EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
29-34
Year: 2017

REDUCTION IN ORGANS CADMIUM RETENTION IN MALE RATS: THE ROLE OF *ALLIUM CEPA*

Serah Funke Ige[1]*, Jelili A. Badmus[2], Kamoli Adekunle Oyedokun[1], Joy Chineye Okeke[1]

[1]Department of Physiology Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

***Corresponding Author: Dr. Serah Funke Ige**
Department of Physiology Faculty of Basic Medical Sciences, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Article Received on 26/04/2017 **Article Revised on 16/05/2017** **Article Accepted on 06/06/2017**

ABSTRACT
Cadmium has been known to be associated with various organs toxicity. Cadmium-induced organs toxicity arises from cadmium retention in those organs. Most of the organs toxicities have been ascribed to cadmium induced oxidative stress. *Allium cepa* is a known antioxidant that has been reported to ameliorate cadmium-induced organ toxicity. Therefore this study was carried out to investigate the effect of *Allium cepa* in cadmium retention of selected organs. Twenty four rats were randomly grouped into four groups of six rats each. Group 1, 2, 3, and 4 were given deionized water, 0.3ml of 0.05 mg/mL $CdSO_4$, 1.0mL/100g BW *Allium cepa* for seven days before 0.3ml of 0.05 mg/mL $CdSO_4$ for 21 days, and *Allium cepa* co-treated with cadmium for 21 days respectively. All the treatments were given orally. Blood, liver, duodenum, kidney and testes were analyzed for cadmium concentration, zinc concentration, catalase activity, superoxide dismutase activity and lipid peroxidation. Treatment with cadmium resulted in significant: decrease in percentage weight gain, increase in the organs cadmium retention, decrease in Zinc/Cadmium concentration ratio, decrease in catalase and superoxide dismutase activities, increase lipid peroxidation, co-treatment of cadmium treated animals with *Allium cepa* ameliorate all these effects of cadmium. This study gives evidence that cadmium retention in organs were reduced by *Allium cepa* as a result of its ability to reduce oxidative stress. The reduction in oxidative stress was brought about by increase in the zinc concentration in organs.

KEYWORDS: Zinc, Oxidative stress, superoxide dismutase, *Allium cepa.*

INTRODUCTION
Cadmium is one of the heavy metals that are highly toxic to both humans and animals. It has been known to be associated with various organs toxicity such as renal, hepatic and gonads.[1,2,3] It accumulates in various organs over time with biological half-life of 20-30 years.[4] Most of cadmium-induced organs toxicities have been ascribed to oxidative stress induction[5] which occurs as a result of high levels of cadmium retention.[6]

Two major routes of cadmium poisoning are the diet and the environment. Examples of diet containing cadmium include cereals and vegetables.[7] meat (liver and kidney).[8] The environment route includes cigarettes smoking[9] and workplace exposure such as plastic industries, refineries of metals (zinc refining, lead refining *e.t.c)*, metal plating with cadmium-containing materials, productions of metals (iron, steel), nickel-cadmium battery manufacturing, production of cadmium alloys, cadmium containing pigment production and combustion of fossil fuels.[10-13]

Cadmium absorption from diet is high in the duodenum and less in the other part of the intestine.[14] After intestinal absorption from diets, it is transported to the liver before distributed to other organs with the highest cadmium burden in liver and kidney.[15]

Allium cepa is a potent antioxidant that is rich in flavonoids, reducing sugars, carbohydrates, glycosides, acid compounds, proteins, alkaloids, saponins and oils[16] and and quercetin.[17] It has been reported to play important roles in the amelioration of hepatotoxicity[2], nephrotoxicity[1] and testicular toxicity.[3]

Zinc (Zn) is also a known cofactor of superoxide dismutase (SOD) enzyme and its deficiency has been shown to cause oxidative stress [18]. Likewise, cadmium-induced organ toxicity arises through oxidative stress when there is deficiency of Zn. Therefore this study was carried out to investigate the effect/s of *Allium cepa* in cadmium distribution and retention in selected organs of

rats after oral exposure with reference to Zinc/Cadmium ratio and SOD activity.

