AS MEN OF HONOUR WE JOIN HANDS
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ORIGINAL RESEARCH

Effect of methanol extract of Sida corymbosa leaves on lead acetate-induced hepatotoxicity, hematological and biochemical alteration in male Wistar rats

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Abstract

Background: Detrimental effects of environmental and industrial lead exposure on biochemical and physiological dysfunctions are well documented. *Sida corymbosa* plant is a common weed in Nigeria with recognized medicinal values. Thus, the present study examined the effects of methanol extract of *Sida corymbosa* leaves (MSC) on lead acetate-induced hepatotoxicity, hematological and biochemical alteration in male Wistar rats.

Methods: Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify different compounds present in MSC. Thirty male Wistar rats were grouped into six (n=5) as follow; Control, 15mg/kg lead acetate (PbAc), 100 mg/kg MSC, 200 mg/kg MSC, PbAc plus 100 mg/kg MSC and PbAc plus 200 mg/kg MSC. All the administration was done orally for 54 days. Blood and liver samples were obtained for hematological, liver biochemical, antioxidant and histopathological assays.

Results: Total phenol content in MSC was significantly ($p<0.05$) high. The GC-MS analysis of MSC showed the presence of twenty-four compounds. The leukocyte, lymphocytes, monocyte, granulocytes counts, aspartate aminotransferase and alanine aminotransferase activities and malondialdehyde were significantly increased ($p<0.05$) in PbAc group. The erythrocyte count, hemoglobin concentration, hematocrit, superoxide dismutase and catalase activities were significantly reduced ($p<0.05$) in PbAc group compared to the control group. The liver histology showed significant alteration in PbAc group. The MSC significantly reversed ($p<0.05$) PbAc alteration on hematological, biochemical, oxidant, antioxidant enzymes activities and histological alterations in the liver.

Conclusion: The methanol extract of *Sida corymbosa* leaves possesses anti-inflammatory and antioxidant compounds and may have a potent *in-vitro* antioxidant capacity that may be responsible for its ameliorative effects on lead acetate-induced hepatotoxicity, hematological and biochemical alterations in male Wistar rats.

Keywords: *Sida corymbosa*; antioxidant; hepatotoxicity; hemolytic anemia; lead acetate.

Introduction

Plants with their derived products are sources of many therapeutic agents, and about 80% of the third world population relies on them for their health care needs[1,2,3]. Though the mechanisms of action and chemical compositions of most medicinal plants have not been fully explored and understood yet, the experience gained with their traditional use over the years cannot be ignored. *Sida corymbosa* is among many plants with potential health benefit used traditionally for a long time without proper scientific investigation.

Sida corymbosa R. E. Fries is a weed that belongs to a Malvaceae family with peltate scale, and it is abundant in Nigeria. The plant is used to facilitate childbirth in Nigerian ethnomedicine, and management of postpartum hemorrhage[4,5]. Also, its antimicrobial activity, oral hygiene inducing potential and anti-ulcer properties have been documented[6,7]. The *Sida corymbosa* plant has been suggested to prevent anemia through its hemostatic activities and postpartum hemorrhage management[5,8].

Lead is a ubiquitous environmental and industrial chemical pollutant that has been detected in every facet of environmental and biological systems[9]. The detrimental effects of lead on physiological, biochemical, and behavioural functions have been documented in animals and humans[10,11,12]. The occupational and environmental lead exposures pose a severe problem in many developing countries[13]. Recently in Niger and Zamfara states of Nigeria, there were series of lead poisonings from illegal gold mining activities which had led to the deaths of at least 180 people[14]. Lead has been scientifically reported to affect the hematopoietic system, leukocytosis, erythrophagocytosis, and heme biosynthesis leading to hemolytic anemia[9,15,16]. It also causes hepatotoxicity and disturbs normal biochemical processes[17]. Lead acetate is one of the models used to induce hepatotoxicity and hemolytic anemia in experimental animals due to its ability to bioaccumulate in blood and liver which usually results in increased reactive oxygen species, depletion of antioxidant reserve and induces oxidative stress[18,19].

This study was aimed to evaluate the effects of methanol extract of *Sida corymbosa* leaves on lead acetate-induced hepatotoxicity, hematological and biochemical alterations in male Wistar rats.

Materials and methods

Plant and extract preparation

Sida corymbosa plant was obtained from the botanical garden of the University of Ibadan, Nigeria. The plant was authenticated in the Forestry Research Institute of Nigeria Herbarium, Ibadan, Oyo state, with authentication number FHI./08886. The plant leaves were air-dried, pulverized and subjected to Soxhlet extraction using pure methanol as the solvent. The solvent was removed from the extract using a vacuum-distilled rotary evaporator. The remaining extract was finally oven-dried at 30^{0}C for 2 hours to ensure the removal of any residual solvent. The percentage yield was 2.6%.

Phytochemical screening

Phytochemical screening of the methanol extract of *Sida corymbosa* leaves (MSC) was carried out using standard procedures as described by Edeoga *et al.*[20].

Characterization and identification of chemical compounds in methanol extract of *Sida corymbosa* leaves using Gas Chromatograph Mass Spectrometry (GC–MS) analysis

Two grams (2g) of the MSC was dissolved in 4.0 mL of absolute methanol. The volatile solution of MSC was partitioned and analyzed using a Varian 450 Gas Chromatograph coupled to a Varian 240–MS ion–trap mass spectrometer (VF-5 MS Column) with injector and oven temperature at 250 °C and 200 °C. The heating rate of GC–MS was programmed at 10 °C/minutes. The injection was performed in the split ratio of 200, and the volume was 10.0 µL. The flow of carrier gasses was constant at 1.0 mL/minutes during the run of volatile MSC solution[21]. The identification of chemical compounds present in MSC was carried out by similarity searches, and mass spectra data in the National Institute of Standards and Technology (NIST) MS Search 2.0 Library. Quantification of components in the MSC was done by Mass Spectrometry workstation software.

