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Research Article

Clinicopathology, Liver Lipid Profiles, Oxidative and Antioxidant Biomarkers of *Datura stramonium* Leaf Extract Toxicity and Ameliorative Potentials of *Moringa oleifera* Leaf Extract in Wistar Rats

Saleh[1]*, MY Fatihu[1], MM Suleiman[2], KAN Esievo[1] and JS Ibrahim[1]

[1]*Department of Veterinary Pathology, Ahmadu Bello University (ABU), Zaria*

[2]*Department of Veterinary Pharmacology and Toxicology, ABU, Zaria*

***Corresponding Author:** Saleh, Department of Veterinary Pathology, Ahmadu Bello University (ABU), Zaria.

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Abstract

There has been growing global concern over *Datura stramonium* toxicity in livestocks, pets and humans following ingestion of the plant. Effects of ethanol leaf extract *D. stramonium* (ELEDS) in liver lipid profiles, oxidative, antioxidant biomarkers and liver tissue of rats were investigated and possible ameliorative effects of ethanol leaf extract *Moringa oleifera* (ELEMO). Fifty adults male Wistar rats were randomly divided into five groups of 10 rats each. Group I were administered distilled water (2 mL/kg), group II; ELEMO (200 mg/kg), group III; ELEDS (170.4 mg/kg), group 1V; ELEDS+ELEMO and group V; ELEDS+ physostigmine (0.02 mg/kg). There was significant ($P < 0.05$) decrease in triglyceride and superoxide dismutase in ELEDS-treated groups compared to controls. Whereas, significant elevations were observed in high-density lipoprotein-cholesterol and malondialdehyde compared to controls. Microscopically, the liver of rats in ELEDS- treated group showed congestion and necrosis. Values in ELEDS+ELEMO-treated groups were comparable ($P > 0.05$) to controls. The ability of ELEMO (200 mg/kg) to ameliorate changes in lipid profile, peroxidation and antioxidant, caused by *D. stramonium* (170.4 mg/kg) is suggestive of a novel finding. This finding may be useful especially in areas where animals are prone to *D. stramonium* toxicity with poor accessibility to antidote.

Keywords: *Datura*; Lipid Profile; Peroxidation; Antioxidant; *Moringa*

Introduction

Datura poisoning is fast becoming a global issue and requires much attention. According to Walelign and Mekuriaw [1], poisonous plants affecting animals became a major concern to both livestock farmers and practicing veterinarians worldwide. Plant is said to be toxic if it detrimentally affects health status when consumed at an amount as would be taken normally or under certain circumstance such as extreme hunger or restriction of choice [2]. *Datura* poisoning is more prevalent among the low- and middle-income countries. There had been widely reports of *D. stramonium* poisoning incidents involving sheep, goats, pigs, cattle [3], chickens [4], dogs [5], horses, mules [6] and humans [7]. The plant contains majorly tropane derivative alkaloids (atropine, hyoscyamine, norhyoscyamine, scopolamine and meteloidine) which are competitive antagonist of muscarinic cholinergic receptors causing classical anticholinergic symptoms that can result in death in complicated cases [8]. In Nigeria, most of the livestock are being moved from one part of the country to another in search of greener pasture, hence the more likelihood of exposure to *Datura* spp. The plant grows easily as weed in cultivated fields, abandoned pastures, roadsides, grazing lands, empty lots and barnyards or wastelands especially near habitations [9]. Being often weedy in nature and invasive to pastures and fields, provided it's potential to be harvested with the desired forage [10]. In times of feed scarcity, farming, deforestation, summer droughts or spring when lush grasses are not available, and high cost of animal feeds, most livestock farmers opt to use any available plants to feed their animals, which increases risk of exposure to *D. stramonium*. Regardless of sufficient forage, some livestock just love to browse and may do so along with *D. stramonium* Linn. Herbicide applications were also shown to increase

the palatability of *D. stramonium,* thereby increasing the toxicity risk factor [11]. According to Das., *et al.* [12], wide distribution and high potential of feedstuff contamination were responsible for the high incidence of *D. stramonium* toxicities. Diagnosis of *Datura* spp. intoxication was complex and difficult due to lack of availability of routine laboratory diagnostic facilities [13]. Therefore, in depth knowledge of the toxicological effects of *D. stramonium* toxicity is pivotal in the diagnosis and management of clinical cases of its toxicity. Information of the chronic effects of *D. stramonium* toxicity on the liver archetecture, liver lipid profile, lipid peroxidation and antioxidant defence system (ADS), appear insufficient. Physostigmine (PS) and neostigmine widely used to reverse the anticholinergic effects of the plant [14] appeared to be controlled drugs, expensive and scarced, aside side effects characterized by ataxia, convulsions, circulatory and respiratory collapse, among others [15]. Hence, the need to scientifically investigate the anticholinesterase potency of *M. oleifera* leaf extract against *D. stramonium* toxicity.

