Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20140504020742

Original Research

GESDAV

Protective effects of selenium on some selective immunological and biochemical parameters in cadmium intoxicated rat

E. El-Boshy Mohamed[1,2], F. Risha Engy[2], M. Abdel Hamid Fatma[2]

[1]Department of Laboratory Medicine, Faculty of Applied Medical Science, Umm Al-Qura University, Makkah, Makkah 21955, Saudi Arabia,
[2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt

Address for correspondence:
Prof. E. El-Boshy Mohamed, Department of Laboratory Medicine, Faculty of Applied Medical Science, Umm Al-Qura University, Makkah, PB 7296, Makkah 21955, Saudi Arabia.
E-mail: dr_elboshy@yahoo.com

Received: October 07, 2013
Accepted: May 04, 2014
Published: October 02, 2014

ABSTRACT

Background and Objective: In this study, we investigate the protective effect of selenium (Se) against cadmium (Cd) intoxication in the rat by monitoring some selective cytokines (interleukin-1β [IL-1β], tumor necrosis factor-α [TNF-α], IL-6, IL10 and interferon gamma [IFN-γ]) and antioxidant enzymes (glutathione [GSH], catalase [CAT], superoxide dismutase [SOD], nitric oxide) and lipid peroxidation (malondialdehyde [MDA]). **Material and Methods:** The Cd was orally administrated at dose 20 and 40 mg/L in drinking water for 30 days. The Se orally administrated at a dose rate 0.1 mg/kg bw/day also for 30 days with Cd. Blood samples were collected from heart puncture at the end of experiment (30 days) and serum was separated for estimation the immunological parameters studies. **Results**: the Se improved elevation of serum IL-1β, IL-6, TNF-α, IFN-γ and MDA as well as enhanced antioxidant enzymes activities GSH, CAT and SOD. Furthermore, Se ameliorate the Cd induce liver and kidney damage by improving hepatic and renal markers. **Conclusions:** We could be concluded that Se interaction with Cd extend our understanding of Se biological functions as a potential countermeasure against immunosuppressive as well as hepatic and renal oxidative damage induced by Cd in rats.

KEY WORDS: Cadmium, immunosuppressive, rat, selenium

INTRODUCTION

Adverse immune effect of chemicals defined as immunotoxicity have been used a sensitive biomarker for assessing health effect of environmental pollution. Immunotoxic effect of heavy metals, as a typical environmental agent and their mechanism are area of interest. Cadmium (Cd) is one of the most toxic heavy metals, an environmental and occupational pollutant endangering human and animal health [1,2]. It has been suggested that Cd may be an environmental risk factor for osteoporosis and fertility as well as nephrotoxicity, hepatotoxicity and immunosuppressive [3,4]. The immunotoxic effects of Cd have been reported on the development of immune organs, on the differentiation of immune cells, and on specific and nonspecific immune responses [5]. This indicates an impact on the immune system as a whole, which can lead to a significant decrease in host resistance. Moreover, some of the specific changes that lead to tissue damage and death in chronic exposure of Cd have been related to oxidative stress [6]. The associated toxic effects of Cd attributed to suppression of free radical scavenging function and the enhancement of reactive oxygen species (ROS) contributes to Cd induced oxidative stress and lipid peroxidation (LPO) [7-9].

Selenium (Se) is an essential trace element for mammals. Through selenoproteins, this mineral participates in various biological processes such as antioxidant defense, thyroid hormone production, and immune responses. Some reports indicate that a deficient in Se may be prone to certain diseases. Adverse health effects following Se overexposure, although very rare, have been found in animals and people. Se interaction with Cd has been reported and may reduce Cd accumulation in the body [10].

For the reasons outlined above, we decided to study the immunosuppressive, renal and hepatic damage as well as

oxidative stress effects of Cd and the putative protective effects of Se in rat.

MATERIALS AND METHODS

Forty male albino rats (200 ± 10 g) 10-12 weeks old were conducted in our study. Rats were randomly divided into five, equal groups. The groups treated as following. The first group was keep as a negative control, whereas the second and third groups were given a 20 and 40 mg Cd/L in drinking water respectively according to [11] for 30 days. While the fourth and fifth groups were administrated Cd 20 and 40 mg/L with Se orally administrated at a dose rate 0.1 mg/kg bw [12].

