European Journal of Medicinal Plants
12(4): 1-12, 2016, Article no.EJMP.23763
ISSN: 2231-0894, NLM ID: 101583475

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Amelioratory Effect of Methanolic Leaf Extract of *Moringa oleifera* on Some Liver and Kidney Function and Oxidative Stress Markers in Lead-intoxicated Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CEO, SCM and CED designed the study. Authors CEO, OE and AJO performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DLA, JKN and CFO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/23763
Editor(s):
(1) Thomas Efferth, Chair, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry Johannes Gutenberg University, Germany.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anthony Cemaluk C. Egbuonu, Michael Okpara University of Agriculture, Umudike, Nigeria.
(2) Pawan K. Singh, National Innovation Foundation, India.
Complete Peer review History: http://sciencedomain.org/review-history/13255

Original Research Article

Received 21st December 2015
Accepted 10th January 2016
Published 10th February 2016

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ABSTRACT

This study was designed to evaluate the effect of *Moringa oleifera* (MO) against lead acetate induced changes in some liver and kidney function parameters and oxidative stress markers in rats. Thirty six acclimatized Wistar rats were divided into six groups of six rats each (groups A-F). Different groups were exposed to 100 mg/kg lead acetate, different doses of MO, and combination of lead acetate and different doses of MO per oral (PO) for 6 weeks. Both their pre-treatment and post-treatment serum alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), bilirubin, albumin, total protein, urea, creatinine and uric acid as well as their post-treatment liver and kidney (homogenates) malondialdehyde (MDA), superoxide dismutase (SOD), glutathione-s-transferase (GST), and catalase (CAT) were estimated using standard methods. Lead acetate administration significantly increased the post-treatment levels of ALP, ALT, AST, GGT, bilirubin, urea, creatinine, and uric acid when compared with their pre-treatment and control levels ($p<0.05$). Lead acetate also significantly increased both liver and kidney MDA, but significantly decreased both liver and kidney SOD, GST and CAT ($p<0.05$). However, MO supplementation was associated with significant decreases in the levels of ALP, ALT, AST, GGT, bilirubin, urea, creatinine, uric acid, liver and kidney MDA and significant increases in the levels of both liver and kidney GST, SOD and CAT ($p<0.05$). This study showed lead induced changes in some liver and kidney function parameters as well as some oxidative markers of these organs and also revealed possible amelioratory effects to these changes after MO supplementation.

Keywords: Antioxidant enzymes; kidney markers; lead; liver enzymes; Moringa oleifera.

1. INTRODUCTION

When inhaled or swallowed lead is a very toxic metal and it affects almost every organ and system in the body [1]. It has been reported to affect liver [2] as well as kidney [3]. Its extensive use has lead to environmental contamination and human exposure with significant health problems [4]. Lead has been linked with high incidence of renal dysfunction with attendant glomerular and tubulointerstitial changes, resulting in chronic kidney disease, hypertension as well as hyperuricaemia [5].

The mechanisms through which these occur are still unclear, but animal studies involving chronic exposure have revealed renal tubular damage with lead intranuclear inclusion bodies [6]. This may be as a result of reactive oxygen species which are the initiators of peroxidative damage to membranes [7]. Studies have shown that lead exposure to laboratory animals raised lipid peroxidation or lowered antioxidant defense mechanism [8,9]. Some researchers further showed that the level of lipid peroxidation has a direct relationship with lead concentration in brain regions [10] and in liver of lead exposed rats [11].

The conventional treatment for lead toxicity involves the use of chelators such as Dimercaptosuccinic acid (DMSA), Ethylene Diamine Tetra Acetate (CaNa2EDTA), British anti-lewisite (BAL), and Penicillamine which have been found to cause mild to severe side effects such as abdominal distress, transient rash, elevated hepatocellular enzyme concentration, neutropenia, kidney failure, very low blood pressure, lung injury, infections, coma and seizures [12-15]. Moreover, treatment with chelators does not reverse or diminish toxicity caused by lead. For instance, Rogan et al, in their work pointed out that although chelation therapy for children with blood lead concentrations of 20-44 ug/dl can be expected to reduce blood lead level, but it could not reverse cognitive impairment or other behavioral effects of lead [16].

