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Original Research Article

Anti-apoptotic, Antioxidant, and Anti-Inflammatory Activities of *Sida corymbosa* Leaf Methanol Extract Ameliorate Lead Acetate-Induced Testicular Functions Alteration in Wistar Rats

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ABSTRACT

Lead acetate (Pb) is an environmental toxicant widely reported to distort testicular functions. Methanol extract of *Sida corymbosa* leaves (SC) possesses antioxidant and anti-inflammatory properties. This study was designed to evaluate the role of SC on lead acetate-induced alteration in testicular functions. Thirty adult male Wistar rats were grouped randomly and equally into six and treated as follows: control, Pb (15 mgkg^{-1}), SC (100 mgkg^{-1}), SC (200 mgkg^{-1}), Pb+SC (100 mgkg^{-1}), and Pb+SC (200 mgkg^{-1}) respectively. Administrations were orally done for 54 days. Computer-aided sperm analyzer, ELISA, spectrophotometry, immunohistochemistry, and PCR techniques were used. Pb significantly reduced follicle-stimulating hormone, luteinizing hormone, testosterone, androgen receptors expression, 3β- and 17β-hydroxysteroid dehydrogenase, sperm concentration, progressive sperm motility, viability, catalase, superoxide dismutase activities, and BCL-2. Pb significantly increased abnormal sperm morphology, malondialdehyde, 8-hydroxydeoxyguanosine, BAD, TNF-α, and IL-6. The combination of SC and Pb significantly reverses the hormones, steroidogenic enzymes, sperm quality, testicular oxidant, antioxidant enzymes, testicular DNA damage, apoptosis, and inflammation when compared with the Pb group. Anti-apoptosis, antioxidant, and anti-inflammatory activities of *Sida corymbosa* improve lead acetate-induced testicular functions alteration in Wistar rats.

Keywords: Apoptosis, Inflammation, Lead acetate, *Sida corymbosa*, Steroidogenic enzymes

Introduction

Growing apprehension exists around the harmful effects of environmental pollution in developing countries. Non-regulation policies of industrial and mining activities in developing countries have contributed immensely to the increase in environmental pollutants. Social habits, lifestyle, living conditions, or occupational hazards are common sources of exposure to these pollutants. Environmental toxicants are well-documented to disrupt testicular functions in human and experimental animals.[1,2] Heavy metals are one of the major environmental toxicants. Thus, it has been reported that cadmium and lead-induced oxidative stress by depleting endogenous antioxidants, disturbance of the hypothalamic-pituitary-testicular axis, inhibition of steroidogenesis, and reduction of steroidogenic enzymes that are involved in androgen synthesis.[3,4]

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One of the mechanisms of heavy metals in disrupting spermatogenesis and steroidogenesis is through the generation of reactive oxygen species (ROS).[2] ROS is one of the major causes of inflammation.[5,6] Inflammatory response involves the generation/release of hydrogen peroxide and, the metabolism of neutrophil and nicotinamide adenine dinucleotide phosphate oxidase.[6] Release of tumour necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) that promote inflammation usually further generates more ROS.[7] Increase in ROS may overwhelm total antioxidant capacity and result in peroxidation damage to spermatozoa.[2] Apoptosis plays a substantial role in spermatogenesis in the human testis.[8] The increase in pro-apoptotic cytokines can cause permanent loss of spermatogenesis.[9] Also, inflammatory cytokines, ROS, and nitric oxide can inhibit Leydig cell function.[10,11] Lead was also reported to disrupt the pituitary-testicular axis, inhibit testicular expression, and reduce steroidogenic enzymes such as 17β-hydroxysteroid dehydrogenase.[12]

Sida corymbosa (broom-weed) is a shrubby semi-woody perennial weed that belongs to the Malvaceae family. Varied claims have been made in traditional medicine on the plant's potency in curing such ailments as stomach ulcers, fever, gonorrhea, inflammation, infertility, and wound healing.[13,14] The plant has also been scientifically reported to facilitate uterine contraction and parturition,[15] possess anti-inflammatory activity,[16] and anti-ulcer.[14] Recently, it was reported that methanol extract of *Sida corymbosa* leaves (SC) contains alkaloids, cardiac glycosides, flavonoids, saponin, sterol, and tannic. Also, some biologically important fatty acids such as hexadecenoic acid methyl ester, hexadecenoic acid, 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid, and methyl heptadecanoic acid are present in SC.[17] Hexadecenoic acid methyl ester, one of the compounds present in SC have been reported to reduce oxidative stress and inflammatory markers like TNF-α, IL-6, IL-10, nitric oxide, COX-2 and PGE_2.[18] It

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was proposed that SC may be a good source of antioxidants due to its high phenol content, total antioxidant capacity, metal-chelating activity, increased superoxide dismutase, catalase activities, and reduced (malondialdehyde) MDA in lead acetate exposed Wistar rats.[17]
Therefore, the purpose of the study was to assess the role of anti-apoptotic, antioxidant, and anti-inflammatory activities of *Sida corymbosa* methanol leaf extract on lead acetate-induced alteration in testicular functions of Wistar rats.

Materials and Methods

Plant and extract preparation
The identification of *Sida* corymbosa leaves and SC preparation was done as previously reported.[17]

Chemicals and reagents
The Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA) produced the lead acetate that was used. Analytical grade reagents and chemicals were utilized in all other instances.

