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Original Article

Exploring the Therapeutic Potential of *Moringa Oleifera* in Lead Poisoning: An Investigation of its Effects on Blood Lead Levels and Oxidative Stress in Rats

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Abstract

Objective: Lead poisoning is a global public health problem that has beenassociated with poor treatment outcomes. We therefore evaluated the ability of *Moringa oleifera* (*M. oleifera*) to reduce blood lead levels (BLL) and lead-induced oxidative stress related to dimercaptosuccinic acid (DMSA) in albino Wister rats. **Methods:** Thirty rats were assigned to five groups each consisting of six rats. The control group (A) received normal rat chow and water ad libitum for 12 weeks. Group (B-E) initially received 100-mg/kg body weight of lead acetate orally for 6 weeks. Thereafter, groups B, C, D, and E received DMSA and various doses of *M. oleifera* and their combination for an additional 6 weeks. Blood samples were taken before treatment, 6 and 12 weeks after treatment for analysis of BLL, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione-s-transferase (GST). **Results:** BLL and MDA increased significantly ($p<0.05$), while serum SOD, CAT and GST activities decreased significantly ($p<0.05$) from their pretreatment valuesafter 6 weeks of lead-acetate administration. However, administration of 400-mg/kg body weight M. oleifera at 12 weeks posttreatment significantly ($p<0.05$) reduced 6-week BLL by (40.5%); MDA (52%); and significantly ($p<0.05$) increased the activities of serum SOD by (35%); CAT (26.3%); and GST (53%). **Conclusion:** *M. oleifera* was observed not only to be effective in lowering blood lead levels, but also to alleviate lead-induced oxidative stress through enhanced antioxidant activities. M. oleifera may therefore serve as an alternative therapeutic approach to lead poisoning, particularly in resource-constrained settings.

KEYWORDS: Antioxidants; lead toxicity; *Moringa oleifera*; dimercaptosuccinic acid; oxidative stress.

Introduction

Lead exposure causes approximately 143,000 deaths each year mostly in developing regions [1]. It is one of the biggest environmental problems considering the number of people exposed and the associated public health burden [2]. In Nigeria, the burden of lead poisoning is high. For instance, lead poisoning killed over 400 children, while more than 2,500 children were enrolled in the intervention program organized by Medicens Sans Frontieres following the lead-poisoning outbreak in Zamfara State, Nigeria [3]. In addition, lead poisoning as a result of illegal mining in Niger State, Nigeria claimed around 28 lives (mainly children under age of five), while many required medical treatment [4].

A significant number of small-scale and medium scale industrial/occupational workers (auto-mechanics, electricians, welders, painters, panel beaters etc.) using lead-based materials are also

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at risk of lead poisoning [5-7]. Their exposure risks are increased due to a lack of work place regulations for lead exposure and poor implementation of waste management policies. According to the WHO, the widespread use of lead in different regions of the world has resulted in widespread environmental contamination, human exposure and high public health burden and currently there is no known level of lead exposure that is considered safe [1].

Chelation therapy is the most commonly used treatment option for lead poisoning [8]. These chelating agents include ethylene diamine tetra acetate (CaNa2EDTA), British anti-lewisite (BAL), Dimercaptosuccinic acid (DMSA), and penicillamine etc. Although DMSA is better than other chelating agent in terms of safety, it is still burdened with high content of essential minerals extraction [9]. In addition, medical condition such as lead poisoning, in which the symptoms are not as obvious and often go undiagnosed especially in the case of chronic and low-dose exposure, as in the case of occupational workers do not always require treatment with these conventional drugs. These side effects and other limitations have prompted the search for a safer alternative therapy.

M. oleifera, also called drumstick tree, or horseradish tree, is a well-known and most widespread species of the Moringaceae family. It is a very important tropical plant widely used as medicine, human food, and in oil production [10]. All parts of *M. oleifera* such as leaves, flowers, gums, roots, seeds and fruits are used extensively to treat diabetes mellitus [11], renal and liver diseases [12], hematological and renal function [13], as well as metal intoxications including arsenic [14], and lead [15]. Previous studies have shown that *M. oleifera* leave are a good metal ion chelator [16].

The ability of *M. oleifera* seeds to remove cadmium from aqueous media has also been demonstrated [17]. This property is very useful in purifying drinking water, especially in low-income countries. It is therefore likely that the leaf extract could also remove lead in vivo. However, this work was designed to investigate the therapeutic potential of *M. oleifera* in the treatment of lead toxicity and to relate the outcome of supplementation to that of DMSA in lowering BLL as well as enhancing antioxidant activities.