It is now clear that majority of the conventional drugs for lead toxicity are burdened with many side effects and limitations, and that there is no safe or effective treatment available for heavy metal poisoning such as lead poisoning. Therefore, there is need to identify safe and effective drug for the treatment of lead toxicity. Interest has shifted to the use of antioxidants, metals (iron, calcium, selenium etc.), vitamins (Vit. C and E), amino acids (methionine) for the treatment of heavy metal toxicities either as individual or as combination therapy [17,18].

Thus, the use of plant extracts such as MO that is rich in antioxidants, vitamins, amino acids, and

essential metals [19,20] might prove a better option in the treatment of lead induced toxicity. Almost all the parts of MO are extensively used for the treatment of inflammation, [21] cardiovascular and liver disease [22], hematological and renal function [23], and metal intoxications including arsenic [24] and lead [25].

2.1 MATERIALS AND METHODS

2.1.1 Plant collection and extraction

Fresh leaves of MO were collected from Obukpa, in Nsukka L.G.A. in Enugu State, Nigeria and was taxonomically identified by a botanist (Mr Ugwuozor P.O) in Nnamdi Azikiwe University (NAU), Awka. The voucher specimen (PCG474/A/037) was deposited at the herbarium of the department of Pharmacognosy, Faculty of Pharmaceutical Sciences, NAU, Akwa for future reference. The fresh leaves were washed severally in clean water, air dried under shade for about 3 days and then pulverized with a blender. This yielded a 500 g dried powder which was subjected to complete extraction by cold maceration method. Briefly, the 500 g dried powder was soaked in 2.5 liters of methanol for one week with intermittent shaking and exchange of solvent after every 48 hours. The percolated extract was dried in rotary evaporator at 45℃, weighed and dissolved in distilled water to give the final concentration of 200 and 400 mg extract/kg body weight. The exact weight of the final extract was 86.4 g which gave a percentage yield of 17.28%.

2.1.2 Procurement and care of animals

Thirty six male Wistar rats of weight 140-160 g were procured from the breeding colony of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Akwa and housed in stainless steel cages in their animal facility. They were fed with clean drinking water and rat chow *ad libitum*. Good laboratory practice was followed and the animals were maintained under laboratory conditions at a temperature of 22±3℃, relative humidity of 50±5% and photoperiod of 12 h (12 h-dark and 12 h-light cycle). In order to avoid diurnal variation all the experiments were carried out at the same time of the day.

2.1.3 Experimental design

The study design was approved by the Ethics Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus with reference number FBMS/EC/004/2014. The feeding was done with the aid of gastric gavage. The animals were grouped into six different groups with each group assigned different alphabets A, B, C, D, E, and F each having 6 animals.

- **Group A** rats were fed with normal rat chow and clean water ad libitum for 6 weeks
- **Group B** rats were administered with 100 mg/kg body weight of lead acetate (PO) for 6 weeks.
- **Group C** rats were fed normal rat chow and 200 mg/Kg body weight MO extract (PO) for 6 weeks.
- **Group D** rats were fed with normal rat chow and 400 mg/Kg body weight MO extract (PO) for 6 weeks.
- **Group E** rats were administered with 100 mg/kg body weight lead acetate and 200 mg/Kg body weight MO (PO) for 6 weeks.
- **Group F** rats were administered with 100 mg/kg body weight lead acetate and 400 mg/Kg body weight MO (PO) for 6 weeks.

2.1.4 Sample collection and biochemical analysis

Blood samples were collected in two stages namely: baseline (pre-treatment) and six weeks following treatment. At end of the experiment, the rats were starved over night and the blood was collected into a sterile lead-free plain bottle by retro orbital puncture using a sterile capillary tube. Thereafter, the serum was separated and stored at -20℃ until the analysis of liver and kidney function parameters.