Blood samples were taken in the test tube without anticoagulant. The samples were centrifuged at 3000 rpm for 10 min and the clear serum was separated carefully for determination of some selective humoral immunological parameters (tumor necrosis factor-α [TNF-α], interleukin 1β [IL-1 β], IL-6, IL-10 and interferon gamma [IFN-γ.] were measurement by Enzyme Amplified Sensitivity Immunoassay [EASIA, R and D Systems, Minneapolis, MN, USA]) performed on microplate according to enclosed pamphlet (Aushon Searchlight Biosystem, Billerica, MA). The lower detection limits of 1.5 pg/ml for IL-1β, 6.3 pg/ml for IL-6, 5.4 pg/ml for IL-10, 3.1 pg/ml for TNF-α, and 6.2 pg/ml for IFN-γ. Were also tested using data are presented as pg cytokine/ml serum.

Antioxidant markers, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA), were determined from undiluted serum samples using commercially available ELISA Kits (Cayman Chemical Co, USA). The plates were read at 450 nm and a correction wavelength of 550 nm on a computerized automated microplate ELISA reader. alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities were assayed by using commercial kits (Human, Diagnostic Co., Germany), while total protein, albumin, uric acid, urea and creatinine (Crescent Diagnostic Co., KSA) were estimated spectrophotometeic (BM Co., Germany, 5010) according to enclosed pamphlets.

Statistical Analysis

Our results were analyzed by one-way (ANOVA) using SPSS software statistical program (SPSS for windows, version 15.00, USA) followed by less significant difference. The groups were significantly different if *P* value was statistically lower than 0.05 [13].

RESULTS

In this study, the serum cytokines IL-1β, TNF-α and IFN-γ revealed highly significant increase in Cd treated groups, while the serum level of IL-6 was a significant increase in higher doses of Cd administrated group as compared with the control group. No significant change was observed in serum level of IL-10 in Cd treated groups compared with the control group. The above cytokines serum level in Cd and Se treated groups were none significant change when compared with control groups [Table 1].

Our results show that marked significantly decreased in antioxidant enzymes, GSH, CAT, in both Cd treatment group, while SOD and nitric oxide (NO) were significantly decreased in higher dose in Cd treatment group when compared with the control group. Furthermore in Cd treatment groups, peroxidation MDA was significantly increased in both Cd treated groups when compared with the control group. The antioxidant markers, GSH, CAT, SOD, NO, and MDA were none significantly decreased when compared with the control group [Table 2].

The serum biochemical parameters in our work revealed alteration in hepatic markers in Cd administrated groups when compared with the control group. There was a significantly increased in ALT and AST serum activities while a significant decreased in total protein and albumin; meanwhile, non-significant changed in alkaline phosphatase in Cd treated groups in compared with the control group. In the Cd and Se group

Table 1: Effect of Cd and Se on some selective cytokines markers (mean±SE) 30 days post-treatment

Group	TNF-α (Pg/ml)	IL-1-β (Pg/ml)	IL-6 (Pg/ml)	IFN-γ (Pg/ml)	IL-10 (Pg/ml)
Control	24.41±1.2[b]	32.62±3.4[c]	21.92±4.9[a]	42.32±4.6[b]	9.19±1.2[a]
Cd 20	45.25±6.2[a]	65.14±4.9[b]	26.44±4.4[a]	57.24±5.1[a]	10.34±1.4[a]
Cd 40	59.34±7.4[a]	89.16±6.4[a]	35.85±3.2[b]	64.71±6.9[a]	9.67±1.01[a]
Cd 20 and Se	25.83±3.4[b]	36.87±5.5[c]	24.18±3.2[a]	46.82±6.7[b]	10.95±1.1[a]
Cd 40 and Se	22.66±2.9[b]	38.19±7.4[c]	23.94±4.1[a]	45.38±5.6[b]	9.94±1.25[a]