In-vitro antioxidants activities of methanol extract of *Sida corymbosa* leaves

Total phenol content

Total phenol in MSC and galic acid (control) was determined by the Folin-Ciocalteu method, and the results were expressed as mg of galic acid equivalent (mgGAE) of extracted compound[22].

Total antioxidant capacity

Total antioxidant capacity was analyzed as reported by Prieto *et al.*[23]. The total antioxidant activities of MSC and ascorbic acid (control) were expressed as an ascorbic acid equivalent (AAE).

Metal chelating ability assay

The ferrous ion chelating (FIC) assay was done according to the method of Singh and Rajini[24]. The concentration of MSC and ethylenediaminetetraacetic acid (control) that caused 50% inhibition (IC_{50}) was evaluated and expressed as mg/mL.

Animal model

Thirty (30) male and five (5) female Wistar rats with a weight range of 150 to 180 g were used for this study. They were obtained from the Central Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. The animals were housed under standard laboratory conditions. They were fed with standard commercial rat pellets and allowed access to drinking water *ad libitum*. The animals were acclimatized for one week before the commencement of the administration of lead acetate or MSC. All the procedures used conformed with the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals[25].

Acute oral toxicity of methanol extract of *Sida corymbosa* leaves

The up and down protocol of organization for economic cooperation and development[26] was used for acute oral toxicity and LD_{50} determination. Females Wistar rats were used because they are generally more sensitive than male[27]. The OECD procedure is as follows, 2,000 mg/kg MSC was administered by oral gavage to five female Wistar rats. The rats were fasted and weighed before administration and for further 3-4 hours after administration. They were observed for 14 days for any signs of toxicity.

Research design

Animals were grouped into six (n=5) and treated, as shown in table 1.

Table 1: Grouping and treatment of experimental animals

Groups	Treatment (n=5)
1	Control
2	15 mg/kg Lead acetate (PbAc)
3	100 mg/kg MSC
4	200 mg/kg MSC
5	PbAc+100 mg/kg MSC
6	PbAc+200 mg/kg MSC

They were administered with the vehicle, lead acetate and MSC for 54 days orally. After the last day (on day 55) of administration, the blood sample was collected from all the animals via cardiac puncture into EDTA tubes for hematological and liver functions assay. The animals were then sacrificed, and liver organs were harvested for biochemical, antioxidant and histology assays.

Determination of full blood cells counts

Complete blood cells count assay was performed as described by Lewis *et al.*[28] using ERMA

Hematology autoanalyzer (Model PCE-210N).

Biochemical Assay

A section (2g) of the harvested liver was washed in ice-cold 1.15% KCl solution blotted with filter paper and homogenized in ice-cold 0.1M Tris-HCl (pH 7.4) using Teflon homogenizer fitted into a microfuge tube chilled in ice. Each sample was centrifuged at 10,000 × g for 10 minutes. The supernatant was used for the estimation of superoxide dismutase (SOD), catalase (CAT) activities and malondialdehyde (MDA).

Alanine amino-transferase and aspartate amino-transferase activities assay

Plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were estimated using the modified method of Reitman and Frankel[29]. The plasma (0.1 mL) was mixed with 0.5 mL of phosphate buffer (L-alanine) and (L-aspartate) for ALT and AST respectively. The mixture was incubated for 30 minutes at 37^{0}C and then 0.5 mL of 2, 4-dinitrophenylhydrazine was added and vortexed. The mixture was allowed to stand for 20 minutes at room temperature, then 5 mL of 0.4 mol/L sodium hydroxide added, and the absorbance of the solution was read after 5 minutes at a wavelength of 546nm.

Superoxide dismutase activity assay

The superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich[30]. It involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in a UV via a spectrophotometer. The rate of autoxidation of epinephrine was noted at 30 seconds intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

Catalase activity assay

The catalase activity was measured as described by Cohen *et al.*[31]. A homogenized sample of liver tissue (0.5 mL) was mixed with an equal volume of 30 M of hydrogen peroxide, 1 mL of 6M H_2SO_4 and 7 mL of 2 mM of potassium permanganate. Absorbance was read at 480 nm within 30 to 60 seconds against distilled water and results expressed in units/min/mg protein.

Malondialdehyde assay - lipid peroxidation

Lipid peroxidation was estimated in terms of thiobarbituric acid (TBA), using malondialdehyde (MDA) concentration according to Buege and Aust [32] method. Two (2) mL of TBA reagent and 1mL of trichloroacetic acid (TCA) were mixed with 2 mL of a liver homogenate. The mixture was heated at 60^{0}C for 20 minutes. It was then cooled and centrifuged at 400 rpm for 10 minutes. The absorbance of the supernatant was read at a wavelength of 540 nm, and results were expressed in nmol/mg protein.

Histopathological analysis

Liver samples were fixed in 10% buffered neutral formalin and processed for embedding in paraffin [33]. Sections of 5-6 µm thickness were stained with hematoxylin and eosin, followed by examination under a light microscope. Liver micrographs were obtained by Omax 10.0MP digital camera for a microscope.

