NISEB Journal Vol. 15, No. 2, June, 2015 1595-6938/2015
Printed in Nigeria (2015) Society for Experimental Biology of Nigeria
http://www.nisebjournal.org

Vitamin A induced Changes in Ocular Tissue Lipid Peroxidation and Antioxidant Enzymes Activities of Rabbits in Cadmium Toxicity

Aguebor-Ogie B.[1], Olubodun SO[1], Odjimogho ES[2], and Eriyamremu GE[3].
[1]Department of Medical Biochemistry, University of Benin, P.M.B. 1154, Benin City, Nigeria.
[2]Department of Optometry, University of Benin, P.M.B. 1154, Benin City, Nigeria.
[3]Department of Biochemistry, University of Benin, P.M.B. 1154, Benin City, Nigeria.

Abstract
The study investigated the effect of oral administration of vitamin A on antioxidant enzyme {superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gln Px)} activities and malondialdehyde (MDA) levels in rabbits exposed to ocular cadmium (Cd). Twenty New Zealand rabbits weighing between 1670 – 1810g were assigned to four groups of animals containing 5 rabbits each. The rabbits were placed in wire meshed cages and allowed to acclimatize to laboratory conditions for two weeks with free access to food and water throughout the study. One group of rabbits which served as control, was fed with chow and administered with eye drops of 2mgkg^{-1} body weight Cd (as $3CdSO_4.8H_2O$). The remaining two groups of animal treatments were carried out in accordance with the principles of laboratory animal care. The study lasted for four weeks and the cornea, retina and lens of the eyes recovered for assay. The result revealed that the significant reduction ($P<0.05$) in the weight of rabbits exposed to cadmium was improved by administration of Vitamin A. However, 500mg vitamin A reduced feed intake. The observed accumulation of cadmium in the eye tissues which resulted in increased levels of malondialdehyde (oxidative damage) was ameliorated to levels comparable to control when treated with Vitamin A supplements. Some antioxidant enzymes activities in the eye tissues were also significantly ($P<0.05$) reduced with exposure to cadmium when compared with control. Vitamin A improved the activities of these enzymes particularly with lens SOD and CAT. The effects of vitamin A was dose dependent with the rabbits given 500mg vitamin showing remarkable recovery of the SOD and CAT activities to levels which compared favourably with those observed in the control. The study therefore reveals that Vitamin A supplement at different doses can enhance the antioxidant enzyme capacity in cadmium toxicity.

Keywords: Vitamin A, ocular, peroxidation, enzymes, rabbits, cadmium, toxicity

Introduction
Metals that belong to the class B border-line transition elements of the periodic table are termed heavy and toxic metals vis-à-vis dose concentration (1). Under experimental conditions, class B metals complexes with sulfur and Nitrogen-containing ligands which make protein a potential partner to such metals (1). Exposure to heavy metals has been shown to cause hazardous health difficulties especially to ocular tissues which happen to be the first hit (2). It is present in cigarette smoke and it is one of the major sources of cadmium exposure for people in the general population (3).

The element is readily distributed in tissues after exposure, where it has consistently been shown to result in protein architecture disorientation and cataractogenesis (4). Cataracts are associated with glutathione deficiency in the lens epithelium. This finding indicate that glutathione normally functions in the protection of the lens and lens epithelium against oxidative injury, suggesting that procedures that increase lens glutathione levels might be useful for prevention of other types of cataract (5). Cadmium generates reactive oxygen species and also inhibits antioxidant enzymes such as glutathione peroxidase, superoxide dismutase (SOD) and catalase (6 and 7). Vitamin A, being a fat-soluble vitamin ingested from diet in two forms, as retinol from animal sources such as milk, meat, fish, liver and eggs or as pro-vitamin of carotene from plant sources such as green plants leafy vegetables, yellow fruits and red palm oil. Vitamin A supplement has been implicated and reported to maintain the integrity of membrane of tissues and spare them from oxidation by reactive species generated by exposure to environmental toxicants owing to its oxidants scavenging property (8). Few reports are readily available on Cd induced cataract and ocular antioxidant enzymes. Recent study have shown that palm oil can alter Cd accumulation, antioxidant enzymes and ATPases in ways which suggested that it offers protection of the eyes from ocular exposure to Cd (9). As a follow up, the present experiment was designed to test for the effect of ocular exposure of Cd and oral administration of vitamin A on SOD, catalase, glutathione peroxidase activities and malondialdehyde (MDA) levels in rabbits.

