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Hepatoprotective Effect of Aqueous Seed Extract of *Moringa* Oleifera against Cadmium and Lead Toxicity in Experimental Rats

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ABSTRACT

Moringa oleifera also known as horse radish tree is a rapid growing deciduous shrub. It is widely cultivated in the East and Southeast Asia, Africa and the West Indies. Different parts of this plant are important sources of proteins, vitamins, phenolics and important minerals, hence high range of nutritional value and medicinal uses. Almost all parts of Moringa oleifera tree have been used for treatment of various ailments. This study is aimed at finding out the potential hepatoprotective effect of aqueous seed extract of the plant. The effect of daily administration of aqueous seed extract of Moringa oleifera for two weeks on the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) was studied. The treatment involved oral administration of 30mg/Kg and 6mg/Kg of Lead and Cadmium respectively, to all the animals with exception of control group in order to induce toxicity and followed by oral administration of low dose (189mg/Kg) and high dose (378mg/Kg) of aqueous seed extract of Moringa oleifera to evaluate its antitoxic effect. The control group was found to have AST, ALT and ALP activities of 23.33 ± 6.35, 20.33 ± 1.53 and 32.22 ± 3.85IU/L respectively. The serum levels of AST, ALT and ALP when compared with control at p=0.05 significantly increased after administration of Lead and Cadmium solutions which indicated hepatotoxicity. Upon administration of both doses of the extract, there was slight and sharp decrease in the serum activities of ALT and ALP respectively w, although AST level did respond to the extract treatment. The result revealed that the aqueous seed extract of Moringa oleifera possess hepatoprotective potential against Lead and Cadmium toxicity in experimental rats at the doses administered.

Key Words: *Moringa oleifera, Lead, Cadmium, AST, ALT, ALP.*

1. INTRODUCTION

Heavy metals are currently the most serious pollutants that we encounter on daily basis, and their toxic effects need not to be neglected. They are toxic at low concentration and exert their toxic effect by generating reactive oxygen species like $OH^{\bullet}$, $H^{\bullet}$ and H_2O_2 which causes oxidative stress [1,2,3]. Heavy metals are serious threat to living organisms because they have a wide range of health effect including carcinogenicity, mutagenicity, immunosuppression, impaired reproduction and general poor body conditions [4,5].

Lead is one of the commonly encountered heavy metals for it occur in manufacturing processes where Lead batteries are produced or Lead is recycled, printing ink, gasoline, fertilizers, Lead containing paints, disposal of municipal sewage sludge, mining and smelting activities as well as explosives [6,7]. Lead chronic exposure may result in problems like mental retardation, birth defects, psychosis, autism, allergies, hyperactivity, dyslexia, muscular weakness, paralysis and weight loss [6].

Cadmium together with Lead made the two most abundant toxic metals in the environment. Cadmium acute exposure occurs in the manufacturing processes of batteries and color pigments used in plastics and paints, electroplating and galvanizing processes [6]. Cadmium exposure affects various organs such as bones, brain, kidney, and nervous system, leading to alopecia, arthritis, anemia, learning disorders, migraine, emphysema, and growth impairment among others [8,6].

Several methods were used to ameliorate heavy metal toxicity including chelating therapy [9] to promote metal excretion and dietary supplements [10]. However, metal chelating agents are themselves reported to have a number of efficacy and safety concern. Use of plant species in reducing the effects of heavy metals' toxicity have gained popularity, due to it being more cost effective, and have fewer side effects than the physical and chemical methods [11]. *Moringa oleifera* is one of the plants that were reported to be used both in vitro and in vivo in reducing toxicity of heavy metals [12].

Moringa oleifera (family: Moringaceae) also known as horse radish tree or drum stick tree is a rapid growing deciduous shrub or small tree, measuring about 13 m height and 35 cm in diameter [13]. It is widely cultivated in the East and Southeast Asia, Africa and the West Indies. Different parts of this plant are important sources of proteins, vitamins, phenolics and important minerals, hence high range of nutritional value medicinal uses [14]. To cut it short, almost all parts of *Moringa oleifera* tree have been used for treatment of various ailments. For instance, its flowers and roots are remedies from tumors, seeds for abdominal tumors; leaves are applied on sores or rubbed on temples for relief from headache [15]. It is also reported to play role in the treatment of inflammation, infectious diseases, cardiovascular diseases, hepatorenal, gastrointestinal and hematological disorders [16].