[a,b,c]Means in the same column not followed by the same letter differ significantly ($P<0.05$). SE: Standard error, TNF-α: Tumor necrosis factor-α, IFN-γ: Interferon gamma, IL: Interleukin, Se: Selenium, Cd: Cadmium

Table 2: Effect of Cd and Se on some selective oxidative stress markers (mean±SE) 30 days post-treatment

Group	GSH (μmol/L)	Catalase (U/ml)	SOD (U/ml)	NO (μmol/ml)	MDH (μmol/ml)
Control	9.42±0.35[a]	5.18±0.25[a]	5.72±0.16[a]	8.51±0.49[a]	8.85±0.82[b]
Cd 20	6.51±0.26[b]	4.89±0.38[a]	5.22±0.29[a]	7.92±0.68[a]	12.1±0.41[a]
Cd 40	4.14±0.12[c]	3.41±0.18[b]	3.69±0.20[b]	5.47±0.41[b]	12.52±0.39[a]
Cd 20 and Se	8.15±0.21[a]	4.92±0.20[a]	5.29±0.32[a]	7.98±0.71[a]	9.12±0.59[b]
Cd 40 and Se	8.92±0.27[a]	4.88±0.17[a]	5.65±0.35[a]	8.14±0.95[a]	9.01±0.42[b]

[a,b,c]Means in the same column not followed by the same letter differ significantly ($P<0.05$). GSH: Glutathione, SOD: Superoxide dismutase, NO: Nitric oxide, MDH: Malate dehydrogenase, SE: Standard error, Se: Selenium, Cd: Cadmium

treated with low dose show none significant change in ALT and AST serum level when compared with the control group. Total plasma protein was none significant change in Cd and Se treated groups when compared with the control group, while albumin serum level was significantly increased in Cd and Se treated groups when compared with Cd treated groups [Table 3].

Regarding to the renal markers, in our work, urea and creatinine blood level elevated in groups intoxicated with Cd in comparison with the control group. Uric acid was none significantly changed in both Cd treated groups when compared with the control group. In this work, the urea, creatinine and uric acid in Cd and Se treated groups, show none significant change when compared with the control group [Table 4].

DISCUSSION

Environmental toxicants such as Cd may produce a variety of clinical manifestations. In man and animals, several organ systems, including the renal, hepatic, and immune system. Several studies have documented immunosuppressive effects of Cd on the immune system [14,15].

In the current study the Cd administrated group show elevation serum cytokines IL-1β, IL-6, TNF-α and IFN-γ in compared with the control group. Cd induced elevation in proinflammatory cytokines IL-1β and IL-6 in human fibroblasts after exposure to 7 μM Cd2+ for 7 h [16]. Cd also induced the release of TNF-α, IL-6 and IL-8, which may indicate some potential to induce deleterious effects through this pathway [17]. In the same aspect treatment of albino rats with CdCl (2.5 mg/kg bw) for 2 weeks resulted in a significant increase in TNF-α and IL-1 [18]. Elevation of IFN-γ observed after incubation of cultivated peripheral blood mononuclear cell with high concentrations of 100 mμmol of Cd chloride [14]. The immunomodulatory response of Se was documented by none significant change cytokines serum level in Se and Cd treated groups when compared with the control group. Regarding the immunomodulatory effects of Se, the immune response in albino rats dietary supplemented with Se 8 and 12 PPM for 14 days enhanced by increasing in antibody response to sheep red blood cell of the supplemented groups [19]. The *in vivo* immuno-enhancement activity of selenium-exopolysaccharide (Se-EPS) in immunosuppressed mice was researched. Se-EPS treatments increased macrophage phagocytosis, spleen and thymus indices and hemolytic complement activity [20]. Moreover, the dietary supplementation of Se in a dose 10^{-3} g Se/kg for the period of 10 days enhanced neutrophils phagocytosis function [21]. Furthermore, IL-1 activity was unaffected by Se administered to female Sprague-Dawley rats for 10 weeks at 0.5 and 2.0 ppm [22].

