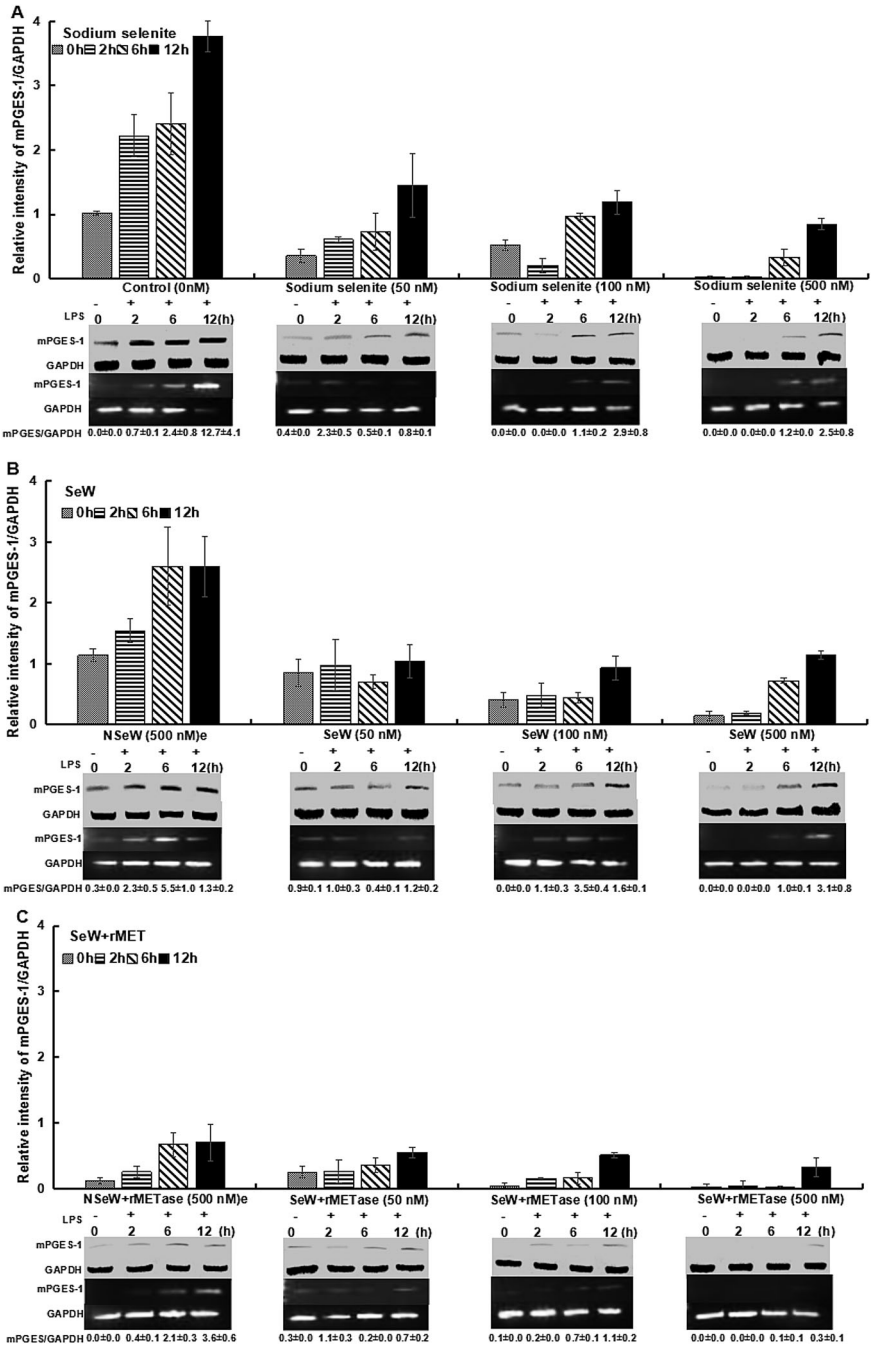


Figure 3. mPGES-1 expression; selenium supplementation modulates the mPGES-1 levels when inflamed with 1 μg/mL LPS up to 12 h. Seleniferous wheat extract in the presence of rMETase incubation (SeW + rMET) shows maximum downregulation in comparison to sodium selenite and seleniferous wheat extract incubation. A noticeable observation of non-seleniferous wheat extract treated with rMETase (NSeW + rMET) showed significant decrease in mPGES-1 protein and mRNA levels (C). Results shown are representative of three independent experiments.

Shunting of the AA pathway from mPGES-1 to H-PGDS by SeSup

Culturing of RAW.264.7 cells in LPS-stimulated SeDef and SeSup cells showed significant increase in H-PGDS expression. A 7.0-fold difference in H-PGDS in SeDef LPS-stimulated cells (control, C) was seen from 0 to 12 h (Figure 4(A)). However, Se supplementation significantly increased the upregulation of H-PGDS with increasing concentration (500 nM > 100 nM > 50 nM) either in the form of SeW + rMET or SS as compared to the control (Figure 4(A, C)).

In addition, SS-supplemented cells without LPS stimulation (0 h) showed increasing levels of H-PGDS (up to 6.4-fold) with increase in Se concentration from 50 to 500 nM. A similar increasing trend was observed in SeW + rMET-treated cells, at a higher concentration (500 nM) with prominent hike in expression levels (4.4-fold) when compared to the SS (2.0-fold) and SeW (1.8-fold)-treated cells w.r.t. their controls, respectively (Figure 4). Similar results were observed in earlier studies with sodium selenite stimulation of macrophages wherein enhanced levels of hematopoietic PGDS (H-PGDS) enzyme catalyse PGH_2 to PGD_2 and 15d-PGJ_2 conversion for the downregulation of inflammation processes. 15d-PGJ_2 acts as an endogenous ligand for peroxisome proliferator-activated receptor (PPAR)γ (Gandhi et al., 2011), a transcription factor that inhibits the expression of NF-kB-regulated genes, while activating the expression of various anti-inflammatory genes (Tyagi, Gupta, Saini, Kaushal, & Sharma, 2011).

iNOS expression

In addition to COX-2 and mPGES-1, expression of iNOS, a well-known NF-kB downstream target gene, was examined at the level of mRNA and product (as nitrite) cell-free supernatant. Upon LPS stimulation, iNOS expression was increased in all treatments. However, in SeDef (i.e. C, NSeW- and NSeW + rMET-treated cells), the mRNA expression was significantly higher than in SeSup cells (Figure 5).

With increasing concentration of Se from 50 to 500 nM, iNOS expression was effectively inhibited within 4 h in all SeSup cells. Although among SS-, SeW- and SeW + rMET-stimulated cells, the SeW + rMET supplementation showed comparatively higher inhibition of iNOS expression (10-fold) as compared to the SS (3-fold) and SeW (3.5-fold)-stimulated cells w.r.t. control (C, 24 h). In SeDef cells, devoid of LPS stimulation, marginal expression of iNOS could be seen in NSeW (0.8-fold) and NSeW + rMET (0.2-fold) as compared to the control (C). It is known that LPS and IFNγ induction upregulates the iNOS expression to augment inflammatory responses in human U937 macrophages (Grkovich, Johnson, Buczynski, & Dennis, 2006). Our results corroborate with a previous report on the ability of Se supplementation to reduce iNOS expression by 3-fold as compared to SeDef LPS-unstimulated RAW 264.7 macrophages (Prabhu et al., 2002). Similar results were seen with the role of *Aster Yomena* (Kim et al., 2017), *Eupartoium makinoi* (Ahn et al., 2015), *Siegesbeckia glabrescens* (Lee, Kang, Hwang, & Kim, 2011) and lansai Cand D (Taechowisan, Wanbanjob, Tuntiwachwuttikul, & Liuin, 2010) in downregulation of iNOS transcription along with NO quenching which further decreases in COX-2 and PGE_2 mRNA levels in LPS-induced macrophages.

