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Testicular Histomorphology Following Datura Stramonium Administration in Adult Male Wistar Rats

O.V. Owolabi[2], L.A. Enye[1], B.O. Omiyale[2], O.S. Saka[1], C.O. Akintayo[3]

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ABSTRACT

Background: Datura contains a mixture of anticholinergic agents (alkaloids such as atropine, scopolamine and hyoscyamine) that are responsible for its pharmacological actions. The objective was to evaluate the effect of *Datura stramonium* in the testes of wistar rats.

Methods: Twenty (20) adult male rats weight ranging from 106g-180g were distributed into five groups (A-E) of four (4) animals each. Animals were administered with *Datura* extract once daily for 21 days as Group A- 50mg/kg, Group B -100 mg/kg, Group C -200 mg/kg ,Group D -400 mg/kg. While, Group E were given distilled water daily. Testes and blood samples were collected from all the groups, for the histological and biochemical activities.

Result: Results showed overall, increase in body weight with siginificant differences in sperm count in all the treatment groups. Administration of high dose of *Datura* extract was found to have adverse effect on the histological findings in the testes. Extract of *Datura stramonium* plant is toxic since single extraction shows presence of scopolamine. Abuse of the flower of the plant pose cholinergic poisoning due to scopolamine, significantly reduces male fertility evidenced by increase in the number of abnormal sperm motility and altered testicular morphology.

Key words: Alkaloid, *Datura Stamonium,* Histomorphology, Testes.

INTRODUCTION

In Nigeria today, the abuse of substance is rampant among the youth, with rates ranging from 3.8 to 40.1% in local studies (Okafor, 2020; Abdulkarim *et al*., 2005). Datura contains a mixture of anticholinergic agents (alkaloids such as atropine, scopolamine and hyoscyamine) that are responsible for its pharmacological actions (Adegoke and Alo, 2013). And this is globally consumed by adolescence.

In the Solanaceae plant family, there are nine kinds of vespertine plants in the genus Datura that are toxic (Adegoke and Alo, 2013). Common names for them include daturas and devil's trumpets (Kanchan and Atreya, 2016). Datura species are all poisonous, particularly the seeds and blooms. The flower is usually purple or white; often double (Kanchan and Atreya, 2016).

Effects of *Datura stramonium* intoxication frequently resemble those of an anticholinergic delirium (usually involving a complete inability to differentiate reality from fantasy) (Freye, 2009) bizarre tachycardia, hyperthermia, severe mydriasis and possibly violent behaviour which causes dilated pupils and can cause excruciating photophobia that lasts for several days. Along with momentary muscular paralysis and pronounced amnesia, these effects are frequently reported (Sassano-Higgins *et al*., 2016). Previous study and research on datura has shown that the alkaloids within datura exert their effects by acting as competitive antagonists at muscarinic acetylcholine receptors, primarily muscarinic acetylcholine receptors M1 and M2. The precise mechanism is not known, however this suppression of acetylcholine causes physical side effects such as acute discomfort and dysphoria, delirium, sedation and vividly realistic hallucinations (Caraceni and Luigi, 2011).

All Datura plant seeds and flowers contain substances like tropane alkaloids, primarily atropine, hyoscyamine and scopolamine which are regarded as a poison substances (Gachande and Khijjare, 2013). The toxicity level of a specific plant is influenced by its age, the environment in which it is growing and weather because it includes a powerful mix of anticholinergic compounds. The current study's aim was to investigate the effects of *Datura stramonium* on the testes of wistar rats.

[1]Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria.
[2]Department of Medical Biochemistry, College of Medicine and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria.
[3]Department of Physiology, College of Medicine and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria.

Corresponding Author: O.V. Owolabi, Department of Medical Biochemistry, College of Medicine and Health Sciences, Afe Babalola University Ado Ekiti, Nigeria. Email: akintehinseoo@abuad.edu.ng.

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MATERIALS AND METHODS

Animal care

Twenty (20) adult male rats weight range from 106 g-180 g was procured from the Department of Biochemistry, Afe Babalola University Ado-Ekiti. The rats were bred in a well-ventilated plastic cage with wire guaze cover for proper aeration, they were kept and maintained under normal temperature, humidity and light. When acquired, they weighted between 101 and 140 g. They were allowed to acclimatize for a period of two weeks and fed with growers mash. The rats were also given water *ad-libidum*. Five groups of rat (A, B, C, D and E) consisting of 4 animals each were housed separately in five cages.

Experimental design

The five groups of four rats each were designated as Group A, Group B, Group C, Group D,and Group E served as the control group. We measured the average body weight of the animals daily (Table 1).

Datura extract administration

Orally, 130 mg/kg of the *Datura stramonium* extract was administered for 21 days using, a pipette to measure. Insulin syringe (5ml) and canula was used to pass liquid through the mouth into the esophagus. Until the conclusion of the study, all rats have access to a conventional meal and water (Soni *et al*., 2012).

Weight of the animals

The animals' body weight were taken and recorded from the first day (day 1) of the experiment and was taken before sacrifice after dosage, using Gallenkamp electronic balance (MP 10001).