The use of *Moringa oleifera* leaf extracts to ameliorate effect of Chromium-induced testicular toxicity in rat testes was reported and was found to significantly enhance the sperm parameters compared to rats exposed to Chromium alone [17]. Accumulation of Lead and Cadmium in different organs and tissues of albino rats were reported. It was found that liver accumulate Lead and Cadmium more than kidney and other tissues [18]. Another study reported a significant increase in the activity of Aspartate aminotranferase, Alanine aminotranferase and increase in concentration of bilirubin after exposure to Cadmium, Lead and Manganese in rats [19].

Because of the facts that, Lead and Cadmium were reported to have caused liver toxicity in different capacities, this study is aimed at determining potential antitoxic effect of seed extracts from *Moringa oleifera* against the damage caused by these two heavy metals on rats' liver. This is planned to be achieved by analyzing serum activities of Aspartate aminotranferase (AST), Alanine aminotranferase (AST) and Alkaline Phosphatase (ALP).

2. MATERIALS AND METHOD

2.1 MATERIALS

2.1.1. Equipments and Apparatus

All the apparatus and equipment's used in this research work were of standard quality and in good working conditions.

2.2 Experimental Animals

A total of twenty five (25) male albino rats of an inbred Novergicus Strain (*Rattusnovergicus*) weighing between 80g to 120g were selected at random and used in this study. They were obtained from the department of biological sciences, Bayero University, Kano, Nigeria. The animals were kept in a good laboratory conditions (Temperature: 25-28^{o}C; 12:12 light: dark cycle), with free access to both food and water. They were housed in aluminium cages (Dimensions 39.50cm by 25.30cm by 14.80cm) with saw dust at the bottom of the cages. They were acclimatized to the conditions for ten (10) days prior to the initiation of experimental treatments.

2.3 Experimental Design

The animals were randomly divided into three groups with group I serving as control and group II and III further divided into three sub-groups each (i.e group IIa, b and c and group IIIa, b and c).The experiment involves oral administration of two different heavy metals (Lead and Cadmium) followed by two different concentrations of *Moringa oleifera* aqueous seed extract to the animals in order to test the plant's antitoxic effect on the liver. After two weeks of administration, the rats were sacrificed by carefully cutting their jugular vein with a very sharp razor. Blood samples were collected into dried and clean centrifuge tubes and were separated. The sera were collected into plain serum bottles for laboratory investigations. For each sample, the level of AST, ALT and ALP were assayed in order to assess liver function.

Table 1. Grouping and Dosage

Group	No. of animals	Dose of heavy metal	Dose of extract
Group I (Control)	3	Nil	Nil
Group IIa	3	Lead (30mg/Kg)	Nil
Group IIb	4	Lead (30mg/Kg)	Low dose (189mg/Kg)
Group IIc	4	Lead (30mg/Kg)	High dose(378mg/Kg)
Group IIIa	3	Cadmium (6mg/Kg)	Nil
Group IIb	4	Cadmium (6mg/Kg)	Low dose (189mg/Kg)
Group IIIc	4	Cadmium (6mg/Kg)	High dose (378mg/Kg)

2.4 Collection and Preparation of *Moringa oleifera* Aqueous Seed Extract

Moringa oleifera seeds were collected from Unguwar Rimi GRA Kaduna, Nigeria. The seeds were removed from the pods and shell. They were dried at room temperature and ground to a fine powder. 20g of the fine powder were weighed and soaked in distilled water for 24hrs. The mixture was then filtered using whatman filter paper. The residue was dried and weighed again. The concentration of the filtrate (seed extract) was determined as the difference in weight (in gramme) between the initial weight of the sample and final weight of the residue per final volume of the filtrate (in ml). The volume of the aqueous seed extract of *Moringa oleifera* to be administered was determined based on the average weight of the experimental rats used.

2.5 Solutions of Heavy Metals

2.5.1 Lead Standard Solution

Accurately weighed 10g of Lead (II) acetate-3-hydrate crystals were dissolved in 100ml of distilled water, 10ml of this was diluted to 100ml for working solution. This later contains 0.1mg of Lead per ml.

2.5.2 Cadmium Standard Solution

Accurately weighed 10g of Cadmium sulphate crystals were dissolved in 100ml of distilled water. 10ml of this solution was diluted to 100ml for a working solution. This later contains 0.1ml of Cadmium per ml.