The enzyme expression through protein levels of GPx-1 , COX-2 , mPGES-1 and H-PGDS showed consistent results in gene expression via mRNA levels. This demonstrates

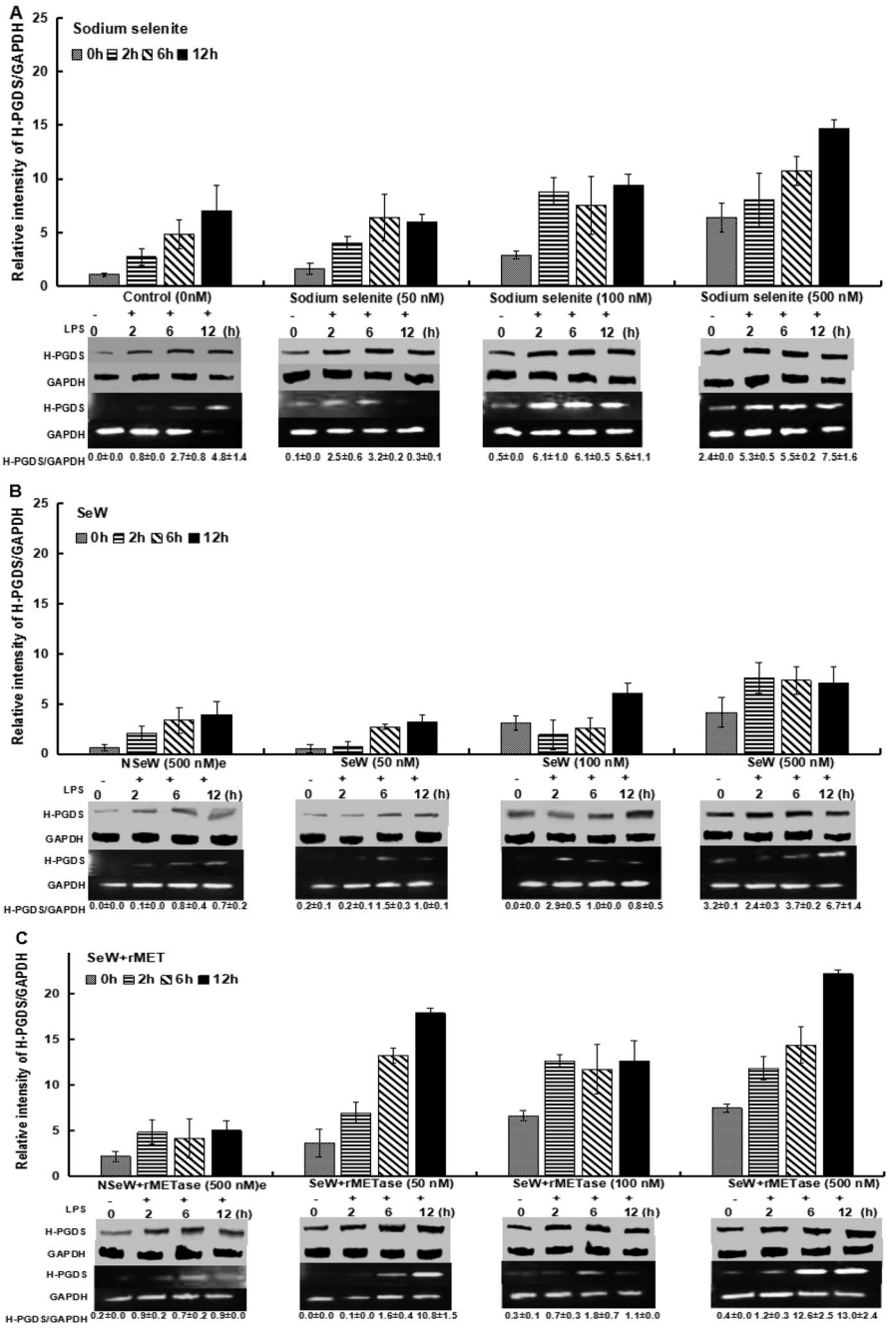


Figure 4. H-PGDS expression; the increase in selenium level in any form (SS, SeW and SeW + rMET) increases the overall H-PGDS expression. A time course study was set up for up to 12 h of 1 μg/mL LPS stimulation, which shows a significant increase in case of SeW + rMET incubation. $n = 3$ shown with mean ± S.D. values.

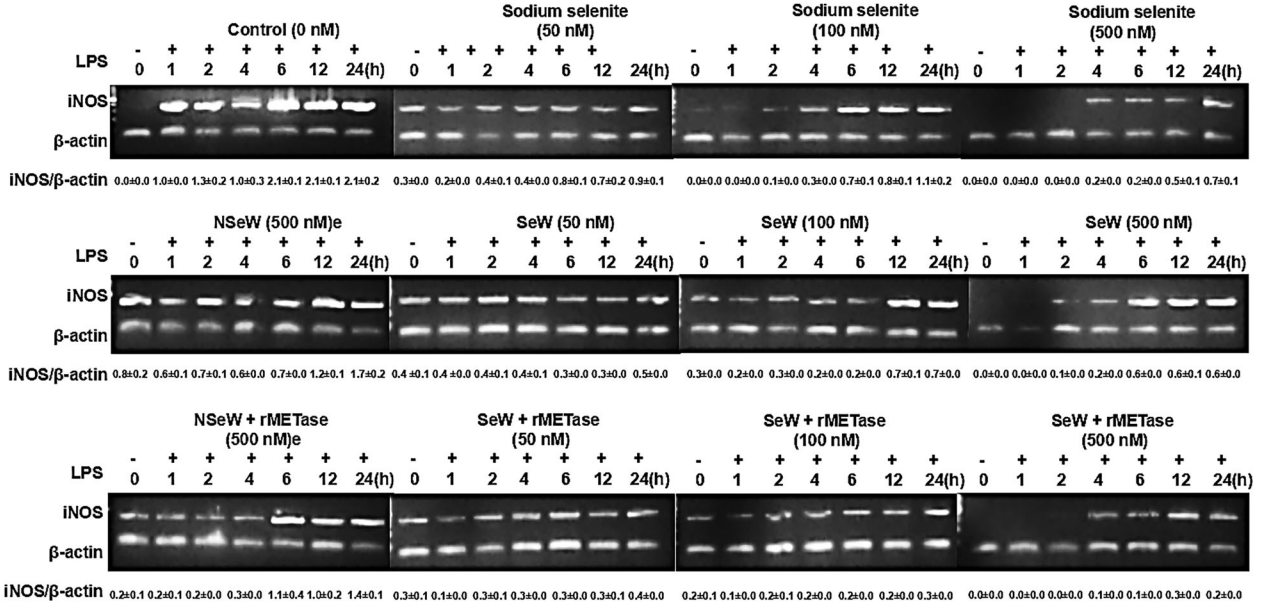


Figure 5. iNOS mRNA levels. Ex-vivo Se supplementation mitigates the iNOS mRNA expression levels by incubating RAW264.7 cells with 1 μg/mL post LPS stimulation for 0–24 h. Densitometry of iNOS and β-actin bands clearly shows that sodium selenite (500 nM) and dietary SeW + rMET (500 nM) reduces the oxidative stress by 3- and 10-fold, respectively. Representative of $n = 3$ shown and quantified by ImageJ software program.