MATERIALS AND METHODS

Animals

Twenty four male Wistar rats weighing 150-180g obtained from Animal Holdings of the Department of Physiology Ladoke Akintola University of Technology, Nigeria were used for this study. All the animals were allowed to rat pellet diet and water *ad libitum.* They were randomly grouped into four groups of six rats each. Control group were given deionized water only. Cadmium group were treated with 0.3ml of 0.05 mg/mL $CdSO_4$ for twenty one days. *Allium cepa* pre-treated (AcE Pre-treat) group received 1.0mL/100g BW *Allium cepa* for seven days follow by twenty one days of 0.05 mg/mL $CdSO_4$. *Allium cepa* co-treated with cadmium group (Ace Co-treat) received 1.0mL/100g BW *Allium cepa* and 0.3ml of 0.05 mg/mL $CdSO_4$ simultaneously for twenty one days. All the treatments were given orally.

Allium cepa extract preparation

Allium cepa extract was prepared following procedure from previous studies.[1-2] Briefly, *Allium cepa* (common onion) bulbs were rinsed thoroughly in distilled water, air dried, and 200g was then blended. Juice was then filtrated and squeezed out of it using a tight sieve. The filtrate juice was prepared daily.

Sample collection and *preparation.*

Blood samples were collected by cardiac puncture. Liver, duodenum, kidney and testes were harvested and weighed. Samples were prepared according to Uyanik *et al.*,[19] method with slight modification. Blood samples were centrifuge at 3500 rev/min for thirty minutes. Then, 2.5mL of nitric acid was added into 0.5mL of serum in a digestion vessel. Organs were also digested by adding 2.5mL of nitric acid into 0.5mg of each sample in a digestion vessel. The vessels were orderly arranged in Q-block wireless digester (Questron Technology Corp, 7-6725, Ontario, Canada.) regulated to 100^0C and the digestion was carried out for three hours. Immediately after the digestion, the samples were diluted with 25.0mL of deionized water then filtered into other separate covered polypropylene bottles.

Determination of cadmium and zinc concentration

Cadmium concentration in each sample were determined using atomic absorption spectrophotometer (AAS) fixed with cadmium hollow cathode lamp. Readings were done at 228.9nm wavelength. Cadmium concentration in each sample was determined by multiplied the readings with the dilution factor (25/0.5). Concentration of zinc was also determined by atomic absorption spectrophotometer (AAS) fixed with zinc hollow cathode lamp.

Biochemical analysis

Superoxide dismutase activity was determined spectrophotometrically according to Mistra and Fridovic[20] method. Catalase activity was determined according to the method of Aebi.[21] Estimation of lipid peroxidation was determined based on the reaction of MDA with thiobarbituric acid (TBA) forming MDA–TBA2 according to the method of Varshney and Kale.[22]

Statistical analyses

Data were expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) was used to analyze for the significance of differences between means followed by Student's t-test. Level of significance was at a *p*-value of less than 0.05.

Ethics

Animals used in this study received care according to the institution guidelines, so also the guidelines for care of animals as outlined in the United State National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication No85-23) was followed.

RESULTS

Effect of Allium cepa on weight gain of cadmium treated rats

There was significant decrease in percentage weight gain of animals treated with cadmium alone when compared with control, however the percentage reduction in weight gain of animals treated with *Allium cepa* did not significantly different from both control and cadmium treated groups, Table 1.

Table 1: Effect of *Allium cepa* on weight gain

Group	Control	Cd	Ace Pre-treat	Ace Co-treat
Initial Weight (g)	159.8 ± 5.2	166.3 ± 4.82	158.13 ± 4.7	153.57 ±4.5
Final Weight(g)	202.0 ± 8.2	187.3 ± 7.8	185.3 ± 6.7	189.2 ± 6.7
Weight Changes (g)	42.2 ± 1.7	21.0 ±1.6*	27.2 ± 6.2	35.6 ± 10.3
% Weight gain (%)	26.41	12.63	17.2	23.18

*p<0.05 vs control
$^+$p<0.05 vs Cd

Effect of Allium cepa on rats organs cadmium concentration.

Cadmium exposure caused significant increase in the organs cadmium retention. Simultaneous treatment of cadmium treated animals with *Allium cepa* significantly reduced the cadmium retention. (Table 2).