Statistical Analysis

The Graphpad Prism (version 7) was used to analyze data using one-way analysis of variance (ANOVA), followed by the Tukey honest significant difference test. The p-value less than 0.05 was considered significant.

Results

Phytochemical screening of *Sida corymbosa*

The phytochemical screening of the MSC showed the presence of alkaloid, tannin, saponin, cardiac glycoside, sterol, and flavonoid.

Gas chromatography-mass spectrometry analysis of methanol extract of *Sida corymbosa* leaves

The characterization and identification of chemical compounds in MSC are shown in table 2 according to their elusion order VF – 5 MS capillary column. Twenty four (24) compounds were identified in MSC (table 2) as tetradecane (4.0%), 3-Butenoic acid, 2,2-diethyl (1.3%), 1H-Imidazole, 4-bromo (1.2%), Hexadecane (6.9%), Dicyclohexyl methylphosphonate (8.9%), 4-Bromo-2,6-di-tert-butylphenol (2.6%), Octadecane (7.3%),

Fumaric acid, ethyl 3-ethylphenyl ester (0.9%), 9-Decen-1-ol, pentafluoropropionate (1.5%), Hexadecanoic acid, methyl ester (7.6%), n-Hexadecanoic acid (6.6%), 2-Thiopheneacetic acid, 2-methyloct-5-yn-4-yl ester (1.5%), Pyrimidine, 2,4-diamino-5-(3-pyridylmethyl) (1.8%), Eicosane (6.6%), Benzenamine, N,N, 3-trimethyl (6.1%), n-Nonadecanol-1 (2.2%), 9,12-Octadecadienoic acid, methyl (3.5%), 9-Octadecenoic acid methyl ester (6.3%), Heptadecanoic acid, 16-methyl-, methyl ester (3.1%), 9,12-Octadecadienoic acid (2.9%), Docosane (5.7%), cis-13-Octadecenoic acid (3.4%), Octadecane (5.5%) and Hexacosane (2.8%).

Table: 2: Chemical Compounds and their Properties of methanol extract of *Sida corymbosa* leaves characterized through GC - MS

S/N	Retention time (min)	Molecular Weight (g/mol)	Chemical Formulae	% Content
1	10.270	198.388	$C_{14}H_{30}$	4.013
2	11.394	114.142	$C_6H_{10}O_2$	1.298
3	11.605	146.973	$C_3H_3BrN_2$	1.228
4	12.997	226.441	$C_{16}H_{34}$	6.909
5	14.279	260.310	$C_{13}H_{25}O_3P$	8.887
6	14.504	285.220	$C_{14}H_{21}BrO$	2.599
7	15.362	254.494	$C_{18}H_{38}$	7.291
8	15.666	248.724	$C_{14}H_{16}O_4$	0.884
9	15.808	302.281	$C_{13}H_{19}F_5O_2$	1.462
10	16.740	270.451	$C_{17}H_{34}O_2$	7.568
11	17.213	256.424	$C_{16}H_{32}O_2$	6.601
12	17.297	264.383	$C_{15}H_{20}O_2S$	1.537
13	17.367	201.228	$C_{10}H_{11}N_5$	1.765
14	17.485	282.547	$C_{20}H_{42}$	6.573
15	17.570	135.206	$C_9H_{13}N_3$	6.077

16	18.353	284.520	$C_{19}H_{40}O$	2.175
17	18.448	294.472	$C_{19}H_{34}O_2$	3.481
18	18.505	294.4721	$C_{19}H_{36}O_2$	6.269
19	18.751	298.504	$C_{19}H_{38}O_2$	3.102
20	18.958	280.446	$C_{18}H_{32}O_2$	2.864
21	19.420	310.601	$C_{22}H_{46}$	5.682
22	20.435	282.461	$C_{18}H_{34}O_2$	3.424
23	21.381	254.494	$C_{18}H_{38}$	5.499
24	24.252	366.707	$C_{26}H_{54}$	2.813

In-vitro antioxidant activities of methanol extract of Sida corymbosa leaves

Table 3 showed the in-vitro antioxidant activities of MSC. The phenol content in MSC was significantly higher ($p<0.05$) than control. There is no significant difference between the total antioxidant capacity of MSC and the control. The IC_{50} metal chelating capacity of MSC was significantly higher than the control.

Table 3: *In-vitro* antioxidant activities of methanol extract of *Sida corymbosa* leaves

Antioxidants	Control	Methanol extract of Sida corymbosa leaves
Phenol content (mgGAE/g)	101.8 ± 3.21	145.20 ± 2.00 *
Total antioxidant capacity (mgAAE/g)	68.1 ± 2.31	65.70 ± 3.40
Metal chelating IC_{50} (mg/mL)	0.015 ± 0.0004	0.038 ± 0.006 0*

Acute oral toxicity of methanol extract of *Sida corymbosa* leaves

The acute toxicity and lethal dose result in female Wistar rats treated with MSC. There was no mortality recorded in all the rats administered with 2,000 mg/kg MSC after 24 hours. Also, no signs of toxicity can be noticed in all the animals after 14 days of observation.

Hematological parameters

The hematological results were presented in table 4. The total leukocytes and lymphocytes counts were significantly increased ($p<0.05$) in animals treated with lead acetate and lead acetate co-treated with MSC when compared with the control group, while significant reduction ($p<0.05$) was observed in lead acetate co-administered with MSC relative to lead acetate alone. Monocyte count was significantly reduced ($p<0.05$) in animals co-treated with lead acetate and MSC relative to lead acetate group.