Materials and Methods

Plant Collection and extraction

Fresh leaves of *M. oleifera* were obtained from Obukpa at Nsukka LGA collected, in Enugu State, Nigeria, and was identified taxonomically by a botanist (UgwuozorPO) at Nnamdi Azikiwe University (NAU), Awka. The specimen copy (PCG474/A/037) has been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, NAU, Agulu for future reference. The fresh leaves were washed in clean water several times, air-dried in the shade for about 3 days, and then pulverized with a blender. This yielded 500 g of dried powder, which underwent complete extraction by a cold maceration process. Briefly, 500 g of dried powder was soaked in 2.5 liters of methanol for a week with intermittent shaking and changing the solvent every 48 h. The percolated extract was dried in the rotary evaporator at 45°C, 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, giving a percentage yield of 17.28%.

Experimental animals

30 male Wistar rats weighing 140-160 g were procured from the breeding colony of the Department of Pharmacology/Toxicology, Faculty of Pharmaceutical Sciences, NAU, Awka. They were maintained under standard laboratory animal conditions at a temperature of 22±3 °C, a relative humidity of 50±5% and a photoperiod of 12 h (12 h dark and 12 h light cycle). All animal experiment was performed in accordance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

Methods

The study design was approved by the Ethics Committee of the Faculty of Basic Medical Sciences, NAU, Nnewi Campus. The animals were assigned to five different groups of six animals each. Group A received normal rat chow and water ad libitum for 12 weeks, while groups B and C received 100-mg/kg body weight lead acetate orally for 6 weeks and then 200 and 400-mg/kg body weight *M. oleifera* for a further 6 weeks. Group D rats were administered 100-mg/kg body weight lead acetate for 6 weeks and then 30-mg/kg body weight DMSA (PO) for 5 days, followed by 14 days 20-mg/kg body weight/day, while group E were administered 100-mg/kg body weight lead acetate for 6 weeks and then continued with 200-mg/kg body weight *M. oleifera* for 6 weeks concomitantly with 30-mg/kg body weight DMSA (PO) for 5 days followed by 14 days 20-mg/kg body weight/day.

Sample collection and biochemical analysis

Blood samples were collected at three stages, namely: baseline (pretreatment), six weeks after administration of lead acetate, and six weeks after administration of *M. oleifera*, DMSA, and their combinations. At the end of the experiment, the rats were fasted overnight and blood was collected by retro-orbital puncture. Blood was collected in lithium heparin containers for blood

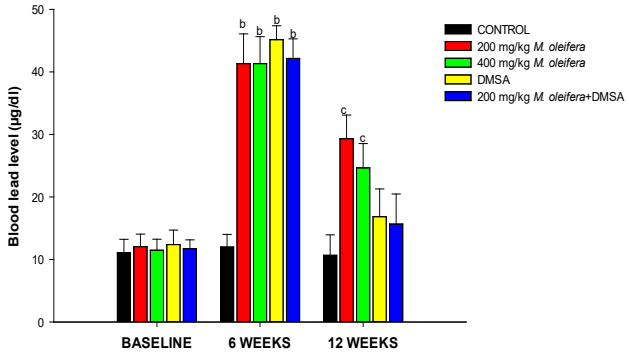
lead determination and also in sterile plain containers. The blood in the plain containers was allowed to clot, collected and the serum separated and stored at −30°C for analysis of oxidative parameters within a week of storage. [18]. SOD was determined using the method of Misra and Fredovich[19]. CAT was tested using the method of Sinha [20]. GST was determined according to the method of Habig, et al. [21].MDA was analyzed using the method of Gutteridge and Wilkins[22].

Statistical analysis

Version 21 of the Statistical Package for Social Sciences (SPSS) (IBM, USA) was used for the statistical analysis. All data were expressed as mean standard deviation. Test of significant difference of one group at different intervals was performed by paired t-test, while mean difference of more than two groups was performed using ANOVA ($p<0.05$ was taken as cut-off point for significant used). The plots were performed using SigmaPlot version 12 software.

