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Histomorphological and Biochemical Assessment of Toxic Effect of Methanol Extract of *Datura Stramonium* Seed on Testicular Integrity of Adult Male Wistar Rat

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Abstract:- This study aimed at exploring the effects of oral administration of a methanolic extract of *Datura stramonium* seed (MEDSS) on the testes of an adult male Wistar rats.

Seventy-nine (79) male Wistar rats, weighing averagely 210±10 g, were randomly selected into seven groups of seven rats each, while the remaining thirty rats were used for the acute toxicity study. Group A served as control, while B, C, D, E, F, and G served as treatment groups. Groups B and E, C and F, D, and G received 50, 100, and 200 mg/kg body weight of MEDSS, respectively, for 56 days, while treatment was withdrawn from groups E, F, and G for 28 days after the initial treatment of 56 days. We weighed the rats weekly during the experimental period and sacrificed them at the end of each experimental period.

Results of oxidative stress marker revealed that Malondialdehyde concentration was significantly ($P< 0.05$) elevated in a dose dependent manner in groups B, C and D compared to the control (A) and was restored significantly ($P< 0.05$) in the recovery groups (E, F and G) compared to groups B, C and D, respectively. Furthermore, SOD and GPx activities were significantly ($P< 0.05$) reduced in a dose dependent manner in Groups B, C and D compared to the control and were restored significantly ($P< 0.05$) in the recovery groups (E, F and G) when compared to Groups B, C and D respectively. Histological studies revealed dose dependent distorted histo-architecture of the testes in groups B, C, and D compared to the normal architecture in control group (A) and was restored to near normal in recovery groups (E, F and G) compared to groups B, C and D respectively.

Results obtained showed that the LD_{50} of MEDSS was 500 mg/kg body weight which was what guided the doses administered to the rats. There was significant increase in the body weight ($P< 0.05$) in all the treatment groups B, C, D, E, F and G compared to control. Relative testicular weight in groups C and D showed significant reduction ($P< 0.05$) compared to control.

MEDSS thus exhibited a reversible anti-fertility effect on male oxidative stress parameters. The observed distortion of the histo-architecture of testes was not reversible at doses lesser than 100 mg/kg body weight as reversibility increased with increasing dosages as seen in this study.

Keywords:- *Histomorphology, Testes, Datura Stramonium, Wistar Rats.*

I. INTRODUCTION

Jimson weed, or *Datura stramonium* (DS), is a member of the *Solanaceae* family and is freely accessible and extensively dispersed. Large, coarse shrub that grows up to 4 feet tall and spreads up to 6 feet in rich soil is its defining feature [1]. The plant's root is large and pale, having a multifiber taproot structure. Typically, the hairless, cylindrical, erect, and leafy stem is green or purple in colour. It has a smooth appearance and frequently forks in two directions [2].

Datura stramonium intoxication usually presents as severe mydriasis with excruciating photophobia that can continue over many days, delirium, hyperthermia, tachycardia, abnormal conduct, and marked amnesia. Atropine, L-hyoscyamine, and L-scopolamine are known to induce anticholinergic syndrome by blocking both peripheral and central muscarinic neurotransmission, which is why these effects are attributed to their presence [3].

According to reports, teens who have a history of abusing many substances, especially those who are in their teens, frequently take *Datura stramonium* on purpose to experience its euphoric and hallucinatory effects. Though

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Datura stramonium intoxication in children has also been documented, this population comprises the bulk of cases documented in the literature [4].

Despite being a potent narcotic, this plant has a unique effect on people that makes it an extremely effective medicinal herb. All sections of the plant are poisonous, albeit the mature seeds have the largest concentration of alkaloids. Numerous reports of inadvertent ingestion of *Datura stramonium* plants have resulted in acute poisoning, characterised by symptoms such as dry mouth with intense thirst, dry skin, pupil dilation, blurred vision, urine retention, fast heartbeat, confusion, restlessness, hallucinations, and unconsciousness [5].

Examining the plant's possible effects on male fertility is crucial because of its recognised toxicity in several regions of the body. The purpose of this study is to investigate how *Datura stramonium* methanol seed extract and withdrawal affect the integrity of the testicles in Wistar rats.

II. MATERIALS AND METHOD

A. Collection and Extraction of Plant Material

Iluju, a community in Oyo State, Nigeria's Orire Local Government Area, is where the plant's seeds were obtained and gathered. The Department of Plant Biology and Biotechnology (PBB), Faculty of Life Sciences, University of Benin, had previously recognised plants. It was subsequently verified by a taxonomist at the Forestry Research Institute of Nigeria (FRIN) in Ibadan, where a voucher specimen (herbarium voucher number FHI110111) was given.

B. Preparation of Methanol Extract

The kernels were separated from the seeds and allowed to air dry for a period of two weeks before being ground up and mixed with methanol to prepare the seeds for extraction. 1600 g of the powdered seed kernels that had been finely milled were weighed and added to the extraction tank during the procedure. After that, 4000 ml of the solvent were added to the extraction tank's contents, and it was left to stand for 48 hours while being periodically stirred. Next, Whatman No. 1 filter paper was used to filter the suspension. To obtain the methanolic extract of *Datura stramonium* seed (MEDSS), the filtrate was concentrated in a water bath at a regulated temperature of 50 degree Celsius. The sterile bottles were marked and stored in a refrigerator (4 degree Celsius) for later use.

C. Animals and Care

For the study, forty-nine (49) adult male Wistar rats weighing 210 ± 10 g on average were employed. These rats were kept in standard hygienic circumstances in the animal house of the School of Basic Medical Sciences, University of Benin, Benin City, Edo State. They were fed a pellet diet and kept at a controlled temperature of 25±2 oC with a 12-hour light/dark cycle. The Canadian Council of Animal Care (CCAC, 2015) has established international, national, and institutional guidelines for the care of laboratory animals in biomedical research and the use of laboratory animals in biomedical research, which were followed during the experimental procedures involving the animals and their care.

