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Impact of *Datura stramonium* Methanolic Seed Extract and Withdrawal on Male Reproductive Parameters in Wistar Rats

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Abstract:-

- ***Background****:*

Male reproductive health is a pivotal component of overall well-being, and its susceptibility to environmental factors, including plant extracts, necessitates thorough investigation. *Datura stramonium*, commonly known as jimsonweed, is renowned for its medicinal properties, yet its influence on male reproductive parameters remains inadequately explored.

- ***Material and Method:***

This study scrutinized the impact of *Datura stramonium* extract (DSE) on male reproductive parameters in adult male Wistar rats. DSE was derived from the plant's seeds via methanol extraction. Forty-nine rats were distributed into seven groups, receiving 50, 100 and 200 mg/kg DSE doses for 56 days, with some groups undergoing withdrawal for an additional 28 days.

- ***Result:***

Proximate analysis unveiled DSE's composition. The investigation encompassed alterations in body weight, epididymis weight, hormone levels (FSH, LH, PRL, PRG, estrogen, testosterone), and semen quality and morphology. Furthermore, histological evaluations of the epididymis were conducted.

Proximate analysis delineated DSE's composition. Administration of diverse DSE doses significantly perturbed body weight, epididymis weight, and hormonal levels, affecting Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), testosterone, and others. Semen analysis exposed changes in sperm count, motility, and morphology. Remarkably, some effects were ameliorated upon DSE withdrawal. Histological examinations authenticated structural modifications in the epididymides, paralleling the functional alterations observed.

In conclusion, *Datura stramonium* seed extract (DSE) considerably impaired male reproductive parameters, yet withdrawal exhibited partial recovery. Subsequent research is imperative for comprehending the enduring repercussions on reproductive health.

Keywords:- *Epididymis, Datura stramonium Extract, Hormonal Imbalance, Semen Quality, Withdrawal Effects*

I. INTRODUCTION

Datura stramonium (DS), commonly known as Jimson weed, belongs to the Solanaceae family and is widely distributed and easily accessible. It is characterized by a large, coarse shrub reaching a height of about 3–4 feet and spreading up to 6 feet in rich soil (Buzzz, 2011; Gaire & Subedi, 2013). The root of the plant is sizeable and whitish, featuring a taproot system with numerous fibers. The stem is typically green or purple, hairless, cylindrical, erect, and leafy. It appears smooth and branches repeatedly in a forked pattern (Gaire & Subedi, 2013).

This plant has been traditionally used in Eastern medicine, particularly in Ayurvedic medicine, to address various human ailments such as ulcers, wounds, inflammation, rheumatism, gout, sciatica, bruises, swellings, fever, asthma, bronchitis, and toothache (Gaire & Subedi, 2013). In Nigeria, its seeds are mixed with palm oil and applied to severe cases of insect bites and stings (Egharevba & Ikhatua, 2008). However, the frequent recreational abuse of *Datura stramonium* has led to toxic syndromes. All parts of the plant contain hazardous levels of tropane alkaloids, including atropine, hyoscyamine, and scopolamine, which are classified as deliriants or anticholinergics (Buzzz, 2011; Gaire & Subedi, 2013).

Intoxication resulting from *Datura stramonium* typically manifests as delirium, hyperthermia, tachycardia, bizarre behavior, severe mydriasis with painful photophobia that can last for several days, and pronounced amnesia. These effects are attributed to the presence of atropine, L-hyoscyamine, and L-scopolamine, which cause anticholinergic syndrome by inhibiting central and peripheral muscarinic neurotransmission (Kurzbaum, Simsolo, Kvasha, & Blum, 2001; Krenzelok, 2010; Freye, 2010). Reports suggest that teenagers, particularly those with a history of polysubstance abuse, often voluntarily

ingest *Datura stramonium* in pursuit of its hallucinogenic and euphoric effects. This demographic represents the majority of cases reported in the literature, although intoxication with *Datura stramonium* has also been observed in children (Kurzbaum *et al*., 2001; Arouko *et al*., 2003; Krenzelok, 2010).

Given the plant's known toxicity in various parts, it is essential to investigate its potential impact on male fertility. This study aims to explore the effects of *Datura stramonium* Methanolic Seed Extract and Withdrawal on Male Reproductive Parameters in Wistar Rats.

II. MATERIALS AND METHOD

A. Collection and Extraction of Plant Material

The plant's seeds were procured and gathered from Iluju, a village located in the Orire Local Government Area of Oyo State, Nigeria. Prior identification of the plant took place at the Department of Plant Biology and Biotechnology (PBB), within the Faculty of Life Sciences at the University of Benin. Subsequently, authentication was conducted by a Taxonomist at the Forestry Research Institute of Nigeria (FRIN) in Ibadan. A voucher specimen was then placed in the Institute's herbarium, assigned the voucher number FHI110111.

B. Preparation of Methanol Extract

Prior to seed extraction, the kernels were removed from the seeds and air dried for two weeks before pulverization and maceration using methanol. In the process, 1600 g of the finely ground powdered seed kernels were weighed and poured into extraction tank. Thereafter 4000 ml of the solvent was added to the content of the extraction tank and it was allowed to stand for 48 hours with periodic agitation. The suspension was then filtered using Whatman No.1 Filter paper. The filtrate was concentrated in a water bath at controlled temperature (50^{o}C) to obtain the *Datura stramonium* Extract (DSE) and stored in labeled sterile bottles which were kept in the refrigerator (4^{o}C) for subsequent use.