Moringa oleifera Lam. is a small to medium sized tree (10 to 15 m in height) belonging to the family *Moringa*ceae. It is believed to be of high medicinal value and with validated anticholinergic inhibitory effect in both *in vitro* and *in vivo* [16] which suggested its role in mitigating the anticholinergic effects caused by *D. stramonium*. Furthermore, *M. oleifera* was reported to high antioxidant activity [17], hepatoprotective [18], neuroprotective [19] and anti-inflammatory [20]. Almost all parts of the plant (e.g. root, gum, bark, leaves, fruits [pods]), flowers, seeds and seed oil are being marketed as herbal remedy for various diseases [21]. However, its usage in mitigation of chronic *D. stramonium* toxicity remain poorly investigated.

Oxidative reactions often leads to lipid H_2O_2 formation which, is further degraded into several aldehydes' compounds including hydroxyalkanals [22]. Malondialdehyde (MDA) is a low molecular weight 3-carbon aldehyde that spontaneously breakdown product of peroxides that can be generated by free radical attack on polyunsaturated fatty acids [23] which makes up the cell membrane. Being the major oxidation product of peroxidized polyunsaturated fatty acids, MDA is considered as an important indicator and estimator of lipid peroxidation (LPx) [24]. Animal cells respond to oxidative stress by either repairing the damage or by decreasing the state of pro-oxidation primarily through enzymatic such as SOD and CAT, and non-enzymatic (e.g. GSH) ADS which have been shown to scavenge free radicals and ROS [25]. Free radicals and ROS removal leads to accumulation of superoxide free radicals ($O_2^{\bullet-}$) which promotes LPx and DNA modulation, alteration of gene expression and cell death [26].

Materials and Methods

Reagents and chemicals

All reagents and solvents used in this research were of analytical grade. Ethanol (95%), atropine (Sigma-Aldrich, UK). A commercial grade Physostigmine Salicylate (PS) was sourced from Burroughs Wellome Co. London. Formaldehyde (37-41%) was sourced from Fisher Scientific, UK. Lithium carbonate (Li_2CO_3), hydrochloric acid (HCl), Xylene, Eosin and Hematoxylene were sourced from Emsure Chemicals Co., Germany. All other chemicals were of reagent grades and were obtained from the local scientific distributors.

Collection, Identification and Preparation of *D. stramonium* and *M. oleifera* Leaves

Fresh leaves of *D. stramonium* and *M. oleifera* were obtained in October 2019 at Shanono (Kano State) and Pioneer Sustainable Agriculture Limited (Kaduna State), respectively. The plants samples were authenticated at the herbarium of the Department of Botany, Faculty of Life Sciences, A.B.U. Zaria with voucher specimen numbers, ABU0108 and ABU0517, respectively. The leaves of both plants were washed 3 times, separately, under running tap water, to remove impurities, and later air-dried under shade at room temperature for 4 weeks until a constant weight was obtained. The dried leaves were then crushed and pulverized with a mechanical grinder to obtain fine homogenous powder. The powdered samples were kept in dried airtight containers at room temperature prior to extraction.

Preparation of Crude Ethanol Leaf-Extracts of *Datura stramonium* and *Moringa oleifera*

The crude extracts were prepared according to Saleh., *et al.* [27]. Five hundred gram of powdered leaf samples of *D. stramonium* and *M. oleifera* were each placed in a 5 L conical flask. They were soaked in 1.5 L of 70% v/v ethanol and then covered with aluminum foil. The mixtures were allowed to stand in the laboratory at room temperature for 72 h with frequent agitation in order to facilitate dissolution of their constituents. The mixtures were then strained using muslin cloth to remove solid material (residue) which was discarded. The extraction was repeated twice by soaking the solid material using 2/3rd of the initial volume of the ethanol for 24 h. The strained liquids were clarified by gravitational filtration using Whatman filter paper size 1. The filtrates were then concentrated to semi-solid crude extracts on a water bath maintained at 50^{o}C and were packaged in a labelled airtight extract bottles and then stored in a desiccator prior to use. Aliquots of the concentrations were prepared immediately before use, using ultra-filtrate water to obtain the required dosages.