Animal model
This study used 14-week-old male Wistar rats (165 ± 15 grams). The rats were acquired from the University of Ibadan, College of Medicine animals' house. The animals were given 14 days to acclimatize before commencing treatments. Five animals per cage were kept in the College of Medicine Central Animals' house, University of Ibadan under standard laboratory conditions. The study was carried out following the National Institute of Health (NIH publication #85-23, revised in 1985) guidelines for the care and use of laboratory animals.

Experimental design
Thirty male Wistar rats were randomly and equally divided into six groups. The groupings and treatments are as follows: Group 1 served as control and received 0.5mL of 10% tween 20; Group 2: (Pb) received 15 $mgkg^{-1}$ lead acetate; Group 3: SC (100 $mgkg^{-1}$) received 100 $mgkg^{-1}$ of SC; Group 4: SC (200 $mgkg^{-1}$) received 200 $mgkg^{-1}$ of SC; Group 5: Pb + SC (100 $mgkg^{-1}$), and Group 6: Pb + SC (200 $mgkg^{-1}$).

All the administrations were done orally for fifty-four days. Blood and testes with epididymis were obtained after sedating the rats with 50 $mgkg^{-1}$ sodium thiopental on the fifty-fifth day.

Hormones assays
Sera were obtained by spinning the blood at 2,500 revolutions per 10 minutes in a cold centrifuge. The serum FSH, LH, and testosterone levels were assessed with the Calbiotech ELISA kits (El Cajon, California). The procedures applied were in accordance with the manufacturer's manual.

Steroidogenic enzymes activities assay
Testicular 3β- and 17β- hydroxysteroid dehydrogenase activities were evaluated according to the method of Talalay.[19] Spectrophotometric method was used to measure the enzyme activities at 340nm.
Testicular androgen receptor expression
Androgen receptor (AR) expression was evaluated using an immunohistochemical staining technique.

Sperm analysis
A computer-aided sperm analyzer (CASA, JH-6004 sperm Quality Analyzer) was used to analyze the sperm samples, while sperm viability and morphology were evaluated.[2]

Determination of testicular zinc and lead ions
The testicular level of lead and zinc ions were evaluated with the Perkin Elmer Absorption Atomic Spectrophotometer (AAS) with Winlab 32 software.

Evaluation of testicular oxidant and antioxidant activities
Testicular nitrite, malondialdehyde levels, catalase, and superoxide dismutase activities were assayed as described by Oyeyemi et al.[2]

Determination of sperm and testicular DNA damage
Sperm DNA condensation was determined by staining the sperm smear with acidic aniline-eosin stain as described by Park *et al*. [20] The testicular DNA damage was assessed with the measurement of 8-hydroxydeoxyguanosine (8-OHdG) using ELISA method (Elabscience ELISA kit).

Amplification of steroidogenic acute regulatory protein, B-cell lymphoma -2- associated death, B-cell lymphoma -2, interleukin-6, and tumor necrosis factor-alpha genes DNA extraction/purification
Zymo Research DNA-tissue miniprep kit was used for testicular DNA purification. The purity of DNA samples was verified with the NanoDrop spectrophotometer method at 260/280 nm wavelength.
Polymerase chain reaction (PCR) was carried out on purified DNA samples. All the DNA samples were amplified with a pair of primers (Table 1). All the reactions (PCR cocktail) were carried out at a primer concentration of 10 pMol in 10 µL containing PCR buffer, 50 mM $MgCl_2$, DMSO, 2.5 mM DNIPs, and Taq 5 µ/L water. The PCR was performed using a thermal cycler (Perkin-Elmer Applied Biosystems). Two µL dye (agarose gel electrophoresis) was added to 4 µL of the PCR product and loaded into the well with 50 base pair ladders, it then ran for 1 hour 30 minutes at 80 volts, 300 mA 60 Watt. The plates were removed and placed in gel-ethidium bromide solution for 5 minutes. It was then viewed under a UV trans-illuminator and the gel picture was saved. The gel plate was then quantified with an imageJ gel analyzer (1.53r Version).

Table 1: Primers used for PCR

NAME (mRNA)	Accession Number	Tm (^{0}C)	Strand position	Primer Sequence	Length	Product size (Amplicon size)	Sequence size (included region)
StAR	NM_031558.3	59.00	Forward-365	GCAAAAGGCCTTGGGCATAC	20	148	1154
		59.02	Reverse-512	TCTGTCCATGGGCTGGTCTA	20		
BAD	XM_006230896.1	58.02	Forward-163	CTTGAGGAAGTCCGATCCCG	21	113	881
		59.88	Reverse-275	GCTCACTCGGCTCAAACTCT	18		
BCL-2	XM_006249630.1	58.95	LEFT-1717	ATCCAGGATAACGGAGGCTG	20	79	3379
		59.03	RIGHT-1795	TCGTCAAGCTGCTCCACTAA	20		
TNF	NM_012675.3	59.02	Forward-868	CATCCGTTCTCTACCCAGCC	20	125	1687
		58.91	Reverse-992	CCCAGAGCCACAATTCCCTT	20		
IL-6	NM_012589.2	59.18	Forward-83	AGAGACTTCCAGCCAGTTGC	20	85	1045
		59.02	Reverse-167	AGTCTCCTCTCCGGACTTGT	20		

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Epididymis and testicular histology
The epididymis and testes sections were de-waxed in xylene for 15 minutes**.** The sections on the slides were fixed in graded ethanol and Carnoy's solution. The fixed slides were rinsed with de-ionized water. The epididymis was stained with Hematoxylin while a periodic acid-Schiff stain was used for the testes. The stained slides were carefully rinsed with tap water. The stained epididymis slides were counterstained with 1% aqueous Eosin for 2 minutes and then rinsed in water. The stained epididymis and testes slides were dehydrated in ascending ethanol solutions and cleared with xylene. The coverslips were mounted onto the stained slides with DPX and examined under a microscope at 200 magnification and a digital camera (Omax 10.0MP) was used for the photomicrograph.