**Corresponding Author's E-mail:* georgeeriyamremu@yahoo.com

Materials and Methods

Experimental design: Twenty (20) New Zealand rabbits weighing between 1670 – 1810g were assigned to four groups of animals containing 5 rabbits each such that the average body difference between the groups was ± 2.0g. The rabbits were housed individually in wire meshed cages and allowed free access to water and food throughout the study. The rabbits were first allowed to acclimatize to laboratory conditions for two (02) weeks before the commencement of the study which lasted four (04) weeks. One group of rabbits was fed with chow and administered with eye drops of $2mgkg^{-1}$ body wt Cd (as $3CdSO_4.8H_2O$) and acted as the test control. All these animal treatment were carried out in accordance with the principles of laboratory animal care of the NIN guide for Laboratory Animal Welfare as contained in the NIN guide for grants and contracts, vol. 14, No. 3, 1985. At the end of the experimental period, the eyes of the rabbits were excised and kept in a freezing compartment at temperature of -20^oC for three days and then dissected to recover the cornea, retina and lens.

Biochemical assay: Each tissue was homogenized in chilled 0.9% NaCl solution, the homogenate was centrifuged at 3,000g for 10mins and the supernatant obtained was used for the estimation of the activities of SOD, catalase, glutathione peroxidase and MDA. The SOD was assayed by the method of Misra and Fridovich (10), while catalase was by the method of Beers and Sizer (11). The level of MDA was assayed by the method of Guttridge and Wilkins (12) which involved the determination of thiobarbituric acid reactive substances (TBARS). Values for TBARS are reported as MDA and quantitated using a molar extinction coefficient of 1.56×10^5 M/cm and expressed as µmol MDA g^1 and glutathione peroxidase by the method of Nyman (13).

Statistical analysis: The results are expressed as Means ± SEM. Statistical difference was determined using ANOVA and differences in the means were tested by Duncan's multiple range tests (14)

Results

The data on effect of vitamin A on food intake, weight gained and faecal output in rabbits exposed to cadmium is presented in Table 1. Statistical analysis of the data revealed significant reduction ($P<0.05$) in the weight of rabbits exposed to cadmium compared to the control animals. Vitamin A administration improved the weight of the animals to levels comparable with the control. Feed intake and faecal output did not significantly change with the administration of cadmium and vitamin A, however, 500mg vitamin A reduced feed intake.

Table 2 presents the effect of vitamin A on cadmium accumulation and lipid peroxidation levels in sections of rabbit eye exposed to cadmium. In the rabbits where cadmium was ocularly administered, all the tissues investigated accumulated the metal compared with the control that cadmium was not administered. Similarly, malondialdehyde level was high in these test animals. Vitamin A in a dose dependent manner reduced this indicator of lipid peroxidation to levels comparable with the control. This study demonstrates that cadmium can accumulate in eye tissues and that Vitamin A supplement has the ability to chelate free radical generated as a result of oxidative damages caused by the exposure to cadmium.

Table 1: Effect of vitamin A on the food intake and weight gain of rabbits ocularly exposed to cadmium.

Parameter	Experimental Group			
	Control Diet	Control Diet + Cadmiumm	Control Diet + Cadmium + 250mg Vitamin A	Control Diet + Cadmium + 500mg Vitamin A
Average Initial weight, g	912.0 ± 5,3	918.0 ± 5.6	913.5 ± 6.0	917.0 ± 16.0
Average Final Weight, g	1469.0 ± 10.6	1258.0 ± 13.4	1267.0 ± 10.4	1409.0 ± 16.0
Weight gain, g/day/rabbit	3.7 ± 0.4^a	2.3 ± 0.5^b	2.5 ± 0.3^b	3.3 ± 0.3^{ac}
Food Intake, g/day/rabbit	64.0 ± 5.5^a	56.4 ± 4.7^{ab}	57.9 ± 5.6^{ab}	52.4 ± 5.0^b
Faecal output, g/day/rabbit	8.7 ± 2.5^a	8.0 ± 2.3^a	8.1 ± 2.6^a	8.1 ± 2.5^a