C. Animals

Forty nine (49) adult male Wistar rats with an average weight of 210 ±10g were used for the research. Rats were housed in animal house of the School of Basic Medical Sciences, University of Benin, Benin city, Edo State at a controlled temperature of 25 ± 2°C with a 12-hour light/dark cycle under standard hygienic conditions and had free access to fresh tap water & pelleted diet. . Experimental procedures involving the animals and their care were conducted in conformity with International, National and Institutional guidelines for the care of laboratory animals in Biomedical Research and the use of laboratory animals in Biomedical Research as promulgated by the Canadian Council of Animal Care (CCAC, 2015).

D. Experimental Design

At the commencement of the experiment, the animals were divided into 7 groups containing 7 rats each: **Group A** served as the Control, and Groups B, C, D, E, F and G were the treatment groups.

Group B and E received 50mg/kg body weight of the extract, **Group C and F** received 100mg/kg body weight while **Group D and G** received 200mg/kg body weight. The treatments lasted for 56 days and were administered by gavage. The dosages were based on the LD 50 of the extract. Rats in **Groups B, C and D** were sacrificed after 56 days of treatment, while treatment was withdrawn from rats in **Groups E, F and G** for 28 days after the initial 56 days treatments before sacrifice.

The rats were weighed twice weekly throughout the experimental period. Also at the last day of the experiment, the body weight of each animal was recorded.

Groups B, C and D were sacrificed after 56 days of treatment upon the commencement of the experiment, while Groups E, F and G were sacrificed after 28 day that treatment was withdrawn from them which was 84 days after the commencement of the experiment along with control animals. Blood was collected in sterile tubes by cardiac puncture. Testis and cauda epididymis of each animal were excised from the surrounding tissue and blotted free of blood for weighing, and preserved in 10% formalin for histological study. Relative organ weight (ROW) for each organ collected, was ascertained using the expression:

$$\textbf{ROW} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100$$

E. Proximate Analysis of Pulverized Seeds

The analysis of the proximate composition of *D. stramonium* seed was carried out using the modified official methods of analysis of the Association of Official Analytical Chemists, (2003).

F. Collection and Analysis of Sperm Samples

For the collection and analysis of sperm samples, each rat was euthanized through cervical dislocation, and their epididymis was carefully harvested. Sperm samples were gathered from the caudal part of the epididymis's reserve, and a smear of these samples was prepared on preheated glass slides to facilitate evaluation.

- *Sperm Motility*

The approach detailed by El-Sherbiny (1987) was adopted to assess sperm motility. Immediately following collection, sperm samples obtained via epididymal washings from each treatment group were evaluated for the presence of progressively motile sperm cells. A drop of the sperm sample was applied to a preheated glass slide, and under light microscopy at magnifications of X10 and X40, the sample was subjectively scored as a percentage. Only sperm cells displaying straightforward movement were included in the motility count, while those exhibiting circular, backward, or pendular movement were excluded. Individual sperm motility was classified according to the following scale: (i) Progressive Motility (PM), (ii) Non-Progressive Motility (NPM), and (iii) Immotile (IM).

➢ *Sperm Viability (Live Proportion)*

The proportion of viable (living) sperm cells was determined by staining a drop of the collected sperm sample with Eosin-Nigrosin stain. The stained slide was allowed to dry for 30 seconds, fixed with ethanol, and observed under a light microscope at X100 magnification (oil immersion). Using a handheld stopwatch manual counter, the percentage of viable sperm cells was counted among a total of 300 sperm cells. Viable sperm cells did not pick up the stain, whereas non-viable ones did (El-Sherbiny, 1987).

➢ *Abnormal Sperm Proportion*

The percentage of abnormal sperm cells was determined following the method outlined by El-Sherbiny (1987). A drop of the sperm sample was stained with Eosin-Nigrosin and smeared on a glass slide. Under lower magnification (X40), the slide was examined to identify primary and secondary abnormal sperm cells. Percentages of various abnormalities, including head, tail, and mid-piece abnormalities, were also quantified (El-Sherbiny, 1987).

G. Hormonal Assay

Quantitative measurements of Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Prolactin, Testosterone, Progesterone, and Estrogen were carried out using the enzyme-linked immunosorbent assay (ELISA) technique with the Accu-Bind Microwell kit, as per Ofem *et al*. (2014).

H. Histological Examinations

Histological examinations were conducted following the method described by Ajonuma *et al*. (2005). Epididymis tissues preserved in 10% formalin were processed into slides for histological analysis. After dehydration in graded ethanol, the tissue samples were embedded in paraffin wax and sectioned to a thickness of 5 µm using a Shandon Finesse Manual Rotary Microtome (model 325, Thermo-scientific). The sections were dried onto super frost microscope slides (Fisher Scientific, Pittsburgh, PA, USA). Hematoxylin and eosin (H&E) staining was performed after dewaxing and dehydrating the slides, enabling subsequent examination under light microscopy. Digital images were captured using a microscope connected to a computer.