Image quantification
The photomicrograph and PCR gel plates were quantified with ImageJ software (version 1.53r).[21]

Statistical analysis
The data were summarized as mean ± SEM (Standard error of the mean). One-way analysis of variance was used to determine statistical significance across groups, followed by the least significant difference test. $p<0.05$ is considered significant. The analysis was done with GraphPad Prism 8.0.1 (244) version.

Results and Discussion

The hypothalamus-pituitary testicular axis plays a major role in regulating spermatogenesis and steroidogenesis. A significant reduction was observed in FSH, LH, and testosterone in the Pb-treated animals for 54 days relative to control (Table 2). The observation is in pact with earlier studies.[2,3] Serum levels of LH and testosterone were reduced in Pb+SC (100 $mgkg^{-1}$) group when compared with control (Table 2). FSH and LH levels were significantly higher in the SC (200 $mgkg^{-1}$) group relative to the control (Table 2). The mechanistic action of Pb in reducing FSH, LH, and testosterone may be through disruption in the hypothalamic-pituitary axis.[2] The observed reduction in testosterone of Pb-exposed rats was directly correlated with serum LH which generally triggers steroidogenesis in the interstitial cells of Leydig via its receptor activation. Table 2 shows that the administration of SC improved the observed decline in FSH, LH, and testosterone of Pb-treated rats. Also, SC alone increased serum levels of FSH (Table 2). Therefore, it can be postulated that SC averts the harmful effect of Pb on the hypothalamic-pituitary-testicular axis probably through its anti-inflammatory, antioxidant, and metal-chelating properties that were earlier reported.[17] Furthermore, the present study explored steroidogenic enzyme activities of SC in the rats' testes after induced reproductive toxicity with Pb. The rats exposed to Pb in this study showed down-regulation of testicular StAR (Figure 1A), and androgen receptor expression (Figure 1B) with a reduction in HSD activities (Table 2). These reductions directly correlated with the observed reduction in the LH and testosterone of Pb-treated rats in the present study. Testicular StAR expression was also reduced in Pb+SC groups compared with control (Figure 1A). Some studies have reported that exposure to Pb triggered a reduction in the StAR expression,[22] androgen receptor, and steroidogenic enzyme activities.[12,23] This observation suggests that Pb disrupts the steroidogenic enzymes in the Leydig cells, consequently affecting the testicular steroidogenesis, and might be accountable for the observed reduction in testosterone of Pb-treated rats. SC (200 $mgkg^{-1}$) increased 3β-HSD activity significantly, while 17β-HSD activity was significantly increased by both doses of SC when compared with the control (Table 2). The SC significantly improved testicular StAR (Figure 1A), androgen receptor expression (Figure 1B), and HSD activities (Table 2) in the Pb-treated rats. It suggests that SC boosts steroidogenic enzyme activity through activation of gonadotropin secretion and mobilization of cholesterol which are required for testosterone biosynthesis in the Leydig cells. Thus, SC may ameliorate Pb-induced testicular steroidogenesis disruption.
The present study showed a significant reduction in sperm count, epididymal sperm volume (Table 3), spermatogenesis arrest, short stratification of spermatogenic cells, and widening of the seminiferous tubular lumen with little spermatozoa (Figure 7) in Pb-treated rats. All these observations are indications of reduced spermatogenesis. Table 3 shows a significant reduction in total sperm motility, progressive sperm motility, sperm average path velocity, amplitude lateral head, cross beat frequency, the line moving and mean move angle, sperm viability, and an increase in immotile and abnormal sperm morphology of Pb treated rats which were in line with previous studies.[2,24] The SC administration with Pb improved spermatogenesis (Figure 7), sperm motility, kinetics, viability, and morphology (Table 3). This prophylactic actions of SC on deleterious effects of Pb on spermatogenesis, kinetics, and viability may be credited to the SC antioxidant properties which may prevent ROS formation and peroxidation of spermatozoa polyunsaturated fatty acids within their plasma membrane. Also, the observed increase in steroidogenesis, zinc ion, antioxidant enzymes, reduction of testicular Pb, oxidant activity, pro-apoptotic and inflammatory markers by SC in this study may be responsible for the improved spermatogenesis, sperm kinetics, viability, and morphology.
Zinc (Zn) is an essential element that is present in all tissues because of its physiological role such as antioxidant, cell growth, immunity, wound curing, and even spermatogenesis.[25,26] Lead administration to rats increased the testicular lead (Figure 2A) and decreased zinc ion level (Figure 2B) when compared to control. This observation may be ascribed to the accumulation of lead in the testes which may compete and displace Zn ion.[27,28] Also, lead influences the absorption and circulation of Zn in blood and other tissues by preventing the operating system of Zn.[27] This may be responsible for the observed decrease in testicular zinc level in this study. The co-administration of SC with lead acetate reversed the testicular Pb and Zn levels (Figure 2A & B). This suggests that SC may prevent testicular lead accumulation and enhance zinc enzymatic reaction through its antioxidant and metal-chelating properties.[17] The elevation of testicular zinc ion by SC in the Pb-exposed rats may be accountable for increased steroidogenic and spermatogenesis in this study.