Erythrocytes count, hemoglobin concentration and hematocrit were significantly reduced ($p<0.05$) in lead acetate group when compared with the control group. The co-administration of lead acetate and MSC significantly increased ($p<0.05$) erythrocytes count, hemoglobin concentration, and hematocrit when compared with lead acetate group alone.

Also, there was a significant reduction ($p<0.05$) in platelets count in lead acetate treated rats compared with the control group, while significant increase ($p<0.05$) was observed in rats co-treated with lead acetate and MSC relative to lead acetate group.

Table 4: Effects of methanol extract of *Sida Corymbosa* leaves on full blood cells counts in lead acetate treated Wistar male rats

	Control	PbAc	100 mg/kg MSC	200 mg/kg MSC	PbAc + 100 mg/kg MSC	PbAc + 200 mg/kg MSC
Leukocytes ($\times10^3/\mu l$)	3.69±0.11	6.15±0.27*	3.82±0.12	4.56±0.17	5.76±0.33*,+	5.18±0.11*,+
Monocytes ($\times10^3/\mu l$)	0.14±0.02	0.77±0.19*	0.11±0.03	0.10±0.02	0.11±0.01+	0.13±0.02+
Lymphocytes ($\times10^3/\mu l$)	0.28±0.04	1.44±0.05*	0.26±0.07	0.15±0.02*	0.53±0.05*,+	0.58±0.12*,+
Erythrocytes ($\times10^6/\mu l$)	6.41±0.31	4.94±0.17*	5.64±0.28*	6.10±0.27	4.33±0.32*,+	6.10±0.03+
Hemoglobin (g/dl)	13.46±0.52	6.32±0.58*	11.58±0.64*	13.04±0.98	10.56±0.16*,+	12.26±0.16+
Hematocrit (%)	42.48±1.49	29.72±1.52*	35.72±1.35*	39.42±1.10	32.1±0.51*	36.26±1.10*,+
Platelet ($\times10^3/\mu l$)	401±44.3	222±9.6*	399±11.3	420±3.4	367±6.8+	348±4.7+

*$p<0.05$ and +$p<0.05$ were considered significant relative to control and lead acetate groups respectively

Superoxide dismutase activity

Figure 1 shows that superoxide dismutase activity was significantly reduced ($p<0.05$) in rats treated with lead acetate when compared with the control group, while significant increase ($p<0.05$) was observed in 200 mg/kg MSC group relative to control group. The co-administration of lead acetate with MSC significantly increased ($p<0.05$) superoxide dismutase activity when compared with the lead acetate group.

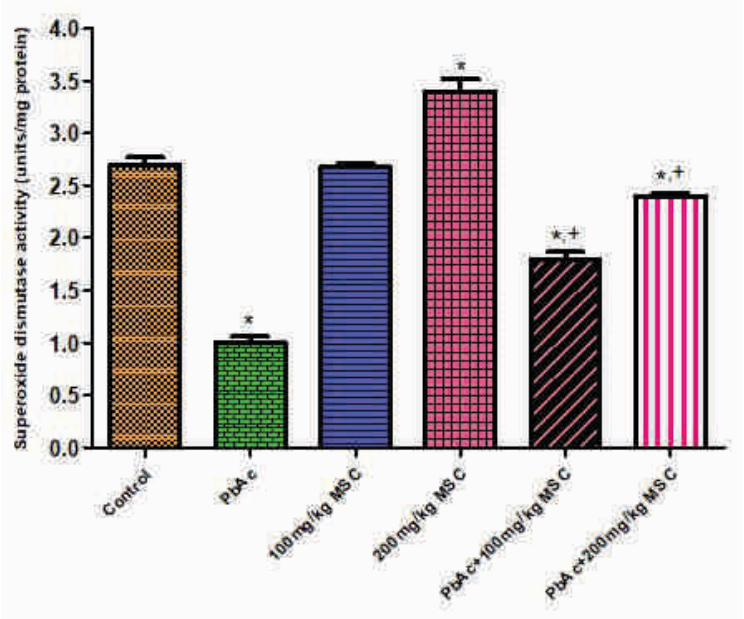

Figure 1: Effect of methanol extract of *Sida corymbosa* leaves on superoxide dismutase activity in lead acetate treated Wistar rats

*p<0.05 and $^{+}$p<0.05 were considered significant relative to control and lead acetate groups respectively

Catalase activity

Figure 2 shows that catalase activity was significantly reduced (p<0.05) in rats treated with lead acetate and lead acetate co-administered with MSC when compared with the control group. The catalase activity was significantly increased (p<0.05) in groups treated with 100 mg/kg and 200 mg/kg MSC when compared with the control group. Also, a significant increase (p<0.05) was observed in the lead acetate co-treated with MSC compared with lead acetate group.

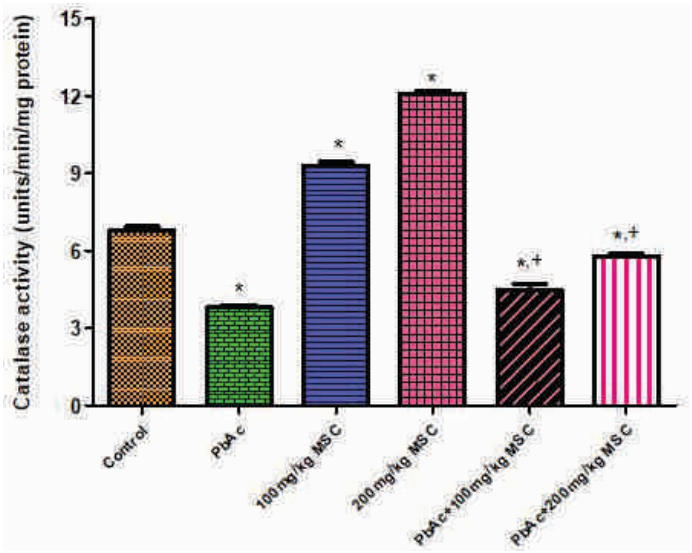

Figure 2: Effect of methanol extract of *Sida corymbosa* leaves on catalase activity in lead acetate treated Wistar rats

