



THE ROLE OF SOME ANTIOXIDANTS ON ABSORPTION, DISTRIBUTION AND ELIMINATION OF LEAD AND IRON: AN IN -VIVO STUDY.

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ABSTRACT

Metals are an integral part of many structural and functional components in living cells, the critical role of metals in physiological and pathological processes can never be underestimated. The present work is aimed at an in-vivo study for the role of some antioxidants on absorption, distribution and elimination of Lead and Iron. Forty healthy albino rats weighing 130-140g were used in the current study. They were divided into Five (5) groups and standard method of sample collection, preparation, digestion and analysis were followed. There were substantial reduction in the level of iron and lead concentration in all the tissues after supplementation. Among the supplements, ascorbic acid was found to be more effective in absorption, distribution and elimination of the metals.

KEYWORDS: Antioxidant, elimination, supplement, toxicity.

1.0.0 INTRODUCTION

Metals are an integral part of many structural and functional components in living cells, and the critical role of metals in physiological and pathological processes can never be underestimated. Heavy metals have been defined based on density, atomic number, toxicity and chemical properties, although the definition given in term of density is the most popular and comprehensive. Therefore heavy metals are those metals having a specific density of more than 5 g/cm³ such as lead, mercury, arsenic, cadmium, zinc, cadmium, nickel e.t.c.. They are widely distributed in the earth's crust and extremely persistent in the environment, but present at very low concentrations in the human body. Potential sources of heavy metals exposure include natural sources (e.g., groundwater, metal ores), industrial processes, commercial products, folk remedies, and contaminated food and herbal products.^[1] The accumulation of these elements can cause severe damage to mucus tissues and intestinal tract, skeletal muscle, central nervous system and reproductive systems.^[1] Heavy metals are known to cause oxidative deterioration of biomolecules by initiating free radical mediated chain reaction resulting in lipid peroxidation, protein oxidation and oxidation of nucleic acid like DNA, RNA and phosphodiester bond of the nucleic acid.^[2] Also studies have demonstrated the reactions of hydroxyl radicals which leads to abstraction of a hydrogen atom from the protein polypeptide

backbone to form a carbon-centered radical, which under aerobic conditions reacts readily with dioxygen to form peroxy radical.^[3] Lead is widely known to be toxic even at low concentration especially in young children and causes hyperactivity, deficits in fine motor function, hand-eye coordination.^[4] Malondialdehyde (MDA) levels were strongly correlated with lead concentration in the tissues of lead exposed rats.^[5] The concentration of thiobarbituric acid reactive substance (TBARS), which is a reflection of endogenous lipid oxidation level, gets increased on lead exposure.^[1] Acute iron toxicosis causes both a direct corrosive effect on the gastrointestinal tract and cellular damage due to circulating unbound iron.^[6] In Addition, increased amounts of iron in the body possess enhanced risk of a variety of diseases including vascular disease, cancer and certain neurological conditions.^[7-8] Numerous studies have focused on the mechanisms associated with heavy metal toxicity, and it has been attributed to generation of reactive free radicals such as oxygen and nitrogen species, which develops imbalance between the prooxidant elements and the antioxidants in the biological system.

The problem of lead and iron toxicities have been widely recognized which needs a special attention and many research have been undertaken for the development either natural or synthetic products having the potential

of reducing the toxic effect of heavy metals; antioxidants were usually employed. Effective antioxidants possess these properties and many of them have been reported from the literatures. Antioxidants are substances capable of preventing or slowing the oxidation of other molecules by free radical scavenging or metal chelation. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen.^[8] An ideal heavy metal chelator should be able to enter the cell easily, chelate the heavy metal from its complex with metallothionein or other proteins, and increase the excretion of the metal without its redistribution to other organs or tissues.^[9] Vitamin E alone or in combination with conventional chelator, CaNa₂EDTA has been reported to decrease the lead-induced lipid peroxide levels in liver and brain in rats.^[10] Vitamin C supplementation in lead-exposed animals significantly reduces blood, liver, and renal lead levels, and associated biochemical changes indicating a significant protective action of vitamin C against toxic effects of lead on heme synthesis and drug metabolism.^[11] The antioxidant effects of *Spirulina fusiformis*, bluegreen algae rich in β -carotene and SOD, against lead toxicity have been examined in the testes of Swiss mice, the antioxidant nutrients scavenged the free radicals after lead administration and ROS generation in mice testes.^[12] Kinetically antioxidants can be classified into six categories as below:

- (1) Antioxidants that break chains by reacting with peroxy radicals having weak O-H or N-H bonds: phenol, naphthol, hydroquinone, aromatic amines and aminophenols.
- (2) Antioxidants that break chains by reacting with alkyl radicals: quinones, nitrones, iminoquinones.
- (3) Hydro peroxide decomposing antioxidants: sulphide, phosphide, thiophosphate.
- (4) Metal deactivating antioxidants: diamines, hydroxyl acids and bifunctional compounds.
- (5) Cyclic chain termination by antioxidants: aromatic amines, nitroxyl radical, variable valence metal compounds.
- (6) Synergism of action of several antioxidants: phenol sulphide in which phenolic group reacts with peroxy radical and sulphide group with hydro peroxide.^[2]

The present work is aimed at an *in vivo* study of the role of some antioxidants on absorption, distribution and elimination of Lead and Iron.

2.0.0 MATERIAL AND METHODS

Analytical (AnalaR) grade reagents and de-ionized water were used for the experiment. All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% (v/v) nitric acid for 24 hrs, cleaned thoroughly with distilled water and dried.

2.1.0 Experimental animals

A total of 40 healthy albino rats weighed (130-140g) were purchased from Biological Sciences Department, BUK and were divided into 5 groups as follows:

GROUP I: Two rats as the normal control un-intoxicated.

GROUP II: Two rats as negative control which were intoxicated with 100cm³ each of lead and iron solution.

GROUP III: Comprises of twelve rats, out of which six were intoxicated with 100cm³ of lead solution for the period of one week after each two were supplemented with 50cm³ each of ascorbic acid, EDTA and vitamin E respectively for two weeks. On the other hand, six were intoxicated with iron solution for the period of one week after which each two were supplemented with 50cm³ each of ascorbic acid, EDTA and vitamin E respectively for two weeks.

GROUP IV: This comprises of twelve rats, each four were intoxicated with 100cm³ each of lead and iron solution for a period of one week after which each of the four were supplemented with 50cm³ each of ascorbic acid, EDTA and vitamin E respectively for two weeks.

GROUP V: It comprises twelve rats as well, each four were intoxicated with 100cm³ each of lead and iron and were supplemented each of the four with 50cm³ each of ascorbic acid, EDTA and vitamin E simultaneously for the period of two weeks.

Animals were maintained and handled according to protocols in animal care and used by the ethical committee of the university.

2.2.0: Sample collection and preparation

2.2.1 Sample collection

After two weeks of supplementation, the rats were sacrificed and blood, liver, kidney and intestine were collected in petridishes, air dry and stored for further use.

2.2.2 Sample Preparation and Digestion

The collected samples were grinded and 1g of each was accurately weighed and placed in crucible and ashed in a muffle furnace for 8 hours at 500^oc and then cooled in a desiccator. Sample digestion given by AOAC was adopted and 5.0cm³ of 1M HNO₃ solution was added and evaporated to dryness on a hot plate and returned to the furnace and heated again at 400^oc for 20 minutes until perfectly grayish – white ash was obtained. The sample was cooled in desiccator followed by the addition of 15cm³ 1:1 (v/v) HCl to dissolve the ash and the solution was filtered into 100cm³ volumetric flask. The volume was made to the mark with de-ionized water.^[13] Triplicate digestions of sample were carried out to ensure precision. The filtered solutions were analyzed using atomic absorption spectrophotometer (210VGP Buck Scientific).