2.5.3 Chemical Reagents

All the chemicals and reagents utilized in the assay of liver enzymes: AST (EC 2.6.1.1), ALT (EC 2.6.1.2) and ALP (EC 3.1.3.1) are of analytical grade. All the three enzymes: AST and ALT and ALP have been analyzed using Randox (brand name) commercially prepared kit produced by Randox laboratories Ltd, UK, together with 0.4N Sodium hydroxide. Other chemical reagents used are Lead (II) acetate-3-hydrate crystals to prepare Lead standard solution and Cadmium sulphate crystals to prepare Cadmium standard solution.

2.6 METHOD

2.6.1 Determination of Liver Enzymes

Aspartate aminotransferase (AST) (EC 2.6.1.1) and alanine transferase (ALT) (EC 2.6.1.2) in serum were analyzed using the procedure described by [20] while serum alkaline phosphatase (ALP) (EC 3.1.3.1) was assayed using the method developed by Roy [21].

3. RESULTS AND DISCUSSION

3.1 Results

The effect of administration of different doses of aqueous seed extract of *Moringa Oleifera* on experimental rats that were previously orally treated with solutions of Lead and Cadmium metals were shown on tables 2 and 3 below.

Table 2: Enzyme activity of Serum AST, ALT and ALP of control rats and rats administered orally with 30mg/Kg of Lead solution, low dose (189mg/Kg) and high dose (378mg/Kg) of aqueous extract of *Moringa oleifera* seed for two weeks.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group I (Control) n = 3	23.33 ± 6.35	20.33 ± 1.53	32.22 ± 3.85
Group IIa (Lead only) n = 3	*31.50 ± 5.20	21.50 ± 1.92	54.17 ± 5.00
Group IIb (Lead+ low dose of extract) n = 4	*33.75 ± 6.08	24.75 ± 0.50	45.83 ± 3.19
Group IIc (Lead+ high dose of extract) n = 4	*44.00 ± 6.98	19.00 ± 2.94	35.00 ± 1.93

n = number of rats

* = statistically observed significant increase when compared with control.

Results were expressed as: mean ± standard deviation

Table 3: Enzyme activity of Serum AST, ALT and ALP of Control rats and rats Administered orally with 6mg/Kg of Cadmium solution, low dose (189mg/Kg) and high dose (378mg/Kg) of aqueous extract of *Moringa oleifera* seed for two weeks.

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group I (Control) n = 3	23.33 ± 6.35	20.33 ± 1.53	32.22 ± 3.85
Group IIIa (Cadmium only) n = 3	*33.75 ± 6.08	23.00 ± 3.83	53.33 ± 4.30
Group IIIb (Cadmium+ low dose of extract) n = 4	*34.75 ± 4.79	22.75 ± 2.99	51.67 ± 1.92
Group IIIc (Cadmium+ high dose of extract) n = 4	*32.50 ± 4.36	21.25 ± 2.22	34.17 ± 3.19

n = number of rats.

* = statistically observed significant increase when compared with control.

Result was expressed as mean: mean ± standard deviation.

From the above table values, statistical comparisons between the results of control and groups that were treated with heavy metal only and those given with low dose and high dose of extract were carried out using student's t-test at 5% level of significance ($p=0.05$).

3.2 Discussion

The clinical suspicion of liver diseases usually leads to the conduct of liver function tests. Several liver enzymes found in the serum are measured in these widely available biochemical tests [22]. The measurement of the activities of marker or diagnostic enzymes in the serum plays a significant role in diagnosis of disease and in the assessment of drugs or plant extract for safety or toxicity risk. The enzymes considered in this study are useful marker enzymes of the liver cells [23]. AST and ALT are normally localized within the cells of the liver, heart, gill, kidney, muscle and other organs in which the enzymes are of major importance in assaying and monitoring liver cytolysis [24]. Their presence in the serum may give information on organ dysfunction [25].

From the results obtained in this research, the rats in the control group have serum AST, ALT and ALP levels of 23.33 ± 6.35, 20.33 ± 1.53 and 32.22 ± 3.85 IU/L respectively. It was observed that there is significant increase, at $p=0.05$, in the serum activities of all the three enzymes especially ALP when group IIa and group IIIa (rats treated with heavy metals only) were compared with control. This shows that, both heavy metals (Lead and Cadmium) might have lead to generation of free radicals, as reported by [26], these free radicals might have attacked the cell membrane of the hepatocytes, thereby causing rapture of the cells and subsequent leaking out of the enzymes into the blood.