D. Experimental Design and Drug administration

The animals were split up into 7 groups at the beginning of the experiment, each with 7 rats: **Group A** was the control group, and Groups B, C, D, E, F, and G were the treatment groups. 50 mg/kg body weight of the extract was given to **groups B and E**, 100 mg/kg body weight to groups C and F, and 200 mg/kg body weight to **groups D and G**. Gavage was the method used to give the 56-day treatment. The extract's LD_{50} served as the basis for the dosages. Rats in **Groups B, C, and D** had 56 days of treatment before being sacrificed, whereas **Groups E, F, and G** did not receive treatment for 28 days following the initial 56-day treatments prior to sacrifice. Throughout the trial, the rats were weighed twice a week. Every animal's body weight was noted on the final day of the experiment as well.

The experiment began with the sacrifice of Groups B, C, and D after 56 days of treatment; Groups E, F, and G were sacrificed after 28 days of treatment withdrawal, which was 84 days after the trial began with the animals in the control group.

E. Sacrificing of Rats/Collection of Testes

Experimental animals were sacrificed using cervical dislocation after the last day of administration. A midline incision was made on the anterior abdominal wall to expose the contents of the abdominal cavity. In order to carry out the histological analysis, the reproductive tract was dissected to collect the whole testis, observing the arrangement of the organs. The testes on both sides were weighed. The right testes were fixed in Bouin's fluid for further histological studies. The left testes were macerated using acid sand for antioxidant estimation.

F. Determination of LD_{50} (Acute Toxicity Study Of The Extract)

Turner (1965) described the application of Karber's approach for the acute toxicity investigation [6]. In short, thirty Wistar strain albino rats, both male and female, weighing between 180 and 230 g, were randomly assigned to five groups, each consisting of six rats, and were kept in clear plastic cages measuring 60 by 30 by 20 centimetres for two weeks before the experiment. The rats had unlimited access to food and drink. Every animal was housed in a typical setting. The test extract was given to the animals 12 hours before they were denied food but not water. Using a stomach gavage needle, the four groups of rats received single oral dosages of the extract: 200 mg/kg, 400 mg/kg, 600 mg/kg, and 800 mg/kg.

Serving as the control group, the fifth group of animals were given distilled water. After an hour of administration, food and drink were given. For a full day, the animals were monitored every hour, and behavioural and clinical indicators of poisoning were noted at each visit. At every dosage level, the hours of death and the percentage fatality were noted and recorded. After converting this data into a dosage response

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curve, the arithmetical approach was used to get the LD50, which is as follows:

$$LD_{50}=LD_{100}-\Sigma(\frac{axb}{n})$$

Where; LD_{50}=median lethal dose, LD_{100}=least dose required to kill 100%, a= Dose Difference, b= Mean Mortality, n= Group Population

G. Phytochemical Analysis

Qualitative screening of the phytochemical components of the seeds was carried out using the modified method described by Sofowora in 2008. Essentially, specific weight of the extracts was made up to 10 ml in a test tube and different reagents were added to specifications. Positive results were indicated by colour change and precipitate formation which were compared against standards. The extracts were tested for the presence of carbohydrates, alkaloids, tannins, saponins, anthraquinones, phenols, steroids and flavonoids [7].

H. Quantitative Phytochemical Composition

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites in the seeds. The following procedures were adopted: Quantitative analysis of alkaloids by the gravimetric method of Harborne [8].

➢ *Determination of Total Phenolic compounds*

Phenol analysis using the follin-ciocaltean colorimetric method of Harborne [8] involved precisely weighing 100 mg of the sample extract and dissolving it in 100 ml of triple distilled water (TDW). 1 ml of this solution was then transferred to a test tube, and eventually the volume was made up to 8 ml with TDW, vigorous shaking, and allowing it to stand for two hours before taking the absorbance at 765 nm.

➢ *Determination of Total Flavonoids*

The process is predicated on the flavonoid-aluminum complex's production, which peaks in absorptivity at 415 nm. Following a mixture of 100µl of 20% aluminium trichloride in methanol, a drop of acetic acid, and 100µl of the plant extracts in methanol (10 mg/ml), the mixture was diluted with methanol to yield 5ml. After 40 minutes, the absorbance at 415 nm was measured. 100 millilitres of plant extracts, one drop of acetic acid, and five millilitres of methanol were used to create the blank samples. Under the same circumstances, the absorption of a standard rutin solution (0.5 mg/ml) in methanol was determined. Every determination was made three times [8].

➢ *Determination of Total Alkaloids*

200 ml of 10% acetic acid in ethanol was poured to a 250 ml beaker containing 5 g of the sample, which was then covered and left to stand for 4 hours. After filtering, the extract was concentrated to a quarter of its original volume in a water bath. The extract was gradually mixed with concentrated ammonium hydroxide until the precipitation was fully formed. After letting the entire mixture settle, the precipitate was gathered, cleaned with diluted ammonium hydroxide, and filtered. The alkaloid, which was dried and weighed, is the residual [8].

➢ *Determination of total Tannins*

Tannin determination using Robinson and Van Buren's approach; a 50 ml plastic bottle was filled with 500 mg of the sample. In a mechanical shaker, 50 ml of distilled water was added and agitated for one hour. This was adjusted to the appropriate level and filtered into a 50 ml volumetric flask. Next, 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide were combined with 5 ml of the filtrate that had been pipetted out into a test tube. In ten minutes, the absorbance at 120 nm was measured [9].

➢ *Determination of Total Saponins*

A conical flask containing around 20 g of the samples was filled with 100 cm^3 of 20% aqueous ethanol. The samples were cooked to approximately 55 °C for four hours while being constantly stirred over a hot water bath. After filtering the mixture, 200 millilitres more of 20% ethanol were used to remove the residue once more. Over a water bath heated to roughly 90 °C, the mixed extracts were reduced to 40 ml. After transferring the concentrate into a 250 ml separatory funnel, 20 ml of diethyl ether was added and thoroughly agitated. The ether layer was disposed of, and the aqueous layer was recovered. It was necessary to repeat the purifying procedure. 60 millilitres of n-butanol were added. Using 10 millilitres of 5% aqueous sodium chloride, the mixed n-butanol extracts were cleaned twice [10].