*p<0.05 and $^{+}$p<0.05 were considered significant relative to control and lead acetate groups respectively

Malondialdehyde (Lipid peroxidation)

Figure 3 shows that malondialdehyde was significantly increased ($p<0.05$) in rats treated with lead acetate and lead acetate co-treated with MSC compared to control group, while significant reduction ($p<0.05$) was observed in 200 mg/kg MSC group when compared with the control group. Malondialdehyde was significantly reduced in rats co-treated with lead acetate and MSC relative to the lead acetate group.

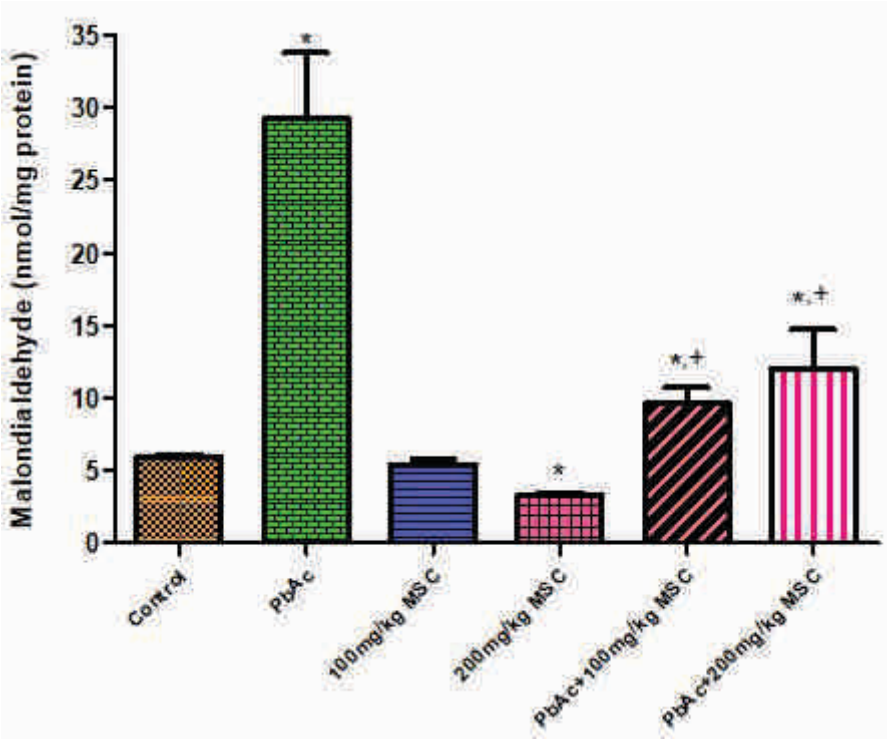

Figure 3: Effect of methanol extract of *Sida corymbosa* leaves on malondialdehyde in lead acetate treated Wistar rats

* $p<0.05$ and $^{+}$ $p<0.05$ were considered significant relative to control and lead acetate groups respectively

Alanine aminotransferase and aspartate aminotransferase activities

Figure 4 shows the results of alanine aminotransferase activity. Alanine aminotransferase activity was significantly increased ($p<0.05$) in lead acetate group when compared with the control group, while significant reduction ($p<0.05$) was observed in rats co-treated with lead acetate and MSC when compared with lead acetate group.

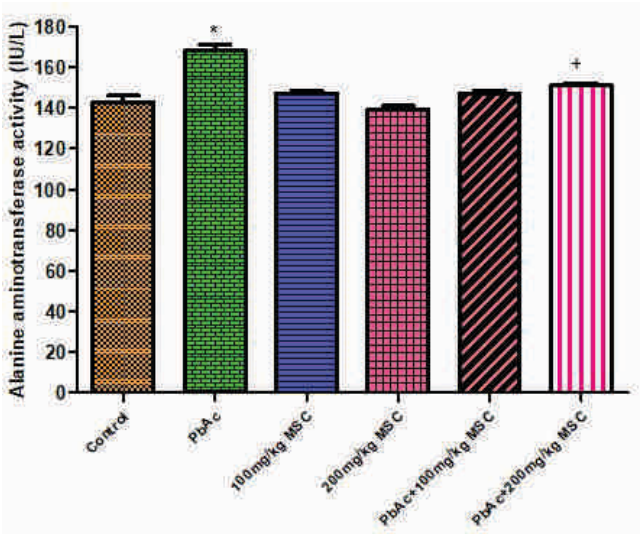

Figure 4: Effects of methanol extract of *Sida corymbosa* leaves on alanine aminotransferase activity in lead acetate treated Wistar rats

*p<0.05 and $^{+}$p<0.05 were considered significant relative to control and lead acetate groups respectively

Figure 5 shows that aspartate aminotransferase activity was significantly increased (p<0.05) in lead acetate group compared with the control group, while significant reduction (p<0.05) was observed in 200 mg/kg MSC group relative to control group. The co-administration of lead acetate with 200 mg/kg MSC significantly reduced (p<0.05) aspartate aminotransferase activity relative to lead acetate group.