Ethical clearance, research animals acquisition and management

Animal experimentation in this study was approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), A.B.U., Zaria with Approval Protocol Number: ABUCAUC/2020/024. Fifty (50) healthy adult male Wistar rats weighing between 180 and 220 g were acquired from the Small Animal Experimental Station, Central Diagnostic Division, National Veterinary Research Institute (NVRI), Vom, Jos. They were kept in aluminium rat' cages 20 × 40 × 70 cm dimensions, with beddings of wood shavings at ambient temperature and light/dark cycle of 12 h. in the laboratory animal room, Department of Veterinary Pathology, Faculty of Veterinary Medicine, A.B.U. The rats were dewormed using albendazole (10 mg/kg) and allowed to acclimatize for four weeks prior to commencement of the experiment. The rats were fed standard pellet diet (Grand cereals Feeds, Jos, Nigeria) and clean water *ad libitum*.

Experimental design

Fifty (50) male Wistar rats were allocated at random into five groups of 10 rats each. Group I (DW) was administered distilled water at 10 mL/kg; Group II (ELEMO) was administered ethanol leaf extract of *Moringa oleifera* at dose rate of 200 mg/kg; Group III (ELEDS) was administered ethanol leaf extract of *D. stramonium* at dose rate of 170.40 mg/kg (4% of the LD_{50}); Group IV (ELEDS+ELEMO) was pre-administered ELEDS (170.40 mg/kg) then followed by ELEMO (200 mg/kg) 30 min later; Group V (ELEDS+PS) was pre-administered ELEDS (170.40 mg/kg) then followed by physostiminne (PS) (prepared in DW to make 10% stock solution) at dose rate of 0.02 mg/kg, 30 min later. The ELEMO and ELEDS were administered orally by gavage using rat stomach cannula, whereas, PS was administered via intraperitoneal route (i.p), for period of 36 weeks. All regimens were administered once daily between 08:00 am and 10:00 am.

Postmortem examination and preparation of liver tissue homogenate

At the end of the experiment (36 weeks), animals were fasted for 24 h before euthanasia. Five (5) rats from each group were humanely euthanized via jugular venesection after light chloroform anesthesia and rapidly dissected to excise the liver, which was washed using normal saline and used for the organ concentration. Ten percent homogenate of the tissue was prepared using 6.7 mM potassium phosphate (K_3PO_4) buffer, pH 7.4 in a Teflon homogenizer (Heidolph Silent Crusher M). The tissue homogenates were centrifuged at 10,000 *g* for 15 min to obtain a clear supernatant which was carefully decanted and kept at 4°C for biochemical analysis. Lipid peroxidation (LPx) and antioxidant enzyme activities; SOD, CAT and GSH were measured spectrophotometrically at 535 nm (MDA), 480 nm (SOD), 240 nm (CAT) and 412 nm (GSH) using the colorimetric kits. Representative tissue samples for histopathology were collected from the organ, fixed in 10% neutral buffered formalin and kept at room temperature for 1 week.

Determination of liver lipid profile

Total cholesterol TC), triglycerides (TG) and high-density lipoprotein, (HDL-c) levels were determined by enzymatic colorimetric methods using commercial kits (AGAPPE DIAGNOSTICS SWITZERLAND GmbH). Low-density lipoprotein-cholesterol (LDL-c) values were obtained by the difference between TC and HDL-c levels.

Determination of malondialdehyde concentration

Determination of malondialdehyde (MDA) was carried out according to the method described by Atawodi [28]. Exactly 2 cW12 of 15% trichloroacetic acid (TCA) was measured into a test tube, 2 cW12 of thiobabitutric acid (TBA) and 100 µL of tissue homogenate were added. The mixture was incubated at 80°C for 30 min on a water bath and allowed to cool for 30 min, followed by centrifugation at 3000 rpm for 10 min. The clear supernatant was collected and the absorbance was measured at 535 nm using a spectrophotometer. Thiobarbituric acid reactive substances (TBARS) concentrations were expressed in nMol/mg protein calculated as follows:

$$\text{Concentration (nMol/mg) protein} = \frac{\text{Absorbance of sample}}{1.5\text{x}10{-}5 \text{ x protein concentration (mg/mL)}}$$

Protein concentration (mg/mL) was done as described by Atawodi [28]. Briefly, 5 cW12 of tissue homogenate was mixed with 4 cW12 of biuret reagent and incubated at room temperature for 30 min and the absorbance was measured spectrophotometrically at 540 nm. The protein concentration was determined from the calibration curve, which was a plot of the absorbance of egg albumin (standard) against reaction mixture.