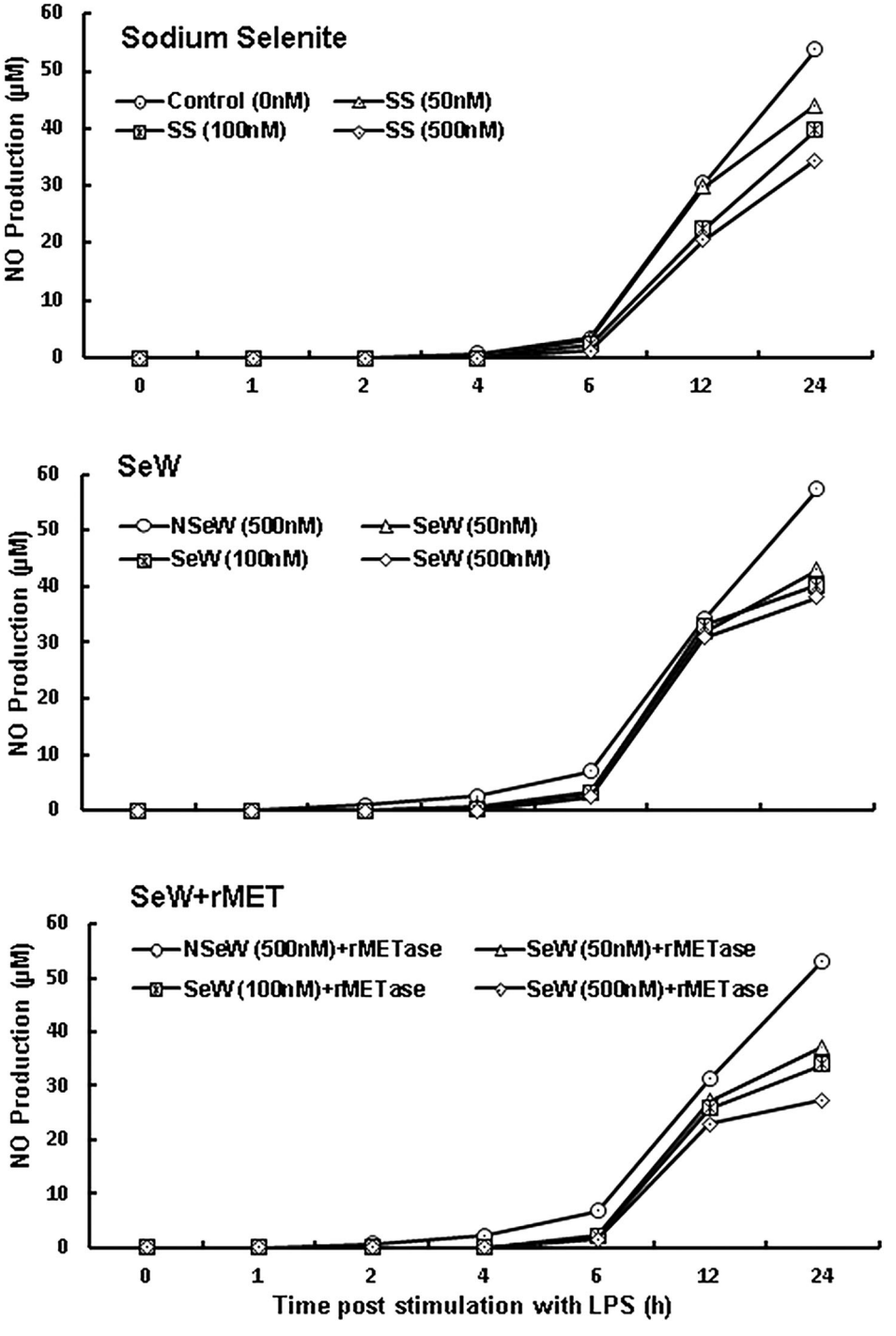


Figure 6. NO production in Se-supplemented and Se-deficient RAW264.7 cells. Nitrite levels were measured in cells with 1μg/mL post LPS stimulation in culture media using Griess reagent. Representative of $n = 3$ shown. Significance at <0.05.

that SeSup regulates the AA metabolism at the transcriptional level. Thus, Se modulates the pro-inflammatory genes transcriptionally to reduced levels of COX-2 , mPGES-1 and iNOS expression. However, the antioxidant capacity of genes like GPx-1 and H-PGDS showed elevated mRNA expression with SeSup as compared to the control.

NO production

NO· production (measured as nitrite) was also analysed by collecting cell-free supernatants over time (Figure 6). The SeDef (C, NSeW and NSeW + rMET) shows significantly higher NO level as compared to the SeSup cells (SS, SeW and SeW + rMET). The observations clearly indicate that upon LPS stimulation, Se deficiency results in exogenous NO production (Hoffmann, 2007), which is part of the inflammatory markers along with other cytokines such as TNF-α, IL-1β and IL-6 in LPS-induced J774.1 mouse macrophages (Safir, Wendel, Saile, & Chabraoui, 2003).

Conclusions

In the present study, we observed the enhanced anti-inflammatory role of bio-available Se facilitated through rMETase-treated seleniferous wheat extracts by inhibiting the expression of COX-2, mPGES-1, iNOS expression and nitrite production. This proves the regulatory effect of bio-available Se in induction of anti-inflammatory GPx-1 and H-PGDS protein levels through downregulation of pro-inflammatory protein levels. Our data highlight the role of rMETase enzyme in dietary Se supplementation in the form of wheat extract as nearly similar to that of sodium selenite supplementation in resolving inflammatory responses in the RAW264.7 macrophage model. To the best of our knowledge, this is the first report that demonstrates the influence of bio-available Se from Se-rich dietary matrices on inflammatory responses. Further studies with *in-vivo* models are to substantiate these observations on bioavailability and bio-activity of Se from Se-rich dietary matrices.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

NTP, SS and NID acknowledge the grant and support provided by the Council of Scientific and Industrial Research (CSIR) sanction no. 38(1341)/12/EMR-II. KSP thanks the Foundation for the National Institutes of Health PHS grant DK 077152 and Office of Dietary Supplements (ODS-NIH) for funding.

Notes on contributors

Noorpreet Inder kaur Dhanjal is a doctoral student at the Department of Biotechnology, Thapar working in the area of selenium biofortification and its bioactivity with specific reference to inflammation.

Dr. Siddharth Sharma, associated with Department of Biotechnology, Thapar University, India, is pursuing research on Pharmacogenetics of individualized cancer chemotherapy.

Dr. K. Sandeep Prabhu is associated with Pennsylvania State University, State College, USA, and has been exploring the role of selenium, selenoproteins, and redox homeostasis, macrophage biology; and pathways of inflammation and resolution.

Dr. N. Tejo Prakash is working in the area of selenium quantification, bioaccessibility and bioactivity, at School of Energy and Environment, Thapar University, Patiala, India.

ORCID

N. Tejo Prakash http://orcid.org/0000-0003-3422-5550

References

Ahn, S-I., Lim, S. J., Gu, G-J., Kim, J-S., Paek, J. H., Kim, S. Youn, H. S. (2015). Eupartoium makinoi suppresses lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase-2 expression. *Food and Agricultural Immunology*, *26*(4), 496–503. doi:10.1080/09540105.2014.968768

Alabi, G. (1979). Bradford's law and its application. *International Library Review*, *11*, 151–158. doi:10.1016/0020-7837(79)90044-X

Behne, D., Alber, D., & Kyriakopoulos, A. (2009). Effects of long-term selenium yeast supplementation on selenium status studied in the rat. *Journal of Trace Elements in Medicine and Biology*, *23*, 258–264. doi:10.1016/j.jtemb.2009.05.001

Bukkens, S. G., de Vos, N., Kok, F. J., Schouten, E. G., de Bruijn, A. M., & Hofman, A. (1990). Selenium status and cardiovascular risk factors in healthy Dutch subjects. *Journal of the American College of Nutrition*, *9*, 128–135. doi:10.1080/07315724.1990.10720361

Cai, Y., Sukhova, G. K., Wong, H. K., Xu, A., Tergaonkar, V., & Vanhoutte, P. M. (2015). Rap1 induces cytokine production in pro-inflammatory macrophages through NFκB signaling and is highly expressed in human atherosclerotic lesions. *Cell Cycle*, *14*, 3580–3592. doi:10.1080/15384101.2015.1100771

Cao, J., Guo, F., Zhang, L., Dong, B., & Gong, L. (2014). Pigs fed camelina meal increase hepatic gene expression of cytochrome 8b1, aldehyde dehydrogenase, and thiosulfate transferase. *Journal of Animal Science and Biotechnology*, *5*, 1–7. doi:10.1186/2049-1891-5-1

Conrado, A. B., D'Angelantonio, M., Torreggiani, A., Pecci, L., & Fontana, M. (2014). Reactivity of hypotaurine and cysteine sulfinic acid toward carbonate radical anion and nitrogen dioxide as explored by the peroxidase activity of Cu, Zn superoxide dismutase and by pulse radiolysis. *Free Radical Research*, *48*, 1300–1310. doi:10.3109/10715762.2014.951839

Cubadda, F., Aureli, F., Ciardullo, S., D'Amato, M., Raggi, A., Acharya, R., … Nagaraja, T. P. (2010). Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. *Journal of Agricultural and Food Chemistry*, *58*, 2295–2301. doi:10.1021/jf903004a

Daham, K., James, A., Balgoma, D., Kupczyk, M., Billing, B., Lindeberg, A., … Dahlen, B. (2014). Effects of selective COX-2 inhibition on allergen-induced bronchoconstriction and airway inflammation in asthma. *Journal of Allergy and Clinical Immunology*, *134*, 306–313. doi:10.1016/j.jaci.2013.12.002

Davis, F. Z., Ying, L. U., Thompson, J. T., Prabhu, K. S., Reddy, P. V., Sordillo, L. M., & Reddy, C. C. (2002). Nuclear factor-κB mediates over-expression of cyclooxygenase-2 during activation of RAW 264.7 macrophages in selenium deficiency. *Free Radical Biology and Medicine*, *32*, 890–897. doi:10.1016/S0891-5849(02)00775-X

Dhanjal, N. I., Sharma, S., & Nagaraja, T. P. (2016). Quantification and in vitro bioaccessibility of selenium from Osborne fractions of selenium rich cereal grains. *Cereal Chemistry Journal*, *93*, 339–343. doi:10.1094/CCHEM-10-15-0199-R