All the biochemical parameters were analyzed using HITACHI 902 fully automated Chemistry Analyzer by Roche Diagnostics (Germany). The appropriate operator's manual for analyzer-specific assay instructions for these parameters were strictly followed to ensure optimal performance of the assay. The quality control materials from Roche Diagnostics and Randox Company (USA) were used during the analysis.

GGT was analyzed using the method of Szasz [26] while AST and ALT estimated using the method of Bergmeyer et al. [27] ALP was estimated by the method of Mc Comb and Bowers [28], Serum bilirubin, albumin and total protein was also quantified using modified Jendrassik-Groff, [29] Doumas et al. [30] and Biuret's Methods [31] respectively. Creatinine by the Jaffé reaction, [32] while the level of urea and uric acid were also assessed using the methods

of Sampson et al. [33] and Kageyama, [34] respectively.

2.1.5 Preparation of homogenate

The organs (liver and kidney) were dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight noted and then homogenized. 10% w/v homogenate was prepared using phosphate buffer saline (pH 7.4). The mixture of tissue and buffer (pH 7.4) was homogenized using a homogenizer and the homogenates centrifuged using TGL-20M Ultra refrigerated centrifuge (China) at 12,000 g for 20 minutes at 4℃ to get the post mitochondrial supernatant which was used for the assay of antioxidant enzymes (SOD, GST and CAT), and MDA. The samples were stored at -30℃, and were analyzed within one week.

SOD was determined using the method adopted by Misra and Fredovich [35]; CAT was assayed using the method of Sinha [36], GST was assessed using the method of Habig et al. [37] while MDA was quantitated using the method of Gutteridge and Wilkins [38].

2.1.6 Statistical analysis

The version 21 of Statistical Package for Social Sciences (SPSS) (IBM, USA) was used in statistical analysis. All data were expressed as the mean ± standard deviations. The test of significant difference of a group at different interval was done by paired t-test while the different in mean of more than two groups was done using ANOVA. The graphical presentations were done using Sigma Plot version 12 software. $P<0.05$ was used as the cut-off point of significant.

3. RESULTS

The pre-treatment serum levels of ALP, ALT, AST and GGT were similar in all the groups ($P>0.05$). All the liver enzymes increased significantly after 6 weeks of lead acetate administration (group B) when compared with their pre-treatment levels and other groups ($P<0.05$). However, all the liver enzymes were still similar after 6 weeks of administration of 200 mg/kg MO (group C) and 400 mg/kg MO (group D) when compared to their pre-treatment levels ($P>0.05$). ALT, AST, and GGT increased significantly after 6 weeks administration of 200 mg/kg MO+Lead (groups E) and 400 mg/kg MO+Lead (group F) when compared to their pre-treatment levels ($P<0.05$), but ALP levels did not differ significantly in these groups ($P>0.05$) (Fig. 1).

The pre-treatment serum levels of TBil, TP and albumin in all the groups did not differ significantly ($P>0.05$). Only the serum TBil levels increased significantly after 6 weeks of lead acetate administration (group B) when compared to their pre-treatment levels and other groups ($P<0.05$). However, all these parameters were still similar after 6 weeks of administration of 200 mg/kg MO (group C) and 400 mg/kg MO (group D) when compared to their pre-treatment levels ($P>0.05$). TBil levels increased significantly after 6 weeks administration of 200 mg/kg MO+Lead (groups E) and 400 mg/kg MO+Lead (group F) when compared to their pre-treatment levels ($P<0.05$), but TP and albumin levels did not differ significantly in these groups ($P>0.05$) (Fig. 2).