Sacrifice of animals

The rats were sacrificed when the experiment was concluded. This was done using cotton wools soaked with ethyl placed inside a desicator to sedate the rats. Both testes were harvested and fixed with Bouin's fluid and sucrose solution for histological process and biochemical analysis. The pituitary gland was collected and fixed in 10% formal saline. Blood samples were taken and aspiration was done to get the serum from the blood.

Histochemical and histological studies

From each group, testes were retrieved, fixed in Bouin's fluid, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in molten paraffin wax. Fine, thin sections was obtained at 5µm using rotary microtome MICROM GmbH 69190 Walldorf, germany serial No 42861, cat no 902100), stained Hematoxylin and Eosin and evaluated for any structural changes under the light microscope. Periodic acid schiff was used to stain the tissues (PAS) for the detection of glycogen, basement membrane and neutral polysaccharides as per (Komolafe *et al*., 2013).

Serum analysis of testosterone, luteinizing and follicle stimulating hormonal level

Blood was collected from the heart and allowed to clot for 2 hours at room temperature. After 5-minute (3000 r/min) centrifugation,collected supernatant (serum) was used for hormone measurement. The testosterone, luteinizing and follicle stimulating hormones were analyzed by ELISA method using rat FSH ELISA kits, Catalog numbers: RSHAKRFS-010R and E-EL-R0391, shibayagico Ltd. 1062-1 Ishihara, shibukawa, Gunma, Japan 377-0007 respectively.

Biochemical tests

Measurement of testicular tissue malondialdehyde level (MDA), Superoxide dismutase (SOD), Tissue reduced glutathione concentration (GSH).

Glutathione concentration (GSH)

To estimate the reduced glutathione (GSH) level followed the Ellman method. In this method thiols react with Ellman's reagent [5, 5?-dithiobis-(2- nitrobenzoic acid], joining with disulfide bond to give 2-nitro-5-thiobenzoate (TNB-), which ionizes to the $TNB2^{-}$alkaline pH and dianion in water at neutral and determining the GSH contents in samples, 15 µL of hemolysates was combined with 260 µL assay buffer (0.1 Msodium phosphate and 1 mMEDTA, pH: 8) and 5 µL Ellman reagents (Delavari *et al*., 2017).

Superoxide dismutase (SOD)

The Superoxide dismutase SOD activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2- (4-iodophenyl) -3- (4-nitrophenol) -5-phenyltetrazolium chloride to form a red formazon dye. Briefly, 300 microlitres of heterogenous substrate was added to 200 microlitres of hemolysates. 75 microliters of xanthine oxidase were added to the reactions after the samples had been thoroughly mixed (Delavari *et al*., 2017).

Malondialdehyde (MDA)

The thiobarbituric acid reaction method was used to quantify the amounts of malondialdehyde in the samples. By comparing the absorption to the standard curve of MDA equivalents produced by the acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane, the reactive compounds for thiobarbituric acid were measured at 532 nm. A working solution with 0.25N hydrochloric acid, 0.375% thiobarbituric acid and 15% trichloroacetic acid was made to measure the MDA level (Delavari *et al*., 2017).

Photomicrography

Olympus binocular microscope was used. A 5.1 megapixel MV550 research camera.

Statistical analysis

One-way ANOVA was used to analyse data, followed by Student Newman-keuls (SNK) test for multiple comparisons. Graph Pad Prism 5 (Version 5.03, Graphpad Inc.) was the

statistical package used for data analysis. Significant difference was set at $p<0.05$.

RESULTS AND DISCUSSION

In comparison to the initial body weights, there was an overall increase in final body weight across all treatment groups, the final body weight of group C rats showed insignificant decrease when the experiment was concluded compared to the corresponding group of control animals. Compared to the initial body weights, the treatment groups' final body weights generally increased (Table 2).

The sperm counts revealed a significant differences between group A and Control ($p<0.05$) (**), group B and control ($p<0.05$) (**), group C and control ($p<0.05$) (**) and group D and control ($p<0.05$) (***). When group A was compared to group E, the motility significantly increased ($p<0.05$) (Table 2). An increase in abnormal morphology between group D and group E (control) ($p<0.05$). There was no difference ($p<0.005$) in the normal morphology between the groups compared significantly (Table 3). There were also no significant difference statistically in follicle stimulating hormone (FSH), testosterone and luteinizing hormone across the groups (Fig 1, 2 and 3). As tabulated in Table 4, a significant increase ($p<0.05$) was observed in group D mean glutathione concentration in testicular tissue compared to group E, it is significantly increase in group E ($p<0.01$) when compared with group C. Testicular tissue MDA showed high concentration that is statistically in group D when

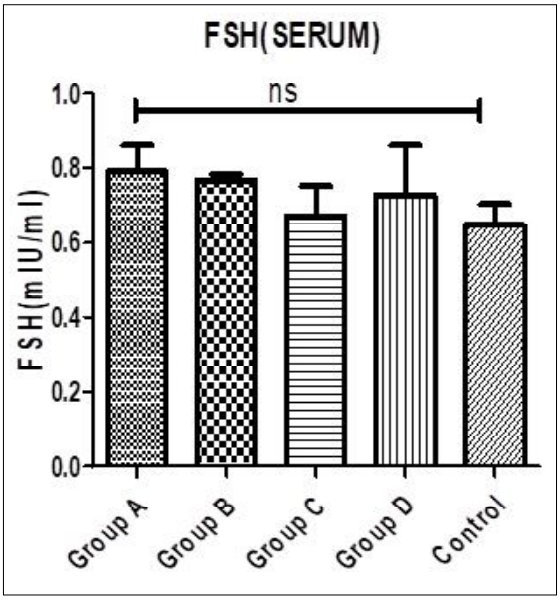

Fig 1: There was no significant changes statistically in serum follicle stimulating hormone level across the group. Bars indicate means±SEM. A: 50mg/kg Datura stramonium, B: 100 mg/kg Datura stramonium C: 200 mg/kg Datura stramonium D: 400 mg/kg Datura stramonium E.