Duntas, L. H. (2009). Selenium and inflammation: Underlying anti-inflammatory mechanisms. *Hormone and Metabolic Research*, *41*, 443–447. doi:10.1055/s-0029-1220724

Finley, J. W. (2006). Bioavailability of selenium from foods. *Nutrition Reviews*, *64*, 146–151. doi:10.1111/j.1753-4887.2006.tb00198.x

Fukumoto, M., Kudou, D., Murano, S., Shiba, T., Sato, D., Tamura, T., … Inagaki, K. (2012). The role of amino acid residues in the active site of L-methionine γ-lyase from pseudomonas putida. *Bioscience, Biotechnology, and Biochemistry*, *76*, 1275–1284. doi:10.1271/bbb.110906

Gandhi, U. H., Kaushal, N., Ravindra, K. C., Hegde, S., Nelson, S. M., Narayan, V., … Prabhu, K. S. (2011). Selenoprotein-dependent up-regulation of hematopoietic prostaglandin D2 synthase in macrophages Is mediated through the activation of peroxisome proliferator-activated receptor (PPAR) . *Journal of Biological Chemistry*, *286*, 27471–27482. doi:10.1074/jbc.M111.260547

Gandhi, U. H., Nagaraja, T. P., & Prabhu, K. S. (2013). Selenoproteins and their role in oxidative stress and inflammation. *Current Chemical Biology*, *7*, 65–73. doi:10.2174/2212796811307010007

Grkovich, A., Johnson, C. A., Buczynski, M. W., & Dennis, E. A. (2006). Lipopolysaccharide-induced cyclooxygenase-2 expression in human U937 macrophages is phosphatidic acid phosphohydrolase-1-dependent. *Journal of Biological Chemistry*, *281*, 32978–32987. doi:10.1074/jbc.M605935200

Higgins, L. G., & Hayes, J. D. (2011). Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents. *Drug Metabolism Reviews*, *43*, 92–137. doi:10.3109/03602532.2011.567391

Hoffmann, P. R. (2007). Mechanisms by which selenium influences immune responses. *Archivum Immunologiae et Therapiae Experimentalis*, *55*, 289–297. doi:10.1007/s00005-007-0036-4

Huang, Z., Rose, A. H., & Hoffmann, P. R. (2012). The role of selenium in inflammation and immunity: From molecular mechanisms to therapeutic opportunities. *Antioxidants and Redox Signaling*, *16*, 705–743. doi:10.1089/ars.2011.4145

Ipp, H., Zemlin, A. E., Erasmus, R. T., & Glashoff, R. H. (2014). Role of inflammation in HIV-1 disease progression and prognosis. *Critical Reviews in Clinical Laboratory Sciences*, *51*, 98–111. doi:10.3109/10408363.2013.865702

Jana, D., Sarkar, D. K., Ganguly, S., Saha, S., Sa, G., Manna, A. K., … Mandal, S. (2014). Role of cyclooxygenase 2 (COX-2) in prognosis of breast cancer. *Indian Journal of Surgical Oncology*, *5*, 59–65. doi:10.1007/s13193-014-0290-y

Kaushal, N., Kudva, A. K., Patterson, A. D., Chiaro, C., Kennett, M. J., Desai, D., … Prabhu, K. S. (2014). Crucial role of macrophage selenoproteins in experimental colitis. *The Journal of Immunology*, *193*, 3683–3692. doi:10.4049/jimmunol.1400347

Kim, J. S., Kim, A. Y., Shin, H. M., Ahn, S. I., Shim, H. J., Nam, K. W., … Youn, H. S. (2017). *Aster yomena suppresses LPS-induced cyclooxygenase-2 and inducible nitric oxide synthase expression*. *Food and Agricultural Immunology*, *28*, 202–210. doi:10.1080/09540105.2016.1251395

Kirkbya, N. S., Chanb, M. V., Zaissd, A. K., Vaze, E. G., Jiaod, J., Berglunde, L. M., … Mitchella, J. A. (2016). Systematic study of constitutive cyclooxygenase-2 expression: Role of NF-κB and NFAT transcriptional pathways. *Proceedings of the National Academy of Sciences*, *113*, 434–439. doi:10.1073/pnas.1517642113

Lee, J. H., Kang, B. S., Hwang, K. H., & Kim, G. H. (2011). Evaluation for anti-inflammatory effects of siegesbeckia glabrescens extract in vitro. *Food and Agricultural Immunology*, *22*, 145–160. doi:10.1080/09540105.2010.549210

Lu, J., Zhang, J., Jiang, C., Deng, Y., Ozten, N., & Bosland, M. C. (2016). Cancer chemoprevention research with selenium in the post-SELECT era: Promises and challenges. *Nutrition and Cancer*, *68*, 1–17. doi:10.1080/01635581.2016.1105267

Maehira, F., Mivagi, I., & Eguchi, Y. (2003). Selenium regulates transcription factor NF-κB activation during the acute phase reaction. *Clinica Chimica Acta*, *334*, 163–171. doi:10.1016/S0009-8981(03)00223-7

Meplan, C., Johnson, I. T., Polley, A. C. J., Cockell, S., Bradburn, D. M., Commane, D. M., … Hesketh, J. (2016). Transcriptomics and proteomics show that selenium affects inflammation,

cytoskeleton, and cancer pathways in human rectal biopsies. *The FASEB Journal*, *30*, 2812–2825. doi:10.1096/fj.201600251R

Nakayama, T., Esaki, N., Lee, W. J., Tanaka, I., Tanaka, H., & Soda, K. (1984). Purification and properties of L-methionine γ-lyase from aeromonas sp.. *Agricultural and Biological Chemistry*, *48*, 2367–2369. doi:10.1080/00021369.1984.10866502

Nelson, S. M., Lei, X., & Prabhu, K. S. (2011). Selenium levels affect the IL-4–induced expression of alternative activation markers in murine macrophages. *Journal of Nutrition*, *141*, 1754–1761. doi:10.3945/jn.111.141176

Nettore, I. C., De Nisco, E., Desiderio, S., Passaro, C., Maione, L., Negri, M., … Macchia, P. E. (2017). Selenium supplementation modulates apoptotic processes in thyroid follicular cells. *Biofactors*, 415–423. doi:10.1002/biof.1351

Onal, S., Naziroglu, M., Colak, M., Bulut, V., & Flores-Arce, M. F. (2011). Effects of different medical treatments on serum copper, selenium and zinc levels in patients with rheumatoid arthritis. *Biological Trace Element Research*, *142*, 447–455. doi:10.1007/s12011-010-8826-7

Prabhu, K. S., Davis, F. Z., Stewart, J. B., Thompson, J. T., Sordillo, L. M., & Reddy, C. C. (2002). Selenium deficiency increases the expression of inducible nitric oxide synthase in RAW 264.7 macrophages: Role of nuclear factor-κB in up-regulation. *Biochemical Journal*, *366*, 203–209. doi:10.1042/bj20020256

Premarathna, L., McLaughlin, M. J., Kirby, J. K., Hettiarachchi, G. M., Stacey, S., & Chittleborough, D. J. (2012). Selenate-enriched urea granules are a highly effective fertilizer for selenium biofortification of paddy rice grain. *Journal of Agricultural and Food Chemistry*, *60*, 6037–6044. doi:10.1021/jf3005788

Rayman, M. P. (2012). Selenium and human health. *The Lancet*, *379*, 1256–1268. doi:10.1016/S0140-6736(11)61452-9

Ricciotti, E., & FitzGerald, G. A. (2011). Prostaglandins and inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *31*, 986–1000. doi:10.1161/ATVBAHA.110.207449

Safir, N., Wendel, A., Saile, R., & Chabraoui, L. (2003). The effect of selenium on immune functions of J774.1 cells. *Clinical Chemistry and Laboratory Medicine*, *41*, 1005–1011. doi:10.1515/CCLM.2003.154

Sakazaki, F., Arakawa, T., Shimizu, R., Ogino, H., Okuno, T., & Ueno, H. (2014). Allergies are aggravated by mild selenium deficiency and abrogated by supplementation with selenomethionine. *Food and Agricultural Immunology*, *25*, 477–485. doi:10.1080/09540105.2013.837866

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*, 671–675. doi:10.1038/nmeth.2089

Shih, R. H., Wang, C. Y., & Yang, C. M. (2015). NF-kappaB signaling pathways in neurological inflammation: A mini review. *Frontiers in Molecular Neuroscience*, *8*, 3244–3248. doi:10.3389/fnmol.2015.00077