I. Statistical Analysis

All quantitative data were analysed using GraphPad Prism® software (Version 6). One way Analysis of Variance (ANOVA) with Significance level set at ($P< 0.05$) (95% Confidence Interval) was used for the comparison of relative expression levels for different groups followed by Turkey Post Hoc test. The outcomes were represented in bar charts with error bars to show the mean and standard error of mean (M ± SEM) respectively.

III. RESULT

A. Proximate Analysis of the Datura Stramonium Extract (Dse)

The moisture content, crude protein, ash value, total carbohydrates, crude fiber, crude fat, and energy value of *Datura stramonium* seed extract (DSE) were estimated to give insights into the seed's potential uses and nutritional value.

Table 1 Proximate Analysis of the *Datura stramonium* Seed Extract (Dse)

Parameters	Composition (Mean ± S.E.M)
Moisture	10.31 ± 0.03
Crude protein	21.80 ± 0.02
Ash value	7.32 ± 0.05
Total carbohydrate	38.83 ± 0.01
Crude Fibre	16.32 ± 0.02
Crude fat	12.01 ± 0.05
Energy Value (KJ/100g)	350.49 ± 0.03

B. Effect of the Extract on the Body Weight and Organ Weight of Experimental Rats

The result revealed that administration of *Datura stramonium* seed extract (DSE) led to a significant ($P< 0.05$) decrease in body weight in the treatment groups (B, C, and D) compared to the control group (A). However, withdrawal from DSE treatment (groups E, F, and G) partially mitigated this weight loss, resulting in statistically significant ($P< 0.05$) weight recovery.

Similarly it was revealed that administration of varied doses of *Datura stramonium seed* extract (DSE) led to a significant ($P< 0.05$) reduction in both right and left epididymis weights in the treatment groups (B, C, and D) when compared to the control group (A). However, in the groups that experienced 28 days of withdrawal from DSE treatment (E, F, and G), the epididymis weights were not significantly ($P< 0.05$) different from those in the control group, indicating a potential for recovery in epididymis weight following withdrawal.

Table 2 Effect of the Dse on the Body Weight of Experimental Rats

Group	Initial Weight	Final Weight
Control (A)	232.00±6.78	294.14±4.44 ***
50mg/kg of DSE (B)	212.64±5.67	291.79±7.10 *
100mg/kg of DSE (C)	206.43±6.06	256.00±5.91 *
200mg/kg of DSE (D)	203.93±5.94	235.57±6.93 *
50mg/kg of DSE + Withdrawal (E)	202.646±.77	250.73±6.99 *
100mg/kg of DSE + Withdrawal (F)	204.50±5.66	274.71±6.73 *
200mg/kg of DSE + Withdrawal (G)	213.21±5.39	274.29±9.25 *

***** represent significant difference ($p <0.005$) when compared to the initial weights.
Where DSE: *Datura stramonium* Extract

Table 3 Effect of the Dse on the Epididymis Weight of Experimental Rats

Group	Right Epididymis Weight	Left Epididymis Weight
Control (A)	0.70 ± 0.06	0.77±0.14
50mg/kg of DSE (B)	0.58±0.02 [b]	0.62±0.05 [b]
100mg/kg of DSE (C)	0.42±0.03 [a,b]	0.45±0.03 [a,b]
200mg/kg of DSE (D)	0.30±0.03 [a,b]	0.30±0.03 [a,b]
50mg/kg of DSE + Withdrawal (E)	0.71±0.11	0.80±0.04
100mg/kg of DSE + Withdrawal (F)	0.67±0.03	0.65±0.04
200mg/kg of DSE + Withdrawal (G)	0.51±0.05 a	0.56±0.09

a: represent significant difference ($p<0.05$) when compared to control rats.
b : represent significant difference ($p<0.05$) when compared to corresponding reversal group
Where DSE: *Datura stramonium* Extract

C. Effect of the Extract on the Reproductive Hormone of Experimental Rats

Results revealed that DSE administration led to decreased levels of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) in a dose-dependent manner, while Prolactin (PRL) levels significantly increased. Additionally, DSE exposure elevated Progesterone (PRG) and Estrogen levels, particularly at higher doses (100 and 200 mg/kg), but decreased Testosterone levels. Importantly, withdrawal from DSE exposure partially reversed these hormonal alterations.

Table 4 Showing The Levels of Follicle Stimulating Hormone (Fsh), Leutenizing Hormone (Lh), and Prolactin (Prl) in Experimental Rats.

Group	FSH (µmol/L)	LH (µmol/L)	PRL (µmol/L)
Control (A)	1.44±0.02 [a]	1.03±0.04 [a]	0.30±0.01 [a]
50mg/kg of DSE (B)	0.57±0.03 [b]	0.84±0.04 [b]	0.49±0.0c,f [a,f]
100mg/kg of DSE (C)	0.12±0.01 [c]	0.24±0.05 [c]	0.25±0.02 [a]
200mg/kg of DSE (D)	0.13±0.02 [c,d]	0.21±0.01 [c,d]	5.89±0.22 [b]
50mg/kg of DSE + Withdrawal (E)	1.12±0.04 [e]	1.17±0.03 [a]	0.91±0.05 [c,f]
100mg/kg of DSE + Withdrawal (F)	1.48±0.05 [a]	0.61±0.03 [e]	1.20±0.04 [c,f]
200mg/kg of DSE + Withdrawal (G)	0.97±0.03 [f]	0.64±0.02 [e,f]	3.51±0.11 [d]

*****All columns were compared with each other. Means with the same superscript are not significantly different from each other ($P>0.05$) while Means with different superscript are significantly different from each other.
Where DSE: *Datura stramonium* Extract

Table 5 Showing the Levels of Progesterone (Prg), Estrogen and Testosterone in Experimental Rats.