Table 1: Experimental design.

Groups	Exposure
A	50 mg/kg of *Datura* extract was given once daily for 21 days
B	100 mg/kg of *Datura* extract was given once daily for 21 days
C	200 mg/kg of *Datura* extract was given once daily for 21 days
D	400 mg/kg of *Datura* extract was given once daily for 21 days
E	0.2 ml of Distilled Water was given

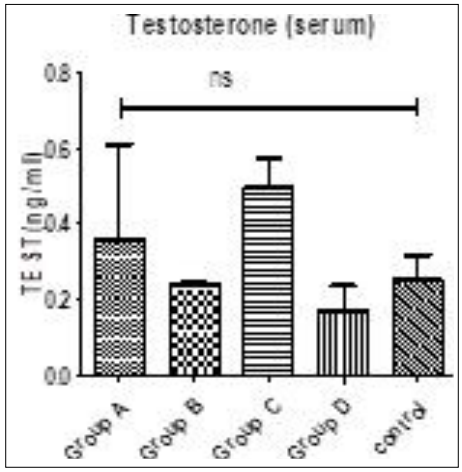

Fig 2: There was no significant changes statistically in the serum Testosterone level across the group. Bars indicate means±SEM. A: 50 mg/kg *Datura stramonium*, B: 100 mg/kg *Datura stramonium* C: 200 mg/kg *Datura stramonium* D: 400 mg/kg *Datura stramonium* E.

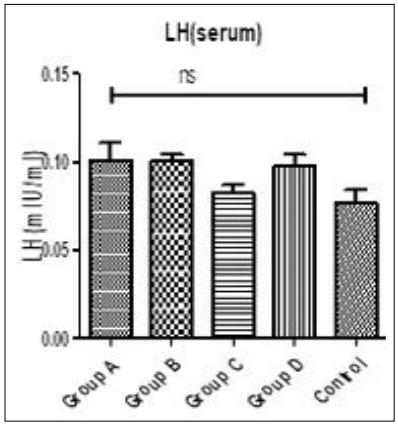

Fig 3: There was no significant changes statistically in the serum Luteinizing hormone level across the group. Bars indicate means±SEM. A: 50 mg/kg *Datura stramonium*, B: 100 mg/kg *Datura stramonium* C: 200 mg/kg *Datura stramonium* D: 400 mg/kg *Datura stramonium* E.

compared with group and there was no significant difference statistically in superoxide dismutase (SOD) (Table 4).

Herbal high is a new trend of drug abusers (Graziano *et al*., 2017) in which plant or organic substances are used for recreation purposes. Most of the new plants used have been documented long ago since ancient times for religious rites and rituals (Halberstein, 2005). Datura metel is a major plant used as herbal high. It is reported to constitute 0.83% of plant abused in Nigeria alone (Kar and Spanjers, 2017). This data is rising has local observation reveals that university students engage more in their use, as it is not detected in routine drug test. The crude extract's

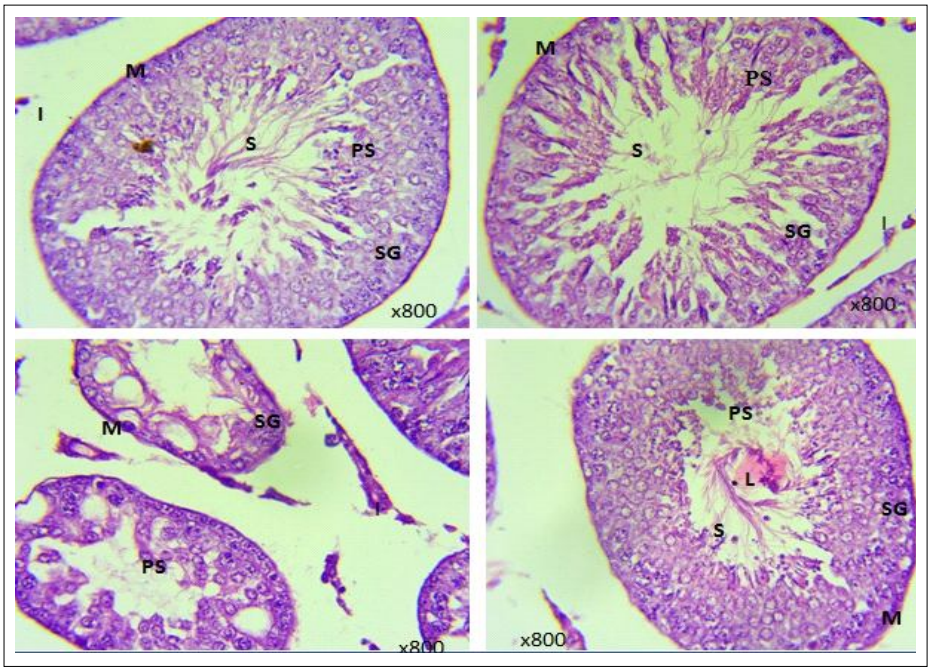

Fig 4: Photomicrograph of histology (H&E) of experimental animal group A (50 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800 mg (seminiferous tubules).