Determination of superoxide dismutase activity

Activity of superoxide dismutase (SOD) in tissue homogenates was measured as described according to Fridovich [29]. The prin-

ciple of the method is the ability of SOD to inhibit auto oxidation of adrenaline. Carbonate buffer 0.05 M (pH 10.2) and adrenaline (0.3 mM) solution were prepared fresh. Tissue homogenate 0.2 cW12 was added to 2.5 cW12 of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 cW12 of 0.3 mM adrenaline. The reference mixture contained 2.5 cW12 of 0.05M carbonate buffer, 0.3 cW12 of 0.3 mM adrenaline and 0.2 cW12 of distilled water. The absorbance was measured at 480 nm at 30 seconds as initial and 170.40 seconds as final absorbance, respectively.

$$\text{Increase in absorbance per min} = \frac{(A2-A1)}{2.5}$$

Where A1 = Initial absorbance

A2 = Final absorbance

$$\% \text{ Inhibition} = 100 - \left[\left(\frac{\text{increase in absorbance for sample}}{\text{increase in absorbance of blank}}\right) \times 100\right]$$

1 unit of SOD activity is the quantity of SOD necessary to elicit 50 % inhibition of the oxidation of adrenaline to adrenochrome in 1 min.

Determination of catalase activity

Activity of catalase in tissue homogenates was assayed according to the method of Abebi [30]. Exactly 10 µL of tissue homogenate was added to a test tube containing 2.80 cW12 of K_3PO_4 buffer (50 mM, pH 7.0). The reaction was initiated by adding 0.1 cW12 of freshly prepared 30 mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240 nm for 5 min using a spectrophotometer. A molar extinction coefficient (Ɛ) of 0.041 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity.

$$\text{Catalase Concentration} = \frac{\text{Absorbance of sample}}{\varepsilon}$$

$$\text{Catalase activity} = \frac{\text{Catalase concentration}}{\text{protein concentration (mg/mL)}}$$

Determination of glutathione concentration

This measurement was carried out as described by Rajagopalan., *et al.* [31]. Exactly 150 µL of tissue homogenate was added to 10% TCA and centrifuged at 1500 rpm for 5 min and 1 cW12 of the supernatant was treated with 0.5 cW12 of Ellman's reagent and 3 cW12 of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured spectrometrically at 412 nm. The quantity of glutathione concentration was deduced as:

$$\text{GSH Concentration} = \frac{\text{Absorbance}}{\varepsilon}$$

Where Ɛ = Molar extinction coefficients

Histopathological study

The tissues for histopathology were processed and stained as described by Baker., *et al*. [32] and Saleh., *et al.* [43]. The tissues were sliced laterally and longitudinally to be placed in cassettes and processed using Leica® rotary histokinette. In the histokinette, the tissues were run through formalin, followed by a series of graded ethanol, xylene and paraffin wax. After processing overnight, they were embedded in molten paraffin. The paraffin embedded tissues were cut at 4 µm of thickness using a Leica® Microtome. The sections were mounted on glass slides and air–dried and were heated at 60 °C for 60 min. Sections were tained with Haematoxilin and Eosin (H&E) for assessment of tissue morphology. They were deparaffinised by immersion in xylene (I, II, III) and then rehydrated in a graded series of alcohol (100% [trice], 90%, 80% and 70%), before washing in running tap water with a final rinsing in distilled water (GAD I and II). Each change was approximately 3 min. The sections were immersed in haematoxylin for 20 sec followed by washing in running tap water and then rinsed in DW (GAD I and II). The sections were decolourized with acid alcohol (0.5% HCl + alcohol) for 2 sec and then neutralized with 0.1 % Li_2CO_3 for 2 sec, before rinsing in water. The sections were counter stained in 0.5% eosin for 5 min. After completion of the staining, sections were dehydrated by sequential immersion in DW (GAD I and II), through graded ascending alcohol solutions (70%, 80%, 90% and 100% [twice]) before being cleared in xylene I, II & III and mounted with dibutylphthalate polystyrene xylene (DPX). The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Japan). Four-field observations were made per slide at 250x magnification. The severity of changes in the sections were scored "1 – 4" according to modified version of Kubiak., *et al.* [33].

Data analysis

Quantitative results of changes in lipid profiles and oxidative stress biomarkers across groups were analyzed by One–Way ANOVA using Statistical Package for Social Sciences (SPSS) version 20 software (SPSS Inc., Chicago, USA). Differences between each group were compared using the Tukey's Post Hoc Multiple Comparison Tests. The mean total histopathology scores were summarized and subjected to a descriptive statistic and Non–Parametric Kruskal–Wallis Test to differentiate the mean total scores. All the results were expressed as mean ± SEM. The probability value, $P < 0.05$ was used as the critical level of significance.