3.0 RESULT AND DISCUSSION

The mean concentrations of iron and lead were presented in table 1 and 2 respectively. The mean iron

concentration of the collected samples in group I was found to be lower when compared with group II. Similarly, the mean iron concentration of the collected samples in group III, IV and V of all the subjects supplemented with ascorbic acid, EDTA and vitamin E were found to be lower when compared with group II (intoxicated control) and group I (normal control). The substantial reduction in the iron concentration of the collected samples in group III, IV and V showed that the supplementation of ascorbic acid, EDTA and vitamin E is effective in chelating or reducing the iron concentration. However, it has been shown that the ascorbic acid is more effective than those of EDTA and

vitamin E which might be due the fact that ascorbic acid is a water soluble vitamin and can dissolve well in liquid medium compared to EDTA and vitamin E. Vitamin E is lipid soluble vitamin.^[14] Furthermore, the mean iron concentration of blood sample was found to be higher when compared with intestine and liver. This might be arise due the fact that the supplements especially ascorbic acid increase the intestinal absorption of iron into the blood stream and the blood being a transporter, distributes it to various organs such liver, spleen etc.^[14] This finding was found to have correlates with the finding of.^[15,16, and 16]

Table 1: Mean iron concentration ($\mu\text{g/g}$) in tissues of rats orally supplemented with ascorbic acid, EDTA and vitamin E for two weeks

GROUPS/SUPPLEMENTS		INTESTINE	BLOOD	LIVER	KIDNEY
Group I		6.13 \pm 0.12	6.07 \pm 0.12	4.74 \pm 0.09	3.92 \pm 0.04
Group II		9.52 \pm 0.09	9.53 \pm 0.04	7.62 \pm 0.11	6.07 \pm 0.12
Group III	A. Acid	4.00 \pm 0.09	6.02 \pm 0.25	3.89 \pm 0.06	5.67 \pm 0.23
	EDTA	4.37 \pm 0.04	7.39 \pm 0.13	2.12 \pm 0.13	4.04 \pm 0.22
	Vit. E	5.86 \pm 0.09	7.19 \pm 0.08	5.69 \pm 0.20	6.05 \pm 0.19
Group IV	A. Acid	3.94 \pm 0.06	6.19 \pm 0.23	4.15 \pm 0.15	7.23 \pm 0.12
	EDTA	5.80 \pm 0.19	7.33 \pm 0.08	5.89 \pm 0.02	7.22 \pm 0.09
	Vit. E	5.76 \pm 0.01	6.05 \pm 0.19	3.62 \pm 0.05	5.62 \pm 0.22
Group V	A. Acid	5.71 \pm 0.22	7.03 \pm 0.12	5.83 \pm 0.08	5.04 \pm 0.07
	EDTA	4.02 \pm 0.09	5.97 \pm 0.14	5.91 \pm 0.27	5.84 \pm 0.09
	Vit. E	5.89 \pm 0.08	7.35 \pm 0.19	3.96 \pm 0.09	6.07 \pm 0.12

Values are mean \pm S.D

Table 2: Mean lead concentration ($\mu\text{g/g}$) in tissues of rats orally supplemented with ascorbic acid, EDTA and vitamin E for two weeks

GROUPS/SUPPLEMENTS		INTESTINE	BLOOD	LIVER	KIDNEY
Group I		4.48 \pm 0.03	4.42 \pm 0.17	4.45 \pm 0.07	2.30 \pm 0.05
Group II		4.51 \pm 0.09	6.66 \pm 0.05	6.76 \pm 0.09	9.11 \pm 0.11
Group III	A. Acid	4.14 \pm 0.51	4.99 \pm 0.06	2.84 \pm 0.08	6.24 \pm 0.07
	EDTA	4.85 \pm 0.15	2.64 \pm 0.10	4.42 \pm 0.08	2.59 \pm 0.07
	Vit. E	2.74 \pm 0.08	6.31 \pm 0.11	6.57 \pm 0.11	4.17 \pm 0.08
Group IV	A. Acid	4.16 \pm 0.07	4.47 \pm 0.06	2.44 \pm 0.07	6.37 \pm 0.05
	EDTA	5.00 \pm 0.06	2.59 \pm 0.06	2.39 \pm 0.06	2.01 \pm 0.08
	Vit. E	3.75 \pm 0.09	4.45 \pm 0.08	2.84 \pm 0.08	4.20 \pm 0.11
Group V	A. Acid	2.77 \pm 0.14	4.79 \pm 0.08	1.95 \pm 0.08	6.55 \pm 0.16
	EDTA	3.81 \pm 0.11	2.84 \pm 0.58	4.44 \pm 0.06	6.66 \pm 0.05
	Vit. E	2.68 \pm 0.10	2.40 \pm 0.08	4.77 \pm 0.09	4.97 \pm 0.04