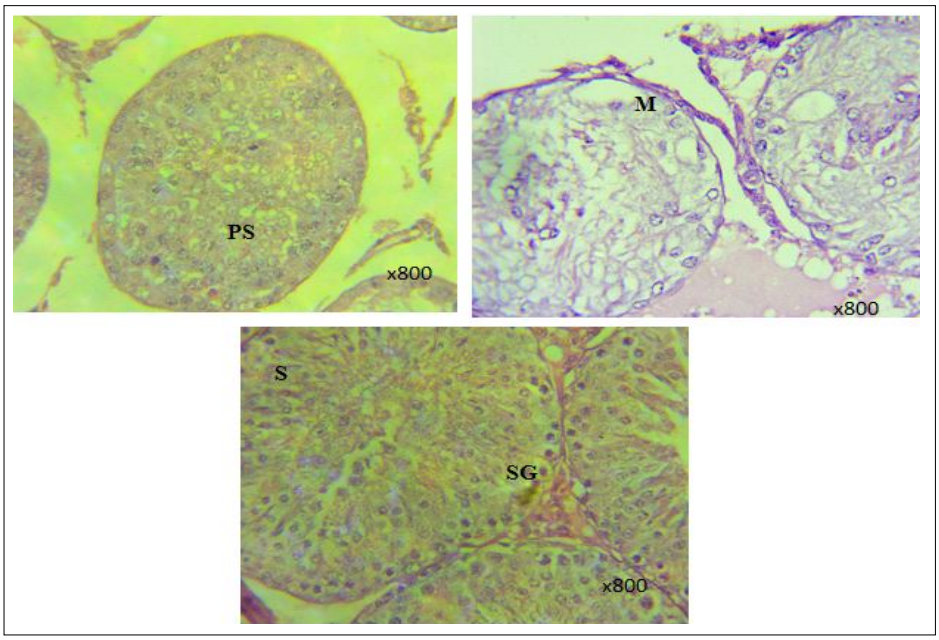

Fig 5: Photomicrograph of histology (H&E) of experimental animal group B (100 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800 mg (seminiferous tubules).

GCMS investigation showed it contains about 3% scopolamine (tropane alkaloid) which is a strong anticholinergic (Menkovska, 2014). This is the primary compound responsible for the plant's stimulatory qualities (Matsuura and Fett-Nato, 2015). Anticholinergic poisoning manifests as the symptoms of accidental or deliberate intake (Walker *et al*., 2014). Scopolamine from fruit extract is also a muscarinic agonist, this means that abusers of the plant are exposing themselves to scopolamine poisoning. The fruit and the flower of the plant is said to contain more alkaloids than the leaves and roots (Jakabova *et al*., 2012). This is also showed from our observation as single extraction

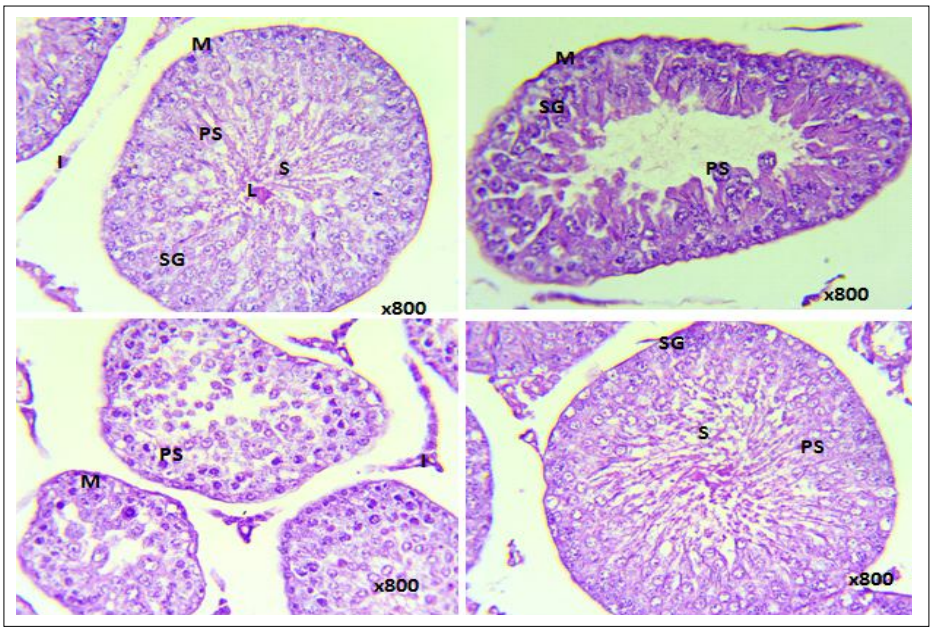

Fig 6: Photomicrograph of histology (H&E) of experimental animal group C (200 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800mg (seminiferous tubules).

