

Effect of *Moringa oleifera*, activated carbon and wood charcoal on biochemical and hematological parameters of Wistar rats exposed to lead acetate

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Summary

The prophylactic efficacy of *Moringa oleifera*, activated charcoal and wood charcoal to reduce lead toxicity in Wistar rats was evaluated. Seventy-five rats were divided into five groups consisting of 15 rats and marked as groups I to V. Group I was given distilled water while groups II to V were given lead acetate at a daily dose of 1000 mg/kg for seven days consecutively. Group II served as positive control while group I was a negative control. Groups III, IV and V were subsequently treated daily with extract leaves of *Moringa oleifera*, activated charcoal and wood charcoal respectively, at a dose of 1000 mg/kg for a period of one week ($n = 5$) and two weeks ($n = 5$). Group II was given distilled water daily for two weeks to study the possibility of natural recovery. The adverse effects of lead toxicity on biochemical and hematological parameters were demonstrated when the positive groups were compared to negative groups. Treatment with *Moringa oleifera*, activated charcoal but not wood charcoal, resulted in improvement in biochemical and hematological parameters. This study demonstrates ameliorating effects of *Moringa oleifera* on lead induced toxicities in the liver, kidneys, and blood, there is potential to exploit these properties for treatment and prevention.

Keywords: Wistar rats, lead acetate, *Moringa oleifera*, activated charcoal, wood charcoal

Lead is considered a major environmental pollutant as it is incorporated into a variety of products including pesticides, discarded batteries, wall paints and tank lining and piping. It is also used extensively in industrial processes and large-scale mining. Lead is absorbed through the digestive, respiratory tracts and skin (14).

A permissible exposure limit for metallic lead, lead oxide and lead salts has been set by WHO and other health organizations (16), all these sources of lead can contribute to an increase in tissue levels.

Lead is a toxic agent that causes symptoms reflecting its multisystemic actions, it affects target organs such as the haematopoietic system, kidneys, liver and nervous system (8, 9, 29).

After lead absorption into the blood, 99% of lead is bound to erythrocytes (RBC), the other 1% remains in the plasma and is distributed to other tissues. The serum half-life of lead is around 25 days (17). Lead has been shown to alter RBC membrane flexibility and to increase RBC fragility, leading to increased risk of hemolysis. Lead remains in soft tissues (brain and kidneys) for less time than in bone. Lead accumulates in bone with time, and lead levels in bone generally increase with age (37). An investigation into lead toxicity using hematological indices revealed a significant decrease in the mean cell hemoglobin (MCH), mean corpuscular volume (MCV) and RBC count and an increase in monocyte count, and platelets in comparison with the control group (12).

An earlier study showed that low-level exposure to lead might accelerate progressive renal insufficiency (27). It has also been reported that exposure to lead may cause Fanconi-like syndrome in humans.

Lead also induces hepato-toxicity, leading to variation in serum alanine amino transferase (ALT), aspartate transferase (AST), γ -glutamyl transferase (GGT) and alkaline phosphatase (ALP). These changes were observed after chronic oral lead acetate administration to albino rats (40).

The ability of activated charcoal or carbon (AC) to mitigate the effects of lead intoxication has been extensively studied (8, 9). AC positively affected parameters altered by lead intoxication such as blood urea nitrogen, creatinine, AST and ALT. Some research work showed that treating cases of lead toxicity with AC, has a number of beneficial effects (27, LEAD Action News). It acts to minimize adsorption by forming complexes with lead and thereby reducing the effect of lead poisoning. Evaluation of biochemical and hematological parameters has demonstrated the potency of AC in ameliorating the effect of lead toxicity in mice (8, 9).

Alteration of the pro-oxidant and antioxidant balance in favor of pro-oxidant actions and generation of oxygen free radicals have been proposed as possible mechanisms for lead toxicity (11). Several intervention studies using reducing agents or substances containing reducing agents have been performed on experimental animals following lead exposure in order to investigate this hypothesis (4, 13).