Values are Mean ± SEM, n = 5. Means of the same row followed by different letters differ significantly ($P<0.05$)

Table 2: Effect of vitamin A on cadmium and lipid peroxidation levels in sections of rabbit eye ocularly exposed to cadmium

Parameter	Experimental Group			
	Control Diet	Control Diet + Cadmium	Control Diet + Cadmium + 250mg Vitamin A	Control Diet + Cadmium + 500mg Vitamin A
Cadmium level				
Cornea	N.D.	5.9 ± 0.6	5.0 ± 0.6	5.4 ± 0.5
Lens	N.D.	15.7 ± 1.7	13.9 ± 1.4	11.7 ± 2.0
Retina	N.D.	8.6 ± 1.5	7.9 ± 1.2	6.8 ± 1.2
Malondialdehyde				
Cornea	10.8 ± 2.0	19.9 ± 2.7	14.2 ± 2.8	13.6 ± 2.7
Lens	8.7 ± 1.3	14.2 ± 1.9	8.9 ± 1.1	6.3 ± 1.5
Retina	8.0 ± 2.2	15.4 ± 1.7	9.6 ± 1.9	6.1 ± 1.7

Values are Means ± SEM, n = 5. Means of the same row followed by different letters differ significantly ($P<0.05$)
Values of cadmium in the tissues are expressed as µg Cd/g tissue. Lipid peroxidation is presented as µmol MDA mg^{-1} protein

The data on the effect of Vitamin A on some antioxidant enzymes activities in rabbit exposure to cadmium are reported in Table 3. We observed a statistical significant ($P<0.05$) reduction in the antioxidant enzymes tested i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gln Px) with the exposure to cadmium compared with the control. Vitamin A improved the activities of these enzymes particularly with lens SOD and CAT. This effect of the vitamin was dose dependent with the rabbits given 500mg of the vitamin showing remarkable recovery of the SOD and CAT activities to levels which compared favourably with that observed in the control. The study therefore shows that Vitamin A supplement at different doses can enhance the antioxidant enzyme capacity in cadmium toxicity.

Discussion

We had earlier demonstrated that palm oil may be beneficial to rabbits in ocular cadmium toxicity (9). In our argument we tried to attribute this quality of palm oil to its B-carotene content. This study however studied the effect of an important vitamin in sight (vitamin A) in ocular cadmium toxicity.

This study and many others have consistently shown that cadmium can affect weights of animal (9 and 15) and the effect of cadmium on weight is irrespective of route of administration. Body weight is been shown to be reliable indicator of chemically induced changes in test animals (16 and 17). The reduction in the weight of the rodents given ocular cadmium is not surprising. It however reiterates that the route of exposure to this heavy metal is immaterial insofar as its effect on weight is concerned. Vitamin A obviously offered some protection from the toxic effect of the metal because it improves the weight of the animals. This effect of vitamin A on weight could not be traced to its effect on food intake, so the mechanism through which the vitamin improve the weight of the animals exposed to cadmium may be linked to its ability to act as an antioxidant vitamin. Cadmium being an inducer and sustainer of lipid peroxidation which oxidizes lipid, protein and nucleic acids (18); lead to their damage and eventually, cell death. This may have profound effect on weight. Vitamin A, being a known antioxidant vitamin can prevent lipid peroxidation by acting as substrate for lipid peroxidation process and spare cells components from oxidative damage and eventual dying. This may impart account for its ability to improve the weight of the animals.

Although, it has been reported by Livchev (19) that cadmium (Cd) does not directly generate free radicals like other heavy metals but does generate non-radical hydrogen peroxide that serves as initiator of other forms of free radicals through fenton chemical reactions. Thus, the increased levels of thiobarbituric acids reactive substances (TBARS),

Table 3: Effect of Palm Oil on Superoxide Dismutase (SOD) and Catalase activities in rabbits exposed to cadmium ocularly.