I. Biochemical Assay

Animals after they were sacrificed, the left testes were washed thoroughly and rinsed with ice. 10% of the homogenate was prepared in 0.05M phosphate buffer (pH 7.4) using a mortar and pestle at 4°C. The homogenate was centrifuged at 3000rpm for 15 min to remove cell debris. The supernatant was used for the estimation of SOD, GPx and MDA. One of the byproducts of lipid peroxidation, malondialdehyde (MDA), was quantified using the Buege and Aust method [11]. The Nyman technique was used to determine glutathione peroxidase (GPx) [12]. The Misra and Fridivich method [13] was utilised to measure the amount of superoxide dismutase (SOD).

J. Histological Slide Preparation

After being removed from the fixative, the right testis was dehydrated for one hour in 70%, 90%, and graded ethanol. It was then moved to three different absolute alcohol changes for an additional hour each. After that, the tissues were cleaned in xylene. After being cleaned, the tissues were submerged in melted paraffin wax and heated to 58 degrees Celsius. The tissues were imbedded in wax and blocked out after two changes of molten paraffin wax were created at one-hour intervals. The testis was orientated at embedded such that they were sectioned perpendicular to the long axes of the testes. Serial sections of 5 µm thick were obtained from the solid block of tissue; the sections were then floated out on a warm water bath, picked by a clean slide on which Mayer's egg albumin had been spread to cement the sections to the slides. After that they were passed through xylene and alcohol. Subsequently, they were stained with hematoxylin

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and eosin, cleared in xylene and dried between 35°C and 40°C and then covered with a cover slip. For light microscopic studies, images were acquired using an Olympus binocular research microscope (Olympus, New Jersey, USA) connected to an AM-scope camera (5.1 MP) [14].

K. Statistical Analysis

The analysis of all quantitative data was conducted using Version 6 of GraphPad Prism® software. A Turkey post-hoc test was employed after a one-way analysis of variance (ANOVA) with a significance threshold set at ($P<0.05$) (95% confidence interval) to compare the relative expression levels for the various groups. The results were displayed as the mean (M SEM) and standard error of mean (SEM) in bar charts with error bars.

III. RESULTS

A. Phytochemical Screening

To gain insight into the possible applications and nutritional value of Datura stramonium seed, the phytochemical components and concentrations of its methanolic extract (MEDSS) were assessed. The result revealed high presence of phenols and Alkaloids. Present in moderate concentration were carbohydrate, flavonoids and tannins. There was low presence of reducing sugar but anthraquinones and saponins were almost absent.

Table 1: Phytochemical Constituents of the Extract

Plant Constituents	Methanol Extract
Carbohydrates	+ +
Reducing sugar	+
Saponins	—
Alkaloids	+ + +
Flavonoids	+ +
Tannins	+ +
Anthraquinones	—
Phenols	+ + +

Key: (--) Absent; (+) Low; (+ +) Moderate; (+ + +) High

Table 2: Phytochemical Concentrations of the Extract

Phytochemicals	Concentration (%)
Carbohydrate	2.01 ± 0.04
Alkaloids	3.58 ± 0.02
Anthrquinones	0.01 ± 0.00
Tannins	1.74 ± 0.05
Saponins	0.00 ± 0.00
Phenol	5.62 ± 0.02
Flavonoids	3.33 ± 0.05

Data are represented as mean ± standard error of Mean (M ± SEM).

B. Acute Toxicity Test of the Extract

The result of acute toxicity testing of the effect of extract on Wistar rats is shown with dose-response curve. The result revealed the LD_{50} of the extract on oral route was 500 mg/kg.