In the present study, *Moringa oleifera* (MO) was used to treat lead poisoning. MO contains many antioxidant compounds such as vitamin C, E and B and flavonoid: each of these compounds has an ameliorating effect on lead poisoning (23). Vitamin C is a known free-radical scavenger and has been shown to inhibit lipid peroxidation in liver and brain tissue of lead-exposed animals (19, 35). Vitamin E has a known protective action in contributing to membrane stability and protecting membrane lipoproteins from oxidative damage (34). Flavonoids have reducing properties or antioxidant effects (3). An earlier study indicated that MO administration reduced levels of lead in blood, muscle, liver, and kidneys in experimentally exposed chicks (3). MO extracts have been shown to be safe in rodent administration trials (1).

The aim of the present study is to investigate the effect of MO, AC and wood charcoal (WC) on lead induced toxicities in Wistar rats.

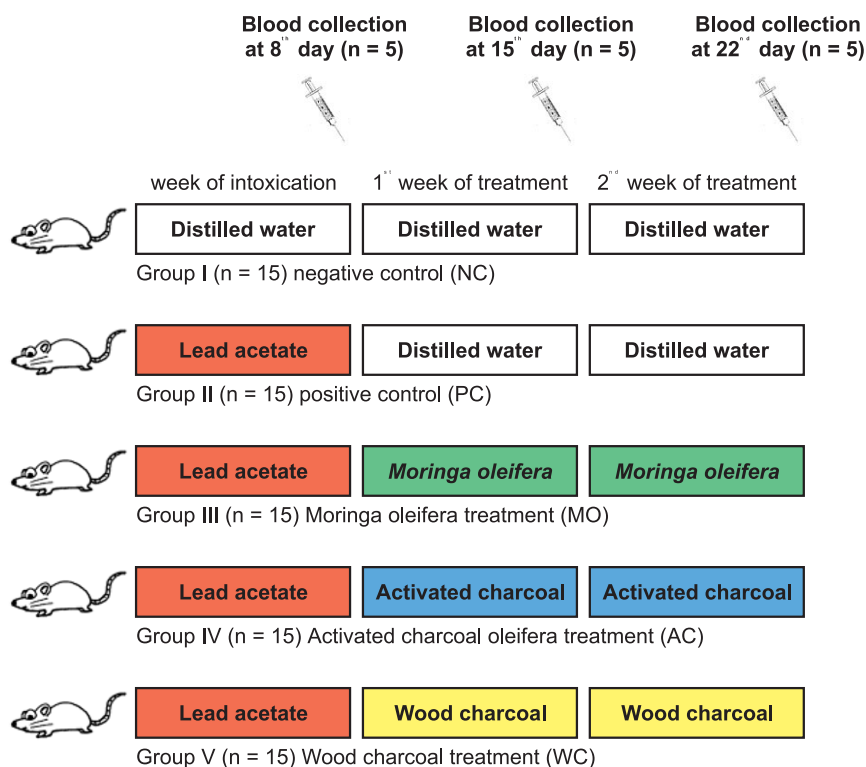


Fig. 1. Treatment scheme for rats: white bar – distilled water (NC); red bar – lead acetate (PC; 1000 mg/kg); green bar – *Moringa oleifera* (MO; 1000 mg/kg); blue bar – activated charcoal (AC; 1000 mg/kg); yellow bar – wood charcoal (WC; 1000 mg/kg). Each treatment was administered once a day. Rats (n = 5) were sacrificed on the 8th, 15th and 22nd day following initiation of lead acetate treatment

Materials and methods

Materials. Crude leaf extract of MO (extracted in biochemistry laboratory of NVRI-Nigeria), lead acetate tri-hydrate (May&Baker Company, England), AC (BDH laboratory, England), WC (prepared by slow pyrolysis of wood from Acacia tree sp., Nigeria, bought in loco), distilled water produced by Milly Q system.

Collection of *Moringa oleifera*. MO is a plant commonly grown in most parts of Nigeria. The leaves of MO were obtained from Angwan Mai Lafiya area, Kaduna South Local Government, Nigeria. The leaves were harvested in June. The fresh leaves from the MO plants were allowed to dry in an oven at a temperature of 40°C for 3 days.

Extraction of phytochemicals from MO leaves. The dried leaves were pounded in a mortar to fine particles. 671.8 g of the powdered leaves was weighed and dissolved in 5.5 liters of distilled water. The mixture was stirred at different time intervals until a homogenous mixture was obtained. The mixture was then kept in a refrigerator at 4°C for 96 hours, after which it was sieved using laboratory sieves of 1000, 500, and 300 micron pore size, consecutively. The filtrate was transferred into an oven at 40°C to remove the water content. Complete drying took 6 days.