Results

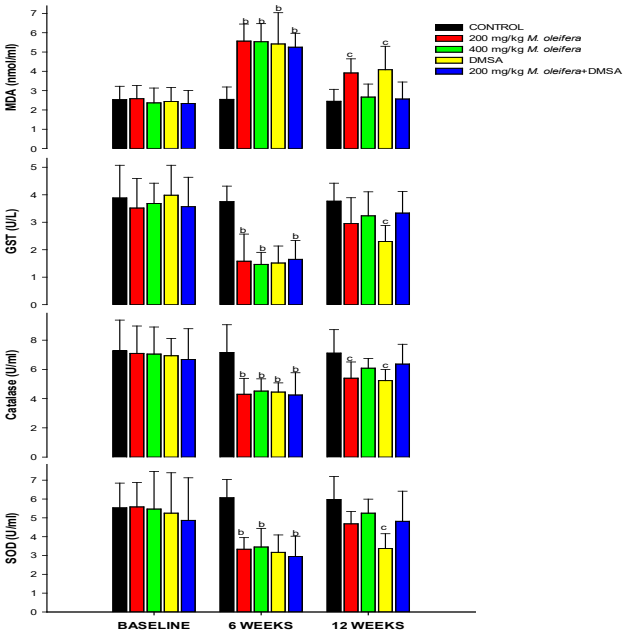
The pretreatment BLL did not differ significantly ($p>0.05$) in all the groups. After six weeks of lead-acetate administration, there was significant ($p<0.05$) increase in the mean BLL in all the groups when compared with their pretreatment, and control group. BLL level decreased significantly ($p<0.05$) after 6 weeks of subsequent *M. oleifera* and DMSA administration when compared to their six weeks (lead acetate administration) level in all the groups. BLL at 12 weeks in 200-mg/kg body weight *M. oleifera* and 400-mg/kg body weight *M. oleifera* groups differed significantly ($p<0.05$) when compared to their pretreatment levels($p<0.05$), while BLL at 12 weeks in DMSA and 200-mg/kg body weight *M. oleifera* + DMSA groups were similar with their pretreatment levels ($p>0.05$) (Figure 1).



b=p<0.05 when compared with their baseline and 12 weeksc=p<0.05 when compared with their baseline

Figure 1. The effects of 6-week administration of lead, and subsequent 6-week administration of different doses of *M. oleifera*, DMSA and their combinations on mean levels of blood lead levels.

The six-week administration of 200-mg/kg body weight *M. oleifera*, 400-mg/kg body weight *M. oleifera*, DMSA, and 200-mg/kg body weight *M. oleifera* + DMSA were able to reduce blood lead level up to 29%, 40.5%, 63% and 63.3% respectively from their six weeks lead acetate levels (Figure 2).



b=p<0.05 when compared with their baseline and 12 weeks. c=p<0.05 when compared with their baseline.

Figure 2. The effects of 6-week administration of lead, and subsequent 6-week administration of different doses of *M. oleifera*, DMSA and their combinations on mean levels of SOD, catalase, GST, and MDA.

The pretreatment levels of serum MDA, SOD, catalase and GST did not differ significantly ($p>0.05$) in all the groups. The mean level of MDA increased significantly ($p<0.05$) while the mean levels of SOD, GST and CAT activities decreased significantly ($p<0.05$) after six weeks of lead-acetate administration when compared with their pretreatment levels in all the groups, except in control group ($p>0.05$). However, the mean level of MDA decreased significantly ($p<0.05$) while the mean levels of SOD, GST and catalase activities increased significantly ($p<0.05$) after 6 weeks of subsequent *M. oleifera* and DMSA administration when compared with their six weeks of lead-acetate administration in all the groups ($p<0.05$), except for SOD and GST in DMSA and control group ($p>0.05$).All the parameters did not differ significantly after 6 weeks of subsequent *M. oleifera* and DMSA administration when compared with their pretreatment levels in all the groups ($p>0.05$), except in DMSA group and for MDA and catalase in 200-mg/kg body weight *M. oleifera* group ($p<0.05$) (Figure 2).

The six-week administration of 200-mg/kg body weight *M. oleifera*, 400-mg/kg body weight *M.*

oleifera, DMSA, and 200-mg/kg body weight *M. oleifera* + DMSA produced serum increase in SOD activities by 29%, 35%, 5%, 38.5% (Figure 3); CAT activities by 20.8%, 26.3%, 14.8%and 34% (Figure 3); GST activities by 48.6%, 53%, 32% and 49.4% (Figure 3); and were able to decrease MDA levels by 29.6%, 52%, 23.7% and 51.9% respectively (Figure 3).