Group	PRG (µmol/L)	ESTROGEN (µmol/L)	TESTOSTERONE (µmol/L)
Control (A)	13.81±0.52 [a]	16.41±0.49 [a]	2.71±0.06 [a]
50mg/kg of DSE (B)	34.94±1.29 [b]	23.58±1.27 [b]	1.70±0.07 [b]
100mg/kg of DSE (C)	39.31±0.26 [c,d]	34.71±1.58 [c]	0.96±0.04 [c]
200mg/kg of DSE (D)	66.09±1.74 [d]	56.01±1.76 [d]	0.34±0.02 [d]
50mg/kg of DSE + Withdrawal (E)	19.57±0.86 [e]	23.97±0.77 [b,e]	2.06±0.04 [e]
100mg/kg of DSE + Withdrawal (F)	31.73±1.25 [b,f]	23.44±0.45 [b,f]	1.24±0.04 [b,f]
200mg/kg of DSE + Withdrawal (G)	43.73±1.37 [c,g]	36.76±0.76 [c,g]	0.81±0.05 [g]

*All columns were compared with each other. Means with the same superscript are not significantly different from each other ($P>0.05$) while Means with different superscript are significantly different from each other.
Where DSE: *Datura stramonium* Extract

D. Effect of the Extract on Semen Quality and Morpholoy of Experimental Rats

The experimental findings reveal significant impacts on semen quality due to *Datura stramonium* Extract (DSE) administration. The control group displayed the highest Total Sperm Count (TSC) and the largest percentage of Normal Semen, along with the lowest percentage of Abnormal Semen. Conversely, groups exposed to DSE at varying doses exhibited a dose-dependent reduction in TSC, an increase in Abnormal Semen, and a decrease in Normal Semen.

Furthermore, Progressive Motile Sperm (PM), Non-Progressive Motile Sperm (NPM), and Immotile Sperm (IM) were assessed. The control group had the highest PM and the lowest NPM and IM percentages. However, DSE exposure led to decreased PM, increased NPM, and IM in varying degrees. Withdrawal from DSE exposure resulted in a partial recovery of these parameters.

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Table 6 Shows Semen Analysis Result of the Various Experimental Groups. Total Sperm Count (Tsc), Normal Semen and Abnormal Semen.

Group	Total sperm count (10^6)	Normal	Abnormal
Control (A)	370.00±23.80 [a]	90.00±2.65 [a]	10.00±2.65 [a]
50mg/kg of DSE (B)	264.29±16.60 [b]	80.00±3.09 [b]	20.00±3.09 [b]
100mg/kg of DSE (C)	350.43±2.57 [a]	85.86±0.80 [a,b]	14.14±0.80 [a,b]
200mg/kg of DSE (D)	200.00±10.47 [b]	60.00±3.09 [c]	40.00±3.09 [c]
50mg/kg of DSE + Withdrawal (E)	290.00±16.18 [a,b]	80.00±1.50 [d,b]	20.29±1.43 [d,b]
100mg/kg of DSE + Withdrawal (F)	82.86±4.74 [c]	50.00±1.38 [e]	50.00±1.38 [e]
200mg/kg of DSE + Withdrawal (G)	200.00±20.35 [b,d]	70.00±0.79 [f]	30.00±0.79 [f]

*All columns were compared with each other. Means with the same superscript are not significantly different from each other (P>0.05) while Means with different superscript are significantly different from each other.
Where DSE: *Datura stramonium* Extract

Table 7 Shows Semen Analysis Result of the Various Experimental Groups. Progressive Motile Sperm (Pm), Non Progressive Motile Sperm (Npm), and Immotile Sperm (Im).

Group	Progressive motile	Non-progressive motile	Immotile
Control (A)	75.71±2.02 [a]	11.43±1.43 [a]	12.86±1.84 [a]
50mg/kg of DSE (B)	58.57±3.40 [b]	28.57±1.43 [b]	12.86±2.86 [a]
100mg/kg of DSE (C)	69.86±0.99 [a,b]	15.86±0.40 [a,b]	18.57±3.40 [a]
200mg/kg of DSE (D)	31.43±3.40 [c]	50.00±0.00 [c]	24.29±2.12 [b]
50mg/kg of DSE + Withdrawal (E)	55.71±4.29 [b,d]	34.29±4.29 [c,d]	14.29±1.08 [a]
100mg/kg of DSE + Withdrawal (F)	10.00±0.00 [e]	65.71±8.12 [d]	10.00±0.00 [a]
200mg/kg of DSE + Withdrawal (G)	40.00±3.09 [c,f]	48.57±2.61 [c]	11.43±1.43 [a]

*All columns were compared with each other. Means with the same superscript are not significantly different from each other (P>0.05) while Means with different superscript are significantly different from each other.
Where DSE: *Datura stramonium* Extract

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

The histological findings indicate that DSE exposure led to changes in the epididymal histology, primarily characterized by reduced spermatozoa within the lumen and increased hypocellular areas. However, withdrawal from DSE treatment showed potential for the partial reversal of these effects, suggesting some recovery in the histological integrity of the epididymides.