Results

The rats in the control and ELEMO-treated groups showed no signs of toxicity. However, the in ELEDS-treated group, the signs of toxicity include relative decrease feed intake which lasted for about 1½ h after treatment, stunted growth, rough hair coat (Plate 1) and dryness of the mouth observed at about W28 of the study

period and 30% mortality. Similar observations were made in ELEDS+ELEMO-treated and ELEDS+AP-treated groups, but less in severity with the mortality rates of 20% and 10%, respectively.

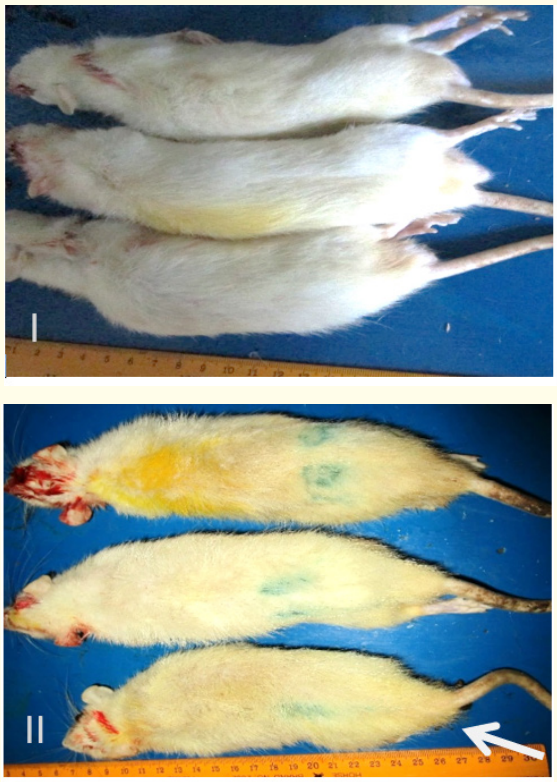

Plate 1: Photographs of Wistar rats; A, administered distilled water (DW) at 10 mL/kg showing normal hair coat. B, administered Ethanol leaf extract of *Datura stramonium* (ELEDS) at170.40 mg/kg showing starry hair coats and stunted (arrow).

Mean liver lipid concentrations

The mean lipid concentration (Figure 1) of TG was found to be significantly ($P < 0.05$) lower in ELEDS-group with value (21.9 ± 2.39 mg/dL) compared to DW (39.28 ± 5.45 mg/dL) and ELEMO (40.74 ± 4.43 mg/dL). In contrast, the HDL-c was significantly ($P < 0.05$) higher in ELEDS-treated (31.4 ± 4.23 mg/dL) group compared to DW-control (16.94 ± 1.39 mg/dL) and ELEMO-treated (15.50 ± 1.33 mg/dL) groups. The LDL-c and TC were similar across all the groups.

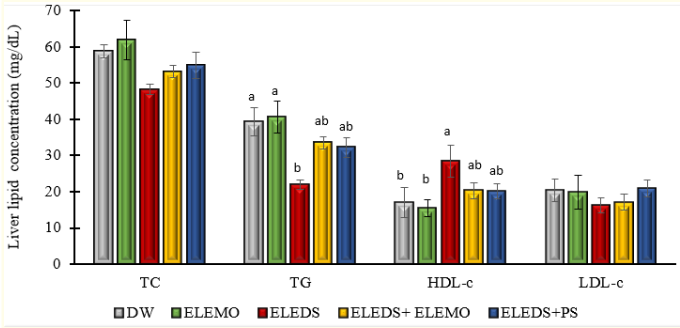

Figure 1: Mean liver lipid profiles of rats in the different treatment groups (n = 5).

*One–Way ANOVA, a different superscript (a, b) within each variable across the groups indicates significant ($P < 0.05$) differences. DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = physostigmine (0.02 mg/kg). TC = Total cholesterol, TG = Triglyceride, HDL-c = High-density lipoprotein-cholesterol, LDL-c = Low-density lipoprotein-cholesterol. Data represents the means ± SEM, n = Sample size

Mean liver lipid peroxidation of rats in the treatment groups

The mean malondialdehyde concentration (MDA) (Figure 2) of rats in the ELEDS- (311.28 ± 30.70 nMol/mg protein) group was significantly ($P < 0.05$) higher compared to DW and ELEMO-treated groups with a mean value of 197.2 ± 20.36 and 173.9 ± 14.21 nMol/mg protein, respectively.