Table 2: Effect of *Allium cepa* on organs cadmium concentration

Group/Organ	Control (mg/kg)	Cd (mg/kg)	Ace Pre-treat (mg/kg)	Ace Co-treat (mg/kg)
Duodenum	0.4±0.03	1.7±0.01*	1.6±0.08*	0.8±0.05*+
Liver	0.6±0.02	2.4±0.05*	2.3±0.06*	1.4±0.03*+
Kidney	0.5±0.02	2.3±0.06*	2.4±0.04*	1.4±0.06*+
Testes	0.2±0.04	0.5±0.02*	0.4±0.02*	0.3±0.04+
Blood	0.1±0.05	2.8±0.06*	2.4±0.2*	0.2±0.03+

*p<0.05 vs control, +p<0.05 vs Cd

Effect of Allium cepa on Zn/Cd concentration ratio in rats organs

Zinc/Cadmium concentration ratio was significantly reduced in organs of cadmium treated animals. Co-treatment of the animals with *Allium cepa* significantly increased the Zn/Cd ratio when compared with the cadmium only treated animals. There was no significant difference in Zn/Cd ratio in the testes of *Allium cepa* co-treated animals when compared with the control, (Table 3).

Table 3: Effect of *Allium cepa* on organs Zn/Cd concentration ratio

Group/Organ	Control (mg/kg)	Cd (mg/kg)	Ace Pre-treat (mg/kg)	Ace Co-treat (mg/kg)
Duodenum	32.9 ± 2.1	17.5 ± 0.5*	8.7 ± 0.6 *+	28.25 ± 1.4+
Liver	64.1 ± 2.4	11.31 ±0.6 *	9.9 ±0.2*	24.6 ±0.6*+
Kidney	42.8 ±1.9	10.5 ±0.3*	11.4 ±0.3*	13.7 ±0.4*+
Testes	107.2 ±5	66.9 ± 1.2*	49.8 ± 5.4*	93.0 ±7.8

*p<0.05 vs control
+p<0.05 vs Cd

Effect of Allium cepa on Antioxidants Activities and Lipid peroxidation in organs of Cd treated rats.

Superoxide dismutase and catalase activities were significantly reduced while lipid peroxidation was significantly increased in organs of cadmium only treated animals. Co-treatment of the animals with *Allium cepa* significantly increased SOD and catalase and reduced lipid peroxidation in duodenum, liver and kidney when compared with the cadmium only treated group. There was no significant difference in testes SOD, catalase and lipid peroxidation when compared with control and cadmium treated animals, (Table 4,5 and 6).

Table 4: Effect of *Allium cepa* on SOD activity in organs

Group/Organ	Control (U/mg protein)	Cd (U /mg protein)	Ace Pre-treat (U/ mg protein)	Ace Co-treat (U/ mg protein)
Duodenum	0.3 ± 0.05	0.1 ± 0.01*	0.2 ± 0.03	0.3± 0.07+
Liver	1.7 ± 0.16	0.2 ± 0.02*	0.4 ± 0.06*+	0.5 ± 0.05*+
Kidney	2.5 ±0.04	0.7 ± 0.04*	0.6 ± 0.11	1.0 ± 0.09*+
Testes	4.1 ± 0.6	1.2 ± 0.01 *	1.9 ± 0.62*	5.6± 3.43

*p<0.05 vs control, +p<0.05 vs Cd

Table 5: Effect of *Allium cepa* on Catalase activity in organs

Group/Organ	Control (µmol/mg protein)	Cd (µmol/mg protein)	Ace Pre-treat (µmol/mg protein)	Ace Co-treat (µmol/mg protein)
Duodenum	0.4 ± 0.05	0.2 ± 0.02*	0.7 ± 0.07*+	0.6 ± 0.03*+
Liver	0.3 ± 0.03	0.1 ± 0.01*	0.2 ± 0.03*+	0.2 ± 0.05*+
Kidney	16.8 ± 0.49	18.5 ± 0.43*	15. 8± 1.94	14..7 ± 0.89+
Testes	2.11 ± 0.31	0.9 ± 0.14*	1.1 ± 0.11*	1.8 ± 0.36

*p<0.05 vs control, +p<0.05 vs Cd

Table 6: Effect of *Allium cepa* on organs lipid peroxidation

Group/Organ	Control (µmol/mg protein)	Cd (µmol/mg protein)	Ace Pre-treat (µmol/mg protein)	Ace Co-treat (µmol/mg protein)
Duodenum	2.8 ±0.67	8.5±0.51*	5.0±0.46*+	6.10±0.38*+
Liver	2.2±0.08	4.1±0.42*	3.0±0.19*	3.3 ±0.14 *
Kidney	0.2± 0.03	0.5 ±0.04*	0.3± 0.06*	0.3 ± 0.04*+
Testes	27.5±3.71	41.1±0.58	36.1 ±2.75	37.0± 4.13