Table 2: Effects of *Sida corymbosa* on reproductive hormones and testicular steroidogenic enzymes activities in lead acetate male Wistar rats treated

	Control	Pb	SC (100 $mgkg^{-1}$)	SC (200 $mgkg^{-1}$)	Pb+SC (100 $mgkg^{-1}$)	Pb+SC (200 $mgkg^{-1}$)
FSH ($mIUmL^{-1}$)	22.0 ± 0.95	10.6 ± 0.93*	20.2 ± 0.80	29.8 ± 0.66*	19.4 ± 0.81$^{\#}$	19.6 ± 1.03$^{\#}$
LH ($mIUmL^{-1}$)	61.5 ± 2.25	42.4 ± 1.57*	64.5 ± 2.04	70.0 ± 2.81	45.6 ± 2.32*	64.7 ± 1.72$^{\#}$
Testosterone ($ngmL^{-1}$)	11.0 ± 0.71	4.2 ± 0.86*	9.6 ± 0.93	10.8 ± 0.86	5.6 ± 0.53*	9.2 ± 0.86$^{\#}$
3ß HSD (unit/mg tissue)	8.6 ± 0.93	5.0 ± 1.30*	9.4 ± 0.81	16.4 ± 2.20*	7.9 ± 1.67$^{\#}$	9.8 ± 1.56$^{\#}$
17ß HSD (unit/mg tissue)	7.6 ± 0.93	2.7 ± 0.22*	14.0 ± 1.45*	13.2 ± 1.16*	6.6 ± 0.93$^{\#}$	8.2 ± 1.39$^{\#}$

Values are represented as Mean±SEM, n=5. *Significant difference from the control at $p<0.05$. $^{\#}$Significant difference from the Pb group at $p<0.05$.

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Table 3: Effects of *Sida corymbosa* on sperm parameters in lead acetate exposed male Wistar rats

	Control	Pb	SC (100 mgkg^{-1})	SC (200 mgkg^{-1})	Pb+SC (100 mgkg^{-1})	Pb+SC (200 mgkg^{-1})
Sperm Count (million/mL)	156.7 ± 9.7	106.8 ± 9.7*	185.3 ± 26.9	189.0 ± 12.0*	148.4 ± 5.7$^{\#}$	141.9 ± 7.9$^{\#}$
Sperm motility (%)	90.2 ± 0.40	62.2 ± 5.06*	90.0 ± 0.49	90.1 ± 0.37	75.3 ± 6.78*,$^{\#}$	82.1 ± 2.18*,$^{\#}$
Progressive Motility (%)	39.2 ± 1.38	11.0 ± 2.00*	41.0 ± 1.31	46.4 ± 1.43*	32.9 ± 2.92$^{\#}$	40.2 ± 2.73$^{\#}$
Non-Progressive Motility (%)	50.0 ± 1.84	51.2 ± 5.68*	49.0 ± 1.64	45.0 ± 2.27	42.2 ± 6.82	44.6 ± 1.68
Immotile (%)	9.8 ± 0.40	37.8 ± 5.06*	10.0 ± 0.49	9.9 ± 0.37	24.7 ± 6.78*	17.9 ± 2.18*,$^{\#}$
Average Path Velocity (µm/s)	10.5 ± 0.17	8.2 ± 0.21*	10.7 ± 0.48	11.1 ± 0.24	10.8 ± 0.53$^{\#}$	11.8 ± 0.68$^{\#}$
Curvilinear velocity (µm/s)	19.4 ± 0.26	17.9 ± 0.32	19.4 ± 0.52	19.8 ± 0.54	19.5 ± 0.61	20.0 ± 0.96$^{\#}$
Straight-line velocity (µm/s)	4.5 ± 0.08	4.1 ± 0.05	4.7 ± 0.25	4.6 ± 0.13	4.7 ± 0.30$^{\#}$	4.7 ± 0.23$^{\#}$
Amplitude Lateral Head (µm)	0.56 ± 0.01	0.52 ± 0.01*	0.58 ± 0.02	0.59 ± 0.01	0.57 ± 0.03$^{\#}$	0.55 ± 0.02$^{\#}$
Beat Cross Frequency (Hz)	4.7 ± 0.04	4.3 ± 0.07*	4.5 ± 0.08	4.7 ± 0.14	4.6 ± 0.13$^{\#}$	4.4 ± 0.06*
Linearity (%)	9.0 ± 0.51	7.0 ± 0.40*	10.1 ± 1.49	10.0 ± 0.99	10.2 ± 1.15$^{\#}$	10.8 ± 0.37$^{\#}$
Wobble (%)	54.1 ± 0.36	55.0 ± 0.87	55.2 ± 0.96	54.4 ± 0.67	55.3 ± 1.37	57.2 ± 1.57
Mean angular displacement (°)	13.9 ± 0.16	11.1 ± 0.48*	13.0 ± 0.59	13.8 ± 0.35	13.5 ± 0.71$^{\#}$	12.7 ± 0.54
Viability (%)	66.0 ± 4.18	29.0 ± 4.18*	62.8 ± 3.03	69.6 ± 3.65	46.8 ± 2.17*,$^{\#}$	56.8 ± 4.97$^{\#}$
Abnormal sperm morphology (%)	19.0 ± 1.87	51.4 ± 2.97*	24.0 ± 4.18	15.0 ± 3.54	30.6 ± 1.52*,$^{\#}$	28.0 ± 1.87*,$^{\#}$

Values are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. $^{\#}$Significant difference from the Pb group at p<0.05.