The enzymatic antioxidant defense system includes mainly SOD, CAT, glutathione peroxidase (GPx) and GSH and this system protects cells against ROS toxicity and LPO. SOD converts the superoxide anion radical to hydrogen peroxide and CAT cleaves this hydrogen peroxide into the molecules of water and oxygen [23]. GPx is a selenoenzyme that catalyzes the oxidation of GSH to glutathione oxidase and thereby scavenges the H_2O_2 [24]. Our results show that marked significantly decreased in antioxidant enzymes, GSH, CAT, and SOD and NO were significantly decreased in Cd treatment groups, while LPO MDA was significantly increased. Cd decreased antioxidant markers activities such as GSH, CAT, SOD, NO, and significant increased MDA in rats received Cd chloride (5 mg/kg bw) for 28 days and (1.5 mg/4 ml/kg) daily for 5 days has been reported [25,26], respectively. Moreover, our findings are consistent with other published reports, which quoted that antioxidant markers concentration decreased during Cd intoxication [27,28]. Cd has been documented to impact the body system damage through inhibited antioxidants markers and induced oxidative damage with ROS generation, which destruct proteins, lipids and DNA by oxidation. LPO involves a broad spectrum of alterations in cells and the consequent degeneration of cell membranes. Free radicals and intermediate products of peroxidation are capable of damaging the integrity and altering the functions of bio-membranes, which can leads to the development of many pathological processes [25]. Cd interferes with intracellular signaling network and gene regulation at multiple levels. Some of the specific changes that lead to tissue damage and death in exposure of Cd have been related to oxidative stress and thiol depletion [6]. Furthermore, *in vitro* Cd2+ exposure caused cell death, ROS generation, activation, apoptosis, and finally inhibitors of cellular signaling pathways [29]. Studies with Cd revealed that the primary route for Cd toxicity is the depletion of GSH and binding of Cd to - SH groups of proteins. The depletion of cellular sulfhydryl reserves seems to be an important indirect mechanism for oxidative stress induced by Cd [30]. Suppression of free radical scavenging function and the enhancement of ROS contributes to Cd induced oxidative stress, LPO and its associated toxic effects [7,8]. LPO is thought to be an important mechanism of the cell membrane injury and MDA is one of its end-products, which is generated during the oxidative degradation of lipids [31]. The enhanced LPO might result from the reduction in the activities of CAT, SOD and GSH observed in our work, since these antioxidant enzymes protect from this process via elimination of ROS. Cd induced liver damage is associated with

Table 3: Some selective liver function markers (mean±SE) 30 days post-treatment with Cd and Se

Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (g/dl)	Albumin (g/dl)
Control	41.2±3.29^{c}	52.8±3.56^{c}	118.2±9.21^{a}	7.56±0.49^{a}	3.72±0.28^{a}
Cd 20	62.5±5.41^{b}	69.1±5.28^{b}	122.4±8.14^{a}	6.25±0.38^{b}	2.54±0.21^{b}
Cd 40	82.4±6.32^{a}	86.1±6.22^{a}	127.1±9.16^{a}	6.12±0.41^{b}	2.32±0.28^{b}
Cd 20 and Se	45.1±5.12^{cd}	56.4±5.21^{cd}	125.6±7.85^{a}	7.41±0.34^{a}	3.51±0.31^{ac}
Cd 40 and Se	54.1±5.22^{d}	62.5±7.12^{d}	121.2±8.15^{a}	7.25±0.29^{a}	3.01±0.25^{c}

^{a,b,c,d}Means in the same column not followed by the same letter differ significantly ($P<0.05$). ALT: Alanine transaminase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SE: Standard error, Se: Selenium, Cd: Cadmium

Table 4: Some selective kidney function markers (mean±SE) 30 days post-treatment with Cd and Se

Group	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	40.1±4.45[b]	0.46±0.12[b]	0.92±0.16[a]
Cd 20	55.9±3.15[a]	0.75±0.11[a]	1.12±0.11[a]
Cd 40	59.1±3.29[a]	0.82±0.13[a]	1.18±0.14[a]
Cd 20 and Se	42.2±3.25[b]	0.51±0.15[b]	1.01±0.18[a]
Cd 40 and Se	44.6±5.26[b]	0.58±0.18[b]	1.10±0.12[a]

[a,b]Means in the same column not followed by the same letter differ significantly ($P<0.05$). SE: Standard error, Se: Selenium, Cd: Cadmium

increased LPO [32-34]. In the current study, GSH, CAT, SOD antioxidant markers and MDA in Cd and Se treated groups show insignificant change when compared with the control group. Se supplementation may prevent the formation of free radicals and the process of LPO [35]. The protective effect of Se against Cd induce tissues damage could be attributed it is own antioxidant activity and enhanced the cellular antioxidant enzymes [36].