Taechowisan, T., Wanbanjob, A., Tuntiwachwuttikul, P., & Liuin, J. (2010). Anti-inflammatory effects of lansai C and D cause inhibition of STAT-1 and NF-κB activations in LPS-induced RAW 264.7 cells. *Food and Agricultural Immunology*, *21*, 57–64. doi:10.1080/09540100903419592

Tanaka, H., Esaki, N., & Soda, K. (1985). A versatile bacterial enzyme: L-methionine γ-lyase. *Enzyme and Microbial Technology*, *7*, 530–537. doi:10.1016/0141-0229(85)90094-8

Tyagi, S., Gupta, P., Saini, A. S., Kaushal, C., & Sharma, S. (2011). The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. *Journal of Advanced Pharmaceutical Technology & Research*, *2*, 236–240. doi:10.4103/2231-4040.90879

Vunta, H., Davis, F., Palempalli, U. D., Bhat, D., Arner, R. J., Thompson, J. T., … Prabhu, K. S. (2007). The anti-inflammatory effects of selenium Are mediated through 15-deoxy- 12,14-prostaglandin J2 in macrophages. *Journal of Biological Chemistry*, *282*, 17964–17973. doi:10.1074/jbc.M703075200

Youn, H. S., Lim, H. J., Choi, Y. J., Lee, J. Y., Lee, M. Y., & Ryu, J. H. (2008). Selenium suppresses the activation of transcription factor NF-κB and IRF3 induced by TLR3 or TLR4 agonists. *International Immunopharmacology*, *8*, 495–501. doi:10.1016/j.intimp.2007.12.008

Yui, K., Imataka, G., Nakamura, H., Ohara, N., & Naito, Y. (2015). eicosanoids derived from arachidonic acid and their family prostaglandins and cyclooxygenase in psychiatric disorders. *Current Neuropharmacology*, *13*, 776–785. doi:10.2174/1570159X13666151102103305

Zeng, H., Botnen, J., & Jhonson, L. (2008). A selenium-deficient caco-2 cell model for assessing differential incorporation of chemical or food selenium into glutathione peroxidase. *Biological Trace Element Research*, *123*, 98–108. doi:10.1007/s12011-008-8097-8

Quantification and In Vitro Bioaccessibility of Selenium from Osborne Fractions of Selenium-Rich Cereal Grains

Noorpreet I. Dhanjal,[1] Siddharth Sharma,[1] and N. Tejo Prakash[2,†]

ABSTRACT

Cereal crops cultivated in the seleniferous belt of Punjab, India, were observed to hyperaccumulate a significantly high concentration of selenium (20–123 μg/g). Selenium concentration (μg/g) in storage proteins of wheat, maize, and rice, namely, albumin (401, 280, and 29, respectively), globulin (264, 192, and 242, respectively), glutelin (563, 359, and 178, respectively), and prolamin (629, 339, and 257, respectively) indicated variable selenium levels, with prolamin contributing significantly higher levels of selenium when compared with other proteins with reference to the total concentration of the protein fraction. The simulated gastric and gastrointestinal digestion studies indicated better accessibility of selenium during intestinal digestion, with variability across proteins and cereal types. The observations provide an insight into the bioavailability of selenium in selenium-rich cereal grains, used in the study, and their potential use as source for selenium supplementation to deficient populations, or as "bioactive" selenium-rich nutraceutical blends for health benefits.

Cereal Chem. 93(4):339–343

Cereal grains contain up to 7–12% proteins, 65–75% carbohydrates, 2–6% lipids, and 12–14% water. Osborne (1907) divided the cereal proteins into four fractions based on their solubility in different solutes, namely, albumins (water), globulins (saline solution), glutelins (alkaline solution), and prolamins (70–80% alcohol). This method is widely used to classify the structural, metabolic, and storage proteins that exhibit the functional properties. The percentages of protein fractions vary significantly in different kinds of cereal grains. Prolamin has the smallest proportion among the total protein present in rice (Danno and Natake 1980) but a dominating fraction in wheat (Shewry and Mifflin 1985). The percentage of protein range varies from 2% prolamin to 78% glutelin in rice, 6% globulin to 46% glutelin in wheat, and 3% globulin to 55% prolamin in maize (Alais and Linden 1991; Eliasson and Larsson 1993).

Albumins and globulins are metabolic proteins (Kruger and Reed 1988) that provide energy and nutrition to the grains during germination via activation of various hydrolyzing enzymes. These are low-molecular-weight proteins with reduced disulfide bonds (Kruger and Reed 1988), whereas prolamins and glutelins are storage proteins having high molecular weight with intra- or interchain disulfide linkages showing biochemical polymorphism (Mandac and Juliano 1978; Alais and Linden 1991). They have sulfur-rich subunits depending upon the cysteine and methionine contents, which are the maximum in wheat, followed by maize and rice (Phillips 1997).

Cereal grains fall under the categories of selenium nonaccumulator plants. In contrast, in selenium accumulators excess selenium is anabolized to nonprotein amino acids such as Se-methylselenocysteine (MeSeCys) or γ-glutamyl-Se-methylselenocysteine (γ-Glu-MeSeCys), resulting in reduced synthesis of selenoproteins, without affecting other plant metabolic processes (Cubadda et al. 2010). Cereals are a rich source of minerals, vitamins, proteins, carbohydrates, phytochemicals, and antioxidants.

Seleniferous food can potentially be used as selenium supplements in supranutritional doses in the case of cancer and inflammation (Ip et al. 1994; Combs et al. 2001). The supranutritional diet provides a sufficient amount of methylselenol, a selenium metabolite that induces apoptosis (Kaeck et al. 1997; Sinha and Medina 1997). Therefore, biofortification with selenium (Wu et al. 2015) has been enhanced in many selenium-deficit countries such as Finland, where 10 μg/g of selenium is being supplemented to soils as sodium selenate (Eurola and Hietaniemi 2000) to enhance its delivery to the human population through food as supranutritional supplementation. Alifar et al. (2014) conducted a greenhouse trial with five different concentrations of sodium selenite (0, 100, 300, 500, and 700 g/ha) on selenium uptake by rice. The authors recommended application of selenium at the rate of 500 g/ha, beyond which there would be no further uptake of selenium in grains.

Dietary organoselenium, from sources of foods that are naturally enriched with selenium, can be a very good dietary source of this nutrient because organic forms are easily bioaccessible (≥90%) in the intestine, where they can bind with albumins along with β-proteins for circulation in the blood stream.

Keeping in mind the growing importance of dietary sources of selenium at supranutritional levels and the bioaccessibility, the objective of the current study was to determine the distribution and bioaccessibility of selenium in Osborne fractions of cereal grains collected from seleniferous fields. The present study represents selenium bioaccessibility, which is influenced not only by the amounts but also by the form of protein to which selenium is bound.

MATERIALS AND METHODS

Sample Collection. Wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*) were collected in November 2014 at sites near the villages of Jainpur and Barwa geographically located at 32°46′N, 74°32′E, in the Nawanshahr-Hoshiarpur districts of Punjab, India. Samples were collected from three different locations of each field, and composite samples were prepared and processed separately. Nonseleniferous samples were collected from agricultural fields of Patiala (30°32′N, 76°40′E), India. Healthy grains were manually separated from damaged ones, cleaned from dust, and ground to fine powder with an electric blender.

Selenium Determination. Selenium was determined in defatted whole grain flour, starch residue, and all protein fractions following the procedure given by Levesque and Vendette (1971) (Fig. 1). This method involved closed vessel digestion with HNO_3 and $HClO_4$ (SD Fine-Chem, Mumbai, India), reduction of selenium from Se^{+6} to Se^{+4} in a microwave digester (MARS 6 240/50:910905, CEM, Matthews, NC, U.S.A.), complexing of Se^{+4} with 2,3-diamino-naphtalene (HiMedia, Mumbai, India), and extraction of the piazselenol in cyclohexane at pH 1.8. Fluorescence was measured after extraction at 360 nm (excitation) to obtain the emission spectrum of piazselenol at 520 nm (emission) with a fluorescence spectrometer (LS-45, Perkin Elmer, Waltham, MA, U.S.A.). Selenium quantification in each sample was carried out by a relative method using the emission spectrum of National Institute of Standards and Technology certified selenium

† Corresponding author. Phone: +91-175-2393318.
E-mail: ntejoprakash@thapar.edu

[1] Department of Biotechnology, Thapar University, Patiala, India.
[2] School of Energy and Environment, Thapar University, Patiala, India.

http://dx.doi.org/10.1094/CCHEM-10-15-0199-R

ICP standard solution (SRM-1349), and standard addition was used and percent recovery was checked for selected samples.