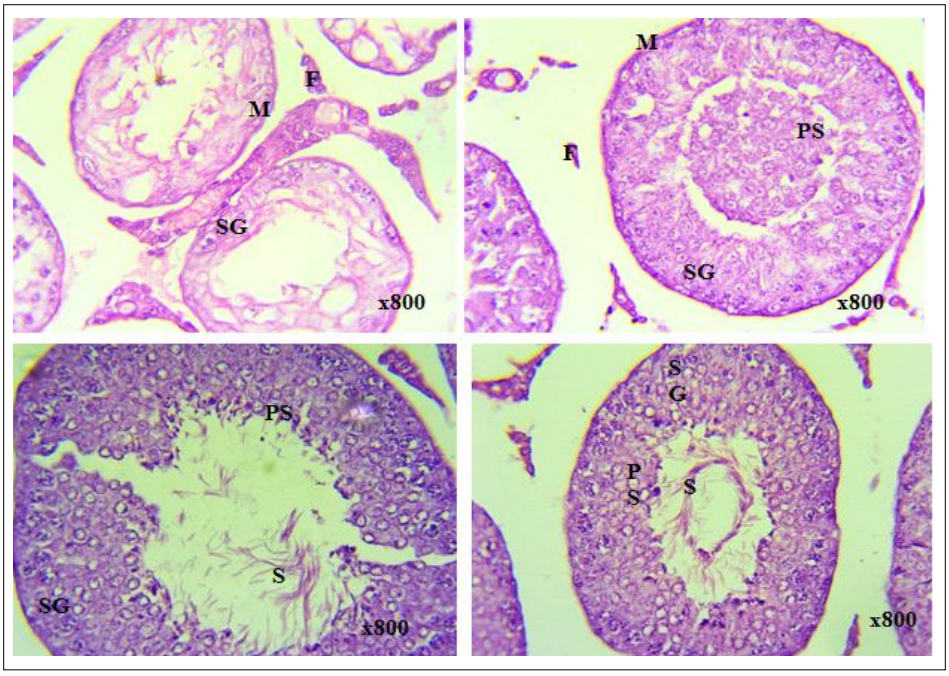

Fig 7: Photomicrograph of histology (H&E) of experimental animal group D (400 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800 mg (seminiferous tubules).

of the leaves with ethanol shows no presence of alkaloids on GCMS study (Inusa *et al*., 2018).

This study showed that animals exposed to *Datura stramonium* extract gained more weight but was less when compared to control, the animals' food consumption changed; they consumed more than usual (Adegoke and Alo, 2013). Ademiluyi *et al*., (2016) reported similar increased in weight in animals exposed to *Datura* fruit extract. Increased weight of animals exposed to *Datura stramonium* in utero has also been documented (Ademiluyi *et al*., 2016). Part the features of drug dependence is grazing appetite which can cause weight gain (Davis, 2016). This shows that the plant shares features with psychoactive agent been abused.

The histology findings (Fig 4 - Fig 13) in this investigation demonstrated degenerative alterations marked by interstitial vacuolization, reduction in the luminal spermatozoa and devoid spermatozoa in cross sections of the seminiferous tubules of rats exposed to various concentration of *Datura stramonium* extract. (50mg, 100mg, 200mg, 400mg for 21days). This is supported by several other previous reports on exposure to drug substances in animals involving cytotoxic chemicals (Whitesell *et al*., 1992).

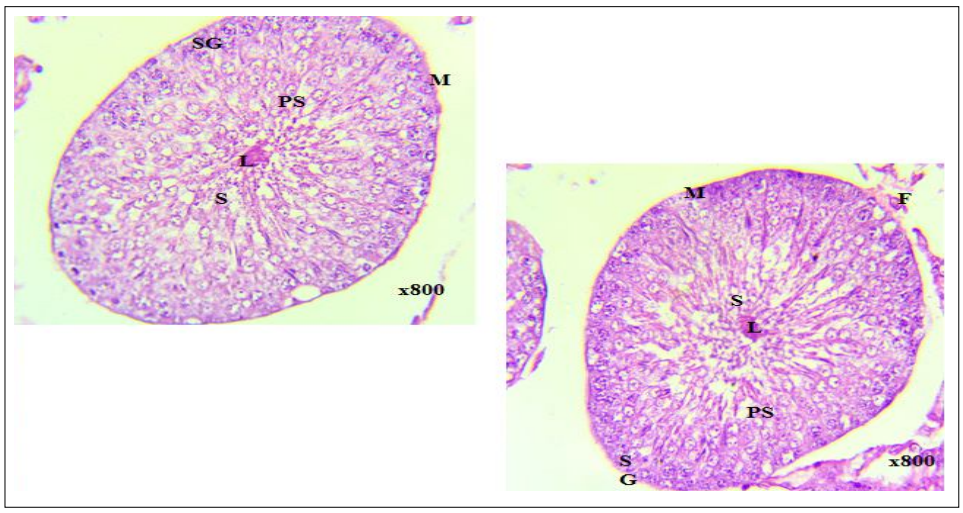

Fig 8: Photomicrograph of histology (H&E) of experimental animal control of *Datura stramonium* extract, S- Spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen an dM- Myoid cells. X 800 mg (seminiferous tubules).