Cd produces a variety of health hazards in humans and animals due to its ability to induce severe alterations in various organs and tissues including nervous system [25]; meanwhile, liver and kidney are considered as the main targets of Cd induced toxicity [26,37]. The hepatotoxicity of Cd demonstrated by alteration of serum hepatic markers in Cd administrated groups. There was a significantly increased in ALT and AST serum activities, while a significant decreased in total protein and albumin; meanwhile, non-significant changed in alkaline phosphatase in Cd treated groups in compared with the control group. A significant increased hepatic markers, ALT, AST, ALP, total bilirubin in rats received Cd chloride (5 mg/kg bw) for 28 days [25]. Furthermore, ALT and AST were elevated in rats intraperitoneal injected with Cd 2 mg/kg/day for 8 days [38]. This elevation could be attributed to liver damage included swollen and ruptured parenchymal cells leukocytes infiltration, and focal necrosis. The tissues damage, could attributed to, during Cd exposure most of the antioxidant enzymes become inactive due to its binding to the active sites of the enzyme containing –SH groups, result in enhances ROS [39]. On the other hand, Obianime and Roberts [40] recorded decrease serum ALP in the rat after 4 h of Cd administration at a dose 40 mg/kg bw. The hypoproteinemia and hypoalbuminemia in the present work could be attributed to liver damage and proteinuria as a renal dysfunction in laboratory animals is commonly reported in Cd toxicity [37].

In the present work, the Se ameliorate Cd hepatotoxicity in Cd and Se treated group. Several mechanisms could be operating in the protective action of Se, which could result, for example, in changed absorption of the Cd or in a change in their action and distribution in the organism and within target organs. The protective mechanism of antioxidants and Se in Cd-induced tissues damage has been documented by several authors [36,38,40].

The kidney is generally recognized as the most critical organ affected by chronic exposure to Cd. Renal dysfunction in laboratory animals is commonly reported in Cd toxicity. Cd reaches the kidney in the form of Cd-metallothionein is filtrated in the glomerulus, and subsequently reabsorbed in the proximal tubules. It then remains in the tubules cells result in tubular damage [41]. Concerning to the renal markers, urea and creatinine blood level elevated in groups intoxicated with Cd. Elevated serum level of urea and creatinine reported in rats received 40 mg/kg bw [40]. Proteinuria and histopathologic damage have been observed at doses ranging from 1.8 to 2.5 mg/kg bw/day Cd in rats [37]. Moreover, chronic environmental oral exposure to Cd in humans leads to renal failure, characterized by proteinuria due to renal tubular dysfunction [42]. The urea and creatinine in the Cd and Se treated groups was none significantly changed when compared with the control group. The evidence of Se ameliorate the renal damage in Cd intoxicated rat by reducing the urea and creatinine serum level demounted by [40]. The protective effect of Se against Cd toxicity could be attributed to it is antioxidant activity. Interactions between nontoxic levels of dietary Se and relatively high levels of dietary Cd apparently resulted in partial amelioration of Cd toxicity in different systems [43].

CONCLUSION

Our results demonstrated that Cd is capable of causing marked oxidative stress in addition inhibiting the activities of antioxidant enzymes. The treatment with Se could significantly attenuate the Cd induced immunosuppressive oxidative stress as well as hepatotoxicity and renal damage.