Parameter	Experimental Group			
	Control Diet	Control Diet + Cadmiumm	Control Diet + Cadmium + 250mg Vitamin A	Control Diet + Cadmium + 500mg Vitamin A
SOD				
Cornea	5.0 ± 0.8+a	2.5 ± 0.2^{b}	3.0 ± 0.6^{bc}	3.8 ± 0.7^{c}
Lens	4.1 ± 0.3^{a}	2.7 ± 0.2^{b}	2.9 ± 0.4^{b}	3.8 ± 0.4^{a}
Retina	4.1 ± 0.1^{a}	2.9 ± 0.3^{b}	3.1 ± 0.2^{b}	3.8 ± 0.2^{a}
Catalase				
Cornea	3.1 ± 0.2^{a}	1.3 ± 0.1^{b}	2.1 ± 0.3^{c}	2.2 ± 0.2^{c}
Lens	2.7 ± 0.2^{a}	1.4 ± 0.1^{b}	1.5 ± 0.3^{b}	2.3 ± 0.4^{ac}
Retina	3.0 ± 0.4^{a}	1.4 ± 0.3^{b}	2.0 ± 0.3^{bc}	2.3 ± 0.3^{ac}
Glutathione peroxidase				
Cornea	1.9 ± 0.4^{a}	1.1 ± 0.2^{b}	1.1 ± 0.3^{b}	1.4 ± 0.1^{b}
Lens	1.5 ± 0.2^{a}	1.0 ± 0.1^{b}	1.3 ± 0.2^{ab}	1.4 ± 0.2^{a}
retina	2.5 ± 0.3^{a}	1.4 ± 0.3^{b}	2.0 ± 0.4^{b}	2.0 ± 0.3^{b}

Values are Means ± SEM, n = 5. Means of the same row followed by different letters differ significantly ($P<0.05$)
Values of SOD, Catalase and Glutathione peroxidase in the tissues are expressed as enzyme Unit mg^{-1} protein.

or malondialdehyde (MDA), a known indicator of lipid peroxidation observed in this study indicates that cadmium increased lipid peroxidation in ocular tissue of animals as a result of hydrogen peroxide formed; as gradual stable ratio of antioxidant enzymes to reactive radical species (RRS) was restored with dose dependent administration of vitamin A. This study is consistent with the findings of Virk *et al*; (20) who reported supplemental vitamin C (antioxidant), Rosemary leaf extract (15, 05, 30 mg/kg) and Amla fruit extracts (100 or 200 mg/kg) markedly alleviated lipid peroxidation. However, vitamin A (a lipid soluble antioxidant) was used as antioxidant while vitamin C was used in the study reported by Virk *et al* (20).

All antioxidant co-treatment with vitamin A of cadmium exposed animal revealed remarkable improvement in superoxide dismutase (SOD), catalase (CAT) activities when compared to control. The activities of antioxidant enzymes were observed to increase with dose increased administration of vitamin A of 250 or 500 mg. The vitamin A treatment used was observed to chelate and in fact significantly suppress cadmium induced generation of free radicals species and oxidative stress. The co-treatment of vitamin A of 250 mg dose indicate mild increased or improved antioxidant enzyme activities (SOD and CAT) when compared to the control. This finding is in agreement with the reports of Eriyamremu *et al*; (9) and Virk *et al*; (20). Eriyamremu *et al;* (9) reported significant improvement in SOD and CAT activities in cadmium exposed animals; that palm oil, being rich in β-Carotene could probably be the active constituent in palm oil responsible for the improved activities of SOD and CAT. Similarly, Virk *et al;* (20) reported improved SOD and CAT activities in liver and kidneys of rats exposed to cadmium, co-treated with vitamin C. Although, the tissues considered for SOD and CAT activities are different from the tissues considered in this study, it however points to the fact that multiple organs and tissues are affected and subjected to oxidative stress when animals are exposed to cadmium but oxidative damages to membrane lipids of tissues and organs can be effectively controlled and avoided by supplemental use of antioxidant vitamins.