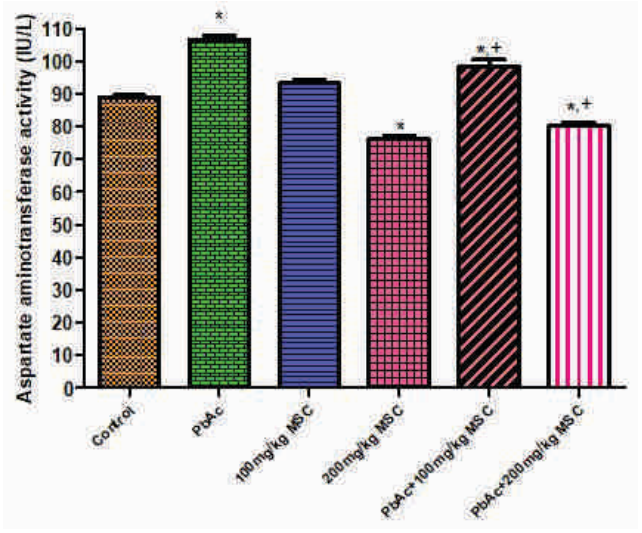

Figure 5: Effect of methanol extract of *Sida corymbosa* leaves on aspartate aminotransferase activity in lead acetate treated Wistar rats

*p<0.05 and $^{+}$p<0.05 were considered significant relative to control and lead acetate groups respectively

Histopathology

Figure 6 shows that the control group liver histology is normal, the central venule (thick white arrow) appears free of congestion, hepatocytes (thick black arrow) and sinusoids (thin black arrow) appeared normal. The lead acetate group liver shows moderate dilatation of the sinusoid with inflammatory cells infiltration (thin black arrow), their liver sinusoids are also moderately attended by red cells (thin black arrow), some hepatocyte has undergone apoptosis (thick white arrow) while some show hyperchromic nuclei (thick blue arrow). The MSC (100 and 200 mg/kg) groups show normal liver sinusoids (white arrow), and morphology of the hepatocytes (thin black arrow) appear normal. The liver histology of PbAc + MSC (100mg/kg) group shows moderate dilatation of the sinusoid with inflammatory cells infiltration (thin black arrow) and moderately attended by red cells (thick white arrow), some hepatocytes have undergone apoptosis (thin black arrow), while others appear normal. The liver cytoarchitecture of PbAc + MSC (200 mg/kg) group show normal central venule (thick white arrow) with very few inflammatory cells in the liver sinusoids (thin black arrow), and the hepatocytes appear normal.

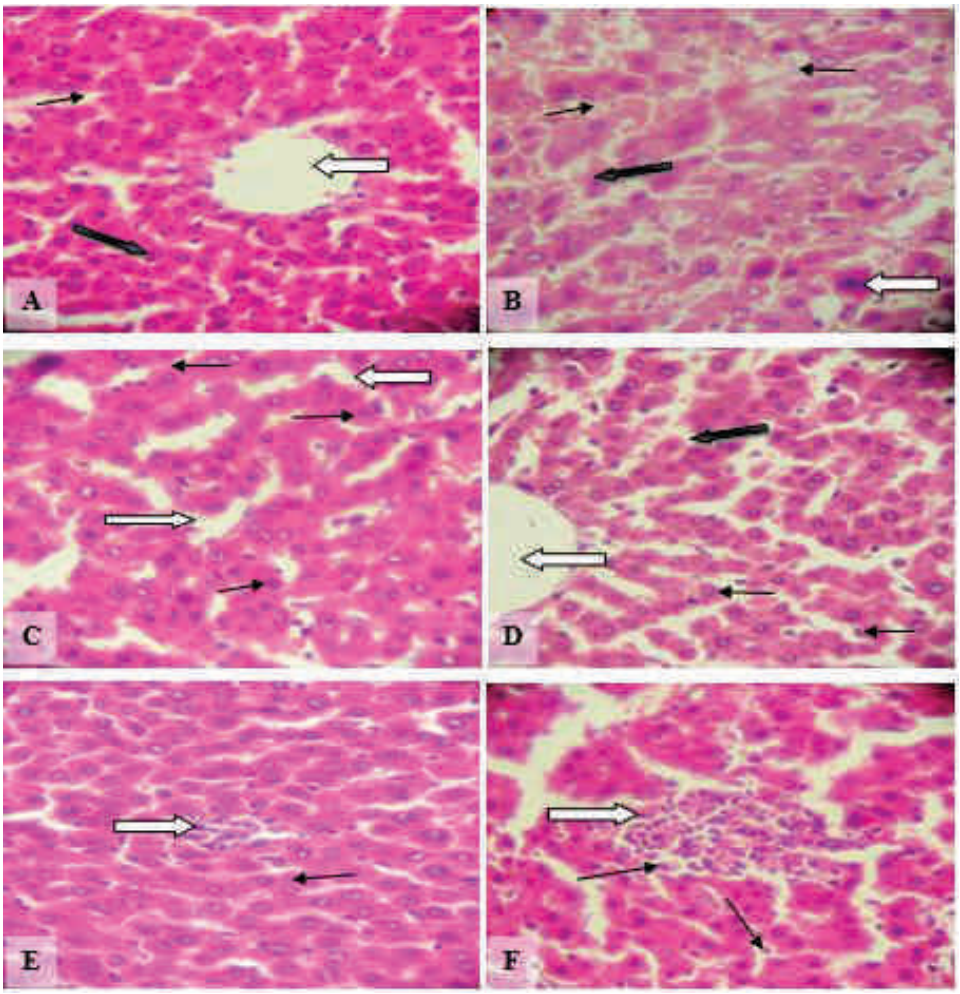

Figure 6: Effect of methanol extract of *Sida corymbosa* leaves on liver architectural in lead acetate treated male Wistar rats (×400).