Protein Extraction. Osborn fractions (namely, albumin, globulin, glutelin, and prolamin) were extracted from cereal grains according to the method of Ju et al. (2001). The systemic scheme is common for wheat, rice, and maize protein extracts and is given in the flow chart (Fig. 1). Fractions were extracted, independently, from defatted sample flour based on their solubility in distilled water (albumin), 5% NaCl (globulin), 0.1M NaOH (glutelin), and 70% ethanol (prolamin) at 25°C. For precipitation of proteins, the pH was adjusted to the isoelectric point (titration of aliquot and as indicated by pH electrode) with 1N HCl and kept overnight (4°C) to ensure complete aggregation. The extracts were then centrifuged at 12,000 rpm for 20 min (4°C), washed with acetone, cryodried, and crushed to powder for further use.

Crude Starch Extraction. To the residue left after extraction of different proteins, four washings were given: aqueous washing with 1 L of distilled water for 24 h, salt washing with 2% NaCl for 24 h, alkali washing twice with 0.1N NaOH for 48 h, and alcohol washing with 80% ethanol at 80°C in a water bath for 1 h to remove and denature the remaining protein, if any. After settling for 4 h at 4°C, the supernatant was discarded. The residue was air dried at ambient temperature (37°C) overnight to get crude starch powder (Reddy and Bhotmange 2013). For wheat and maize, to reduce the interference of unwanted materials such as fiber, fine cell residues, and other soluble materials, the upper white starchy layer was collected manually and resuspended in 1 L of 0.1N NaOH for 48 h and in diethyl ether for 4 h at room temperature before the alcohol washing step.

Enzymatic Digestion. In vitro gastrointestinal digestion was performed according to the method of Kulkarni et al. (2007) (Fig. 1). Gastric digestion was mimicked with 1% w/v pepsin (HiMedia) in 0.15M NaCl, pH 1.75, and intestinal digestion with 3% w/v pancreatin (Sisco Research Laboratories, Mumbai, India), 1.5% w/v α-amylase (HiMedia), and 1% w/v bile salts (SD Fine-Chem) in 0.15M NaCl, pH 6.8, to estimate the selenium bioavailability in gastric and gastrointestinal juices. Samples were centrifuged at 5,000 × *g* for 10 min (4°C); supernatant was filtered through a 0.45 µm pore size filter and subjected to selenium quantification.

Statistical Analysis. The observations were further analyzed for descriptive and comparative interpretations with GraphPad Prism 5.1 software (GraphPad Software, La Jolla, CA, U.S.A.).

RESULTS AND DISCUSSION

Selenium in Soil and Cereal Whole Grains. The mean (± standard deviation) selenium concentration in soil samples collected during different cropping seasons was 3.32 ± 0.15 µg/g. Our earlier estimates of the selenium concentration in soils of the same region ranged from 2.7 to 6.5 µg/g, as analyzed by neutron activation

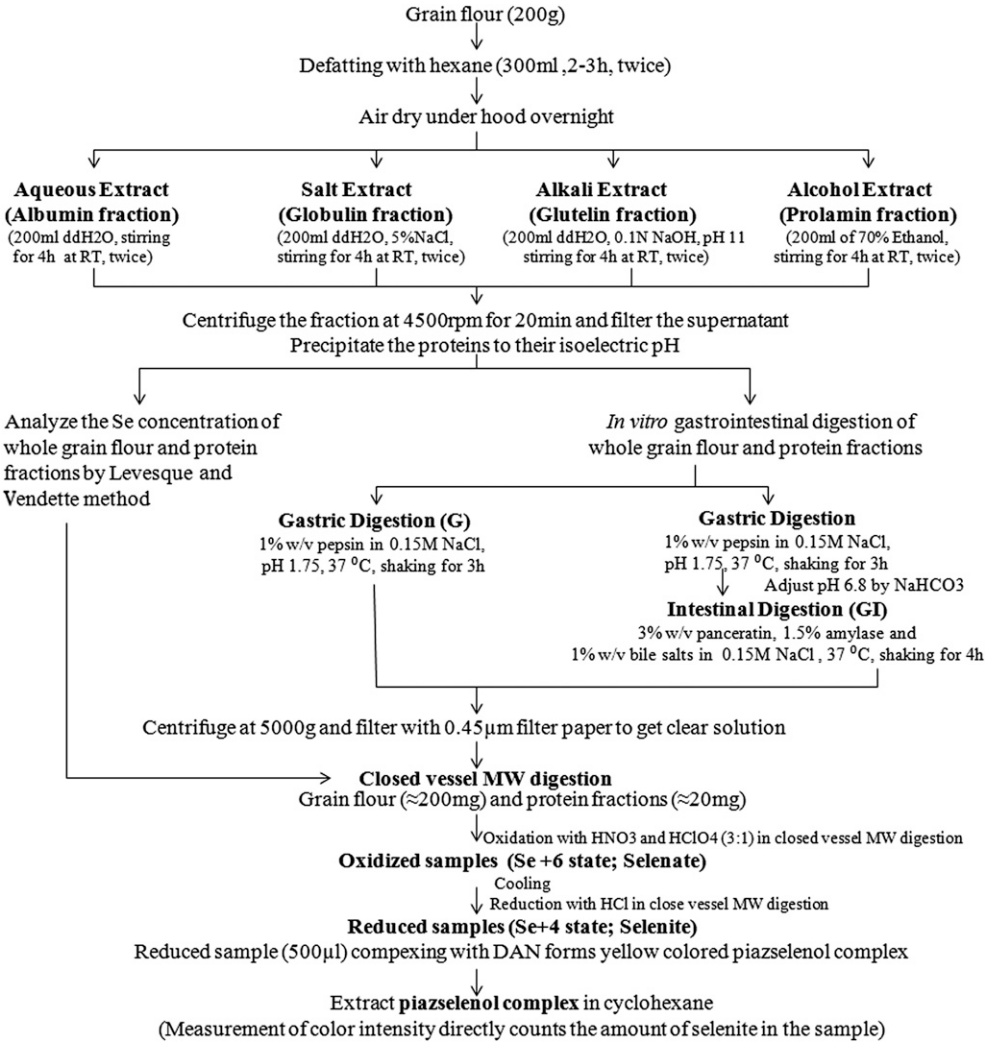


Fig. 1. The scheme for the quantification of the different protein fractions, in vitro gastrointestinal digestion, and estimation of selenium in wheat, rice, and maize samples.

analysis (Sharma et al. 2009). Dhillon and Dhillon (1991) reported that soil in the said region had 4–5 times more selenium than nonseleniferous regions. The concentration varied from 0.23 to 4.55 μg/g of selenium up to 180 cm depth of soil. This area is significantly impacted by the selenium (340 μg/L) (Bajaj et al. 2011) in irrigation water and soil, resulting in hyperaccumulation of selenium in cereal grains (Dhillon and Dhillon 2003). Soil with selenium concentration of ≥1 μg/g is seleniferous in nature, whereas at 0.4 μg/g the soils are generally considered selenium deficient (Plant et al. 2005).

The concentrations of selenium in whole grains of wheat, maize, and rice were 122.9 ± 0.6, 26.5 ± 0.2, and 19.7 ± 0.2 μg/g, respectively, when compared with selenium levels in grains (1.1–1.3 μg/g) from a nonseleniferous area (Fig. 2). The selenium levels in the nonseleniferous area under study also indicated moderately higher levels when compared with global averages (Jaiswal et al. 2015). Selenium concentration in most of the world's wheat ranges between 0.02 and 0.6 μg/g (Alfthan and Neve 1996). Referring to the selenium levels, wheat from seleniferous areas of South Dakota, U.S.A., contained selenium to the extent of 30 μg/g. Thus, studies from this region reported selenium concentrations in wheat and maize at much higher levels than those reported elsewhere. Mostly selenium (~90%) is present in organic form, selenomethionine in wheat (Stadlober et al. 2001) and rice (Guzmán Mar et al. 2009). Among the inorganic forms, selenate is identified as the major species present in wheat and rice, but it is absent in maize grains (Aureli et al. 2012). Selenate is more mobile up to the xylem and is less readily transformed into organic forms (Terry et al. 2000; Sager 2006), whereas selenite adsorbs on humic material present in soil, forms hydroxides and oxyhydroxides with iron, manganese, or aluminum, and gets rapidly metabolized into SeMet or SeCys forms (Séby et al. 1998).