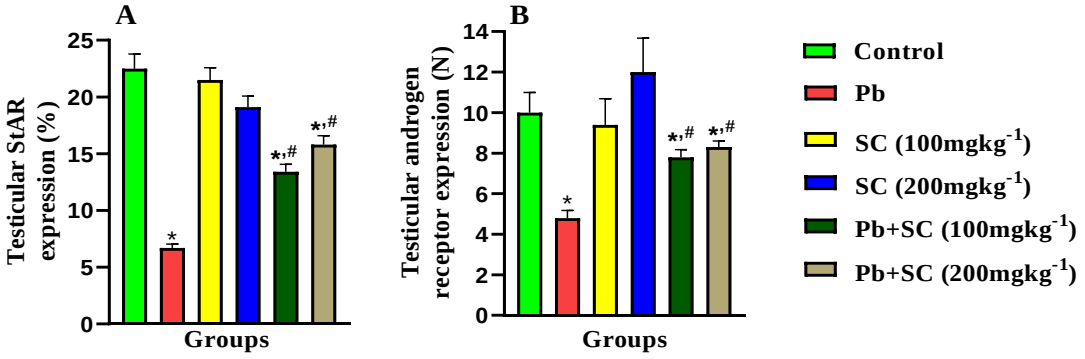

Figure 1: Effects of methanol extract of *Sida corymbosa* leaves on (A) steroidogenic acute regulatory protein and (B) testicular androgen receptor expression in male Wistar rats treated with lead acetate.
Bars are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. $^{\#}$Significant difference from the Pb group at p<0.05.

Malondialdehyde, a biomarker of lipid peroxidation and NO is known to damage DNA, proteins, and lipids. They also inhibit Leydig cell steroidogenesis and spermatogenesis through free radical generation.[29] The present study showed an increase in testicular nitrite (Figure 3A) and MDA (Figure 3B) levels in animals treated with Pb relative to the control group. These observations could be due to the increasing generation of inducible nitric oxide synthase and reactive oxygen species as a result of testicular lead accumulation. The present observation is consistent with previous reports.[2,30] The SC extract reduced MDA generation in dose dose-dependent manner, it also modulated testicular levels of nitrite and MDA in Pb-treated animals (Figure 3A & B). The SC ability to control testicular nitrite and MDA levels may be due to its antioxidant and metal chelating capacities which may avert testicular lead accumulation.[17] Several scientific reports showed that Pb has inhibitory effects on endogenous antioxidant enzymes and increased generation of lipid peroxidation in the testes.[30] The present results show that Pb administration reduces catalase and SOD activities relative to the control group (Figure 3C & D). The co-treatment of lead acetate with SC in the present study improved catalase and SOD activities in the testes relative to the Pb group (Figure 3C & D). The ability of SC to increase the activities of these two enzymes in the Pb-treated rat could be ascribed to preventing testicular lead ion accumulation, reduced nitrite and MDA levels which may prevent the overwhelming of endogenous antioxidant enzymes by lipid peroxidation.

Sperm DNA condensation and testicular 8-OHdG were used in the present study to measure sperm and testicular DNA damage. Figure 4A shows significant increases in sperm DNA chromatin condensation of Pb and Pb+SC groups relative to control. Testicular 8-hydroxydeoxyguanosine level (Figure 4B) was increased significantly in the Pb group, while significant reduction was observed in SC (200mgkg^{-1}) and Pb+SC groups relative to control. The observed increases in sperm DNA chromatin abnormality and testicular 8-OHdG of Pb-treated rats in this study may be related to ROS generation due to an increase in MDA, NO, and lead ions. The observed decrease in testicular 8-OGdG in the Pb-treated rats is similar to the report of He et al.[31] The reduction in the testicular zinc ion after Pb exposure in this study may be responsible for an increase in sperm DNA chromatin abnormality. Since Pb has been proposed to restrict the reform and tight packaging of sperm DNA during sperm formation by competing with zinc on protamine binding sites.[32] The sperm DNA chromatin condensation and testicular 8-hydroxydeoxyguanosine level were reduced significantly in Pb+SC groups when compared with the Pb group (Figure 4A & B). The SC was able to reverse the observed sperm DNA chromatin abnormality and testicular 8-OHdG induced by lead. The ability of SC to prevent sperm and testicular DNA damage may be

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related to its metal-chelating property.[17] The chelating activity of SC may prevent lead ions from displacing zinc ions, oxidize 20-deoxyguanosine, and maintain chromatin condensation maintenance by binding to thiol groups of cysteine residues of protamine.