Phytochemical analysis. The crude aqueous extracts of MO were qualitatively screened for the following phytochemical ingredients: alkaloids, anthraquinones, cardiac glycoside, steroids and tannins (41), flavonoids (28) and saponins (31), by TLC, HPLC and GC/MS techniques.

Therapeutic agents. The therapeutic agents tested were aqueous extracts (solutions) of MO, and suspensions of AC

and WC. These agents were selected following a literature search on their medicinal values.

Preparation of solutions. Water solutions of lead acetate and MO, and suspensions of AC and WC were prepared each at 150 mg/mL and were administered to experimental rats at 1000 mg/kg body weight. Stock solutions were stored at room temperature for a maximum period of 15 days after the preparation.

Animals. Seventy five adult male Wistar rats were obtained from the Small Animal Production Unit of NVRI-Vom, Nigeria, these had a mean body weight of 160 ± 30 g. The rats were maintained on a normal diet, their environment was kept at the standard temperature (25.0°C), 12/12-hr light/dark cycle, ventilation, and hygienic conditions of the experimental animal house. The rats were allowed to acclimatize for seven days in the animal facility before the commencement of the experiment. All animals were handled in accordance with the standard guide for the care and use of laboratory animals (32).

Experimental design. The rats were randomly divided into 5 groups of 15. All the rats were fed daily with rat feed and had access to mineral water *ad libitum*. The grouping was as follows. Group I (NC): rats served as negative controls (NC). They received distilled water by gavage daily for 21 days. Group II (PC): rats served as positive controls (PC). They received lead acetate solution (1000 mg/kg) by gavage daily for 7 days and then were treated for 14 days with distilled water daily. Group III (MO): rats received lead acetate solution (1000 mg/kg) by gavage daily for 7 days and then MO solution at 1000 mg/kg daily (five rats for 7 days and five rats for 14 days). Group IV (AC): rats received lead acetate solution (1000 mg/kg) by gavage daily for 7 days and then AC at 1000 mg/kg daily (five rats for 7 days and five rats for 14 days). Group V (WC): rats received lead acetate solution (1000 mg/kg) by gavage daily for 7 days and then WC at 1000 mg/kg daily (five rats for 7 days and five rats for 14 days). Five animals of each group were randomly sacrificed at three different times (8th, 15th, and 22nd day after the initiation of lead acetate administration) (fig. 1). Three ml of blood was withdrawn directly from the heart of each animal. They were then sacrificed. About 1 ml of blood was collected into tubes containing EDTA (hematological screening) and about 2 ml into tubes without anticoagulant (for biochemistry tests).

Hematological screening. Total leukocyte count (TLC), Hemoglobin content (Hb%), Packed cell volume (PCV), White blood Cell (WBC) Count, Mean Cell Volume (MCV), Hemoglobin (HGB), Red Blood Cell (RBC) Count, Mean Cell Haemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Neutrophils % (N%), and Lymphocytes % (L%) were measured. The samples were analyzed using Abacus Junior Diatron GmbH, Wien Austria, S/N 111175.

Biochemical parameters. The biochemical parameters were determined according to the method described by the particular manufacturer. Enzyme activities were expressed in U/l. All reagents used were from Randox.

The biochemical parameters measured were: glutamyl-transferase (GGT), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), urea and creatinine (36).

Statistical analysis. Statistical values were expressed as mean \pm standard deviation (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered to be significant.

Results

Phytochemical screening of the aqueous extract of MO revealed the presence of saponins, alkaloids, cardiac glycosides, steroids, tannins and flavonoids, however anthraquinones were absent. Analyses were qualitative, and the concentration of the different compounds was not calculated.

Rats administered with 1000 mg/kg of lead acetate daily for one week showed several variations in their liver, kidney and hematological parameters.

Liver (fig. 2a, b, c). GGT, AST and ALT levels were significantly different in the lead acetate treatment group compared to the controls.