*p<0.05 vs control, +p<0.05 vs Cd

DISCUSSION

Ingestion of contaminated water is one of the routes through which humans and animals can be exposed to the cadmium. In this study, animals were exposed to cadmium through oral route, from where it can be absorbed from gut and then transported by blood to other organs of the body. This study revealed a significant reduction in weight gain in the animals as a result of cadmium administration. This is in agreement with the previous study by Nadir *et al.*,[23], where administration of cadmium resulted in reduction in weight gain.

The reduction in weight gain as a result of cadmium administration in the current study can be suggested to be as a result of toxic effect of cadmium and consequently increase in metabolic demand. Heydarnejad and co-workers has also shown reduction in fish growth to be due to the toxicity effect of cadmium[24] and consequent increase metabolic demand. Other study by El-Missiry and Shalaby[25] also gave evidence that exposure to cadmium affect basal metabolic rate. In present study, feed and water intake of cadmium only treated rats decreased per day (data not shown) compared to the control which may be responsible for the observed decrease in their weight gain (Table 1).The decrease in feed and water intake of cadmium only treated group is in tandem with the previous work which might be one of the plausible reasons for increase in metabolic demand that cannot be met by food supply. Since previous study has made it clear that cadmium causes an increase in metabolic rate and our own study and that of other[26] established reduction in feed intake in cadmium treated rats. Therefore from our study and that of other, reduction in weight gain can be ascribed to increase in metabolic demand which cannot be met by feed supply.

The distribution pattern of organs cadmium retention in this study reveals that liver and kidney have the highest retention potential which is in agreement with previous studies.[27,28] Although, previous study averred with evidence that organs cadmium retention do not follow the same pattern.[29] However, in this study, liver has the highest cadmium retention followed by kidney, duodenum and the testis (Table 2). This supports reports from previous studies where cadmium was found to be concentrated mainly in the liver.[30] Liver being the major metabolic organ in the body which metabolizes every ingested substance can be one of the reasons why we have high content of cadmium in the liver. Likewise absorption of cadmium takes place in the liver before transported to other organs.

Transportation of cadmium in the circulation is by binding with albumin or metallothionein (Cd-MT).[31] Cadmium – methallothhionein Cd-MT) complex is non-toxic; however, organ toxicity begins when the production of MT is not enough to detoxify cadmium.[32]

Result of the present study also reveals significant reduction in Zinc concentration of cadmium treated rats compared to control, though the Zinc concentration of cadmium treated group was increase in testis compared to other organs which shows that testis has the highest Zn/Cd ratio out of the organs studied. This is in agreement with previous study of Amara et al.,[33] Previous study of Susuki et al.[34], has also reported decrease organ Zinc content by accumulation of cadmium.

Cadmium is known to alter antioxidant-oxidant balance in the body system and thereby induced oxidative stress which culminated into cadmium-induced organ toxicity.[1, 35,] It has been reported to generate reactive oxygen species (ROS) and lipid peroxidation in the organs.[36] In the present study, cadmium administration significantly decreased the catalase and superoxide dismutase activities and increased the lipid peroxidation in organs studied. Though lipid peroxidation was non - significantly increased in the testis, this may be due to high content of Zinc in the testes which had been earlier reported[33] and reveals by this study. Co-treatment of the animals with the *Allium cepa* ameliorates cadmium effects better than pre-treatment by significantly increased the catalase and superoxide dismutase activities and decreased the organs lipid peroxidation. This effect of *Allium cepa* can be attributed to the quercetin component of it because it has been reported to contain quercetin which can scavenge free radicals and chelating transitional metal ions - catalysts of oxidative processes.[17]

Significant reduction of SOD an antioxidant enzyme and increase in lipid peroxidation occasioned by cadmium treatment in this study may be useful evidence of increase oxidative stress in those organs.[37] This is in agreement with previous study where cadmium caused reduction in SOD activities.[1]

Zinc deficiency is related to oxidative stress and DNA damage and its supplement has been found to alleviate cadmium-induced oxidative stress.[17, 33, 36] Zinc is a known co-factor of SOD and competes with cadmium binding site of enzymatic protein and aids the elimination of the metalloid thereby ameliorate its toxicity.[36] It has also been shown to induce the synthesis of metallothionein[36] a protein with high affinity for cadmium which can cause detoxification of cadmium by binding with it.[36] The present study shows a significant decrease in Zn/Cd ratio in the selected organs while co-treatment of the animals with *Allium cepa* and cadmium significantly increased the Zn/Cd ratio when compared with the cadmium only treated animals.