References

1. Naumann B, Eberius M, Appenroth K-J. Growth rate based dose–response relationships and EC-values of ten heavy metals using the duckweed growth inhibition test (ISO 20079) with Lemna minor L. clone St. J Plant Physiol 164:1656–1664, 2007.
2. Avunduk A.M., Yardimci S., Avunduk M.C. and Kurnaz L. Cadmium and iron accumulation in rat lens after cigarette smoke exposure and the effect of vitamin E (alpha-tocopherol) treatment. Curr Eye Res, 18(6):403-407, 1999.

3. Ramakrishnan. S., Sulochana, K.N., Selvaraj, T., Abdulrahim, A., Lakshmi, M. and Aunagiri, K. Smoking of beedies and cataract: Cadmium and Vitamin C in lens and blood. Br. J. Ophthalmol. 79: 202 – 206, 1995.
4. Moreau K.L and King J.A. Protein Misfolding and Aggregation in Cataract Disease and Prospects for Prevention Trends Mol Med., 18(5): 273–282, 2012.
5. Mårtensson, J., Steinherz, R., Jain, A. and Meister, A. Glutathione esters prevents buthionine sulfoximine-induced cataracts and lens epithelial cell damage. Proc. Natl. Acad. Sci. U.S.A. 86(22): 8727 – 8731, 1989.
6. Gupta, S., Athar, M., Behari, J.R. and Scrivastava, R.C. Cadmium mediated induction of cellular defence mechanism: A novel example for the development of adaptive response against a toxicant. Indian Health. 29: 1 – 9, 1991.
7. Bagchi, D., Bagchi, M., hassoun, E.A. and Stohs, S.J. Cadmium induced excretion of urine lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-Dawley rats. Boil. Trace Elem. Res. 52: 143 – 140, 1996.
8. Banala R.R and Karnati P.R. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. Int J Dev Neurosci. 47:298-303. 2015.
9. Eriyamremu G. E., Ojimogho S. E., Asagba S. O. and Osagie V. E. 'Palm oil induced changes in ocular tissue lipid peroxidation, antioxidant enzymes and ATPases of rabbits in cadmium toxicity'. Food and Chemical Toxicology, 46: 3155-3158, 2008.
10. Misra, H.P. and Fridovich, I. The role of superoxide ion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170 – 3175, 1972.
11. Beers, R.F. Jr. and Sizer, I.W. Spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195: 133 – 140, 1952.
12. Guttridge, J.M.C. and Wilkins, S. Copper-dependent hydroxyl radical damage to ascorbic acid. FEBS Lett. 137: 327 – 329, 1982.
13. Nyman, M. Serum haptoglobin. Scand. J. Clin. And Lab. Invest. 11, Suppl. 39, 1959.
14. Sokal, R.R. and Rohlf, F.J. The principles and practice of statistics in biological research. San Francisco: Freeman and Co. Pp. 469 – 484, 1969.
15. Asagba, S.O. and Eriyamremu, G.E. Oral cadmium exposure and levels of superoxide dismutase, catalase, lipid peroxidation and ATPases in the eye. Res. J. Environ. Toxicol. 1(4): 204 – 209, 2007.
16. Ezemonye L.I, Ogeleka D.F, Okieimen F.E. Lethal toxicity of industrial chemicals to early life stages of Tilapia guineensis. J Hazard Mater. 30: 157 (1):64-68, 2008.
17. He, X., Chin, M.G. and Ma, Q. Activation of Nrf2 in defence against cadmium-induced oxidative stress. Chem. Res. Toxicol., 21: 1375 – 1383, 2008.
18. Zhou, Q.-X., Zhang, Q.-R, and Liang, J.-D (2006) Toxic effects of acetochlor and methamidophos on earthworm Eisenia fetida in phaiozem, northeast China. Journal of Environmental Sciences, 18(4): 741–745.
19. Livchev, S.I. The mechanism of fenton-like reaction and their importance for niological systems. A biologist's view. Metal Ions Biological System. 36: 1 – 39, 1999.
20. Virk, P., Elobeid, M., Hamad, S., Korany, Z., Al-Amin, M., Daghestani, M., Omer, S., Alolayan, E., Iqbal-Siddiqui, M. and Mirghani, M. Ameliorating effects of Embilica officinalis and Rosmarinus officinalis on cadmium-induced oxidative stress in Wistar rats. J. Medicinal Plant Res. 7(14): 805 – 818, 2013.

.