The mean superoxide dismutase activity (SOD) is shown in Figure 3. The SOD of ELEDS- (8.54 ± 0.72 U/mg protein) group was significantly ($P < 0.05$) lower than in the DW- and ELEMO-treated groups with mean values of 16.2 ± 1.96 and 17.5 ± 2.21 (U/mg protein), respectively.

The mean CAT (Figure 4) and GSH (Figure 5) of the rats in all the groups did not vary significantly ($P > 0.05$).

Mean microscopic changes scored for some organs in rats

The mean histopathological changes scored for the liver was significantly ($P < 0.05$) higher in the ELEDS-treated (2.00 ± 0.44) group compare to the control (DW)- and ELEMO-treated groups

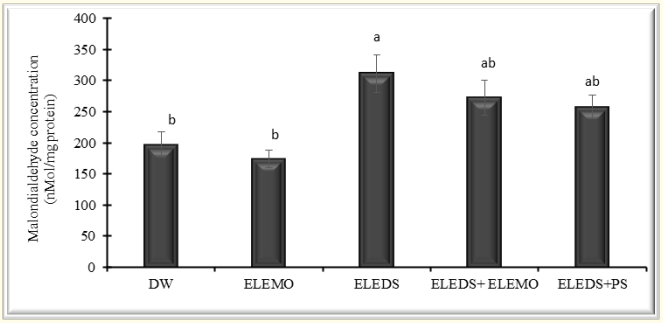

Figure 2: Mean liver malondialdehyde concentration of rats in the different treatment groups (n = 5).

*One–Way ANOVA, a different superscript (a, b) within each variable across the groups indicates significant (P < 0.05) differences. DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = physostigmine (0.02 mg/kg). Data represents the means ± SEM, n = Sample size.

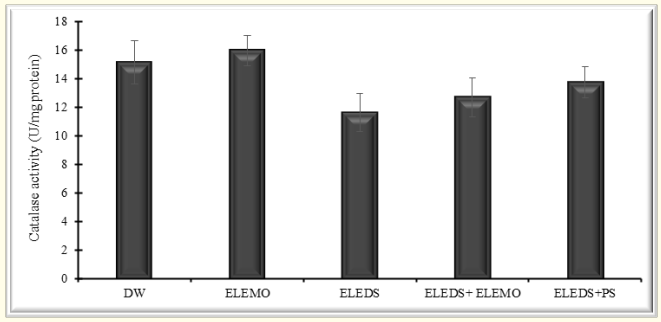

Figure 4: Mean liver catalase concentration of rats in the different treatment groups (n = 5).

*One–Way ANOVA, a different superscript (a, b) within each variable across the groups indicates significant differences (P < 0.05). DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = physostigmine (0.02 mg/kg). Data represents the means ± SEM, n = Sample size.

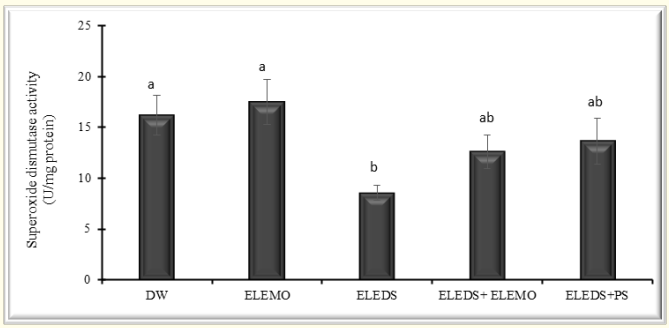

Figure 3: Mean liver superoxide dismutase concentration of rats in the different treatment groups (n = 5).

*One–Way ANOVA, a different superscript (a, b) within each variable across the groups indicates significant differences (P < 0.05). DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = physostigmine (0.02 mg/kg). Data represents the means ± SEM, n = Sample size.

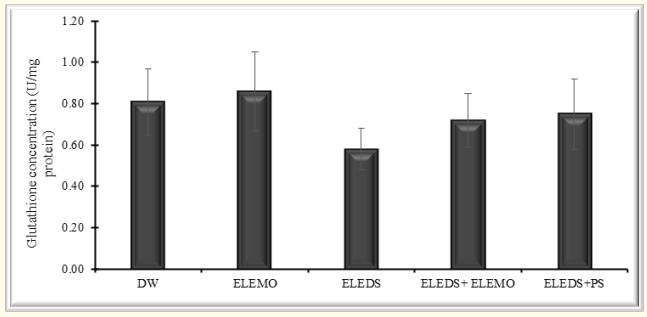

Figure 5: Mean liver glutathione concentration of rats in the different treatment groups (n = 5).