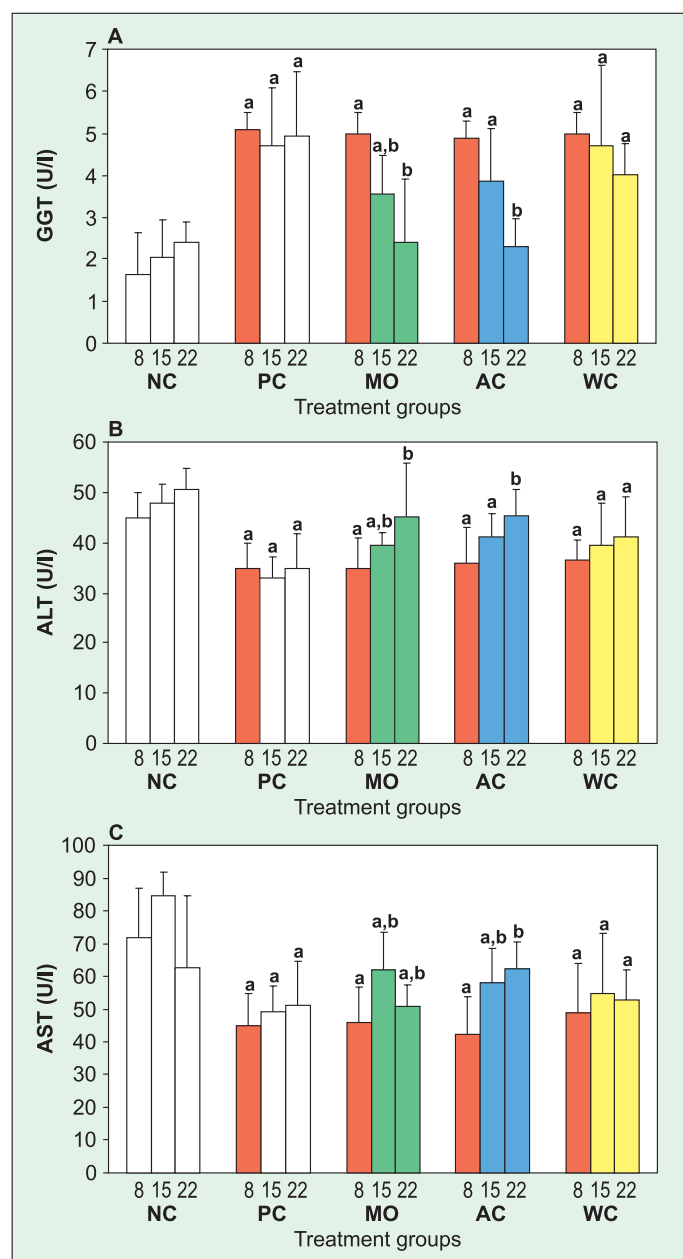


Fig. 2. Liver parameters on the 8th, 15th and 22nd day following initiation of lead acetate treatment. A) GGT; B) ALT; C) AST

Explanations: NC = negative group; PC = positive group; MO = *Moringa oleifera* group; AC = activated charcoal group; WC = wood charcoal group

Moringa oleifera. MO treatment resulted in a significant decrease and increase in GGT and ALT values respectively on the 15th day. Although they did not return to their pre-treatment levels, by the 22nd day, GGT and ALT levels returned to within the normal range ($P < 0.05$). MO partially improved AST values taken on the 15th and 22nd day.

Activated charcoal. AC treatment resulted in normal GGT and ALT levels by the 22nd day of treatment. AC resulted in decreased AST values by the 15th day, by the 22nd day ($P < 0.05$), AST values had returned to normal.

Wood charcoal. WC did not affect any of the liver parameters tested.

Kidney (fig. 3a, b). Urea was the only kidney parameter significantly affected by lead acetate treatment compared to the negative control. MO treatment decreased urea values by the 15th day and both MO and AC treatment restored urea values to normal by the 22nd day. WC did not cause any significant variation in urea values at any time point.

Hematological parameters. MCV, MCH and MCHC were significantly affected by lead acetate treatment compared to the negative controls (tab. 1). MO and AC treatment groups had normal MCV and MCH values restored by the 15th day, while MCHC values demonstrated an incomplete but significant recovery. The WC group did not show any improvement in the above mentioned parameters. By the 22nd day, hematological parameters for all the groups (including the PC) returned to normal values.