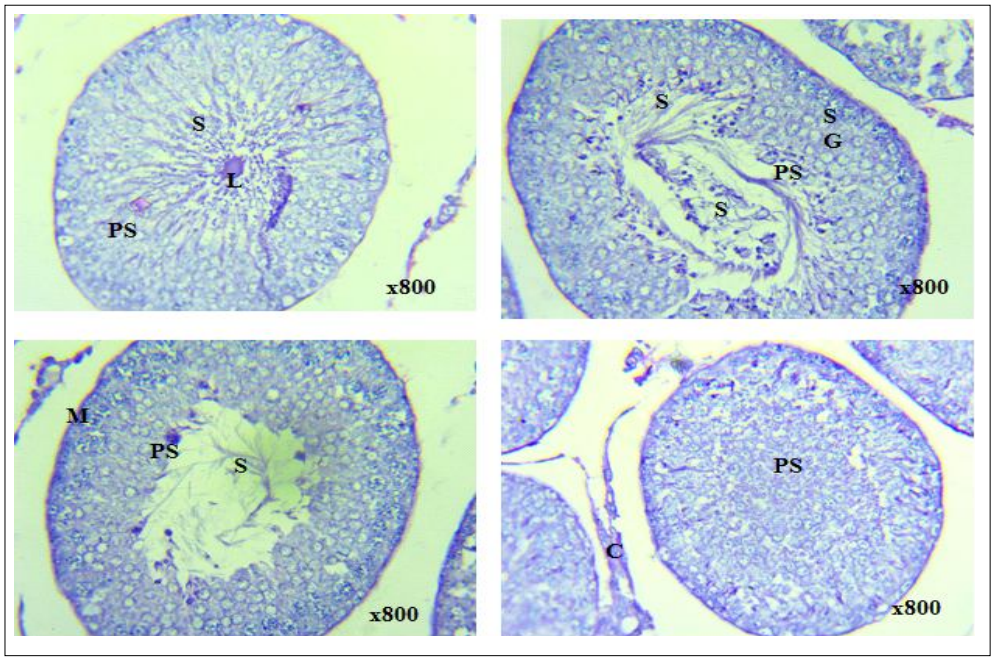

Fig 9: Photomicrograph of histology (PAS) of experimental animal group A (50 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800 mg (seminiferous tubules).

The results got agree with studies which previously reported that Datura effect on germinal cells leads to necrosis and disruption of spermatogenesis (Osman *et al*., 2015).

Follicle stimulating hormone is a heterodimetric glycoprotein which acts on spermatogonia in male stimulates sperms' production in sexually mature male. The action of FSH together with testosterone stimulates all the phases of spermatogenesis (Kerr *et al*., 1992). A biologic marker for determining the functions of sertoli cell is thought to be FSH (Kerr *et al*., 1992). In the study, there was an insignificant

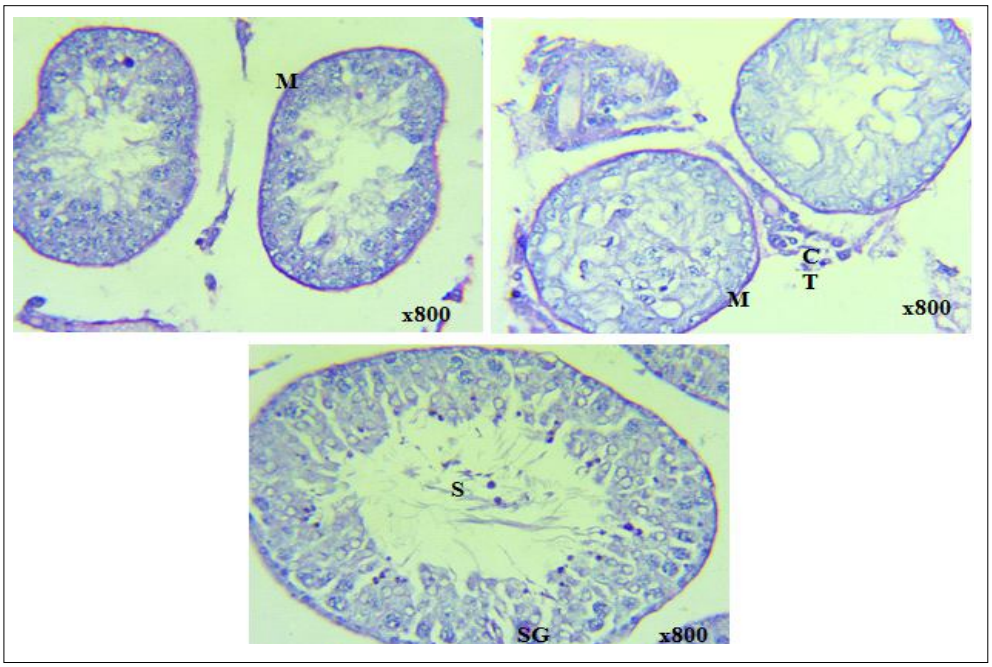

Fig 10: Photomicrograph of histology (PAS) of experimental animal group B (100 mg/kg) of *Datura stramonium* extract, S- Spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells and C- connective tissue. X 800 mg (seminiferous tubules).