Osborn Fractions and Selenium Content. The amounts of various Osborne fractions with reference to percentage of total protein and selenium concentration are given in Table I. Referring to the selenium levels met in various proteins, the glutelin fraction dominated over other proteins across all the grains tested. Prolamin was observed to contribute significant selenium levels despite being the lowest among all protein fractions across all grains (Fig. 3). The overall selenium content in prolamin and glutelin fractions was higher than in other proteins, except in the case of rice. A multiple comparison test showed significant variation ($P < 0.05$) in selenium levels across grains but not significant across proteins. Nearly similar observations were also obtained in wheat crop samples obtained from other locations in the seleniferous belt (Jaiswal et al. 2015). Correspondingly, selenium levels in the residual starch were below detectable limits with respect to the presence of 2,3-dihydroxypropionyl-selenolanthione (*m/z* 345), hexose moiety (*m/z* 317 and *m/z* 358), and hexose pentose moiety (*m/z* 407 and *m/z* 408) as selenosugars in cereal grains (Aureli et al. 2012). In the spent residue, selenium is still present with 46.5 ± 0.7, 6.4 ± 0.8, and 10.6 ± 0.1 μg/g concentration in wheat, maize, and rice, respectively, as residual percentage loss.

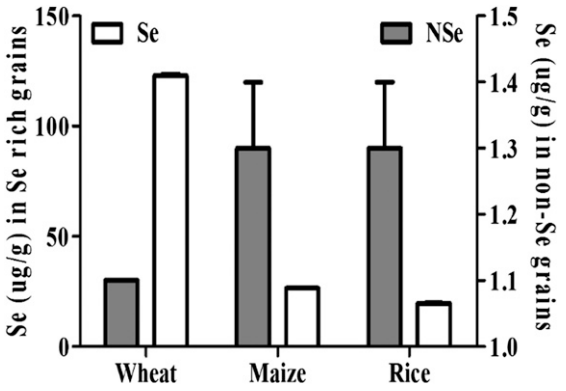


Fig. 2. Concentration of selenium in cereal grains from selenium-impacted (Se, scale at left) and normal soils (NSe, scale at right).

Abundance of methionine and cysteine in each fraction represents nonspecific incorporation of selenomethionine and selenocysteine, respectively, into proteins (Strub et al. 2003). Aureli et al. (2012) reported that the total selenium in rice is present in organic form (SeMet) dominantly in the glutelin fraction (31.3%) and only 2.85% as inorganic selenium. In turn, Se-methyl-selenocysteine and selenocystathionine are present in nonprotein fractions of selenium-accumulating plants. It would, therefore, be of further interest to understand the profile of selenium moieties in these selenium-rich Osborne fractions.

Selenium Bioavailability. The bioavailability of selenium in different protein fractions is directly proportional to the concentration present in them. This bioavailability indicates that the intestinal fluids break down the selenium-containing proteins, which are then easily absorbed. Generally, selenium is higher in cereal grains (Jaakkola et al. 1983) than in animal foodstuffs (Combs 1988). Hakkarainen (1993) earlier reported the selenium bioavailability from wheat (83–100%), barley (78–85%), oats (41–45%), fish (64–80%), and meat meal (22–30%). With reference to the present study carried out on the selenium fractionation into Osborne fractions, the proteins were subjected to in vitro gastric and gastrointestinal digestion. Table II presents the selenium levels in the bioaccessible fractions with reference to various proteins and cereal grains.

The bioaccessible fractions across the cereal grains showed significant variation, with wheat providing higher bioaccessibility of selenium through digestion, followed by rice and maize. In rice, the small proportion of selenium-containing protein was still bound to the starch matrices (Fang et al. 2010). Bioaccessibility of selenium through intestinal digestion was higher compared with gastric digestion in all the selenium-rich protein samples investigated, indicating that the intestinal fluids sufficiently break down the selenium-containing proteins, which are then easily absorbed. Selenium from cereal grains in organic forms (selenomethionine and selenocysteine) are more bioaccessible and are efficient reservoirs for long-term storage (Behne et al. 2009). Further, the glutathione peroxidase activity, generally considered an index of selenium levels in the liver, kidney, and whole blood, revealed that bioavailability of selenium in wheat was greater than in tuna (Alexander et al. 1983).

TABLE I
Percent Protein Yield and Corresponding Selenium Concentration of Osborne Fractions

	Wheat		Maize		Rice	
Fraction	**Protein Yield (%)**	**Se Conc. (μg/g)**	**Protein Yield (%)**	**Se Conc. (μg/g)**	**Protein Yield (%)**	**Se Conc. (μg/g)**
Albumin	11 ± 0.1	401 ± 1.9	20 ± 0.8	280 ± 2.6	8 ± 0.9	29 ± 1.5
Globulin	24 ± 2.6	264 ± 4.2	34 ± 0.8	192 ± 0.8	26 ± 2.2	242 ± 1.6
Glutelin	49 ± 2.1	563 ± 2.1	38 ± 1.1	359 ± 2.3	59 ± 1.8	178 ± 1.3
Prolamin	16 ± 1.1	629 ± 6.9	8 ± 0.6	339 ± 1.3	7 ± 0.5	257 ± 1.3

CONCLUSIONS

Selenium fractionation in Osborne proteins indicated that the nonaccumulator cereal crops deposited most of the selenium in proteinaceous matrices, possibly as selenoproteins and selenoenzymes. Prolamins and glutelins had significantly higher selenium levels across all grains except in rice, for which globulin had a higher selenium level than glutelin. The bioaccessibility of selenium in proteins correlated with their selenium concentrations. These seleniferous grains can potentially be used as selenium supplements in supranutritional doses in countries where the food system does not provide enough selenium to support nutritional requirements. However, it is important to understand that the intake of the organic form of selenium is multifold when compared with inorganic intake. The majority of the studies, to date, have been associated with low-selenium dietary proteins; thus, the present observations on naturally enriched sources such as cereals are important to understand the accessibility of selenium.

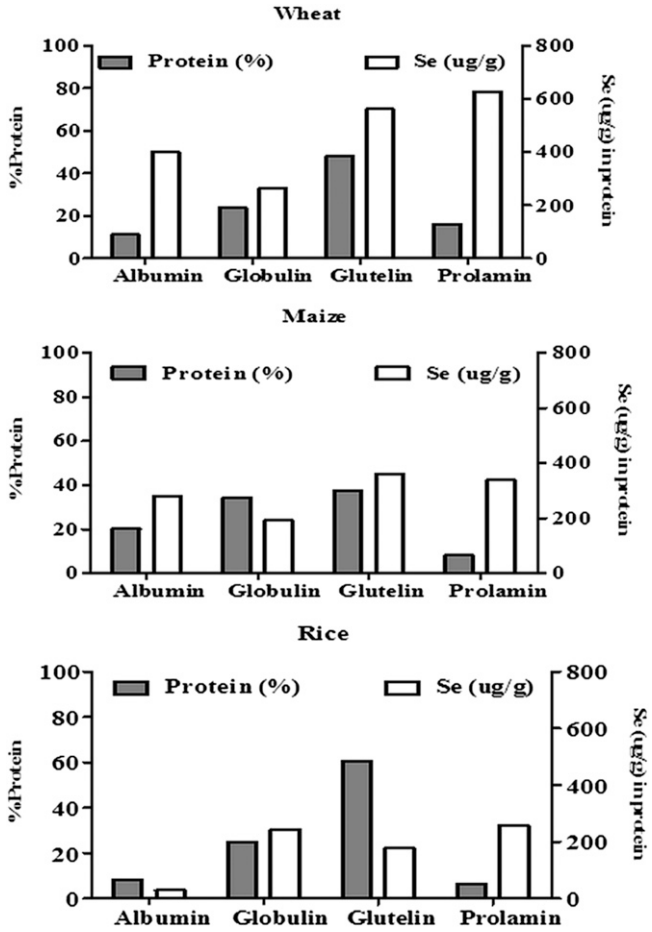


Fig. 3. Levels of selenium in different protein extracts of various cereal grains.

TABLE II
Bioaccessibility of Selenium from Osborne Fractions of Selenium-Rich Cereal Grains During Gastric (G) and Gastrointestinal (GI) Digestion

	% Bioaccessibility (G/GI)		
Fraction	**Wheat**	**Maize**	**Rice**
Whole grain	75/98	59/95	51/82
Albumin	78/88	79/99	42/71
Globulin	74/79	75/98	91/95
Glutelin	79/85	60/95	71/82
Prolamin	78/82	45/80	94/97

ACKNOWLEDGMENTS

The CSIR, India, research grant to N. Tejo Prakash utilized for the study is duly acknowledged.