Discussion

Lead is a significant public health issue in developing countries where there are various sources and pathways of exposure. Awareness of the public health impact of exposure is growing, but relatively few of these countries have introduced policies and regulations for significantly combating the problem. Exposure to lead from lead mining, smelting, battery factories, leaded petrol and cottage industries is a significant environmental hazard in developing countries. Water and soil in Nigeria are widely contaminated by lead (20, 30, 33). In this country, lead pollution produces a high number of deaths, primarily among children, and many health organizations have been alerted and are actively trying to address the problem using a variety of approaches.

Biochemical parameters

Lead acetate administration. The administration of lead acetate at 1000 mg/kg increased levels of the liver-specific enzyme GGT. A rise in plasma GGT levels is usually suggestive of hepatocellular oxidative damage. The initial increase in GGT noted in this study concurs with earlier findings reported by Jalan and Hayes (22) and Ishak (21). There were significant variations ($P < 0.05$) in serum levels of AST, ALT, and urea while creatinine did not change significantly ($P > 0.05$) compared to negative control group after one week of lead exposure. The decrease in ALT and AST agrees with

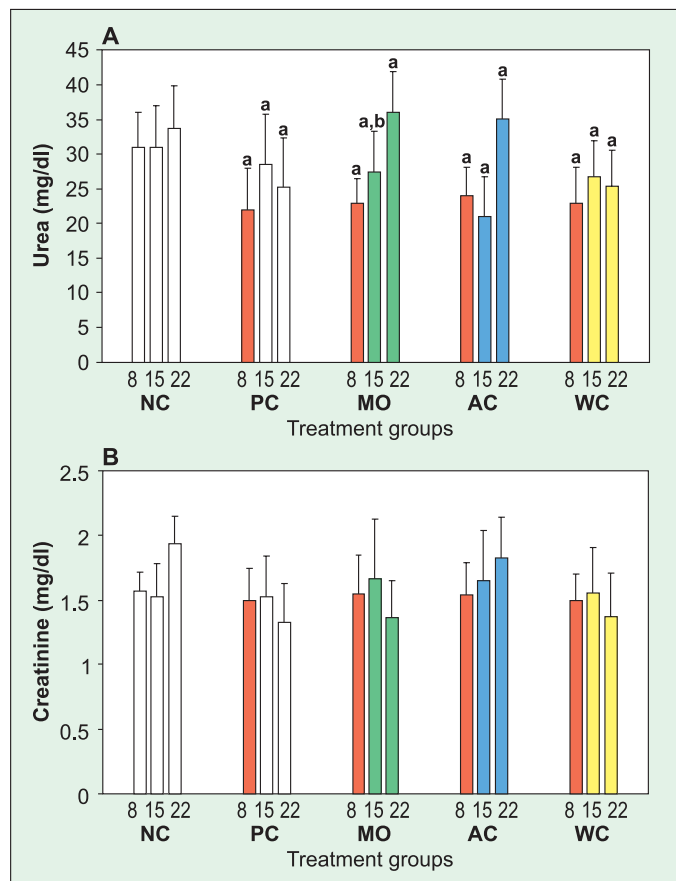


Fig. 3. Kidney parameters on 8th, 15th and 22nd day following initiation of lead acetate treatment. A) Urea; B) Creatinine
 Explanations: NC = negative group; PC = positive group; MO = *Moringa oleifera* group; AC = activated charcoal group; WC = wood charcoal group. a – Significantly different from the NC group; b – Significantly different from the PC group

findings of the study carried out by Falke et al. (12). This study showed that these parameters could be decreased with severe liver damage resulting from severe hepatitis, advanced cirrhosis or following an overdose of liver toxin as a result of a marked reduction in the number of hepatocytes. Hence, in the final stage, the total amount of transaminases released may be so reduced that plasma activities fall, despite the continuing damage to remaining cells. The decrease in urea levels may be the result of kidney damage and impaired hepatic deamination of amino acid. It is possible for there to be a net decrease in urea levels even though the animal has experienced renal damage if the decrease in production by the liver is more marked than the increase in levels due kidney damage and subsequent decreased glomerular rate filtration (GRF). This is consistent with earlier findings (24).