The pre-treatment serum levels of creatinine, urea and UA were similar in all the groups ($P>0.05$). All these parameters increased significantly after 6 weeks of lead acetate administration (group B) when compared to their pre-treatment levels and other groups ($P<0.05$). However, creatinine and urea were still similar after 6 weeks of administration of 200 mg/kg MO (group C) and 400 mg/kg MO (group D) when compared to their pre-treatment levels ($P>0.05$), but UA levels decreased significantly in these groups when compared with its pre-treatment level ($P<0.05$). All the parameters increased significantly after 6 weeks administration of 200 mg/kg MO+Lead (groups E) and 400 mg/kg MO+Lead (group F) when compared to their pre-treatment levels ($P<0.05$), except for urea that did not differ significantly after 6 weeks administration of 400 mg/kg MO+Lead (group F) ($P>0.05$) (Fig. 3).

The mean levels of kidney SOD, GST, and catalase decreased significantly while MDA levels increased significantly in group B when compared with group A ($P<0.05$). All these parameters in groups C, D, E and F did not differ significantly when compared with group A ($P>0.05$), except for GST in group E ($P<0.05$). However, all these parameters differed significantly in group B when compared with groups C, D, E, and F ($P<0.05$) (Fig. 4).

The mean levels of liver SOD, GST, and catalase decreased significantly while MDA levels increased significantly in group B when compared with group A ($P<0.05$). All these

parameters in groups C, D, E and F did not differ significantly when compared with group A (P>0.05). However, all these parameters differed significantly in group B when compared with groups C, D, E, and F (P<0.05) (Fig. 5).

4. DISCUSSION

Lead is a common environmental toxic heavy metal that has no known biological function in the body. It has very good qualities/properties and these have earned it a wide industrial application, thus making lead toxicity to remain an important public health problem because of its variety of sources in any household and environment [39].

In this study, the activities of serum liver enzymes before and following lead acetate and MO administration were investigated. The results showed that the liver enzymes (ALT, AST, ALP, and GGT) and bilirubin were raised after six weeks of lead acetate administration when compared to the pre-treatment and control levels. These results were in line with the works of other researchers [40-42] who also reported increased liver enzymes after lead acetate administration.