*One–Way ANOVA, a different superscript (a, b) within each variable across the groups indicates significant differences (P < 0.05). DW = 10 mL/kg, ELEMO = 200 mg/kg, ELEDS = 170.40 mg/kg, PS = 0.02 mg/kg. DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = physostigmine (0.02 mg/kg). Data represents the means ± SEM, n = Sample size.

with mean values of 0.00 ± 0.00 each (Table 1). While all the micrograph of the liver in the DW- (Plate IIa), ELEMO- (Plate IIb), (ELEDS+ELEMO)- (Plate IId) and (ELEDS+PS)-treated groups were normal to near normal. There was, however, congestion and necrosis in ELEDS- (Plate IIc)-treated rats.

Organ	Mean score ± SEM				
	DW	ELEMO	ELEDS	ELEDS+ELEMO	ELEDS+PS
Liver	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.00 ± 0.44^{b}	0.94 ± 0.29^{ab}	0.44 ± 0.14^{ab}

Table 1: Mean microscopic changes scores of the liver sections of the Wistar rats (n = 3).

*Kruskal Wallis test, a different superscript (a, b) within each variable across the groups indicates significant differences (P < 0.05), SEM = Standard error of mean, n = Sample size, DW = Distilled water (10 mL/kg), ELEMO = Ethanol leaf extract of *Moringa oleifera* (200 mg/kg), ELEDS = Ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), PS = Physostigmine (0.02 mg/kg).

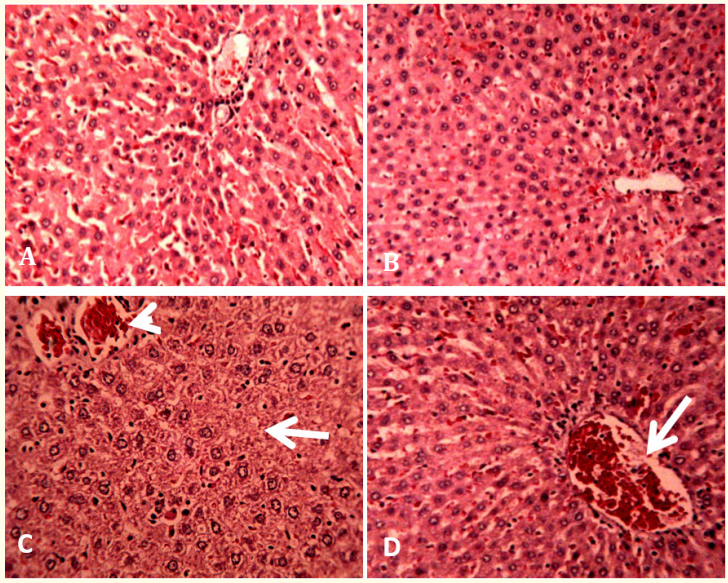

Plate II: Photomicrographs of the liver of rat administered; A, distilled water (10 mL/kg), showing normal hepatic tissue. B, ethanol leaf extract of ethanol leaf extract of *Moringa oleifera* (200 mg/kg), showing normal hepatic tissue. C, ethanol leaf extract of *Datura stramonium* (170.40 mg/kg), showing necrosis (arrow) and congestion (arrow head). D, ELEDS+ELEMO showing mild congestion (arrow). H&E stain x 250.

Discussion

The stunted growth observed in the ELEDS-treated rats could be attributed to accumulation or high doses of the extract or compounds in the body inducing stress in the animals, thereby leading to reduced appetite and intake of water due to hepatorenal toxicity, consequently leading to reduced body weight and stunted growth [34,35]. The starry hair coat and dryness of the mouth recorded in the ELEDS-treated rats might be due to dehydration which is in consistent with the known pharmacological properties of tropane alkaloid (atropine) [36], further supported by the presence of starry hair coat.