Moringa oleifera administration. MO had an ameliorative effect on lead induced damage to the liver and kidney. The levels of ALT and urea were increased while GGT levels were decreased when compared with the PC. There was ongoing improvement with time. This supports recent findings (<http://www.moringasource.com/2011>). It was shown that MO is effective in reducing the circulating level of lead and reduces levels of

Tab. 1. Hematological parameters recorded in the different groups at 8th, 15th and 22nd day after the beginning of lead acetate treatment

Parameters	Treatmet groups				
	NC	PC	MO	AC	WC
HB (g/L)	133.50 ± 10.23	130.40 ± 7.23	125.80 ± 2.03	133.75 ± 2.90	128.00 ± 6.51
HCT (%)	48.25 ± 4.54	47.23 ± 3.56	46.85 ± 0.89	44.31 ± 0.76	43.23 ± 2.41
MCV (fL)	52.80 ± 0.58	45.00 ± 0.97 ^a	44.60 ± 1.12 ^a	41.29 ± 1.96 ^a	44.00 ± 10.02 ^a
MCH (pg)	15.34 ± 1.82	11.88 ± 0.40 ^a	8.36 ± 0.62 ^a	8.79 ± 0.93 ^a	10.37 ± 3.37 ^a
MCHC (g/L)	291.40 ± 33.29	256.40 ± 14.21 ^a	231.20 ± 24.97 ^a	231.49 ± 15.67 ^a	217.67 ± 14.37 ^a
WBC (x 10 ⁹ /L)	2.76 ± 0.52	3.06 ± 0.58	3.19 ± 0.60	3.71 ± 0.98	3.67 ± 2.26
N (%)	24.99 ± 7.89	24.67 ± 5.45	25.80 ± 4.08	24.77 ± 3.41	23.80 ± 5.52
L (%)	61.45 ± 16.12	65.45 ± 15.00	61.40 ± 13.46	61.43 ± 11.96	64.80 ± 15.59
HB (g/L)	134.40 ± 11.03	128.40 ± 7.04	127.80 ± 2.51	123.88 ± 3.30	125.88 ± 7.59
HCT (%)	47.86 ± 4.51	48.13 ± 2.34	46.85 ± 1.54	44.28 ± 0.84	44.23 ± 2.65
MCV (fL)	52.80 ± 0.54	44.80 ± 1.99 ^a	55.12 ± 2.54 ^b	57.25 ± 2.06 ^b	44.25 ± 10.54 ^a
MCH (pg)	16.01 ± 2.23	12.01 ± 1.25 ^a	18.87 ± 1.52 ^b	18.90 ± 0.82 ^b	13.54 ± 3.85 ^a
MCHC (g/L)	297.40 ± 35.45	203.40 ± 18.43 ^a	250.25 ± 24.01 ^{a,b}	225.01 ± 15.55 ^{a,b}	217.45 ± 24.32 ^a
WBC (x 10 ⁹ /L)	2.98 ± 0.72	2.75 ± 0.60	2.67 ± 0.60	2.37 ± 0.71	2.76 ± 2.57
N (%)	25.67 ± 7.31	25.67 ± 8.54	25.65 ± 4.08	24.75 ± 3.20	23.41 ± 5.54
L (%)	59.33 ± 5.21	63.00 ± 8.01	61.22 ± 13.46	61.50 ± 8.75	62.94 ± 9.84
HB (g/L)	134.25 ± 5.44	126.60 ± 2.73	143.25 ± 9.81	154.00 ± 4.00	151.40 ± 4.58
HCT (%)	47.98 ± 1.32	46.12 ± 0.91	44.87 ± 3.05	48.09 ± 1.70	45.44 ± 1.23
MCV (fL)	53.00 ± 1.68	53.60 ± 0.87	54.75 ± 0.75	57.67 ± 0.88	58.00 ± 1.70
MCH (pg)	15.35 ± 0.42	15.36 ± 0.23	17.45 ± 0.36	16.53 ± 0.33	17.14 ± 0.40
MCHC (g/L)	300.75 ± 23.20	310.80 ± 21.83	311.25 ± 23.09	309.33 ± 12.85	311.60 ± 15.66
WBC (x 10 ⁹ /L)	2.89 ± 0.63	2.98 ± 1.86	2.97 ± 1.74	2.49 ± 0.95	2.65 ± 0.76
N (%)	26.40 ± 4.11	25.00 ± 7.22	25.40 ± 1.78	26.25 ± 0.85	24.60 ± 4.40
L (%)	62.80 ± 8.18	60.80 ± 8.45	63.20 ± 7.56	62.25 ± 8.03	5.20 ± 6.63