CONCLUSION

This study shows that *Allium cepa* has ability to reduce organ cadmium retention and increase organ Zn/Cd ratio which leads to possible amelioration of cadmium toxicity.

ACKNOWLEDGEMENTS
The authors sincerely acknowledge the financial assistance of Oyedokun and Okeke families.

Conflict of Interest*:* Nil.

REFERENCES

1. Ige SF, Akhigbe RE, Adewale AA, Badmus JA, Olaleye SB, Ajao FO, Saka WA, Owolabi OQ Effect of *Allium cepa* (Onion) extract on cadmium-induced nephrotoxicity in rats. Kidney Res. J., 2011; 1(1): 41–47.
2. Ige SF, Akhigbe RE, Edeogho O, Ajao FO, Owolabi OQ, Oyekunle OS, Ajayi AF Hepatoprotective activities of *Allium cepa* in cadmium-treated rats. Int J Pharm Pharm Sci., 2011; 3(*5): 60-63.*
3. Ige SF, Olaleye SB, Akhigbe RE, Akanbi TA, Oyekunle OA, Udoh US Testicular toxicity and sperm quality following cadmium exposure in rats: Amelorative potentials of *Allium cepa*. J Hum Repro Sci., 2012; 5: 37-42.
4. Flora SJ, Mittal M, Mehta A Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Indian J Med Res., 2008; 128: 501–23.
5. Anetor JL Rising environmental cadmium levels in developing countries: Threat to genome stability and health. Niger. J. Physiol. Sci., 2012; 27: 103-115.
6. [6]. Reynders H, Van Campenhout K, Bervoets L, De Coen WM, Blust R Dynamics of cadmium accumulation and effects in common carp (Cyprinus carpio) during simultaneous exposure to water and food (Tubifex tubifex). *Environ. Toxicol. Chem.*, 2006; 25: 1558–1567.
7. Baldini M, Stacchini P, Cubadda F, Miniero R, Parodi P and Facelli P Cadmium in organs and tissues of horses slaughtered in Italy. Food Addit. Contam, 2000; 17: 679-687.
8. Coni E, Baldaini M, Stacchini P and Zanasi F Cadmium intake with the diet in Italy: apilot study. J. Trace Elem. Electrolytes Health Dis., 1992; 6: 175-181.
9. Jarup L, Berglund M. Elinder CG, Nordberg G. and Vahter M Health effects of cadmium exposure--a review of the literature and a risk estimate. Scand. J. Work Environ. Health, 1998; 24(1): 1-51.
10. Ellis KJ, Cohn SH, Smith TJ Cadmium inhalation exposure estimates their significance with respect to kidney and liver cadmium burden. J Toxicol Environ Health, 1985; 15: 173-87.
11. Ellinder CG, Edling C, Linddberg E, Kagedal B, Vesterberg O Assessment of renal function in workers previously exposed to cadmium. Br J Ind Med., 1985; 42: 754-60
12. Zwennis WC, Franssen AC Assessment of occupational exposure to cadmium in the Netherlands, 1980-1989. Am J Ind Med., 1992; 21: 793-805
13. Taylor MD Accumulation of Cadmium derived from fertilizers in New Zealand soils. Sci Total Environ, 1997; 208: 123-6.
14. Reeves PG, Chaney RL, Simmons RW, Cherian MG Metallothionein Induction is not involved in cadmium accumulation in the duodenum of mice and rats fed diets containing high-cadmium rice or sunflower kernels and a marginal supply of zinc, iron and calcium. J. Nurt, 2005; 135: 99.
15. Akyolcu MC, Ozcelik D, Dursun S, Toplan S, Kahraman R Accumulation of cadmium in tissue and its effects on live performance. J. Phys.1V France, 2003; 107: 33-36.
16. Ugwoke CEC, EzugweCO Phytochemical screening and promiximate composition and onion bulb (*Allium cepa* L). Journal of Pharmaceutical and Allied Sciences, 2010; 7(2). ISSN:1596-8499.
17. Tsanova-Savova *S.* Biologically active composition and health impact of *Allium cepa*. *Acta Medica Bulgarica*, 2011; *XXXVIII: 1.*
18. Ho E, Courmanche C and Ames BN. Zinc deficiency induceds oxidative DNA damage, and increases, 53 expression in human lung fibroblasts. J. Nutr., 2003; 133: 2543-8.
19. Uyanik F, Even M, Atasever A, Tuncoku G, Kolsuz AH. Changes in some Biochemical Parameters and organs of broilers exposed to cadmium and effect of zinc on cadmium induced alterations. Israel J Vet Med., 2001; 56: 128–34.
20. Fridovich I. Superoxide dismutases. Adv Enzymel Relat Area Mol Biol., 1986; 58: 61-97.
21. Aebi, H. Catalase in vitro. Methods Enzymol, 1984; 105: 121–126.
22. Varshney R, Kale RK. Effects of calmodulin antagonists on radiationinduced lipid peroxidation in microsomes. Int J Radiat Biol., 1990; 58: 733-43.
23. Nadir R, and Suat E. Oral administration of lycopene reverses cadmium-suppressed body weight loss and lipid peroxidation in rats. Biol Trace Elem Res., 2007; 118: 175-183
24. Heydarnejad MS, Khosravian-Hemamai M, and Nematollahi A. Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). Ir Vet J., 2013; 66(1): 11.
25. El-Missiry MA and Shalaby F. Role of β-carotene in ameliorating the cadmium-induced oxidative stress in rat brain and testis. J Biochem Mol Toxicol, 2000; 14: 238–243.
26. Borgmann U, Ralph KM. Effects of cadmium, 2,4-dichlorophenol and pentachlorophenol on feeding, growth and particle-size-conversion efficiency of white sucker larvae and young common shiners. Arch Environ Cont Toxicol, 1986; 15: 473–480.
27. Munga A, Xhaxhiu D, Laci D, Dova I. Cadmium accumulation in the main organs during a chronic exposure. Natura Montegrina Podgorica, 2010; 9: 719–30.