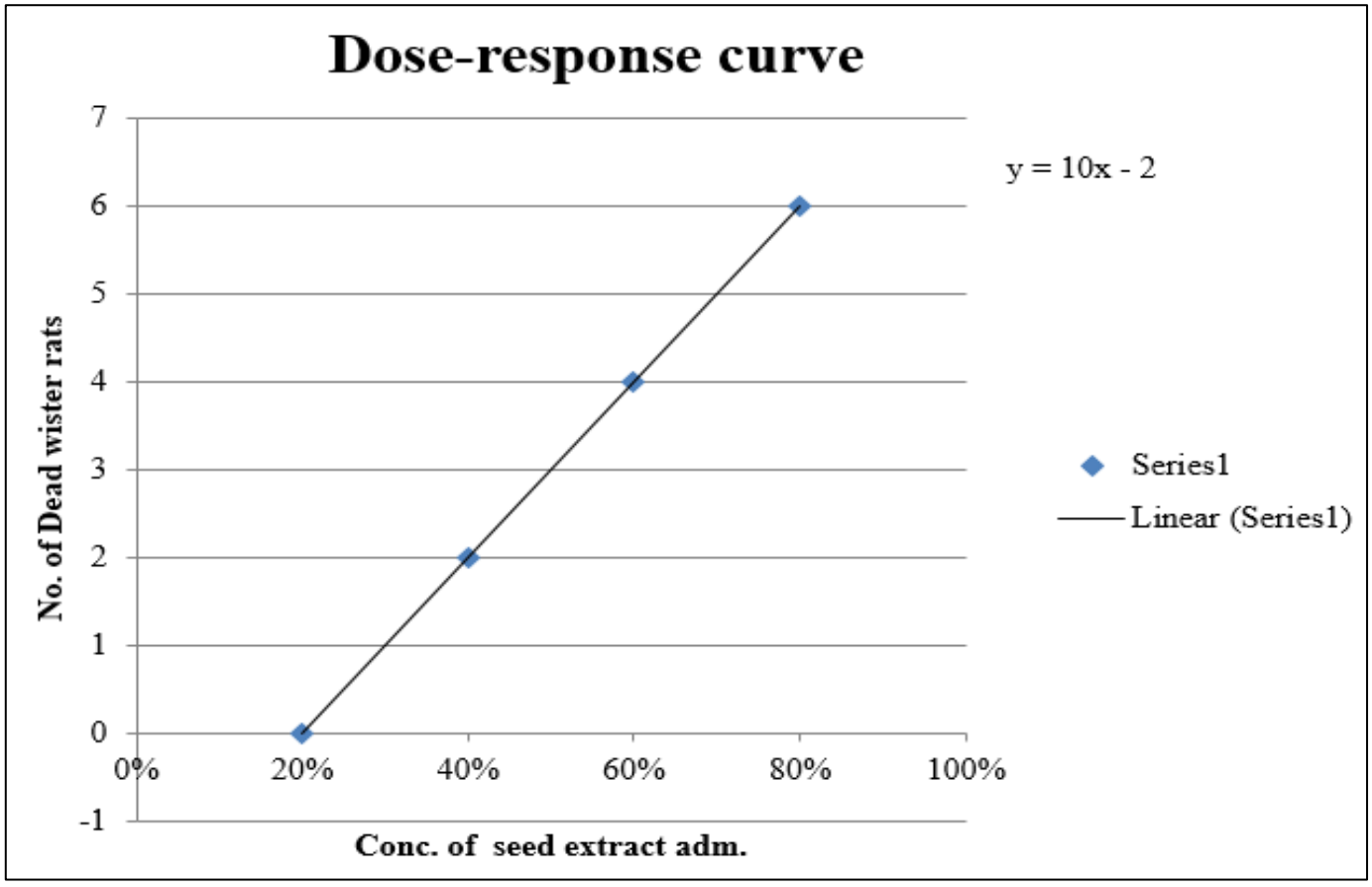

Fig 1: Showing LD_{50} Dose Response Curve of Seed Extract Administered to Experimental Animals

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C. Effect of the Extract on the Body Weight and Organ Weight of Experimental Rat

The findings showed that when methanolic extract of Datura stramonium seed (MEDSS) was administered, body weight decreased significantly (P<0.05) in the treatment groups (B, C, and D) when compared to the control group (A). But groups E, F, and G's withdrawal from the DSE treatment somewhat offset this weight loss, leading to a statistically significant (P<0.05) weight rebound.

Similarly, it was shown that when compared to the control group (A), the administration of different doses of methanolic extract of Datura stramonium seed (MEDSS) resulted in a substantial (P<0.05) decrease in the right and left testicular weights in the treatment groups (B, C, and D). Nevertheless, testicular weights in the E, F, and G groups (which underwent a 28-day withdrawal from MEDSS treatment) did not differ from those in the control group statistically (P<0.05), suggesting that testicular weights could recover after withdrawal.

Table 3: Effect of the MEDSS on the Body Weight of Experimental Rats

Group	Initial Mean Body Weight	Final Mean Body Weight
Control (A)	232.00±6.78	294.14±4.44 ***
50 mg/kg of MEDSS (B)	212.64±5.67	291.79±7.10 *
100 mg/kg of MEDSS (C)	206.43±6.06	256.00±5.91 *
200 mg/kg of MEDSS (D)	203.93±5.94	235.57±6.93 *
50 mg/kg of MEDSS + Withdrawal (E)	202.64±6.77	250.73±6.99 *
100 mg/kg of MEDSS + Withdrawal (F)	204.50±5.66	274.71±6.73 *
200 mg/kg of MEDSS + Withdrawal (G)	213.21±5.39	274.29±9.25 *

*represent significant difference (p<0.005) when compared to the initial weights.
Where MEDSS: Methanolic Extract of *Datura stramonium* Seeds

Table 4: Effect of the MEDSS on the Testicular Weight of Experimental Rats

Group	Right Testes Weight	Left Testes Weight
Control (A)	0.70±0.03	0.85±0.04
50 mg/kg of MEDSS (B)	0.64±0.02	0.76±0.14
100 mg/kg of MEDSS (C)	0.42±0.06 [a]	0.47±0.11 [a]
200 mg/kg of MEDSS (D)	0.30±0.06 [a]	0.36±0.09 [a]
50 mg/kg of MEDSS + Withdrawal (E)	0.87±0.02 [a]	0.78±0.05
100 mg/kg of MEDSS + Withdrawal (F)	0.75±0.02	0.71±0.05
200 mg/kg of MEDSS + Withdrawal (G)	0.66±0.02	0.44±0.06 [a]

a: Represent Significant Difference (p<0.05) when compared to control rats.
Where MEDSS: Methanolic Extract of *Datura stramonium* Seeds

D. Antioxidant Effect of the Extract on Experimental Rats

MDA level was significantly higher in the low dose, medium dose, medium reversal, high dose and high dose reversal groups when compared with the control group but there was no significant difference in MDA level between low dose reversal groups and the control group. The MDA level was significantly lower in the low dose reversal group compared to the low dose group, significantly lower in the medium dose reversal group compared to the medium dose group, and significantly lower in the high dose reversal group compared to the high dose group, according to a comparison of the treated groups and their corresponding reversal groups.

When compared to the control group, GPx activity was significantly lower in the medium dosage, medium reversal, high dose, and high dose reversal groups; however, there was no significant difference in GPx activity between the low dose and low dose reversal groups. When GPx activity was examined between the treatment groups and the equivalent reversal groups, it was found that the medium dosage reversal group had significantly higher GPx activity than the medium dose group, and the high dose reversal group had significantly higher GPx activity than the high dose group. The GPx activity did not, however, change statistically significantly between the low dosage reversal group and the low dose group.

When compared to the control group, SOD activity was significantly lower in the medium dose, medium reversal, high dose, and high dose reversal groups; however, there was no statistically significant difference in SOD activity between the low dose and low dose reversal groups. SOD activity was considerably higher in the medium dosage reversal group compared to the medium dose group and significantly higher in the high dose reversal group compared to the high dose group when the treatment groups and their matching reversal groups were compared. Nonetheless, no statistically significant distinction in SOD activity was seen between the low dosage group and the low dose reversal group.

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Table 5: Antioxidant Effect of the MEDSS on Experimental Rats

Group	Malondialdehyde (MDA)	Superoxide dismutase (SOD)	Glutathione peroxidase (GPx)
Control (A)	7.51±0.09	97.06±0.38	680.25±3.79
50 mg/kg of MEDSS (B)	10.05±0.24	96.38±0.85	658.22±9.22
100 mg/kg of MEDSS (C)	15.15±0.23	78.24±0.38	436.7±4.23
200 mg/kg of MEDSS (D)	22.39±0.46	53.37±2.07	324.06±7.63
50 mg/kg of MEDSS + Withdrawal (E)	6.91±0.26	98.57±0.33	647.36±13.40
100 mg/kg of MEDSS + Withdrawal (F)	10.83±0.26	92.57±0.36	600.30±1.63
200 mg/kg of MEDSS + Withdrawal (G)	13.10±0.23	80.91±0.30	548.20±2.22

E. Histological Effect of Extract Treatment on the Testes of Experimental Rats

According to the histological results, exposure to DSE altered the testicular histology, which was mainly characterised by an increase in hypocellular regions and a decrease in spermatozoa inside the lumen. Withdrawing from DSE treatment, however, may be able to partially reverse these effects, indicating some restoration of the testes' histological integrity.