(A) Control; (B) PbAc (15 mg/kg) group; (C) MSC (100 mg/kg) group; (D) MSC (200mg/kg) group; (E) PbAc + MSC (100mg/kg); (F) PbAc + MSC (200 mg/kg) group.

Discussion

The phytochemical compounds present in MSC are alkaloids, flavonoids, cardiac glycosides, saponins, sterol, and tannins. The identified compounds in MSC were similar to other *Sida* species such as *acuta* and *cordifolia*[34]. The anti-inflammatory property of *Sida corymbosa* plant previous reported by Kayode and Omotoyinbo[6] may be due to the presence of alkaloid[35]. Furthermore, MSC may possess antioxidant properties via flavonoids which are capable of scavenging reactive oxygen species (ROS)[36]. Tannins possess astringent, antimicrobial, antifungal, anti-inflammatory properties, and hasten wounds healing[37]. It also possesses anti-diabetic, anti-ulcer, and cholesterol-lowering activities[38]. Saponins and sterols in MSC have been suggested to be responsible for utero-contractile properties of MSC[4].

Gas Chromatography-Mass Spectrometric analysis of MSC showed compounds of important biological activities. Dicyclohexyl methylphosphonate is the most abundant compound identified in MSC, although information of the biological activity of this compound is scarce in the literature. Hexadecanoic acid methyl ester, (PAME; Palmitic acid methyl ester) is the second abundant compound present in MSC. Anti-inflammatory activity of PAME and its ability to reduce tumour necrosis factor-alpha, interleukin-6, neutrophil infiltration and NF-$_k$B expression in the liver has been reported[39,40]. Also, the protective role of PAME on CCl_4-induced liver fibrosis has been studied[41]. Furthermore, n-hexadecanoic acid, 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid, methyl, heptadecanoic acid, 16-methyl-, methyl ester, 9,12-octadecadienoic acid, and cis-13-octadecenoic acid were also identified in MSC. Hexadecanoic acid (palmitic acid) is a saturated fatty acid[42] and commonly sourced from palm oil. Several saturated fatty acids are known to have antibacterial and antifungal properties; they also control immune reactions through T-cells[43,44]. The other significant compounds detected in MSC were alkanes; they are octadecane, eicosane, tetradecane, hexadecane, octadecane, and hexacosane.

The *in-vitro* antioxidant properties of MSC showed the presence of high phenol content. This observation is similar to phenol content in other *Sida* species such as *acuta*, *rhomboide*, *cordata, cordifolia*, *Spinosa*, *mysorensis*[45,46,47,48]. It may be suggested that MSC possess antioxidant activity since some investigators have reported a direct correlation between phenol content and antioxidant activity of natural products[49,50]. Also, Total antioxidant capacity (TAC) was high in MSC; this observation may be as a result of the synergic effects of other compounds present in the plant. The exogenous antioxidants have been found to be a promising way of opposing the detrimental effects of oxidative stress, and also endogenous booster antioxidant[51]. Hence, MSC may be a good source of exogenous antioxidant. In the present study, the MSC has moderate metal-chelating potential. This observation supports the antioxidants activities of MSC. Thus, MSC may restore the normal physiology of the body through chelating toxic heavy metals and prevent metallotoxicity.

Acute toxicity results showed that oral administration of MSC at a dosage of 2,000 mg/kg body weight neither gives any sign of toxicity nor death in Wistar rats. This observation suggests that the lethal dose (LD_{50}) of MSC is more than 2,000 mg/kg. The MSC may be considered non-toxic via oral route since its LD_{50} is more than 2,000 mg/kg. The result suggested that the MSC may be non-toxic when taken orally.

Evaluation of hematological parameters in rats following the oral administration of chemical compounds and plants extract can be employed to measure the toxic abilities of such compounds and also it may provide useful information on the effect of such compound on blood[52]. It was reported that lead acetate might disturb hematopoietic stem cells by directing progenitors from the erythrocyte route into the granulocyte route of production and/or directly suppress erythropoiesis and stimulating granulocyte production[53]. The observed increased in total white blood cells, neutrophil, lymphocytes, and monocytes counts of lead acetate treated rats in this study is an indication of leukocytosis. The mechanism of lead acetate-induced leukocytosis can be a reaction to inflammation