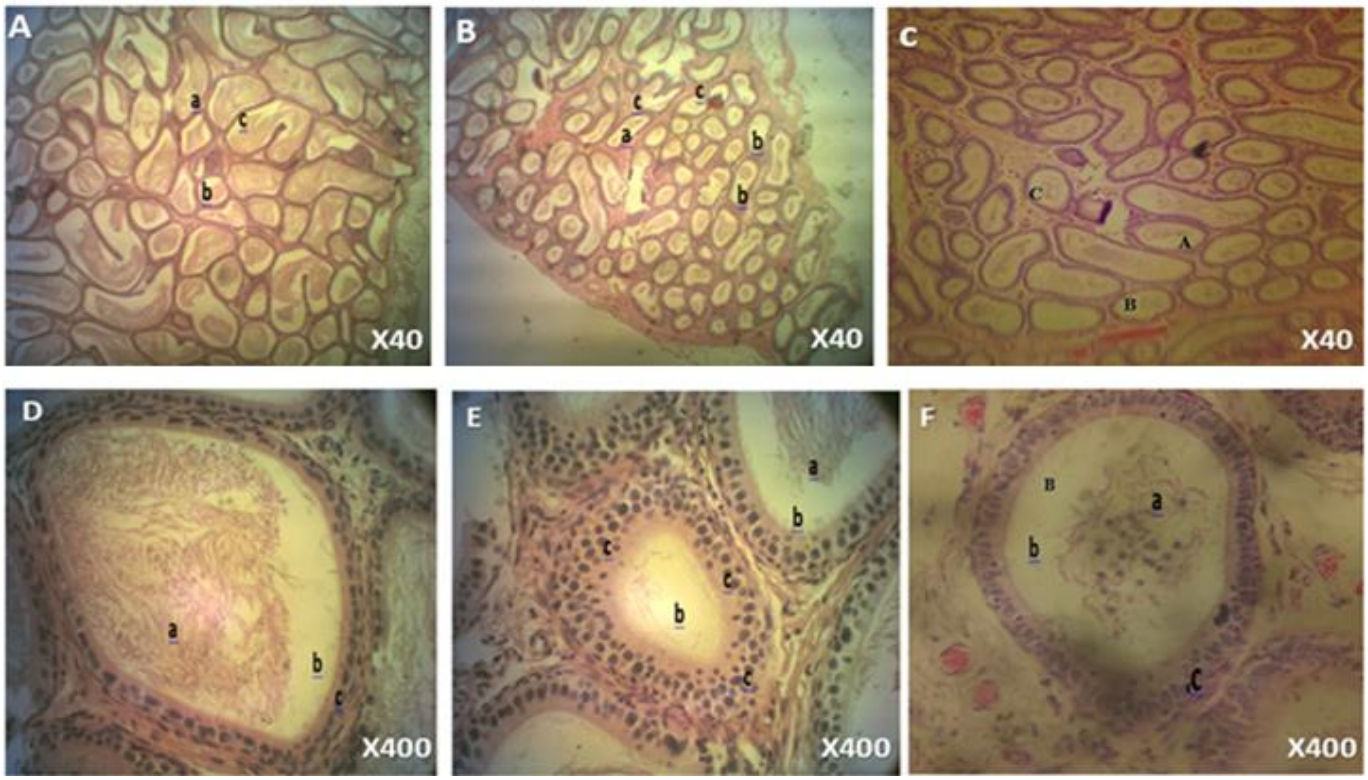

Plate 1 H&E Stain Photomicrographs of the Effects of 50mg/kg Methanolic Extracts of *Datura stramonium* and a Month Withdrawal on the Histology of Epididymis of Experimental Animals.

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➢ *Plate 1A & 1D (Control Group A) :*

The epididymis slide shows **(a)** Lumen with spermatozoa, **(b)** Hypocellular region without spermatozoa, and **(c)** Epithelial lining with columnar cells.

➢ *Plate 1B & 1E (Group B):*

Epididymis of Wistar rats treated with *Datura stramonium* extract, showing **(a)** Lumen with relatively scanty spermatozoa, **(b)** Hypocellular region without spermatozoa, and **(c)** Epithelial lining with columnar cells. Some epididymides have larger hypocellular spaces compared to the control.

➢ *Plate 1C & 1F (Group E):*

Epididymis after one month of withdrawal from treatment with *Datura stramonium* extract, displaying **(a)** Lumen with spermatozoa, **(b)** Hypocellular region without spermatozoa, and **(c)** Epithelial lining with columnar cells.