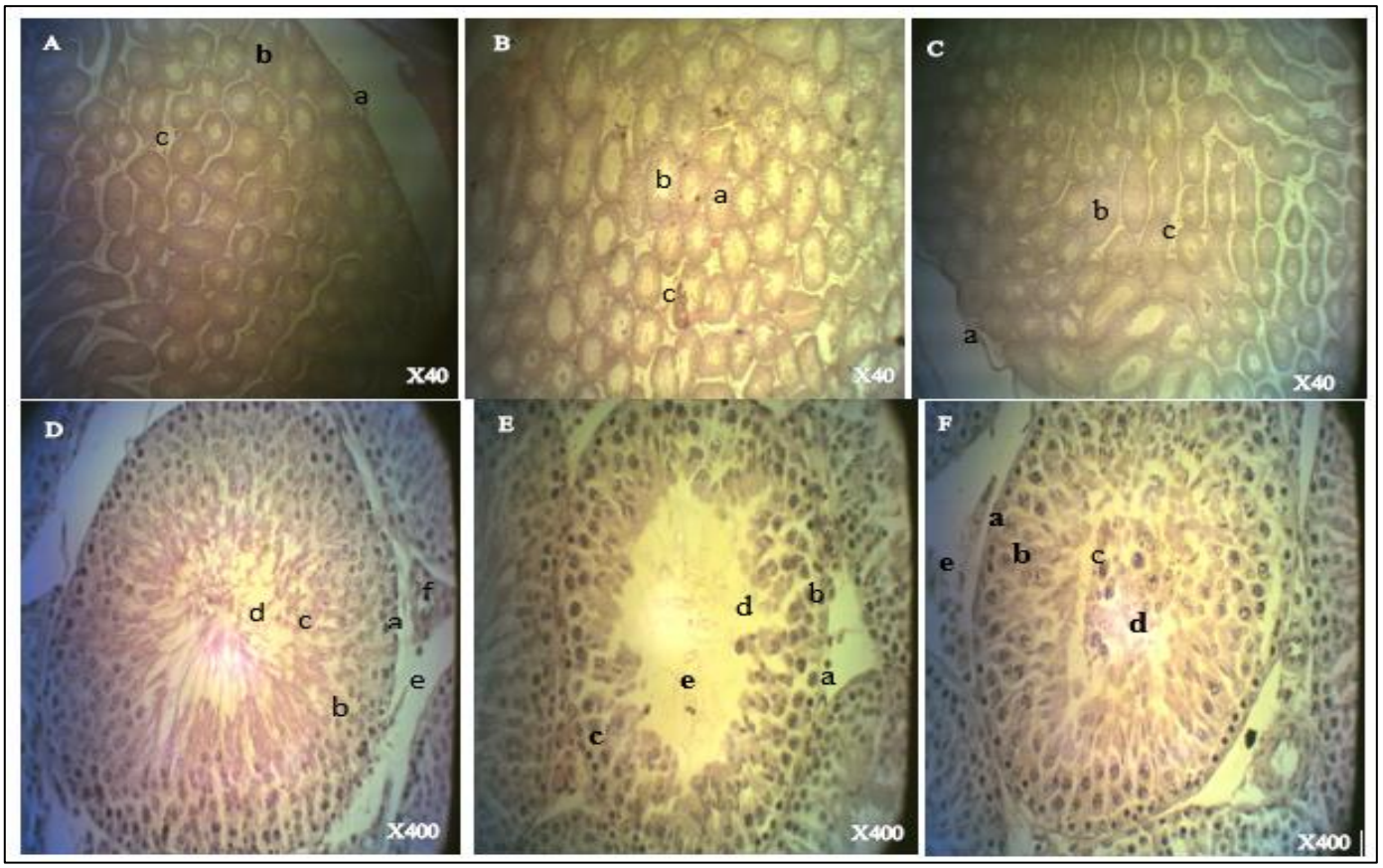

Plate 1: H&E Stain Photomicrographs of the Effects of Datura Stramonium seed 50 mg/kg Methanolic Extracts and a Month Withdrawal on the Histology of the Testes in Experimental Animals

➢ *Control (Group A)*

- Plate 1A**:** The testes slide shows (a) Tunica albuginea (b) Seminiferous tubule (c) Interstitial space with Leydig.
- Plate 1D**:** showing (a) basement membrane (b) early germ cells (c) late germ cells series (d) tails of matured spermatozoa in the lumen (e) testicular interstitium with (f) Leydig cells. The outline shows seminiferous tubules with normal spermatogenesis progressing from spermatogonium and their progressive differentiation into spermatids and spermatozoa.

➢ *Group B*

- Plate 1B**:** The testes slide shows (a) Seminiferous tubules (b) Lumen of seminiferous tubule with scanty spermatozoa and (c) Interstitial space with Leydig cells.
- Plate 1E**:** showing seminiferous tubule with (a) Basement membrane (b) early germ cells series (c) Late germ cells (d) Disruption of seminiferous epithelium resulting in spermatogenic arrest and (e) Lumen of seminiferous tubule with scanty spermatozoa in the lumen.

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➢ *Group E*

- Plate 1C**:** The testis slide shows (a) Tunica albuginea (b) Seminiferous tubule and (c) Testicular interstitial space with Leydig cells.
- Plate 1F**:** showing seminiferous tubule with (a) basement membrane, (b) early germ cells series, (c) Late germ cells (d) aggregates of indeterminate cells in the lumen and (e) interstitial space with Leydig cells.