REFERENCES

1. Järup L. Cadmium overload and toxicity. Nephrol Dial Transplant 2002;17 Suppl 2:35-9.
2. Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PE, Williams DJ, *et al*. A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 2003;137:65-83.
3. Brzóska MM, Moniuszko-Jakoniuk J. Low-level exposure to cadmium during the lifetime increases the risk of osteoporosis and fractures of the lumbar spine in the elderly: Studies on a rat model of human environmental exposure. Toxicol Sci 2004;82:468-77.
4. Pillet S, D'Elia M, Bernier J, Bouquegneau JM, Fournier M, Cyr DG. Immunomodulatory effects of estradiol and cadmium in adult female rats. Toxicol Sci 2006;92:423-32.
5. Koller LD. Cadmium. In: Zelikoff JT, Thomas PT, editors. Immunotoxicology of Environmental and Occupational Metals. London: Taylor & Francis; 1998. p. 41-61.
6. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: Mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem 2001;1:529-39.
7. Thévenod F, Friedmann JM, Katsen AD, Hauser IA. Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappaB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. J Biol Chem 2000;275:1887-96.
8. Szuster-Ciesielska A, Stachura A, Slotwinska M, Kaminska T, Sniezko R, Paduch R, *et al*. The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell cultures. Toxicology 2000;145:159-71.
9. Agency for Toxic Substances and Disease Registry ATSDR. Toxicological Profile for Cadmium. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 1999.
10. Zwolak I, Zaporowska H. Selenium interactions and toxicity: A review. Selenium interactions and toxicity. Cell Biol Toxicol 2012;28:31-46.
11. Galicka A, Brzóska MM, Sredzinska K, Gindzienski A. Effect of cadmium on collagen content and solubility in rat bone. Acta Biochim Pol 2004;51:825-9.