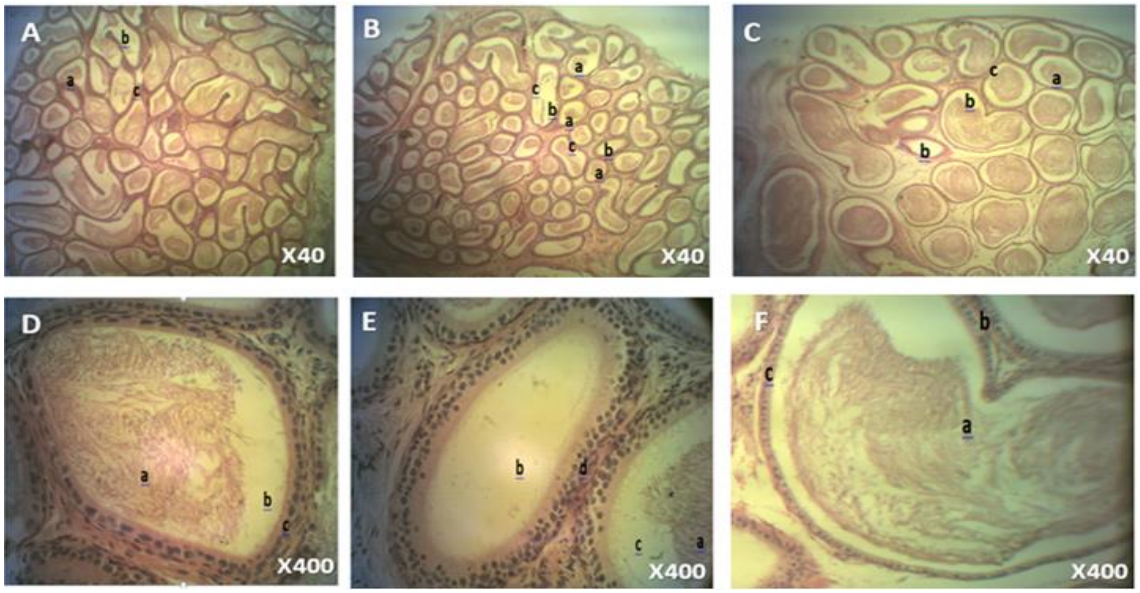

Plate 2 H&E Stain Photomicrographs of the Effects of 100mg/kg Methanolic Extracts of *Datura stramonium* and a Month Withdrawal on the Histology of Epididymis of Experimental Animals.

➢ *Plate 2A & 2D (Control Group A) :*

This epididymis slide reveals **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region without spermatozoa, and **(c)** an epithelial lining with columnar cells.

➢ *Plate 2B & 2E (Group C) :*

Shows the epididymis of Wistar rats treated with 100mg/kg body weight of *Datura stramonium* extract, it shows **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region devoid of spermatozoa, and **(c)** an epithelial lining with columnar cells.

➢ *Plate 2C & 2F (Group F) :*

After one month of withdrawal from treatment with 100mg/kg body weight of *Datura stramonium* extract, this slide shows **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region without spermatozoa, and **(c)** an epithelial lining with columnar cells.

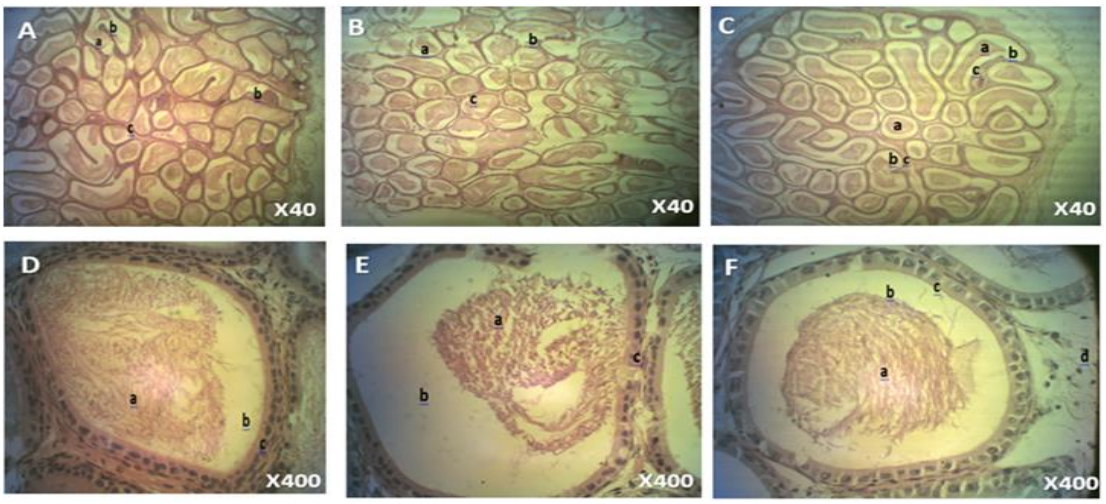

Plate 3 H&E Stain Photomicrographs of the Effects of 200mg/kg Methanolic Extracts of *Datura stramonium* and a Month Withdrawal on the Histology of Epididymis of Experimental Animals.

- *Plate 3A & 3C (Control Group A):*

This epididymis slide shows **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region without spermatozoa, and **(c)** an epithelial lining with columnar cells.

- *Plate 3B & 3D (Group D):*

Depicting the epididymis of Wistar rats treated with 200mg/kg body weight of *Datura stramonium*, it showed **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region devoid of spermatozoa, and **(c)** an epithelial lining with columnar cells.