Values are mean \pm S.D

The mean lead concentration of the collected samples followed similar trend as for iron above. Nutritive substances like glucose, amino acids, lipid and vitamins derived from digested food are absorbed from gastrointestinal tract and are carried by blood to different part of the body. The substantial decrease in lead concentration in the liver after the supplementation of ascorbic acid, EDTA and vitamin of group III, IV and V agreed with the works of.^[10,16]

The liver helps to clear toxic substances such as heavy metals, drugs and alcohol from the bloodstream. The significance reduction observed in the level of lead

concentration in the liver showed that the supplementation of ascorbic acid, EDTA and vitamin is very effective in the chelation and elimination of lead in the liver. However, ascorbic acid showed significant reduction of lead concentration compared to EDTA and vitamin E.

CONCLUSION

The result of the study revealed that ascorbic acid, EDTA and vitamin E supplements have great effect on absorption, distribution and elimination of iron and lead. However, it shows that ascorbic acid supplement has apparent effectiveness as compared to EDTA and

vitamin E. Nevertheless, further study should be carried out while increasing the number of subjects, administered dose, period of study and comparison with various other antioxidants.

REFERENCES

1. Sharma B, Singh S, Siddiqi NJ. Biomedical Implications of Heavy Metals Induced.
2. Imbalances in Redox Systems: Review Article, *BioMed Res. Int.*, 2014; 26. <http://dx.doi.org/10.1155/2014/640754>
3. Swaran JSF. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloids exposure: Review. *Oxid. Med. Cell. Long.*, 2009; 2(9): 191-206.
4. Stadtman ER. Protein oxidation and aging. *Science*, 1992; 257: 1220-4.
5. Mustapha BI, Khalid DK, Zakari A. Comparative assessment of heavy metals concentration in the soil in the vicinity of tannery industries, Kumbotso old dump site and river challawa confluence, at Challawa industrial estate, Kano state, Nigeria. *Intl. J. of Inno. Res., Dev*, 2015; 4(6).
6. Aykin-BN, Franklin EA, Ercal N. Effects of N-acetylcysteine on lead exposed PC-12 cells. *Arch Environ Contam Toxicol*, 2005; 49: 119-23.
7. Greentree WF, Hall JO. Iron toxicosis. In: Bonagura JD, ed. *Kirk's current therapy XII small animal practice*. Philadelphia, Pa: WB Saunders Co, 1995; 240-242.
8. Berg D, Gerlach M, Youdim MBH, Double KL, Zecca L, Riederer P, Becker Brain G. Iron pathways and their relevance to Parkinson's disease. *J Neurochem*, 2001; 79: 225-36.
9. Siah CW, Trinder D, Olynyk JK. Iron overload. *Clin Chim Acta*, 2005; 358: 24-36.
10. Jones MM, Cherian MG. The search for chelate antagonists for chronic cadmium intoxication. *Toxicology*, 1990; 62: 1-25.
11. Patra RC, Swarup D, Dwivedi SK. Antioxidant effects of α tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats; *Toxicology*, 2001; 162(2): 81-88
12. Vij AG, Satija NK, Flora SJS. Lead induced disorders in hematopoietic and drug metabolizing enzyme system and their protection by ascorbic acid supplementation. *Biomedical and Environ. Sci.*, 1998; 11(1): 7-14.
13. Shastri D, Kumar M, Kumar A. Modulation of lead toxicity by *Spirulina fusiformis*, *Phytotherapy Res.*, 1999; 13(3): 258-260
14. Horwitz W. Association of Official Analytical Chemist (AOAC). 14th Edit, 19990, Allentown, Washington D.C. Sunday AM., Musa O., Wilson OH., Augustine OI., Uduak MU. Preventive activity of ascorbic acid on lead acetate induced Cerebellar damaged in adult Wistar rats, *Med. Health Sci. J.*, 2012; 13(1): 99-104.
15. Oputiri EA. Hepatoprotective Effect of Vitamin C (Ascorbic Acid), *J. Sci. Res.*, 2012; 4(1): 84-92
16. Samir, D., Kechrid, Z., Djabar, MR.. Combined protective effective of zinc and vitamin C (ascorbic acid) on Nickel-induced oxidative liver injury in rats, *Annal of Bio. Res.*, 2010; 3(1): 3278-3286.