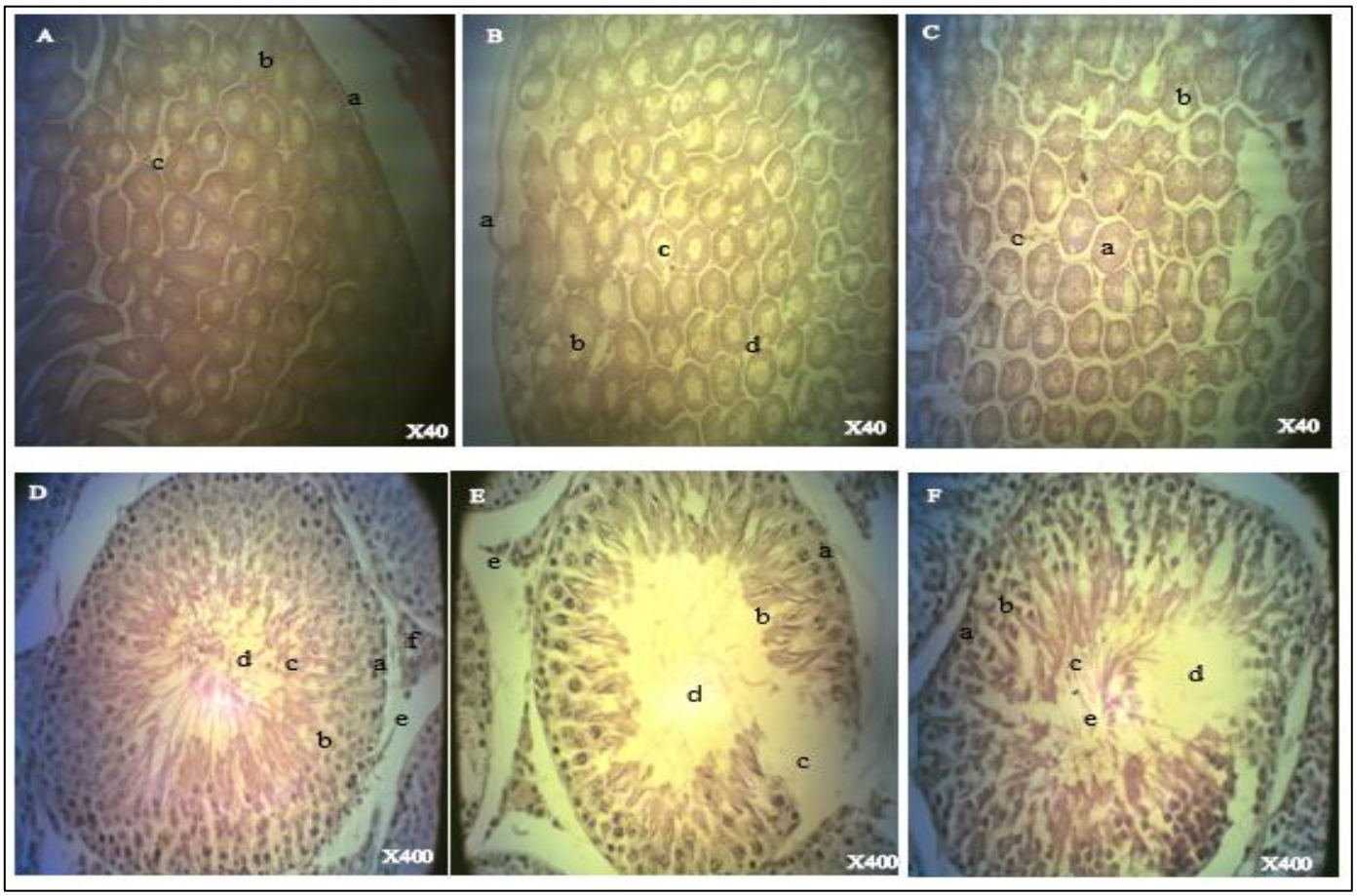

Plate 2: H&E Stain Photomicrographs of the Effects of Datura Stramonium Seed 100 mg/kg Methanolic Extracts and a Month Withdrawal on the Histology of the Testes in Experimental Animals

➢ *Control (Group A)*

- Plate 2A: The testis slide showing (a) Tunica albuginea (b) Seminiferous tubule (c) Interstitial space with Leydig cells.
- Plate 2D: Slide showing the (a) Basement membrane (b) early germ cells (c) late germ cells series (d) tails of matured spermatozoa in the lumen (e) testicular interstitium with (f) Leydig cells. The outline shows seminiferous tubules with normal spermatogenesis progressing from spermatogonium and their progressive differentiation into spermatids and spermatozoa.

➢ *Group C*

- Plate 2B: The testes slide showing (a) Tunica albuginea (b) Seminiferous tubule with germ cells in maturation division. (c) Adluminal compartment of seminiferous tubules depleted of germ cells and spermatozoa (d) interstitial space with Leydig cells.
- Plate 2E: Showing the (a) Basement membrane (b) Adluminal compartment of seminiferous tubules with gross evidence of spermatogenic arrest evidenced by the empty lumen seen in most of the seminiferous tubule (c) Evidence of disruption of seminiferous epithelium (d) Scanty or almost empty lumen devoid of spermatozoa due to arrest of spermatogenesis (e) Testicular interstitium with Leydig cells.

➢ *Group F*

- Plate 2C: The testes slide showing (a) Seminiferous tubule with germ cells (b) Regions of cellular depletion due to spermatogenic arrest (c) Testicular interstitium with Leydig cells.
- Plate 2F: Showing (a) Basement membrane (b) early germ cells series (c) Late germ cells (d) region of disruption of seminiferous tubule with moderate spermatogenesis (e) Lumen with moderate spermatozoa. Comparatively, there is evidence of epithelial regranulation to restore spermatogenesis.