- *Plate 3C & 3F (Group G):*

After one month of withdrawal from treatment with 200mg/kg body weight of methanolic extract of *Datura stramonium*, this slide shows **(a)** a lumen containing spermatozoa, **(b)** a hypocellular region without spermatozoa, and **(c)** the epithelial lining with columnar cells.

IV. DISCUSSION AND CONCLUSION

The male reproductive system plays a pivotal role in the perpetuation of species and the transmission of genetic information. Its proper functioning is essential for human and animal populations' reproductive success and overall health (Gurung *et al*., 2023). Over the years, there has been growing concern about the potential adverse effects of environmental factors, including exposure to various plant-derived substances, on male reproductive parameters. One such plant, *Datura stramonium*, commonly known as Jimson weed or Thornapple, belongs to the Solanaceae family and is known for its toxic properties (Soni *et al*., 2012). It has been traditionally used for medicinal and psychoactive purposes in different cultures. However, its potential impact on male reproductive health has raised significant scientific interest and concern.

Investigation from this study on the effects of *Datura stramonium* seed extract (DSE) on male reproductive parameters, such as sperm quality, hormone levels, and histological changes in the epididymis, would provide valuable insights to the effect of *Datura stramonium* seed extract on the male reproductive system.

The methanolic *Datura stramonium* seed extract (DSE) demonstrates a complex chemical composition with key attributes unveiled in Table 1. Notably, it exhibits a moisture content of 10.31 ± 0.03%, signifying a significant water presence with crucial implications for the extract's stability and shelf life (Isengard, 2001). Moreover, the DSE boasts a substantial crude protein content at 21.80 ± 0.02%, and richness in total carbohydrates (38.83 ± 0.01%) suggests a significant energy source within the extract, promising substantial energy provision to the subjects under study (Silva *et al*., 2021).

The ash value of 7.32 ± 0.05% 16.32 ± 0.02% crude fiber, indicating the presence of dietary fiber that can influence digestion and nutrient absorption, with potential implications for the overall health of the rats (Lattimer & Haub 2010) and sheds light on the mineral composition within the DSE. Finally, the energy value of 350.49 KJ/100g (or 350.49 kJ per 100 grams) accentuates the DSE's potential to impact the metabolic processes of the rats, further highlighting its dietary implications (Gong *et al*., 2016).

Accordingly all groups of animal administered the extract at 50,100, and 200 mg/kg and groups that had the withdrawal treatments increased from their initial weight significantly similar to the control groups (Table 2). This is similar to the report of Joshua and others, who reported increase in animal body weight after the administration of aqueous extract of *Datura stramonium* seeds (Joshua *et al*., 2021).

As seen in Table 3, administration of DSE at increasing dosage from 50mg/kg to 200mg/kg, there was a significant decrease in both right and left epididymis weights. This suggests a dose-dependent relationship between DSE and epididymis weight, with groups administered 200mg/kg showing the least epididymis weight. However group E, F and G who had reversal treatment from the 50, 100 and 200mg/kg of DSE respectively, showed a significantly increased epididymis weight as compared to their corresponding group B, C, D and the Control group. This implies that the adverse effects of DSE on the epididymis could potentially be reversed or mitigated by withdrawal of the extract.

Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are produced by the anterior pituitary gland, FSH stimulates spermatogenesis, ensuring the production and maturation of sperm, while LH triggers testosterone production, responsible for male secondary sexual traits and overall sexual health (Oduwole *et al*., 2021). This study revealed as reported in Table 4 that administration of 50, 100 and 200 mg/kg of DSE resulted in a significant decrease of both FSH and LH levels in a dose dependent manner when compared to the control group. On the contrast Prolactin (PRL), a 23 Kd hormone, synthesized in the adenohypophyseal lactotrophs, whose high concentration in the male interferes with the function of the testicles, the production of testosterone (the main male sex hormone) and sperm production decrease. was observed to be significantly higher in the groups administered the varied doses of DSE in a dose dependent manner, this effect can be caused by the some constituent of *Datura stramonium* seeds such as atropine which as been reported to impair male infertility (Sato *et al.*, 2005). However the 28 days withdrawal of the Extract administration in Group E, F and G revealed a significant increase in the level of FSH and LH hormone as compared to groups administered the extract only i.e. Group B, C and D respectively, this suggests that the withdrawal treatment had a mitigating effect on the DSE-induced hormonal alterations, although the Prolactin concentration remained significantly high in the groups with the Withdrawal treatment.

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Similarly as seen in Table 5, administration of 50, 100 and 200 mg/kg of DSE (Group B) resulted in a significant increase in Progesterone (PRG) and Estrogen levels in a dose dependent manner when compared to the control groups, suggesting a substantial disruption in hormonal balance as the elevated levels of these hormones can inhibit testosterone production (Rochira & Carani 2023). Testosterone levels were significantly reduced, in Group B, C and D as compared to the control group pointing to an adverse impact on male reproductive function. Group E, F and G who were administered 50, 100 and 200 mg/kg respectively followed by the withdrawal treatment showed a reversed decreased level of Progesterone (PRG) and Estrogen with Group E (50mg/kg of DSE + Withdrawal) showing the fastest recovery as compared to Group F and G. Similarly, testosterone level in Group E, F and G improved in a dose dependent manner as compared to their corresponding DSE only groups. This shows that the withdrawal of DSE can improve male reproductive hormone thereby mitigating DSE- Induced male infertility.