The results of comparison, at $p=0.05$, of AST activity of control group with that of group IIb, IIc, IIIb and IIIc revealed significant increase in activity of the enzyme despite treating the animals with low and high dose of the extract. Comparison of ALT values of

group IIb, IIc, IIIb and IIIc (rats treated with the extract) with control group revealed no significant difference, but there is slight and steady decrease in the enzyme activity in rats treated with both low and high dose of the extract when compared with those treated with heavy metals only. Statistically significant decrease at $p=0.05$ was observed when ALP values of group IIa and IIIa (rats treated with heavy metals only) were compared with those of group IIb, IIc, IIIb and IIIc (rats treated with the extract)

AST is not liver specific, therefore increase in its activity could be as a result of leakage from other tissues like heart and muscle and it could also be possible that, the doses administered lower than the curative dose. The aqueous seed extract of *Moringa oleifera* might have contain some phytochemicals that have an antioxidant property which neutralized the free radicals generated by the heavy metals and thereby curing the damage done to the liver cells as it was observed with slight decrease in ALT and sharp decrease in the ALP activities of rats treated with the aqueous extract.

ALT and AST are important in the diagnosis of liver damage caused by drug toxicity or infection. Following liver damage, these aminotranferases leak from the damaged hepatocytes into the blood stream. Measurement of the blood serum concentration of the two enzymes can provide information about the severity of the damage. ALP is produced in bone in addition to liver, and blood activity can be increased in some bone disorders [23].

As it is stated in the introduction, different parts of *Moringa oleifera* are used to cure a wide range of ailments as reported by [15,16], therefore, this study has revealed that the seed of *Moringa oleifera* contain phytochemicals that posses hepatoprotective effect against toxicity of heavy metals under study. It is also in conformity with many findings already in the literature.

4. CONCLUSION

The result obtained in this research shows that, administration of doses of heavy metals: Lead and Cadmium caused hepatotoxic effect in the experimental rats thereby leading to increase in the activities of liver enzymes: serum AST,ALT and ALP with respect to control animals. After oral administration of low (189mg/Kg) and high (378mg/Kg) doses of aqueous seed extract of *Moringa oleifera* for two weeks, decrease in activities of serum ALT, AST and ALP were observed. As these enzymes are plasma specific and increase in cases of damage as a result of injury or diseases or toxicity, it can be concluded that, within the limit of experimental error, the seed extract play a hepatoprotective role against toxicity caused by the concentrations of Lead and Cadmium administered.

REFERENCES

1. A.W. Raymond and E.O. Felix. Heavy metals in contaminated soils. A review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecol.* 2011, pp. 1-20.

2. A. Chezhian, N. Kabilan, K. Suresh, and S. Senthamil. Influence of different Calcium Levels and Low pH of Water on the Plasma Electrolyte Regulation of a Fresh Water Teleost Fish *Cyprinuscarpio* var. communies, (Linnaeus, 1958). *Curr. Res. J. Biol. Sci*. 2011. Vol. 3(2) pp. 147-154.

3. N. Shaista, A.N. Saeed, S. Yasar, and P. Armit. Determination of Heavy Metals in Fresh Water Fish Species of the River Ravi, Pakistan Compared to Farmed Fish Varieties. *Environ. Monit. Assess.*2010, vol.167 (1-4) pp. 461-471.

4. A. M. Scheuhammer. The chronic toxicity of Aluminium, Cadmium, Mercury, and Lead in Birds: A review. *Environ. Pollut.* 1987, vol. 46, pp. 263-295.

5. A.M. Florea and D. Busselberg. Occurrence, use and potential toxic effects of metals and metal compounds. *Biometals*, 2006, vol. 19(4) pp. 419-427.

6. Life Extension Foundation. Heavy Metal Toxicity. Retrieved January 12, 2018 from http://www.lef.org/

7. S. K. Yadav. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany,* 2010, vol. 76 pp. 167–179

8. A. Suresh, B. Sivaramakrishna, and K. Radhakrishnaiah. Patterns of Cadmium Accumulation in the Organs of Fry and Fingerlings of Freshwater Fish (*Cyprinuscarpio)* Following Cadmium Exposure. *Chemoshpere,* 1993, Vol. 26, No.5, pp 945-953.