and oxidative stress which are mediated by many factors and cytokines such as interleukin 1, 3, 6, 8 and tumour necrotic factor alpha[54]. This study showed that MSC did not alter total white blood cell, neutrophil, lymphocyte and monocyte count, which is in agreement with the reports of John-Africa and Aboh[8]. The MSC reduces the observed increased in the total white blood cell, neutrophil, lymphocyte, and monocyte counts in lead acetate treated rats. The GC-MS analysis of *Sida corymbosa* leaves revealed presences of many anti-inflammatory compounds such as palmitic acid methyl ester[39,40] which is capable of reducing tumour necrosis factor-alpha, interleukin-6, neutrophil infiltration and NF-$_k$B expression[41] that strongly linked to leukocytosis. The potent antioxidant property of MSC may be responsible for the reduction in lead acetate-induced leukocytosis observed in this study. The reduced erythrocyte count in lead acetate treated rats, as observed in this study is consistent with previous studies[55,56]. The erythrocyte is regarded as a primary target of lead toxicity since it is the site of bioaccumulation of the heavy metals[16]; this may inhibit erythrocyte pyrimidine 5' nucleotidase and result in hemolytic anemia[57]. Furthermore, the observed reduction in erythrocyte of lead acetate treated rats may be attributed to phosphatidylserine exposure in erythrocyte and erythro-phagocytosis, which is usually involved in pathophysiological clearance of damaged erythrocyte[16]. The methanol extract of *Sida corymbosa* improved the observed reduction in erythrocyte of lead-treated animals in a dose-depended manner. It suggests that MSC may prevent hemolytic anemia induced by lead through prevention of lead accumulation in erythrocyte since the plant possesses high metal chelating activity which may clear the lead in the circulatory system. Also, the antioxidant capacity of MSC may assist in inhibiting erythro-phagocytosis in order to prevent premature termination of erythrocyte and prolong its lifespan. The present study result on the reduction of hemoglobin concentration in lead acetate treated rats is in agreement with the already established lead acetate-induced anemia [58,59]. Lead interferes with erythropoiesis, especially the heme biosynthesis through the inhibition of three key enzymes that are critical to the heme synthesis pathway[60]. The MSC increased hemoglobin concentration when co-administered with lead acetate in a dose-dependent manner. This is an indication that MSC may reverse lead-induced anemia in male rats. The mechanism of action of MSC in elevating hemoglobin in the lead treated rats may be through activation of ferrochelatase enzymes since this plant has high metal chelating property.

In line with the observed reduction in both erythrocyte and hemoglobin concentration in rats exposed to lead acetate, the hematocrit level was also reduced. A linear relationship between hemoglobin and hematocrit has been reported[61]. The erythrocyte lead concentration has been implicated in hematocrit reduction[62]. The methanol extract of *Sida corymbosa* leaves was able to reverse hematocrit concentration in lead acetate treated rats; this may be as a result of the extract to prevent erythro-phagocytosis and increase hemoglobin concentration.

Superoxide dismutase and catalase are well-known endogenous antioxidant enzymes that involve in the removal of ROS, while malondialdehyde is a by-product of lipid peroxidation and also an indicator of reactive oxidative stress (ROS)[63,64]. In this study, lead acetate compromised the antioxidant system, as the content of SOD and catalase were reduced in association with increased MDA. These observations are in agreement with AL-Megrin *et al*[65,] and Abdel Moneim[66] reports. The reduction in SOD, catalase and increased in the liver MDA level in the Pb-treated group could be due to the accumulation of the metal in the liver[67]. The lead also competes with and substitutes some essential antioxidant metals, such as zinc and copper[9]. The co-treatment with MSC improved the lead acetate oxidative damage by increased SOD, catalase activities and reduced MDA level in the liver. The ability of MSC to increase these antioxidant activities and reduce MDA in lead acetate treated rat could be ascribed to its antioxidant and metal chelating properties which may enhance endogenous antioxidants and prevent the generation of reactive

oxygen species.

The elevation of plasma ALT and AST activities is an indication of hepatotoxicity. The vital hepatocyte functions include metabolism, storage, and detoxification of toxic substances. This study shows increased plasma levels of ALT and AST in lead acetate group. This observation is in agreement with the existing body of evidence[17,68]. The MSC reduced the plasma levels of ALT and AST that were elevated by lead acetate; this observation shows that MSC might prevent hepatotoxicity from environmental heavy metals exposure such as lead. An attribute suspected to be due to the metal chelating ability of MSC, which may prevent the deposition of lead in the liver since about 33% of exposed lead is a repository in the liver tissue[18]. Also, MSC may contribute to the availability of calcium ion for maintaining the stability of the hepatocytes membrane and prevent lead from disturbing liver function such as enzymes and protein synthesis[68]. Accumulation of lead in the liver may directly cause hepatotoxicity, mainly by altering the hepatocytes membrane permeability, which may result in the increased release of cytosolic enzymes AST and ALT into the circulation[64].

Furthermore, the MSC protective activity against lead acetate-induced hepatotoxicity may be due to its anti-inflammatory and antioxidant abilities. Palmitic acid methyl ester, chemical components in MSC is well documented to prevent inflammation through inhibition of Kupfer cell functions, TNF-α, IL-6, neutrophil infiltration through NF-$_k$B expression pathway[41,70]. The generations of ROS and free radicals have been implicated in the pathogenesis of hepatotoxicity[69]. The evidence of hepatotoxicity of lead acetate from the present study was seen in hepatocyte histology with inflammatory cells infiltration into moderately dilated sinusoid, with the presence of erythrocytes, hepatocytes have undergone apoptosis, pleomorphism, and hyperchromic nuclei. These observations are similar to the indicators of hepatotoxicity and hepatocytes damage[17,71]. The MSC, especially at a dosage of 200 mg/kg was able to ameliorate the lead acetate-induced hepatotoxicity. There were few infiltrations of inflammatory cells into liver sinusoids, but generally, the hepatocytes appeared normal. The MSC ameliorating effect against lead-induced hepatotoxicity and hepatocyte damage may be ascribed to its anti-inflammatory, antioxidant and metal chelating properties[41,70].

Conclusion

The methanol extract of *Sida corymbosa* leaves possesses anti-inflammatory and antioxidant compounds and may have a strong *in-vitro* antioxidant capacity that may be responsible for its ameliorative effects on lead acetate-induced hepatotoxicity, hematological and biochemical alterations in male Wistar rats.

Conflicts of interest

No conflict of interests in this study.

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