28. Johri N, Jacquillet G, Unwin R. Heavy metal poisoning: The effects of cadmium on kidney. Biometals, 2010; 23: 783–92.
29. Grosicki A. Influence of vitamin C on cadmium absorption and distribution in rats. J Trace Elem Med Biol., 2004; 18: 183–187.
30. Damek-Poprawa M and Sawika-Kapusta K. Damage to the liver, kidney, and testis with reference to burden of heavy metals in yallo-necked mice from areas around steel workers and zinc smelters in Poland. Toxicology, 2003; 186: 1–10.
31. Yang H and Shu Y. Cadmium Transporters in the Kidney and Cadmium-Induced Nephrotoxicity. *Int. J. Mol. Sci.*, 2015; *16:* 1484-1494. doi:10.3390/ijms16011484
32. Klaassen CD, Jie L, and Diwan BA. Metallothionein Protection of Cadmium Toxicity. Toxicol Appl Pharmacol. Aug 1, 2009; 238(3): 215–220.
33. Amara, S.; Abdelmelek, H.; Garrel, C.; Guiraud, P.; Douki, T.; Ravanat, J.L.; Favier, A.; Sakly, M.; Ben, R.K. Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J. Reprod. Dev.*, 2008; *54:* 129–134.
34. Suzuki, C.A.; Ohta, H.; Albores, A.; Koropatnick, J.; Cherian, M.G. Induction of metallothionein synthesis by zinc in cadmium pretreated rats. *Toxicology*, 1990; *63:* 273–284.
35. Manca D, Ricard AC, Trottier B andChevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology, 1991; 67: 303-23.
36. Jamakala O and Rani UA. Amelioration Effect of Zinc and Iron Supplementation on Selected Oxidative Stress Enzymes in Liver and Kidney of Cadmium-Treated Male Albino Rat. Toxicol Int., 2015; 22(1): 1–9.
37. Zhang J, Shen H, Wang X, Wu J, Xue Y. Effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of fresh water fish Carassius auratus. Chemosphere, 2004; 55: 167–74.