9. S. J.S. Flora, M. Mittal and A. Mehta. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res*2008, vol. 128, pp 501-523

10. Z. Qixiao, N. Arjan and C. Wei. A Review: Dietary Strategies for the Treatment of Cadmium and Lead Toxicity. *Nutrients* 2015, vol. *7*pp. 552-571

11. H. Ali, E. Khan and M.A. Sajad. Phytoremediation of heavy metals - Concepts and applications. *Chemosphere* 2013, vol. 91 pp. 869-881.

12. K. Jiraungkoorskul and W.Jiraungkoorskul. *Moringa oleifera*: A new challenge reducing heavy metal toxicity: A review. *Indian J. Agric. Res.*, 2016, vol. 50 (3) pp. 199-205

13. S.T. Anjorin, P. S. Ikokoh and A. Okolo. Mineral Composition of *Moringa oleifera* Leaves Pods and Seeds from Two Regions in Abuja. Nigeria. *International Journal of Agriculture and Biology*. 2010, vol. 12 (3), pp. 1560-1569.

14. S. Veena and P. Ritu. Isolation and Characterization of Saponins from *Moringa oleifera* (moringaeceae) Pods. *Int. J Pharm Pharm Sci*, 2013, Vol 4, Suppl 1, pp. 179-183

15. F. Anwar, S. Latif, M. Ashraf and A. H. Gilani. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytother. Res.* 2007, vol. 21, pp. 17–25

16. Y. Morimitsu, K. Hayashi, Y. Nakagama, F. Horio, K. Uchida and T. Osawa Antiplatelet and anticancer isothiocyanates in Japanese horseradish, wasabi. *Biofactors*, 2000, vol. 13, pp. 271–276

17. G.G. Akunna, O. S. Ogunmodede , C. L. Saalu, B. Ogunlade, A. J. Bello and E. O. Salawu. Ameliorative Effect of *Moring aoleifera* (drumstick) Leaf Extracts on Chromium- Induced Testicular Toxicity in Rat Testes. *World J Life Sci. and Medical Research*, 2012, vol. 2 pp. 20-26

18. P. Josthna, T. Geetharathan, P. Sujatha and G Deepika. Accumulation of Lead and Cadmium in the organs and tissues of albino rat. *Int. J. of Pharm. & Life Sci. (IJPLS)* 2012, vol. 3 (12) pp. 2186-2189

19. M. Iwona, J. Lidia, M. Aleksandra, P. Adam, J. Ewa P. Natalia and P. Krystyna. Effects of Chronic Exposure to Lead, Cadmium, and Manganese Mixtures on Oxidative Stress in Rat liver and Heart. *ArhHigRadaToksikol*, 2015, vol. 66, pp. 51-62.

20. S. Reitman, and S. Frankel. A Colorimetric Method for the Determination of Serum Glutamate-Oxaloacetate and Pyruvate Transaminase. *Am. J. Clin. Path,* 1957, vol. 28 no. 56.

21. A.V. Roy, *Clin. Chem.* 1970. 16:431

22. N.D.C. Finlayson, I.A.D. Bouchier, C. Haslet, C.R.W. Edwardand E.R. Chilvers. Davidson's Principle and Practice of Medicine, 17th Ed. 1995. Churchill Livingstone, pp.484-545.

23. E. Schmidt and F.W. Schmidt. Enzyme diagnosis in disease of the liver and biliary system. *Advanced Clinical Enzymology*, 1979, pp. 239-242.

24. H. Wada and E.E. Shell. Enzymatic transamination of pyrodoxamine-puyruvate transaminase. *J. Biol. Chem,* 1962, Vol. 237, pp. 133-137.

25. R.M. Wells, R.H. McLntyre, A.K. Morgan and P.S. Davis, P.S. Physiological Stress Response in Big game Fish Exposure: Observation on Plasma Chemistry and Blood Factors," *Comp. Biochem. Physiol,* 1986, 64A, pp. 565-571.

26. J. Habeebu, J Liu, C.D. Klaassen. Cadmium-induced apoptosis in mouse liver. *Toxicol. Appl. Pharmacol*. 1998;149:203–209.