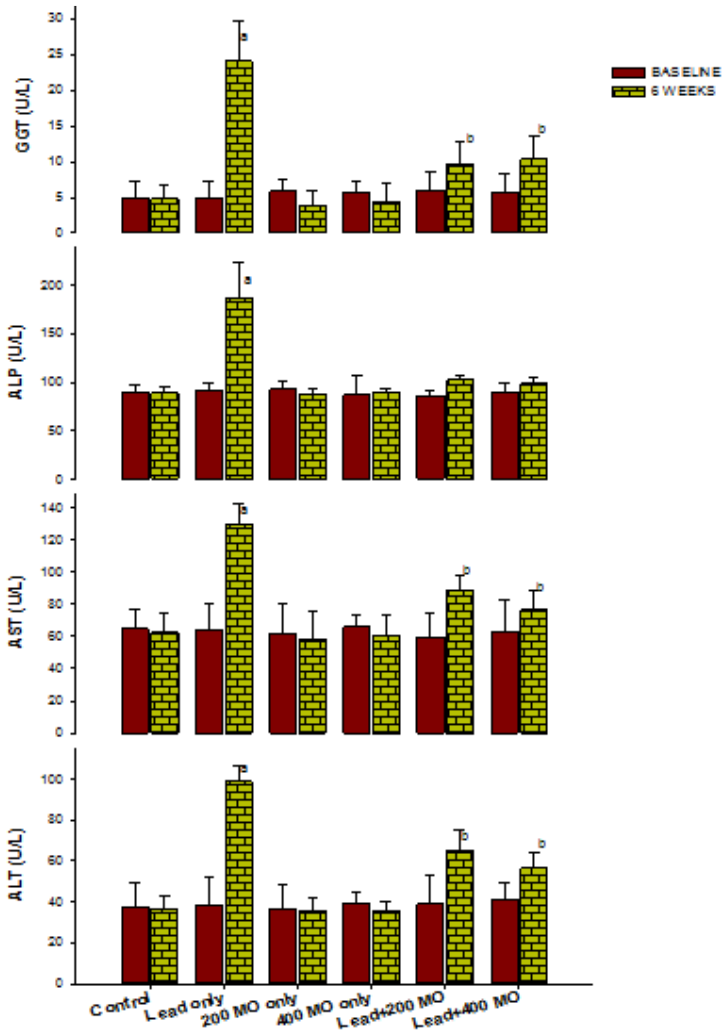

Fig. 1. The effects of six weeks administration of Lead acetate, *Moringa oleifera* and their combinations on mean levels of some liver enzymes

a = P<0.05 when compared with other groups and its baseline
b = P<0.05 when compared with its baseline

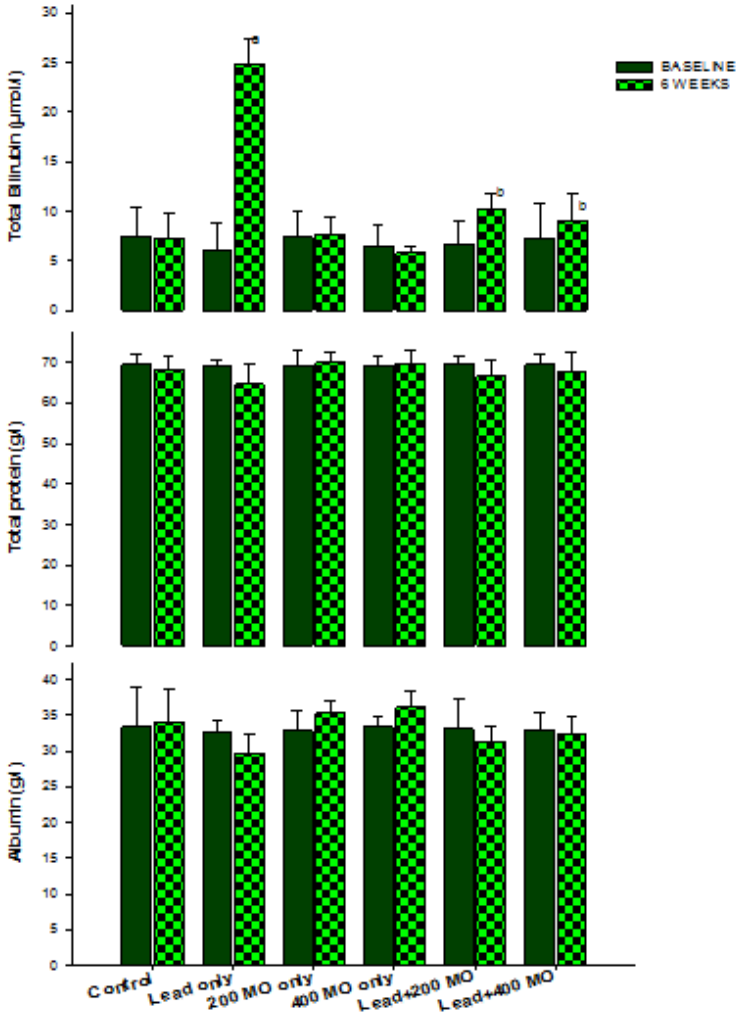

Fig. 2. The effects of six weeks administration of Lead acetate, *Moringa oleifera* and their combinations on mean levels of total protein, albumin and total bilirubin

a = P<0.05 when compared with other groups and its baseline

b = P<0.05 when compared with its baseline

Liver is the major organ for detoxification of many toxic chemicals; consequently its hepatocytes are liable to injury by various chemicals including lead exposure. Cheng et al. has found that lead could activate signals that increase tumor necrosis factor-α, present in the liver which can cause inflammation, malignancy and cell death [43]. Therefore, the raised levels of AST and ALT might be due to the leakage of these enzymes from the hepatocytes into the blood stream after cellular damage [42]. It has also been postulated that the stimulation of lipid peroxidation and depletion of antioxidant reservoirs are the major contributors to hepatic tissue damage related to lead exposure [44-46]. High serum ALP level is an indicator of altered biliary flow and damage to the liver cell membrane [47], while raised GGT level is an indication of liver damage [48] as well as a supportive fact that raised ALP is originating from the liver.