Semen analysis is a critical component in assessing male reproductive health, as it provides valuable information about sperm quality, quantity, and motility. the results presented in Tables 6 and 7, detailed the effects of varying doses of *Datura stramonium* Extract (DSE) and DSE combined with withdrawal on semen parameters, including total sperm count, sperm morphology (normal and abnormal), progressive motile sperm, non-progressive motile sperm, and immotile sperm.

In Table 6, we observe significant alterations in total sperm count (TSC) across the experimental groups. The control group (Group A) displayed a TSC of 370.00±23.80 million sperm per milliliter (10^6/ml). Exposure to DSE, especially at higher doses (Groups B, C, and D), resulted in significant reductions in TSC. Notably, the withdrawal of DSE (Groups E, F, and G) led to partial recoveries in TSC. The evaluation of sperm morphology, specifically the proportion of normal and abnormal sperm, is crucial for assessing sperm quality. In Group A (Control), 90% of sperm exhibited normal morphology, while 10% were abnormal. Exposure to 50, 100 and 200 mg/kg of DSE (Groups B, C, and D) respectively led to a reduction in the percentage of normal sperm and an increase in abnormal sperm. Withdrawal of DSE (Groups E, F, and G) often resulted in varying degrees of recovery in sperm morphology in a dose dependent manner.

Table 7 provides insight into sperm motility. Progressive motile sperm (PM) are those with forward movement, while non-progressive motile sperm (NPM) exhibit movement without forward progression, and immotile sperm (IM) do not move at all. In the control group (Group A), the majority of sperm were PM (75.71%), while a smaller proportion were NPM (11.43%) or IM (12.86%).

Exposure to varied doses of DSE (Groups B, C, and D) resulted in decreased PM and increased NPM and IM percentages, indicating impaired sperm motility as compared to the control groups. Withdrawal of DSE (Groups E, F, and G) often led to partial recoveries in sperm motility, as compared to their corresponding groups that were administered varied doses of the extract only. This results suggest that *Datura stramonium* Extract (DSE) has a significant and dose-dependent impact on semen parameters in male rats and its withdrawal of DSE partially mitigated some of these effects, highlighting the potential for recovery. These findings are consistent with previous studies that have reported the adverse effects of plant extracts on male reproductive health.

Histological analysis of epididymal tissue provides essential insights into the impact of *Datura stramonium* Extract (DSE) exposure and withdrawal on the structural integrity of the epididymis, a critical component of the male reproductive system.

Plate 1A, 2A, 3A, 1D, 2D, and 3D (Control Group A) serve as a reference, showing the typical histological features of the epididymis, including a lumen filled with spermatozoa, a hypocellular region without spermatozoa, and an intact epithelial lining with columnar cells. However the administration of 50mg/kg, 100 mg/kg, and 200 mg/kg as shown in Plate 1B, 2B, 3B, 1E, 2E, and 3E, at different magnification revealed alterations in the epididymal tissue following DSE exposure. Notably, the lumen contains relatively scanty spermatozoa, the hypocellular region is enlarged, and some epididymides exhibit larger hypocellular spaces compared to the control. These observations suggest that DSE exposure affects sperm transit and may result in reduced sperm storage capacity within the epididymis.

While Plate 1C, 2C, 3C, 1F, 2F, and 3F, at both 40X and 400X, showed the histological changes after 28 days of *Datura stramonium* Extract (DSE) withdrawal. While the lumen contains spermatozoa, indicating partial recovery, the hypocellular region and the epithelial lining appear similar to the DSE-exposed group. This suggests that some histological changes may persist even after DSE withdrawal. The histological findings indicate that *Datura stramonium* Extract (DSE) exposure can induce significant structural alterations in the epididymis, potentially affecting sperm transit, storage, and overall reproductive function. While some recovery is observed after withdrawal, certain histological changes may persist, suggesting potential long-term effects on male reproductive health.

V. CONCLUSION

These findings collectively highlight the significant impact of *Datura stramonium* seed extract (DSE) on male reproductive parameters. While exposure to DSE led to adverse effects on hormonal balance, semen parameters, and histological integrity, the withdrawal treatment demonstrated the potential for recovery in some aspects of male reproductive health. However, certain changes persisted, warranting further investigation into the long-term consequences of DSE exposure on male fertility and reproductive function. This study contributes valuable insights into the complex interactions between plant extracts

and the male reproductive system, emphasizing the importance of understanding environmental factors that can affect reproductive health.

➢ *Ethics Approval*

All animals used were approved by the Animal usage committee of Basic Medical Sciences, University of Benin, Benin city, Edo State.

➢ *Availability of Information and Resources*

The corresponding author can provide the datasets used in and/or analyzed during the current investigation upon request.

➢ *Competing Interests*

The authors affirm that they have no known financial conflicts of interest or close personal ties that would have seemed to affect the work reported in this current study.

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The Authors of this article contributed equally in this present study in the conception, design, execution, interpretation of research findings and article writing.

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