Apoptosis is an essential physiological mechanism that regulates cell proliferation. However, excessive apoptosis can cause damage to physiological processes. Reactive oxygen species are well documented to induce oxidative damage to mitochondrial DNA which has been implicated in cell apoptosis.[33] The BAD expression was significantly increased while a significant reduction was observed in the expression of BCL-2 in Pb and Pb+SC (200mgkg^{-1}) groups when compared with the control (Figure 5A & B). The increase in testicular expression of BAD and a reduction in BCL-2 in rats exposed to Pb; may be an indicator of cell death in lead acetate-exposed animals. The testicular apoptotic effect of lead acetate observed in this study is similar to previous studies.[23,34] BAD significantly reduced in SC and Pb+SC groups while BCL-2 significantly increased in SC (200mgkg^{-1}) and Pb+SC (100mgkg^{-1}) relative to control. Expression of BAD in Pb+SC groups was significantly reduced whereas BCL-2 expression was increased significantly in Pb+SC groups when compared with the Pb group (Figure 5A & B). The testicular expression of BAD in rats treated with SC and Pb was reduced, while testicular BCL-2 expression was improved especially in lower doses of SC with lead acetate. This suggests that the SC modulates the testicular apoptotic process in rats treated with Pb through the BCL-2 signal. Also, SC may prevent excessive apoptotic processes in the rat's testes exposed to lead acetate through improved testosterone secretion, antioxidant enzyme activities, reduced testicular lead ion, and ROS observed in this study.

The present study also reveals amplification of testicular pro-inflammatory cytokines (TNF-α and IL-6) expression in the Pb-treated rats relative to control (Figure 5C & D). Similar to this study's observation, an increase in TNF-α and IL-6 in lead-exposed rats was earlier reported.[35] The observed decrease in LH secretion, 3β-HSD, and StAR protein in lead acetate-exposed rats in the present study may be due to the observed increase in proinflammatory markers. Inflammation is known to inhibit LH secretion, P450sec, P450c, and inhibit Leydig cell steroidogenesis through the activation of the NF_k-B signalling pathway.[36] The co-administration of SC with lead acetate significantly reduced the level of elevated TNF-α and IL-6 compared to the Pb group (Figure 5C & D). These observations may be attributed to the anti-inflammatory property of palmitic acid methyl ester, a main fatty acid compound present in SC.[17] Previously, anti-inflammatory properties of PAME and its ability to reduce tumor necrosis factor-alpha, interleukin-6, neutrophil infiltration, and NF-$_k$B expression in the liver were reported.[37,38]

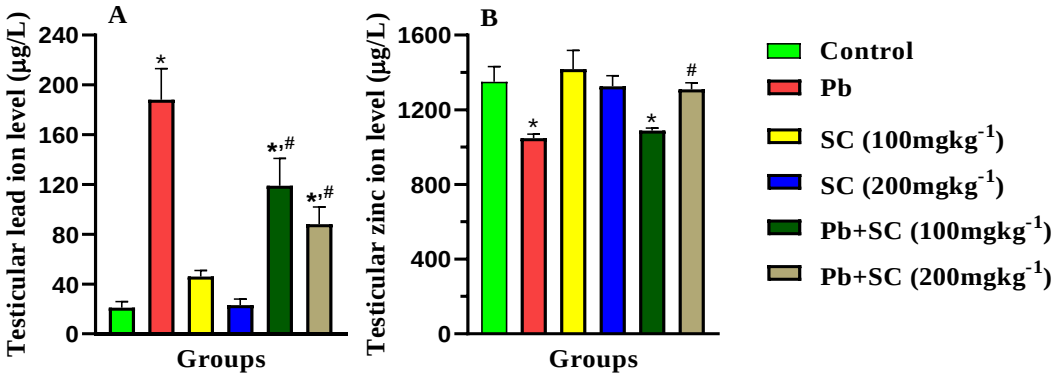

Figure 2: Effects of methanol extract of *Sida corymbosa* leaves on (A) testicular lead and (B) zinc ions level in male Wistar rats treated with lead acetate.
Bars are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. #Significant difference from the Pb group at p<0.05.

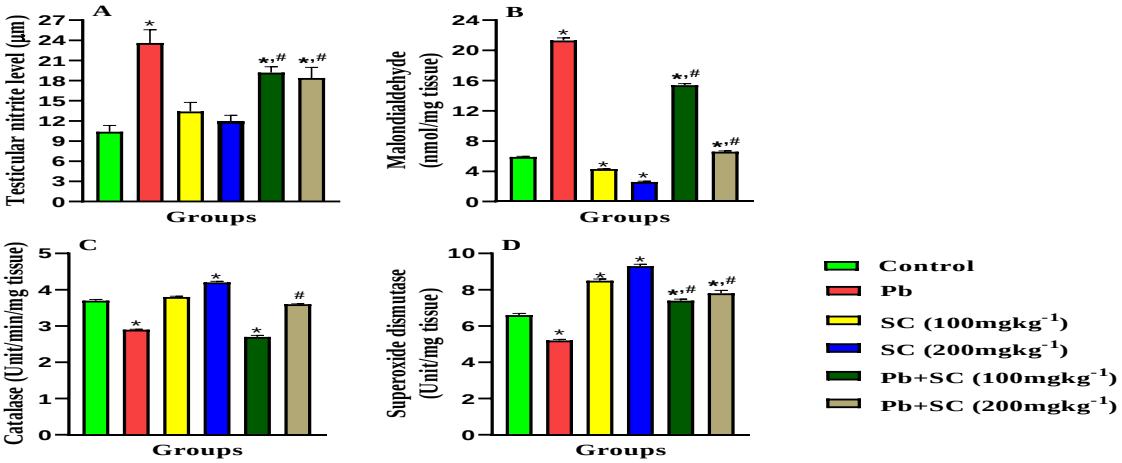

Figure 3: Effects of methanol extract of *Sida corymbosa* leaves on (A) testicular nitrite, (B) malondialdehyde, (C) catalase and (D) superoxide dismutase activities in male Wistar rats treated with lead acetate.
Bars are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. #Significant difference from the Pb group at p<0.05.