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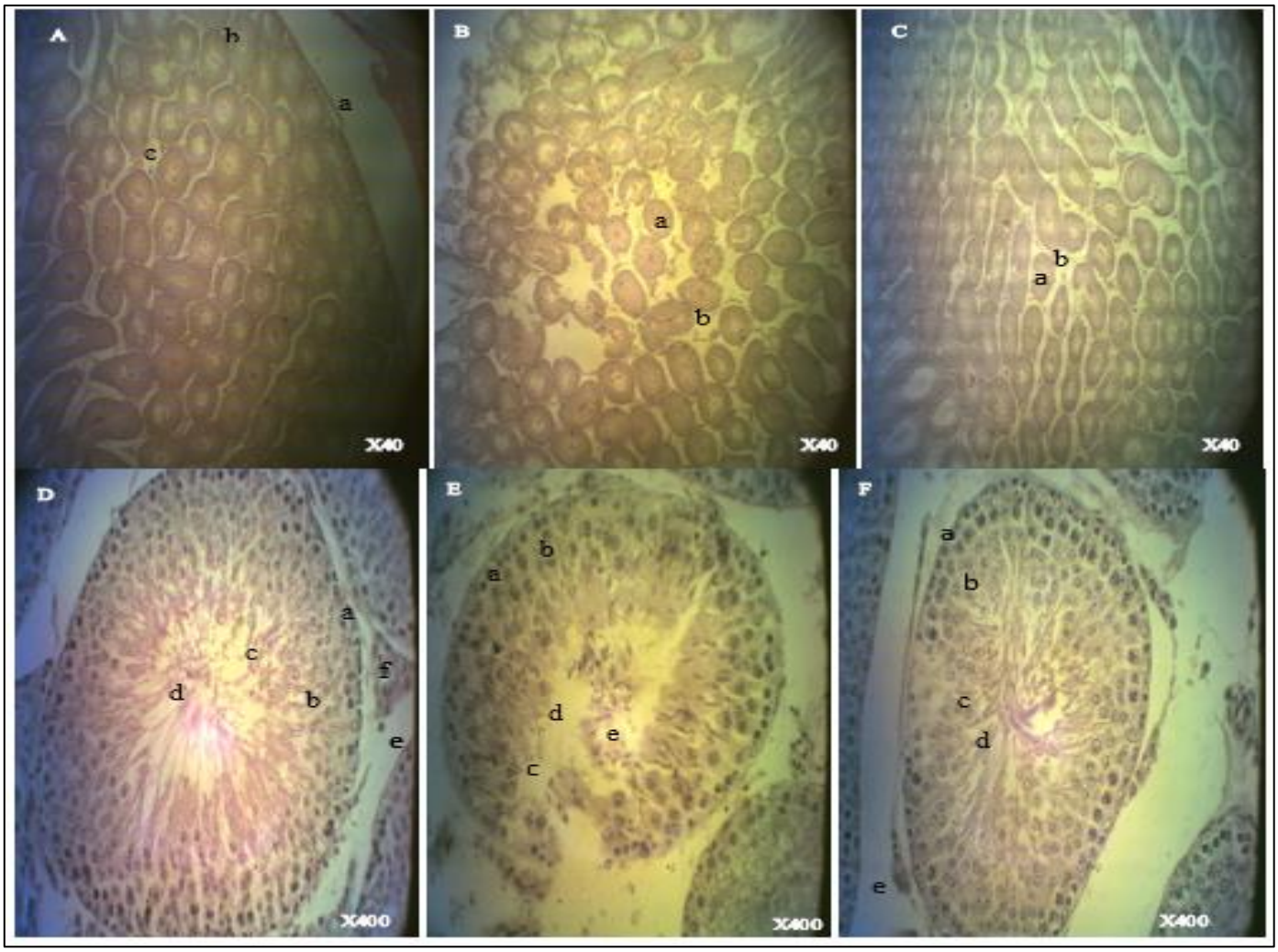

Plate 3: H&E Stain Photomicrographs of the Effects of *Datura Stramonium* seed 200 mg/kg Methanolic Extracts and a Month Withdrawal on the Histology of the Testes in Experimental Animals

➢ *Control (Group A)*

- Plate 3A: Slide of Control (group A) showing normal testis with (a) Tunica albuginea (b) Seminiferous tubule (c) Interstitial space with Leydig cells.
- Plate 3D: Slide of Control (A) testis showing (a) basement membrane (b) early germ cells (c) late germ cells series (d) tails of matured spermatozoa in the lumen (e) testicular interstitium with (f) Leydig cells. The outline shows seminiferous tubules with normal spermatogenesis progressing from spermatogonium and their progressive differentiation into spermatids and spermatozoa.

➢ *Group D*

- Plate 3B: Slide of testes showing evidence of degenerative changes in (a) Seminiferous tubule and (b) Interstitial space containing few Leydig cells.
- P Plate 3E: Slide showing (a) basement membrane (b) early germ cells (c) late germ cells series (d) disrupted seminiferous epithelium resulting in spermatogenic arrest and (e) lumen of seminiferous tubule with indeterminate cells.

➢ *Group G*

- Plate 3C: Slide of Testis showing (a) Seminiferous tubule (b) Testicular interstitial space with Leydig cells.
- Plate 3F: Slide showing (a) Basement membrane (b) early germ cells (c) Late germ cells (d) Lumen of seminiferous tubule rich with spermatozoa (e) Testicular interstitium with Leydig cells. Compared to high dose treated group, there are no remarkable disruption of seminiferous tubule and arrest of spermatogenesis. The slide shows evidence of fairly restored spermatogenesis.

IV. DISCUSSION

Toxicological or medicinal potential of a test compound can be ascertained by subsequent behavioral observation, physical or visual examination of the experimental animals, food and water consumption by the animal, organ's functionality by carrying out biochemical tests as well as body and organ weights of experimental animals [15].

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The median dose of methanolic extract of *Datura Stramonium* seed from this study is 500mg via oral route and this served as a guide to the various doses administered to the experimental rats. The range of toxicity of *Datura Stramonium*is highly variable and unpredictable when ingested, smoked and absorbed topically, as smaller amounts may cause profound central nervous system disturbance [16].

This study revealed that the experimental rats showed significant ($P< 0.001$) gain in their final body weights compared with their respective initial body weights across the groups. The result obtained is consistent with the earlier report of Abdelouahab *et al.,* in 2011 [17], who reported increased final body weights of experimental animals compared with initial body weight in acute toxicity study of methanolic extract of *Datura stramonium* seed in rats but contrary to the report of Dugan *et al.,* in 1989 [18] who reported a decrease in final body weights of animals after treating experimental rats with feed mixed with *Datura stramonium* for three months. Furthermore, Gidado *et al.,* in 2007 [19], also reported an insignificant increase in final body weights of experimental rats in the toxicity studies of ethanol extract of the leaves of *Datura stramonium* in rats. Presence of significant carbohydrate as revealed in the phytochemical screening of methanolic extract of *Datura stramonium* might have enhanced corresponding macromolecules in the diet to boost the final body weight of the rats compared with the initial body weights in various treatment groups.