LITERATURE CITED

Alais, C., and Linden, G. 1991. Food Biochemistry. Ellis Horwood: New York.

Alexander, A. R., Whanger, P. D., and Miller, L. T. 1983. Bioavailability to rats of selenium in various tuna and wheat products. J. Nutr. 113: 196-204.

Alfthan, G., and Neve, J. 1996. Selenium intakes and plasma selenium levels in various populations. Pages 161-167 in: Natural Antioxidants and Food Quality in Atherosclerosis and Cancer. J. Kumpulainen and J. Salonen, eds. Royal Society of Chemistry: Cambridge, U.K.

Alifar, N., Zaharah, A. R., Ishak, C. F., Awang, Y., and Khayambashi, B. 2014. Selenium enrichment of paddy rice grains in Malaysia. Asian J. Crop Sci. 6:345-353.

Aureli, F., Ouerdane, L., Bierla, K., Szpunar, J., Prakash, N. T., and Cubadda, F. 2012. Identification of selenosugars and other low-molecular weight selenium metabolites in high-selenium cereal crops. Metallomics 4:968-978.

Bajaj, M., Eiche, E., Neumann, T., Winter, J., and Gallert, C. 2011. Hazardous concentration of selenium in soil and groundwater in North-West India. J. Hazard. Mater. 189:640-646.

Behne, D., Alber, D., and Kyriakopoulos, A. 2009. Effects of long-term selenium yeast supplementation on selenium status studied in the rat. J. Trace Elem. Med. Biol. 23:258-264.

Combs, G. F. 1988. Selenium in foods. Pages 85-113 in: Advances in Food Research. C. O. Chichester and B. S. Schweiger, eds. Academic Press: San Diego, CA.

Combs, G. F., Clark, L. C., and Turnbull, B. W. 2001. An analysis of cancer prevention by selenium. Biofactors 14:153-159.

Cubadda, F., Aureli, F., Ciadullo, S., D'Amato, M., Raggi, A., Acharya, R., Reddy, R. A. V., and Prakash, N. T. 2010. Changes in selenium speciation associated with increasing tissue concentrations of selenium in wheat grain. J. Agric. Food Chem. 58:2295-2301.

Danno, G., and Natake, M. 1980. Isolation of foxtail millet proteins and their subunit structure. Agric. Biol. Chem. 44:913-918.

Dhillon, K. S., and Dhillon, S. K. 1991. Selenium toxicity in soil, plants and animals in some parts of Punjab. Int. J. Environ. Stud. 37:15-24.

Dhillon, K. S., and Dhillon, S. K. 2003. Quality of underground water and its contribution towards selenium enrichment of the soil-plant system for a seleniferous region of northwest India. J. Hydrol. (Amst.) 272: 120-130.

Eliasson, A.-C., and Larsson, K. 1993. Cereals in Breadmaking: A Molecular Colloidal Approach. Marcel Dekker: New York, NY.

Eurola, M., and Hietaniemi, V. 2000. Report of the selenium monitoring programme 1997–1999. Agricultural Research Centre of Finland: Jokioinen, Finland.

Fang, Y., Catron, B., Zhang, Y., Zhao, L., Caruso, J. A., and Hu, Q. 2010. Distribution and in vitro availability of selenium in selenium-containing storage protein from selenium-enriched rice utilizing optimized extraction. J. Agric. Food Chem. 58:9731-9738.

Guzmán Mar, J. L., Reyes, L. H., Rahman, G. M. M., and Kingston, H. M. S. 2009. Simultaneous extraction of arsenic and selenium species from rice products by microwave-assisted enzymatic extraction and analysis by ion chromatography-inductively coupled plasma-mass spectrometry. J. Agric. Food Chem. 57:3005-3013.

Hakkarainen, J. 1993. Bioavailability of selenium. Nor. J. Agric. Sci. 11: 21-35.

Ip, C., Lisk, D. J., and Scimeca, J. A. 1994. Potential of food modification in cancer prevention. Cancer Res. 54:1957-1959.

Jaakkola, K., Tummavuori, J., Pirinen, A., Kurkela, P., Tolonen, M., and Arstila, A. U. 1983. Selenium levels in whole blood of Finnish volunteers before and during organic and inorganic selenium supplementation. Scand. J. Clin. Lab. Invest. 43:473-476.

Jaiswal, S. K., Prakash, R., and Tejo Prakash, N. 2015. Selenium in storage proteins of wheat cultivated on selenium impacted soils of Punjab, India. Acta Aliment. 44:235-241.

Ju, Z. Y., Hettiarachchy, N. S., and Rath, N. 2001. Extraction, denaturation and hydrophobic properties of rice flour proteins. J. Food Sci. 66:229-232.

Kaeck, M., Lu, J., Strange, R., Ip, C., Ganther, H. E., and Thompson, H. J. 1997. Differential induction of growth arrest inducible genes by selenium compounds. Biochem. Pharmacol. 53:921-926.

Kruger, J. E., and Reed, G. 1988. Enzymes and color. Pages 441-500 in: Wheat: Chemistry and Technology, 3rd Ed., Vol. I. Y. Pomeranz, ed. American Association of Cereal Chemists: St. Paul, MN.

Kulkarni, S. D., Acharya, R., Rajurkar, N. S., and Reddy, A. V. R. 2007. Evaluation of bioaccessibility of some essential elements from wheatgrass (*Triticum aestivum* L.) by in vitro digestion method. Food Chem. 103:681-688.

Levesque, M., and Vendette, E. D. 1971. Selenium determination in soil and plant materials. Can. J. Soil Sci. 51:85-93.

Mandac, B. E., and Juliano, B. O. 1978. Properties of prolamin in mature and developing rice grain. Phytochemistry 17:611-614.

Osborne, T. B. 1907. The Proteins of the Wheat Kernel. Carnegie Institution: Washington, DC.

Phillips, R. D. 1997. Nutritional quality of cereals and legume storage proteins. Food Technol. 51:62-66.

Plant, J. A., Kinniburgh, D. G., Smedley, P. L., Fordyce, F. M., and Klinck, B. A. 2005. Arsenic and selenium. Pages 17-66 in: Treatise on Geochemistry, Vol. 9, Environmental Geochemistry. B. S. Lollar, ed. Elsevier: London, U.K.

Reddy, D. K., and Bhotmange, M. G. 2013. Isolation of starch from rice (*Oryza sativa* L.) and its morphological study using scanning electron microscopy. Int. J. Agric. Food Sci. Technol. 4:859-866.

Sager, M. 2006. Selenium in agriculture, food and nutrition. Pure Appl. Chem. 78:111-133.

Séby, F., Potin-Gautier, M., Giffaut, E., and Donard, O. 1998. Assessing the speciation and the biogeochemical processes affecting the mobility of selenium from a geological repository of radioactive wastes to the biosphere. Analysis 26:193-198.

Sharma, N., Prakash, R., Srivastava, A., Sadana, U. S., Acharya, R., Prakash, N. T., and Reddy, A. V. R. 2009. Profile of selenium in soil and crops in seleniferous area of Punjab, India by neutron activation analysis. J. Radioanal. Nucl. Chem. 281:59-62.

Shewry, P. R., and Mifflin, B. J. 1985. Seed storage proteins of economically important cereals. Pages 1-83 in: Advances in Cereal Science and Technology, Vol. 7. Y. Pomeranz, ed. American Association of Cereal Chemists: St. Paul, MN.

Sinha, R., and Medina, D. 1997. Inhibition of cdk2 kinase activity by methylselenocysteine in synchronised mouse mammary epithelial tumor cells. Carcinogenesis 18:1541-1547.

Stadlober, M., Sager, M., and Irgolic, K. J. 2001. Effects of selenate supplemented fertilization on the selenium level of cereals—Identification and quantification of selenium compounds by HPLC-ICP-MS. Food Chem. 73:357-366.

Strub, M. P., Hoh, F., Sanchez, J. F., Strub, J. M., Bock, A., Aumelas, A., and Dumas, C. 2003. Selenomethionine and selenocysteine double labeling strategy for crystallographic phasing. Structure 11:1359-1367.

Terry, N., Zayed, A., D'Souza, M., and Tarun, A. 2000. Selenium in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51:401-432.

Wu, Z., Banuelos, G. S., Lin, Z. Q., Liu, Y., Yuan, L., Yin, X., and Li, M. 2015. Biofortification and phytoremediation of selenium in China. Front. Plant Sci. 6:1-8.

[Received October 6, 2015. Accepted December 16, 2015.]