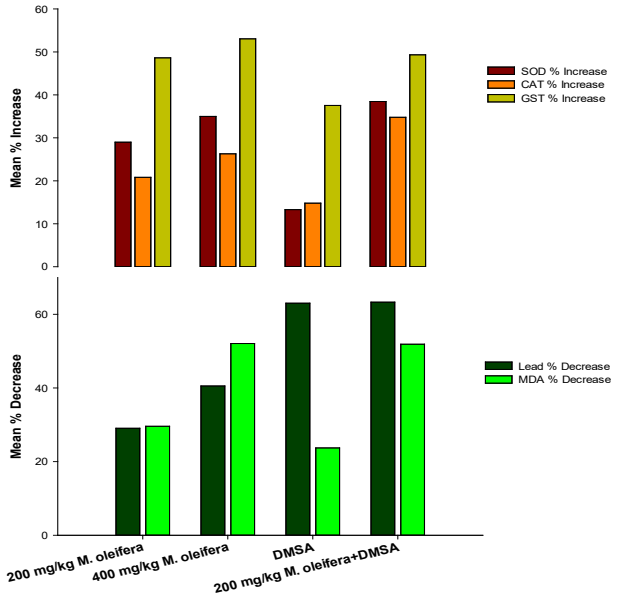


Figure 3. The percentage changes in the levels of BLL, MDA, SOD, CAT, and GST following *M. oleifera* and DMSA treatment on lead-induced toxicity in rat.

Discussion

Lead is a toxic metal when ingested or inhaled, and it affects almost every system in the body [23]. Six weeks oral lead administration, as observed in this study could result in significantly elevated blood lead levels (Figure 1), which correlated with the recorded lead-induced toxicity. A previous study reported an association between elevated blood lead and deleterious effects on antioxidant activities [24].

However, six weeks oral administration of *M. oleifera* leave extract at different doses significantly reduced BLL though DMSA alone and in combination with *M. oleifera* were more effective (Figure 1). The significant reduction in the blood lead level as observed in this study may have been mediated through chelation by the *M. oleifera* leave extract. Studies have shown that the leave extract of *M. oleifera* was rich in essential amino acids especially the sulfur containing amino acids [25]. These proteineous amino acids have been shown to exhibit variety of structurally related pH-dependent properties, which generate negatively charged atmosphere that play important role in binding to metals [17].

Lead induced oxidative stress has been described as the primary contributory agent in the pathogenesis of lead toxicity [26]. This study showed that lead exposure for six weeks significantly decreased antioxidant enzymes (SOD, CAT, and GST) and increased lipid peroxidation product (MDA) (Figure 2). These findings were in agreement with other previous works that reported increased lipid peroxidation [27], and decrease in antioxidant levels [28]. More so, previous studies have shown that lead can cause oxidative stress in many ways. For instance, by inducing the generation of reactive oxygen species; reduction in the antioxidant defense system of cells via depleting glutathione; interference with some essential metals; inhibition of sulfhydryl dependent enzymes or antioxidant enzymes activities; or by increased susceptibility of cells to oxidative attack by altering membrane integrity and fatty-acid composition [29].

After six weeks of administration of different doses of *M. oleifera* (200 and 400-mg/kg body weight), DMSA, and their combinations, the altered oxidative markers induced by lead exposure were variably restored. Though, DMSA alone increased the antioxidant enzymes activities (SOD, CAT and GST) and decreased the raised lipid peroxidation, *M. oleifera* at both concentrations were more effective in restoring the altered oxidative markers (Figure 2). This showed that the *M. oleifera* leave extract can reverse the oxidative cell damage caused by lead toxicity. These findings were in line with a previous study that reported significant higher activities of SOD, CAT and GST and a significant lower level of lipid peroxidation after administration of *M. oleifera* leave extract in diabetic rats [30]. They attributed the protection against oxidative damage by *M. oleifera* to increased level of flavonoid and phenolic contents presence in the *M. oleifera* leave extract.

Figure 3 showed that the combined administration of DMSA and *M. oleifera* was better than the individual treatments in the recovery of the antioxidant parameters and reduction in lipid peroxidation product as well as depletion of blood lead levels. Though, the combination did not appreciably decrease BLL more than DMSA alone, increasing the concentration of *M. oleifera* used in the combination may be accompanied by more pronounced reduction in BLL.

Due to high safety profile of *M. oleifera*[31], and its desirable nutrient contents such as vitamins, essential minerals, amino acids, fatty acids [32], extending the duration of supplementation may increase the degree of blood lead reduction and enhance antioxidant activities in subject exposed to lead. *M. oleifera* may also serve as a desirable therapeutic approach in chronic to low dose lead exposure, such as in the case of occupational workers in which case the conventional chelating

agents may not be recommended. Availability and cost-effectiveness also speakin favor of *M. oleifera* especially in low income countries like Nigeria.

Conclusion

M. oleifera not only significantly reduced blood lead levels, but also protected against oxidative damage after six weeks of administration in lead exposed rats.

Conflict of interest: None

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