This study revealed significant increase in lipid peroxidation index (MDA) with concomitant significant decrease in the levels of antioxidant enzymes (CAT, SOD and GST) of liver after the administration of lead acetate when compared to the control levels. These results were in line with the work of Sharma et al. [49] and Osman, [42] who reported raised liver MDA with decreases in some liver antioxidant enzymes. These

observations are suggestive of possible involvement of oxidative stress in the pathophysiology of lead toxicity. This further supports the raised levels of liver enzymes found in this study, since it has been earlier postulated that oxidative stress can lead to hepatic tissue damage [45].

However, MO at the concentration of 200 and 400 mg/Kg PO was able to significantly decrease the lead-induced levels of liver enzymes and bilirubin as well as lead-induced hepatic lipid peroxidation. This showed a hepatoprotective ability of MO against lead-induced hepatic damage as determined by decreases in AST, ALT, ALP, GGT, bilirubin, lipid peroxidation levels with concomitant increases in the levels of hepatic CAT, SOD, and GST. This present findings were in agreement with the works of Fakurazi et al. [50]; Fakurazi et al. [51]; Uma et al. [52] who showed that MO leaves protected against acetaminophen-induced liver damage by decreasing liver enzymes and hepatic lipid peroxidation as well as increasing antioxidant enzymes levels. Sharifudin et al. [53] also showed that MO leaves and flowers at 200 and 400 mg/kg prevented acetaminophen-induced hepatotoxicity.