Analysis at 8th day after the beginning of lead acetate treatment

Analysis at 15th day after the beginning of lead acetate treatment

Analysis at 22nd day after the beginning of lead acetate treatment

Explanations: NC = negative group; PC = positive group; MO = *Moringa oleifera* group; AC = activated charcoal group; WC = wood charcoal group. a – Significantly different from the NC group; b – Significantly different from the PC group

lead in muscles, liver, and kidneys. Hence, this plant seems to possess an ameliorating effect on lead-induced liver and kidney damage. This effect may be attributed to its fatty acid content or more importantly, to the presence of protective antioxidants. It has been reported to contain large amounts of vitamin C, a known free-radical scavenger and has been shown both to inhibit lipid peroxidation in liver as demonstrated by its ameliorative effect on GGT and ALT levels in lead-exposed animals (40). Additionally, MO contains vitamin E, which is well known for its ability to enhance membrane stability and prevent membrane lipoproteins from oxidative damage by lead (34). In addition, large amounts of the essential amino acid methionine are contained in this plant. Methionine is the preferred substrate for glutathione production by hepatocytes and acts as a precursor for glutathione production in the liver (35). MO also contains zinc, which competes with lead for binding by a metallothionein-like transport protein in the gastrointestinal tract. MO contains also delta-aminolevulinic acid dehydrogenase known to be

inhibited by lead (5). A combination of some or all of these constituents of MO may have contributed to the improvement reported in liver and kidney parameters evident after MO treatment. Surprisingly, the AST values were not completely restored to pre-treatment levels: the reason for this is unknown for the moment. However, the values taken on the 22nd day were within the range reported for normal Wistar rats (32).

Activated charcoal administration. Activated charcoal had some ameliorating effect on lead-induced liver toxicity. GGT was reduced and ALT, AST and urea levels were significantly increased ($P < 0.05$). These findings are in line with the study performed by Cheong and Roh (8, 9). These authors conclude, that activated charcoal may protect against lead-induced kidney and liver damage.

Wood charcoal administration. There was not a significant reduction in liver or kidney parameters ($P > 0.05$) in the group treated with charcoal when compared to the lead and control groups respectively. WC has not been widely investigated for lead intoxication

and it is possible that it has no ameliorating effect on liver and kidney damage.

Hematological parameters. The significant decrease in MCV, MCH and MCHC demonstrated after lead administration is considered an early response to lead intoxication. This hypochromic microcytic anemia has been reported to be the result of decreased of bone marrow activity in both animals (15) and humans (18, 26). MO and AC groups showed a more rapid improvement (at 14th day) in MCV and MCH parameters. This was not the case in the WC group. The MO and AC groups also recorded incomplete restoration of pre-treatment MCHC values. These values were significantly different from both the PC and NC group and were still outside of the normal range for Wistar rats (32). At day 22, every treatment group, including the positive control, returned normal values demonstrating that intoxication with lead acetate at 1000 mg/kg in rats cannot be detected after 14 days post lead treatment using these parameters.

The data illustrated in the present study has been developed in rodents. Recent studies in lead intoxication have shown that animal data can be largely extrapolated to human beings (6). No adverse effects have been noted in people following long term consumption of MO (2, 10, 39). These reasons support the assumption that MO may also have a protective effect for lead intoxication in humans. MO has already shown promise in mitigation of tissue damage in experimentally induced fluoride (38) and arsenic toxicity (7).

In conclusion, the antioxidant properties of MO and adsorptive ability of AC account for their ameliorative action in cases of lead toxicity and as no adverse effects have been noted with long term consumption, they may provide a safe and effective preventative measure against lead toxicity. On the contrary, WC was not shown to be an effective protective agent against lead induced tissue damage.

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