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The present study also reveals amplification of testicular pro-inflammatory cytokines (TNF-α and IL-6) expression in the Pb-treated rats relative to control (Figure 5C & D). Similar to this study's observation, an increase in TNF-α and IL-6 in lead-exposed rats was earlier reported.[35] The observed decrease in LH secretion, 3β-HSD, and StAR protein in lead acetate-exposed rats in the present study may be due to the observed increase in proinflammatory markers. Inflammation is known to inhibit LH secretion, P450sec, P450c, and inhibit Leydig cell steroidogenesis through the activation of the NF_k-B signalling pathway.[36] The co-administration of SC with lead acetate significantly reduced the level of elevated TNF-α and IL-6 compared to the Pb group (Figure 5C & D). These observations may be attributed to the anti-inflammatory property of palmitic acid methyl ester, a main fatty acid compound present in SC.[17] Previously, anti-inflammatory properties of PAME and its ability to reduce tumor necrosis factor-alpha, interleukin-6, neutrophil infiltration, and NF-$_k$B expression in the liver were reported.[37,38]

The observed reduction in sperm concentration and increase in testicular inflammatory markers of animals exposed to Pb in this study can be supported by the epididymis and testicular histology. The epididymal and testicular histology showed hyperplasia and infiltration of inflammatory cells, mild vacuolation in the seminiferous tubule, limited disorganized germinal cell layer, lumen appeared wide with lack of spermatozoa, spermatozoa maturation arrest, and the interstitial space fat deposit were observed after lead acetate administration (Figure 6B & 7B). These observations are similar to the findings of El-Khadragy *et al*.[39], Behairy *et al*.[40], and Adedokun *et al*. [41] in which Pb exposure caused advanced interstitial, tubular, and vascular testicular damage with a drop in spermatids and pachytene spermatocytes ratio. The lead acetate co-treated with SC showed restoration of epididymal (Figure 6E & F) and testicular histology to normal (Figure 7F). The meliorative effects of SC on Pb-induced epididymis and testicular damage may be due to its ability to increase endogenous antioxidant enzymes, Zn ion level, reduce testicular Pb ion accumulation, MDA, 8-OHdG, pro-apoptotic and inflammatory cytokines observed in the present study.

In conclusion, anti-apoptotic, anti-inflammatory, antioxidant, and steroidogenic activities of methanol extract of *Sida corymbosa* leaves ameliorated lead acetate-induced testicular impairment in male Wistar rats.

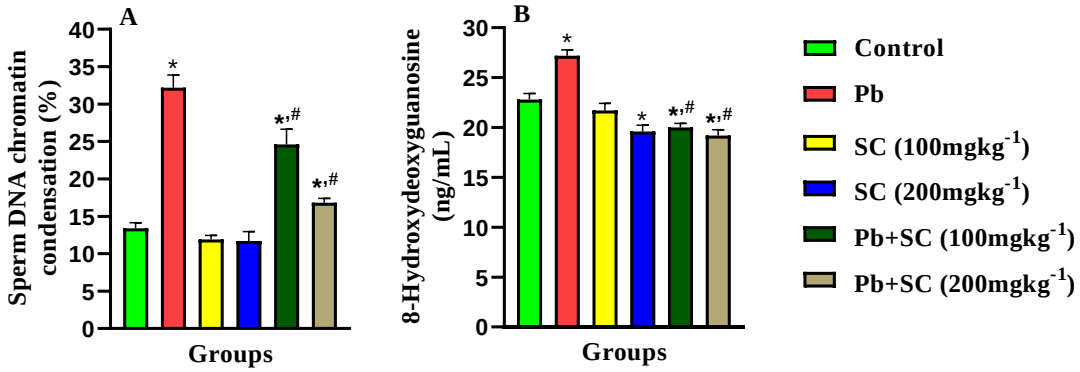

Figure 4: Effects of methanol extract of *Sida corymbosa* leaves on (A) sperm DNA chromatin condensation and (B) testicular 8-hydroxydeoxguanosine in male Wistar rats treated with lead acetate.
Bars are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. #Significant difference from the Pb group at p<0.05.