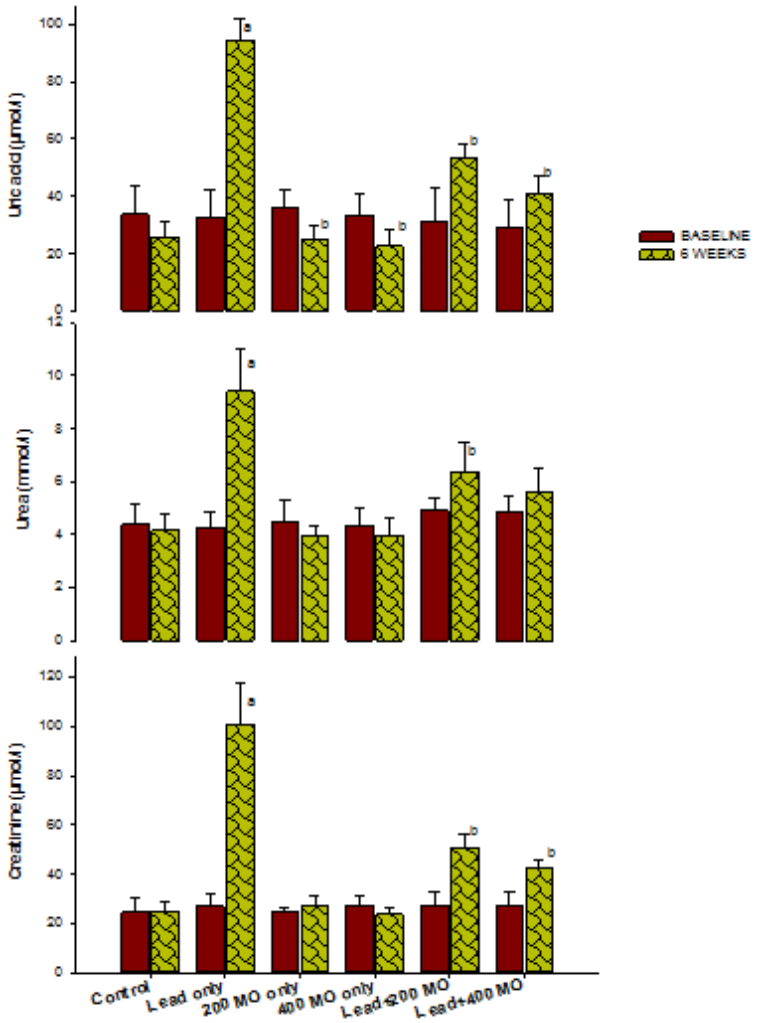

Fig. 3. The effects of six weeks administration of Lead acetate, *Moringa oleifera* and their combinations on mean levels of some kidney markers (creatinine, urea and uric acid)

a = P<0.05 when compared with other groups and its baseline

b = P<0.05 when compared with its baseline

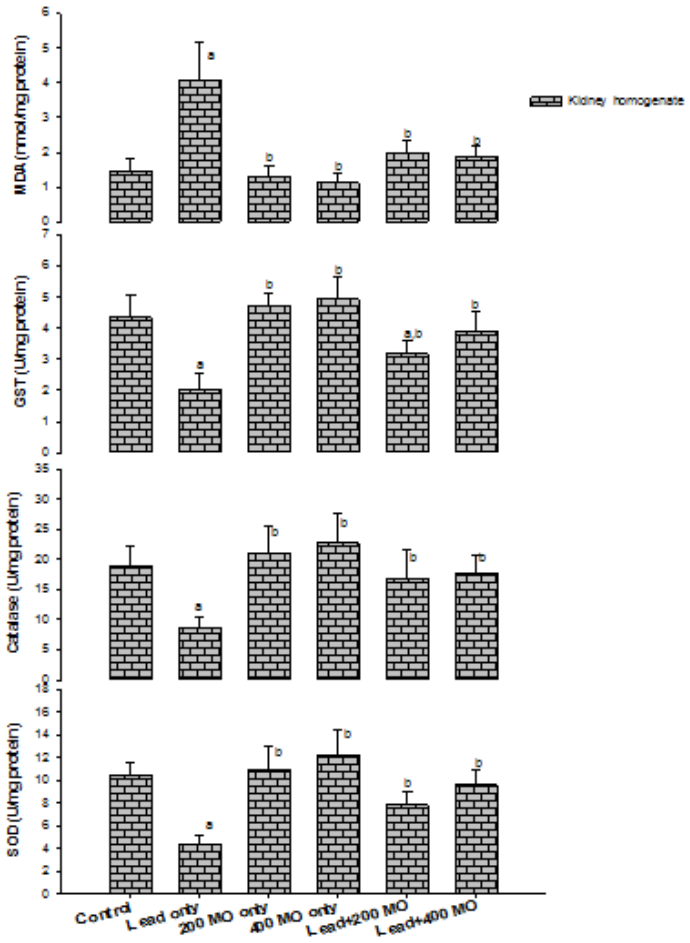

Fig. 4. The effects of six weeks administration of Lead acetate, *Moringa oleifera* and their combinations on mean levels of kidney SOD, GST, MDA and catalase

a = P<0.05 when compared with control
b = P<0.05 when compared with lead only

The study also revealed lead-induced nephrotoxicity as determined by significant increased levels in the creatinine, urea and uric acid in rats that were fed with lead acetate for six weeks compared to the pre-treatment and control levels. This findings were augumented by the significant increase in the kidney lipid peroxidation after six weeks of lead acetate administration as well as decrease in the kidney antioxidant enzymes levels (CAT, SOD, and GST) when compared with their pre-treatment and control levels. These findings were in agreement with the work done by Elgaml et al. [54] who reported an increase in the levels of creatinine, urea, MDA and decrease in SOD in Oreochromis niloticus treated with lead acetate. Increase in the serum levels of urea and creatinine is an indication of impaired kidney function [55]. The increase in the levels of markers of kidney damage in lead exposed group might be due to glomerular insufficiency [56] as well as increase in oxidative stress [57].