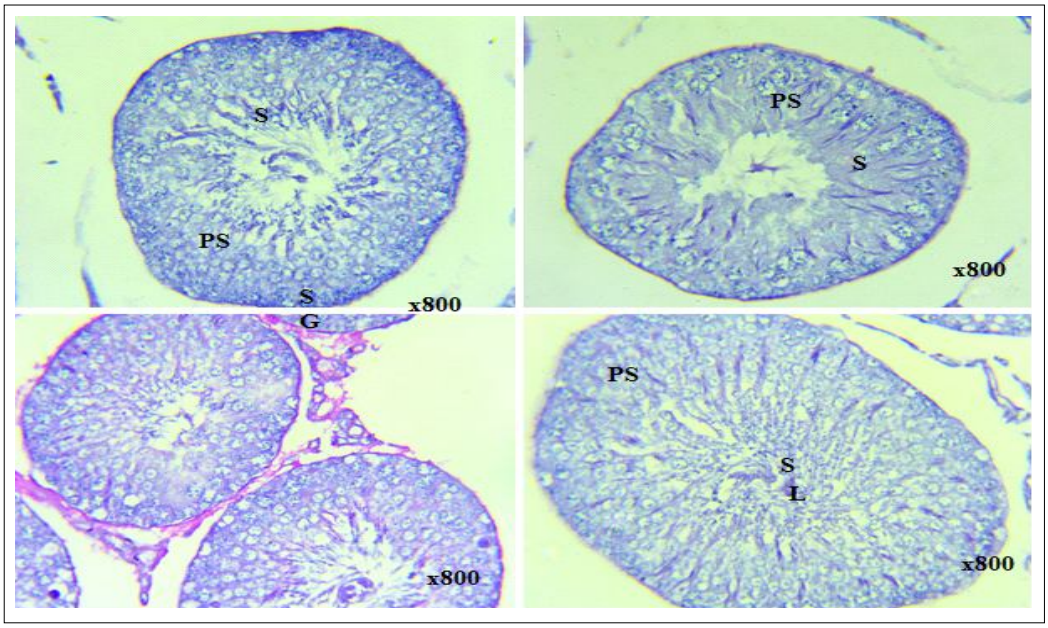

Fig 11: Photomicrograph of histology (PAS) of experimental animal group C (200 mg/kg) of *Datura stramonium* extract, S-spermatocytes, PS- Primary spermatocytes, SG- Spermatogonium, L- Lumen and M- Myoid cells. X 800mg (seminiferous tubules).

increase in FSH in group 1. In cases of infertility, level of FSH is used as aid to determine the reason for low sperm count. A high percentage of it could be caused by primary testicular failure, which may be the result of testicular damage (Hu *et al*., 2013).

MDA is an oxidative stress marker that can be used to measure lipid peroxidation (Tsiropoulou *et al*., 2016). In the current study, MDA level increased in 400 mg Datura extract group when it was compared with the control. This result is in accord once with the report of Bagewadi *et al*., (2019) indicating an increase MDA level after treatment of herbal high (Datura).

SOD is an important antioxidant which plays critical role in the prevention of cellular damage from ROS (Al-Snai *et al*., 2019). The lowest concentration of testicular SOD was found in group D (400 mg/kg), which signifies that the higher intake of *Datura stramonium* causes cellular damage.

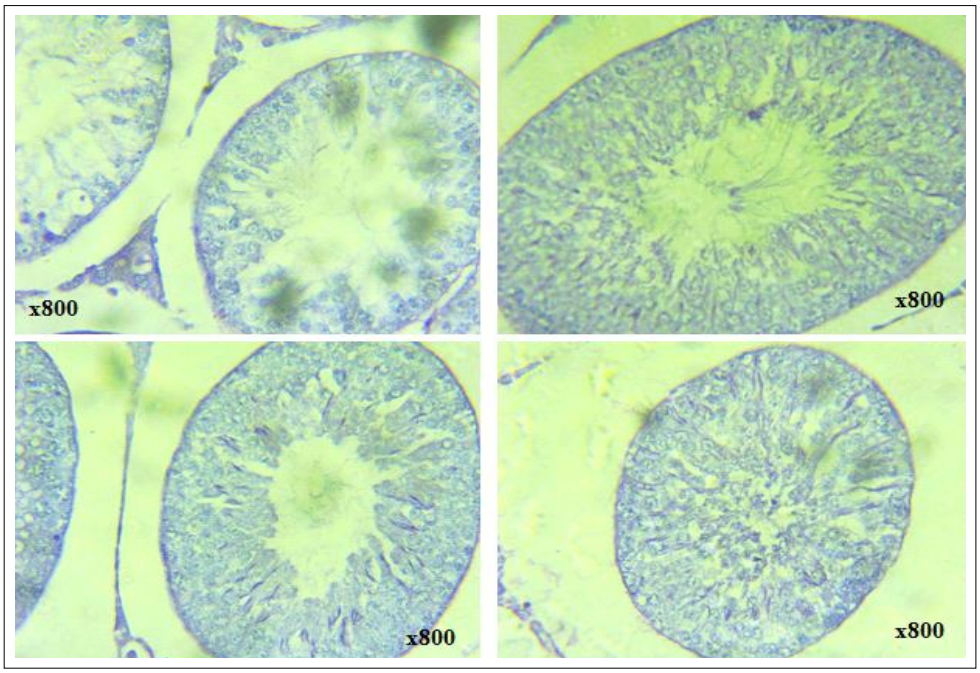

Fig 12: Photomicrograph of histology (PAS) of experimental animal group D (400 mg/kg) of *Datura stramonium* extract, x 800 mg (seminiferous tubules).