12. Aboul-Soud MA, Al-Othman AM, El-Desoky GE, Al-Othman ZA, Yusuf K, Ahmad J, *et al*. Hepatoprotective effects of vitamin E/selenium against malathion-induced injuries on the antioxidant status and apoptosis-related gene expression in rats. J Toxicol Sci 2011;36:285-96.
13. SPSS. Statistical Package for Social Science, Computer Software, Ver. 16. Company. London, UK: SPSS; 2008.
14. Marth E, Barth S, Jelovcan S. Influence of cadmium on the immune system. Description of stimulating reactions. Cent Eur J Public Health 2000;8:40-4.
15. Blakley BR. The effect of cadmium chloride on the immune response in mice. Can J Comp Med 1985;49:104-8.
16. Låg M, Rodionov D, Ovrevik J, Bakke O, Schwarze PE, Refsnes M. Cadmium-induced inflammatory responses in cells relevant for lung toxicity: Expression and release of cytokines in fibroblasts, epithelial cells and macrophages. Toxicol Lett 2010;193:252-60.
17. Freitas M, Fernandes E. Zinc, cadmium and nickel increase the activation of NF-κB and the release of cytokines from THP-1 monocytic cells. Metallomics 2011;3:1238-43.
18. Abbès S, Salah-Abbès JB, Nahdi K, Younes RB, Hetta MM, El-Kady AA, *et al*. Inactivation of cadmium induced immunotoxicological alterations in rats by Tunisian montmorillonite clay. Int Immunopharmacol 2007;7:750-60.
19. Eze JI, Ngene AA, Anosa GN, Ezeh IO. Immunomodulatory effect of dietary selenium supplementation on serum antibody response and leucocytic profile of *Trypanosoma brucei brucei* infected rats. Afr J Biotechnol 2011;10:12098-106.
20. Guo Y, Pan D, Li H, Sun Y, Zeng X, Yan B. Antioxidant and immunomodulatory activity of selenium exopolysaccharide produced by *Lactococcus lactis* subsp. lactis. Food Chem 2013;138:84-9.
21. Musik I, Koziol-Montewka M, Toś-Luty S, Pasternak K, Latuszyńska J, Tokarska M, *et al*. Immunomodulatory effect of selenosemicarbazides and selenium inorganic compounds, distribution in organs after selenium supplementation. Biometals 1999;12:369-74.
22. Koller LD, Exon JH, Talcott PA, Osborne CA, Henningsen GM. Immune responses in rats supplemented with selenium. Clin Exp Immunol 1986;63:570-6.
23. Rana SV, Allen T, Singh R. Inevitable glutathione, then and now. Indian J Exp Biol 2002;40:706-16.
24. Husain SR, Cillard J, Cillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987;26:2489-91.
25. Milton SP, Shagirthab K, Renugadevia J. Quercetin in combination with vitamins (C and E) improve oxidative stress and hepatic injury in cadmium intoxicated rats. Biomed Prev Nutr 2011;1:1-7.
26. Tandon SK, Singh S, Prasad S, Khandekar K, Dwivedi VK, Chatterjee M, *et al*. Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. Toxicol Lett 2003;145:211-7.
27. Morales AI, Vicente-Sánchez C, Sandoval JM, Egido J, Mayoral P, Arévalo MA, *et al*. Protective effect of quercetin on experimental chronic cadmium nephrotoxicity in rats is based on its antioxidant properties. Food Chem Toxicol 2006;44:2092-100.
28. Pharikal K, Das PC, Dey CD, Dasgupta S. Tissue ascorbate as a metabolic marker in cadmium toxicity. Int J Vitam Nutr Res 1988;58:306-11.
29. Kim SJ, Jeong HJ, Myung NY, Kim MC, Lee JH, So HS, *et al*. The protective mechanism of antioxidants in cadmium-induced ototoxicity *in vitro* and *in vivo*. Environ Health Perspect 2008;116:854-62.
30. Pari L, Murugavel P. Role of diallyl tetrasulfide in ameliorating the cadmium induced biochemical changes in rats. Environ Toxicol Pharmacol 2005;20:493-500.
31. Thampi BS, Manoj G, Leelamma S, Menon VP. Dietary fiber and lipid peroxidation: Effect of dietary fiber on levels of lipids and lipid peroxides in high fat diet. Indian J Exp Biol 1991;29:563-7.
32. Jin T, Nordberg M, Frech W, Dumont X, Bernard A, Ye TT, *et al*. Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad). Biometals 2002;15:397-410.
33. Shimada H, Takamure Y, Shimada A, Yasutake A, Waalkes MP, Imamura Y. Strain differences of cadmium-induced hepatotoxicity in Wistar-Imamichi and Fischer 344 rats: Involvement of cadmium accumulation. Toxicology 2004;203:189-97.
34. El-Sharaky AS, Mohammed GB, Kandeel KM, Wahby MM. An electrochemical approach to characterization of metallothione: Direct evidence of interaction of cadmium with cysteine residue. In: Proceedings of the XXV-International Conference on Co-ordination Chemistry, Nanjing, China; 1987, July 26-31.
35. Rana SV, Verma S. Protective effects of GSH, alpha-tocopherol, and selenium on lipid-peroxidation in liver and kidney of copper fed rats. Bull Environ Contam Toxicol 1997;59:152-8.
36. Saïd L, Banni M, Kerkeni A, Saïd K, Messaoudi I. Influence of combined treatment with zinc and selenium on cadmium induced testicular pathophysiology in rat. Food Chem Toxicol 2010;48:2759-65.
37. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Cadmium. Draft for public comment. Atlanta, US: US Department of Health and Human Services; 2008.
38. Koyuturk M, Yanardag R, Bolkent S, Tunali S. The potential role of combined anti-oxidants against cadmium toxicity on liver of rats. Toxicol Ind Health 2007;23:393-401.
39. Quig D. Cysteine metabolism and metal toxicity. Altern Med Rev 1998;3:262-70.
40. Obianime AW, Roberts II. Antioxidants, cadmium-induced toxicity, serum biochemical and the histological abnormalities of the kidney and testes of the male Wistar rats. Niger J Physiol Sci 2009;24:177-85.
41. Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, *et al*. The toxicity of cadmium and resulting hazards for human health. J Occup Med Toxicol 2006;1:22.
42. Environment Agency (EA). Contaminants in soil: Updated Collation of Toxicological Data and Intake Values for Humans. Cadmium. Science Report: SC050021/TOX 3. Environment Agency; Bristol, UK: 2009.
43. Meyer SA, House WA, Welch RM. Some metabolic interrelationships between toxic levels of cadmium and nontoxic levels of selenium fed to rats. J Nutr 1982;112:954-61.

Source of Support: Nil, Conflict of Interest: None declared.