When MO and lead acetate were administered simultaneously, MO significantly prevented lead-induced kidney damage as determined by the reduction in the levels of markers of kidney damage (creatinine, urea and uric acid) as well as decrease in the kidney lipid peroxidation and increase in the levels of kidney antioxidant enzymes (CAT, SOD, and GST). This work agreed with the findings of Ouedraogo et al. [58] who reported that MO at doses of 150 and 300 mg/kg body weight prevent gentamicin-induced nephrotoxicity in rabbits by significantly decreasing the markers of kidney damage including lipid peroxidation, serum creatinine and urea as well as histological changes.

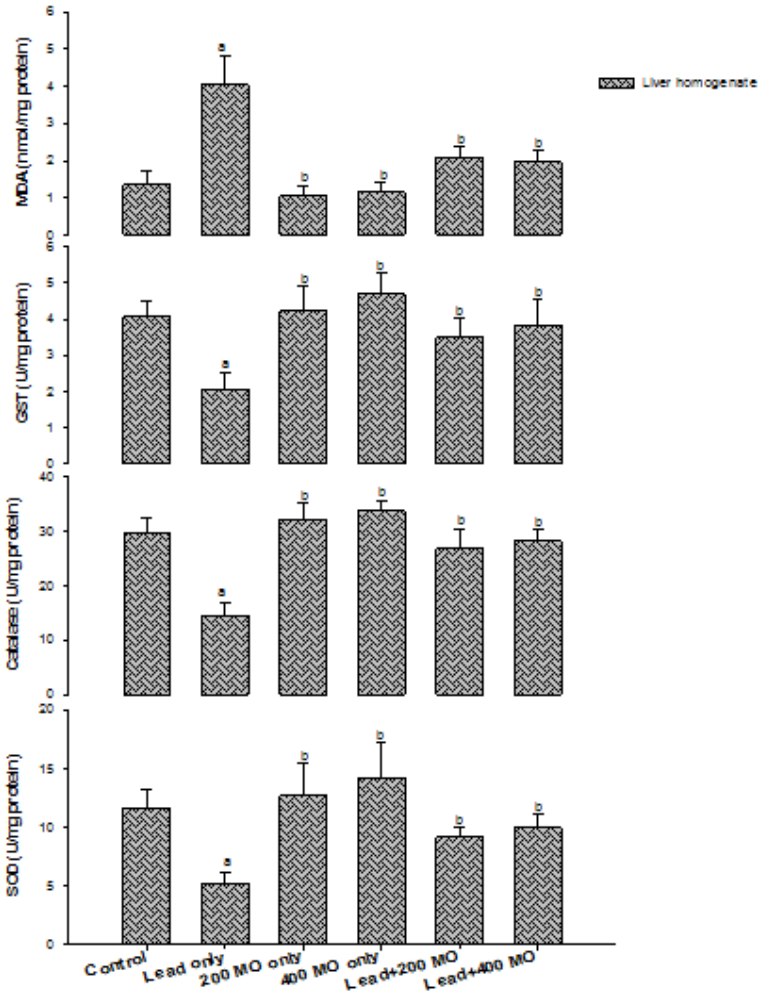

Fig. 5. The effects of six weeks administration of Lead acetate, *Moringa oleifera* and their combinations on mean levels of liver SOD, GST, MDA and catalase

a = P<0.05 when compared with control
b = P<0.05 when compared with lead only

This study revealed both hepatoprotective and nephroprotective activity of MO against lead toxicity. The reduction in the markers of both liver and kidney damage by MO might be attributed to the stabilizing ability of the cell membrane thus preventing enzyme leakage and restoring the integrity of the cells [59]. The protective effect of MO might be due to the presence of Quercetin and kaempferol, [60] vitamin A, and ascorbic acid [61].

5. CONCLUSION

This study showed lead induced changes in some liver and kidney function parameters as well as some oxidative markers of these organs and also revealed possible amelioratory effects to these changes after MO supplementation.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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