The result of the effect of methanolic extract of *Datura stramonium seed* on testicular weights of the experimental rats showed that methanolic extract of *Datura stramonium* caused a significant decrease in testicular weights in a dose dependent manner in group B (low dose 50mg/kg), group C (medium dose 100mg/kg) and group D (High dose 200mg/kg) compared to the control (A). However, the reduction in testicular weights were reversed in withdrawal groups E, F and G compared to treatment groups B, C and D respectively. Groups E and F showed appreciable weight gains compare to group G with values lower than the control. It can be inferred that *Datura stramonium* does not adversely affect the general body weights of the animals but rather affects specific organs targeted by its secondary metabolites. The above obtained result is consistent with the report of Benaouadah *et al.,* in 2016 [20] who reported decrease in organs weight after treatment of experimental rats with alkaloids extracted from *Datura stramonium* species.

Moreover, Bouzidi *et al.,* in 2011 [21] also reported significant ($P< 0.05$) decrease in the weight of the liver, spleen, testes, heart and brain after treating male rats with alkaloids extracted from the seed of *Datura stramonium.* Decreased testicular weights in the experimental rats may be ascribed to the effects of phytochemicals or secondary metabolites present in the methanolic extract of the seed of *Datura stramonium* which might have elicited various biochemical pathways that underlies significant reduction in weights of the testes that further affected the structure and functionality of these organs. One of these secondary metabolites is atropine which has been reported to have a very strong anticholinergic effect as reported by Madungurum and Muhammad in 2015 [22].

Superoxide dismutase SOD is one of the antioxidant enzymes which performs the function of converting singlet oxygen ($O^{\cdot}$) to hydrogen peroxide (H_2O_2) and which will later be converted to water and oxygen by the action of catalase enzyme. Glutathione peroxidase (GPx) is also an antioxidant enzyme that causes reduction in hydrogen peroxide to give water and oxygen, hence, suppress their deleterious effects [23]. Results obtained showed that, the extract caused a reduction in SOD and GPx activities in a dose dependent manner. Group C (medium dose) and group D (high dose) were significantly decreased when compared with the control (A). However, decrease in the activities of these enzymes were restored significantly in groups F (Medium dose reversal) and G (High dose reversal) compared to group C (Medium dose) and group D (High dose) respectively. Group E (low dose reversal) showed insignificant level compared to group B (Low dose) and control (A). This result agrees with earlier reports of Ogunmoyole *et al.,* in 2019 [24] and Salah and Agarwal in 2012 [25]. The methanolic extract of *Datura stramonium* might have elicited oxidative stress by generating free radicals which might have disrupted the structure and functionality of organs like the testes, resulting in severe reduction in *in-vivo* antioxidants enzymes SOD and GPx. The underlying principle behind decrease in these antioxidant enzymes may be traced to various *Datura stramonium* active compounds or their metabolites eliciting or interrupting various biochemical pathways that inhibit elevation of antioxidant status of the rats.

Malondialdehyde (MDA) is the peroxidation end product of polyunsaturated fatty acids in cells. Excessive production of MDA is caused by increased free radicals. MDA remains one of the markers of oxidative stress [26]. In the result obtained, the extract caused a significant increase in MDA concentration in group B (low dose), group C (Medium dose) and group D (High dose) compared to the control (A) group. The increment in these groups was dose dependent. In addition, there were significant reductions in group E (low dose reversal), group F (medium dose reversal) and group G (high dose reversal) compared to group B (low dose), group C (medium dose), and D (High dose) respectively. The result obtained agrees with the earlier reports of Kam and Liew, in 2002 [27] who reported cytotoxic, genotoxic and apoptotic effect of *Datura stramonium* on cells. Furthermore, Pan *et al.,* in 2007 [28] also reported that, methanolic extract of *Datura stramonium* and *Datura metel* seed have cytotoxic effects. The lipid peroxidation effects might be traced to the alkaloids, 'scopolamine and atropine' earlier reported to have various toxicological effects via generation of free radicals that mediated oxidative stress on various reproductive tissues. Thus, phytochemical screening of methanolic extract of *Datura stramonium* as revealed in this present study, showed abundant presence of alkaloids, flavonoids and phenols in the seed.

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The histological findings on the effects of *Datura stramonium* treatment on testes of the rats were observed to be dose dependent. Group B (plates 1B and 1E), group C (plates 2B and 2E) and group D (plates 3B and 3E) revealed hypo-spermatozoa in the lumen of the seminiferous tubules with numerous germinal epithelial deformations in dose dependent manner. The altered structures were gradually restored when the extract was withdrawn from the rats in the recovery phases. This is consistent with the early report of Adekomi *et al.,* 2011 [29] who reported similar morphological changes in liver, lungs, kidneys, and testes of male Sprague dawley rats exposed to the smoke extract of *Datura stramonium.* The above result also corroborated earlier report of Benaouadah*et al.,* in 2016 [20], who observed severe congestion and hepatocyte degeneration as well as severe congestion and inflammation in the renal tissues of mice treated with alkaloids extracted from *Datura stramonium.* Reported potentially toxic alkaloids from seeds of *Datura stramonium* (atropine, lyoscyamine and scopolamine) might have exhibited lipid peroxidation effects on the membranes of the cells of testes and epididymis, disrupting their membranes functionality and altered their morphological structures which apparently resulted in the high level of Malondialdehyde, an end product of lipid peroxidation in biological system estimated in the serum.

V. CONCLUSION

In conclusion, methanolic extract of *Datura stramonium* seed possesses secondary metabolites that have significant antifertility potential. The extract causes induction of lipid peroxidation and compromised architectural integrity of testes which are dose dependent of the extract. Hence, *Datura stramonium* extract is a potential antifertility agent but with some degree of reversibility upon withdrawal of treatment. Illicit use of the seed of *Datura stramonium* as a drugespecially among young people due to their ignorance of its adverse effects should be totally discouraged while its development as a male contraceptive should be advocated.

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