In the present study, decrease in TC and TG in ELEDS-treated groups relative to the controls, with a corresponding increased HDL-c observed in liver tissue homogenates, suggests a possible compromise of membrane integrity and fluidity function. Cholesterol is one of the prominent lipids that maintain the integrity of the cell membrane. A noticeable increase in HDL-c (also known as good cholesterol) in the liver in this study suggested hepatotoxicity. Lipoproteins transport proteins in the blood. High-density lipoprotein-cholesterol is mainly synthesised by the liver and intestinal cells and plays an important role in cholesterol efflux from the tissues and returns it to the liver where it is converted into bile and subsequently removed from the body [37]. El-Banna., *et al.* [38] reported that cholesterol ester and the TG component of the lipoprotein fraction can all be oxidized by free radicals thereby losing their function and chemical structures. The non changes observed in ELEDS+ELEMO-treated group in the present study, depicts ameliorative effects of the ELEMO. Several plants, bioactive components such as alkaloids, tannins, cardiac glycosides, had been associated with hypolipidaemic activity [39,40]. However, phytoconstituents such as β-sistostero might be the principal reducing components of ELEMO which worked either singly or in synergy with other bioactive components [41]. It is, therefore, being suggested that the β-sistostero phytoconstituent may be responsible for the improved lipid profile concentration observed in ELEMO-treated rats in this study as similarly reported in rats administered different concentrations of *M. perigrina* dry seed by El-Hak., *et al.* [42]. It is therefore, being suggested that the protective effect of phytoconstituents accounts, partly, for the nontoxic properties of ELEMO and possibly responsible for the hepatoprotective effect of the ELEMO.

Increase in liver MDA levels recorded in this study in ELEDS-treated rats is an indicator of high level of LPx due to oxidative membrane damage as a result of the adverse effects of ELEDS on the hepatocellular membranous components. These effects may have occurred through ROS production and/or impaired antioxidant system leading to damage to the hepatocellular membranous. Oxidative stress which occurred when there was imbalance between the antioxidants and ROS, leading to alterations of cellular macromolecules, disruption of cellular functions and eventual tissue damage [43] gives more support to the findings in the present study. However, the non alteration found in ELEDS+ELEMO –treated group may be due to potent inhibitors of LPx in ELEMO which improve the ADS by elevating the total antioxidant capacity and decreasing the levels of MDA towards normal value.

The decrease in liver SOD, CAT and GSH in the ELEDS-treated groups is suggestive that the first line of ADS against the free radicals and ROS has been depleted due to their excessive utilization for neutralizing $O_2^{\bullet-}$ and ROS generated by the ELEDS toxicity that led to increased levels LPx as observed in this study and supported by earlier reports [44,45]. These impaired antioxidant systems could cause a shift in the delicate balance between antioxidants and free radicals in favour of the latter, resulting in oxidative stress in body tissues, cellular damage leading to low level of ADS as observed in this study and others [46,47]. The increase in the activity of ADS at low doses may reflect a compensatory response to elevated oxidative stress. However, Oruc and Usta [48]; reported that that activity of ADS could be elevated or inhibited by xenobiotics depending on the intensity and the duration of the stressor, as well as the susceptibility of the exposed animal species.

Microscopic lesions have been widely used as biomarkers in various toxicological studies [49,50]. In this study, the lesions observed in the liver was mainly necrotic changes. This substantiated the observations made in the lipid profile and oxidative stress biomarkers. The hepatic damage observed in ELEDS-treated group is supported by study conducted by Alebiowu., *et al.* [51] who observed mild architectural disarray, mild central venous congestion, destruction of the limiting plates of hepatocytes and hepatic degeneration following oral administration of suspension of powdered leaf of *D. stramonium* in experimental mice. The liver has been reported to be particularly extremely susceptible to ROS or free radicals and oxidative damage [52].

The non elevted levels of the lipid profile and oxidative stress biomarkers in ELEMO-treated group could be attributed to its vitamins (carotenoids) and phytochemical content including flavonoids, phenols, alkaloids, isothiocyanates, glucosinolates, tannins and saponins, kaempferol, quercetin, lectins and phytates which contained potent antioxidant activities and other cellular enhancing biological properties [42,53]. Further suggestive of hepatoprotective effects of *M. oleifera* against ELEDS toxicity as observed also in ELEDS+ELEMO-treated group in this study and as earlier reported [54].

Conclusion

The study revealed that chronic exposure to repeated doses of ELEDS (170.2 mg/kg) induced alterations in lipid profile, LPx, decreased oxidative stress biomarkers gross and microscopic chang-

es in the liver of male Wistar rats. The amelioration of these changes by ELEMO on chronic exposure to ELEDS, highlights its potential antidotal effects to *D. stramonium* toxicity. Veterinarians should have enough information about the physiopathology, clinical and paraclinical features of *D. stramonium* toxicity to make their best diagnosis and management. In patients with anticholinergic signs, *Datura* poisoning should always be considered in the differentials.

Conflict of Interests

None.

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