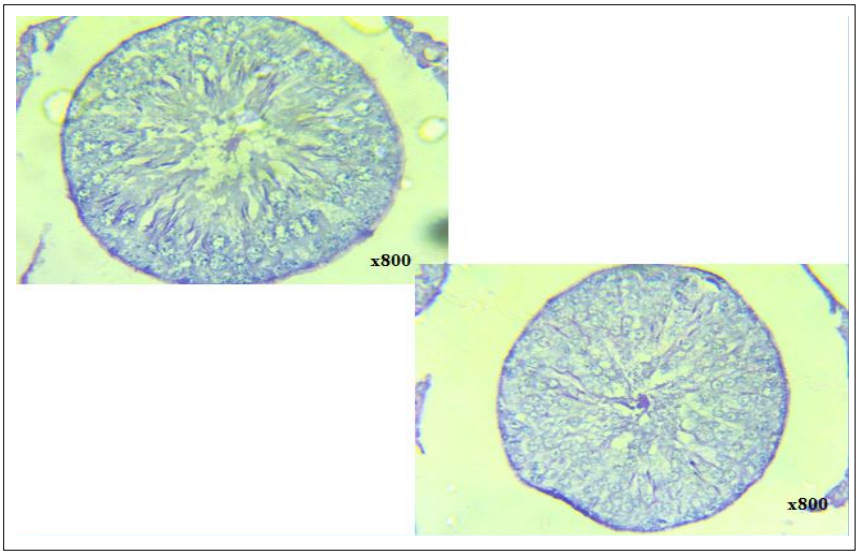

Fig 13: Photomicrograph of histology (PAS) of experimental animal control of *Datura stramonium* extract, X 800 mg (seminiferous tubules).

Table 2: Body weight of different groups.

Groups	Mean of initial (IBW)(g)	Mean of final (FBW) (g)	FBW-IBW (g)	FBW/IBW (g) × 100
A	111.7 g	138.0 g	26.3 (g)	123.5%
B	116.5 g	133.5 g	17 (g)	114.5%
C	109.6 g	115.5 g	5.9 (g)	105.3%
D	131.8 g	152.2 g	20.4 (g)	115.5%
E	127.5 g	145.8 g	18.3 (g)	114.4%

Values are represented as mean Group A; (50 mg/kg *Datura stramonium*), B; (100 mg/kg *Datura stramonium*), C; (200 mg/kg *Datura stramonium*), D; (400 mg/kg *Datura stramonium*), E; Control.

Table 3: Hanges in sperm parameters.

Groups	Sperm count (×10^6/mL)	Sperm motility (%)	Sperm normal (%)	Morphology abnormal (%)
Group A	147±2.0	37±12	28±6.8	72±5.0
Group B	151±3.0	34±6.2	23±3.0	77±3.0
Group C	153±1.0	27±2.4	19±0.5	82±0.4
Group D	126±2.5	16±3.0	15±1.8	85±1.8b
Group E	242±17	40±4.5	29±2.8	68±3.5

Values are represented as mean±SEM. A: 50 mg/kg *Datura stramonium,* B: 100 mg/kg *Datura stramonium,* C: 200 mg/kg *Datura stramonium,* D: 400 mg/kg *Datura stramonium,* E: Control.

Table 4: Testicular level of GSH, MDA and SOD.

Group	GSH (mM)	MDA (uM)	SOD (u/ml)
A	1.0±0.12	9.0±2.8	0.59±0.040
B	1.4±0.042	8.3±0.87	0.78±0.13
C	0.99±0.038	6.5±1.8	0.94±0.0
D	1.5±0.15	11±3.3	0.63±0.13
E	1.0±0.067	9±1.9	0.78±0.083

Values are expressed as mean±SEM. A: 50 mg/kg Datura stramonium, B: 100 mg/kg *Datura stramonium* C: 200 mg/kg *Datura stramonium* D: 400 mg/kg *Datura stramonium* E: Control. Group compared are as follows: A, B, C, D vs E; A vs D. (P<0.005) C vs D. (P<0.005) D vs E. (P<0.005).

CONCLUSION

Extract of *Datura stramonium* plant is toxic since single extraction shows presence of scopolamine. Abuse of the flower of the plant pose cholinergic poisoning due to scopolamine, significantly reduces male fertility evidenced by increasing the number of abnormal sperm motility and altered testicular morphology. This is in addition to many other derangement in histological and biochemical investigation.

Conflict of interest

The authors declare no conflict of interest.

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