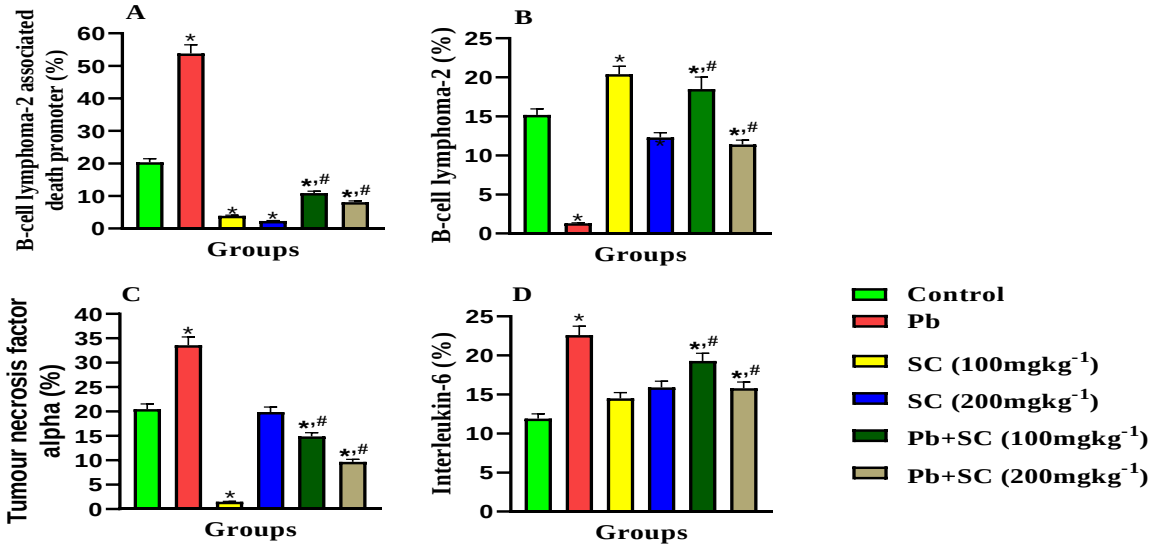

Figure 5: Effects of methanol extract of *Sida corymbosa* leaves on testicular (A) B-cell lymphoma-2-associated death promoter, (B) B-cell lymphoma-2, (C) Tumour necrosis factor and interleukin-6 in male Wistar rats treated with lead acetate.
Bars are represented as Mean±SEM, n=5. *Significant difference from the control at p<0.05. #Significant difference from the Pb group at p<0.05.

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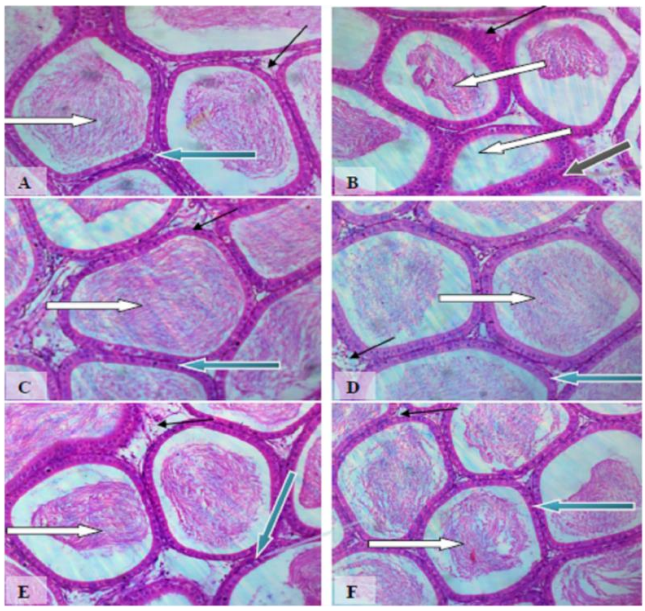

Figure 6: Effect of methanol extract of *Sida corymbosa* leaves on epididymal histology of lead acetate treated male Wistar rats (H&E stained) × 200

Control (A), SC (100 & 200 mgkg-1) (C & D), and Pb+SC (200 mgkg-1) (F) epididymal ducts, epithelial layers, smooth muscle layer (blue arrow), spermatozoa in lumen (white arrow), and interstitial spaces (slender black arrow) appear normal. Pb (B) group shows that some epididymal ducts, epithelial hyperplasia, thick muscles (black arrow), mildly fibrotic interstitial spaces, slightly infiltrated by inflammatory cells (slender black arrow), and scanty spermatozoa in lumen (white arrow). Pb+SC (100 mgkg-1) (E) shows normal epididymal ducts with epithelial layers and smooth muscle (blue arrow), small spermatozoa in lumen (white arrow), and scanty inflammatory cells infiltrating interstitial space (slender black arrow).

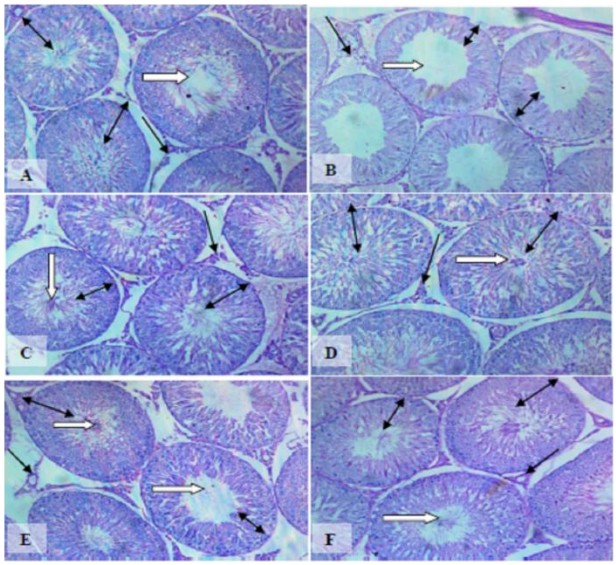

Figure 7: Effect of methanol extract of *Sida corymbosa* leaves on the testicular histology of lead acetate exposed male Wistar rats (PAS stained) × 200

Seminiferous tubules with regular stratification of spermatogenic cells (spanned), Leydig cells appear normal (black arrow), lumen (white arrow) contains curls of spermatozoa strands in the testicular section of the control (A), SC (100 mgkg-1) (C), SC (200 mgkg-1) (D) and Pb+SC (200 mgkg-1) (F) groups. The maturation arrest and short stratification of spermatogenic cells (spanned) were seen in Pb (B) and Pb+SC (100 mgkg-1) (E) groups, though Leydig cells appear normal (slender arrow) in the two groups. Scanty and moderate spermatozoa strands were seen in seminiferous tubules lumens (white arrow) of Pb and Pb+SC (100 mgkg-1) groups respectively.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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