Histomorphological and Biochemical Evaluation of the Effect ofHydroethanolic Extract of*Datura metel* Seed on the Testes and Ovaries of Male and Female Albino Rats.

ABSTRACT

Aim: To evaluate the effect of *Datura metel* seed extracts on reproductive parameters of albino rats.
Study Design: Twenty-five albino rats were divided into 5 groups with 5 animals in each. Group 1 served as negative control, group 2 served as the positive control receiving 100mg/kg of Lead acetate. Groups 3-5 received 150mg/kg, 300mg/kg and 600mg/kg of *Datura metel* seed extracts respectively.
Place and Duration: Department of Medical Laboratory Science, Department of Pharmacology, Niger Delta University Teaching Hospital Bayelsa State, Nigeria, between May 2023 and July 2023.
Methodology: Histological analysis of the Ovaries and Testes of the albino rats was done using Haematoxylin and Eosin Staining method while antioxidant and hormone analysis were evaluated using Enzyme linked immunosorbent assay (ELISA) method.
Results: The parameters were assayed using standard methods of analysis. Statistical analysis was done with Statistical Package for Social Sciences (SPSS), version 23. Results were expressed as mean ± SD and p- values less than or equal to 0.05 were considered statistically significant. The results revealed the reduced levels of SOD, CAT, GST and increased MDA levels when compared to the negative control groups.Group 3 gave an SOD mean value of 7.51±0.72, CAT of 5.41±0.43, MDA of 22.97±0.27 and GST of 1.98±0.26. Group 4 gave an SOD mean value of 7.82±0.29, CAT of 5.92±0.28, MDA, 22.15±0.22 and GST, 1.96±0.14 while Group 5 gave an SOD of 6.40±0.39, CAT 4.98±0.21, MDA 24.47±0.28, and GST of 1.96±0.99. The outcome of this study indicates toxic effect of seed extract on the parameters. There was also a statistically significant reduction in LH, FSH, testosterone, E2 with evident alterations in the histomorphology of the ovary and testes.
Conclusion:this study showed scientific evidence of the toxic effect of *Datura metel* seed extract on reproductive parameters.

Keywords: Datura metel, Lead acetate, Histomorphology, Biochemical Evaluation, Ovary, Testes.

1. INTRODUCTION

The World Health Organization defines infertility as "a disease of the reproductive system which is seen as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility in a couple who have never given birth is known as primary infertility while remaining infertile after a prior pregnancy is known as secondary infertility. Infertility could be due to a number of contributing factors which include; infection, hormonal imbalance, diseases of the reproductive

system and substance misuse. Substance misuse can negatively impact the reproductive health of both males and females in different ways. It may contribute to serious health problems such as infertility, infections, organ damage and cancer. There is notable decline in male fertility as evidence points at a steadily progressive decline of sperm concentration over the past 35 years [2]. Female infertility on the other hand, has been seen to affect an estimated 48 million women [3], with women experiencing the highest rates of infertility in Central/Eastern Europe, North Africa/Middle East, South Asia, and Sub-Saharan Africa. The interest in the possible effects of lifestyle choices and environmental factors on fertility has been reignited by these studies. It is crucial to discover avoidable factors in order to reduce the burdens on public health and the social expenses associated with male infertility. According to a study by [4],nearly 25% of men under 35 use recreational drugs, which have been shown to have negative effects on reproductive health. For example, cannabis smoking can harm male fertility by affecting the hypothalamus-pituitary-gonadal axis, spermatogenesis, and sperm function [5]. Understanding the effect of substance misuse on the reproductive system of both sexes is crucial to making informed health decisions.

Plants have the ability to treat and manage certain diseases and have been utilized for this purpose in various countries. Their medicinal properties come from their bioactive phytochemicals, which have specific effects on the body. These chemicals include tannins, phenolic chemicals, terpenoids, flavonoids, alkaloids, and essential oils. However, some of these chemicals can be harmful if consumed in large amounts [6] [7].

Datura metel is an herbaceous plant in the family Solanaceae, which is used traditionally for its medicinal properties. The plant is found in different parts of the world and is also known as Angel's trumpet or Devil's trumpet. In many parts of Nigeria, although it is occasionally grown, *Datura metel* is mostly found growing as a weed in waste sites and/or abandoned farmlands[8]. The plant's many parts; leaves, fruits, and seeds can be utilized in a variety of ways and for a wide range of applications, largely due to its psychoactive properties. This may lead to certain young people who use *Datura metel* more frequently and are more vulnerable to the risks associated with drug and smoking misuse and abusing its various forms. *Datura metel* is a strong poison, and using the plant components carelessly can cause acute poisoning that can be fatal as well as delirium[8]. *Datura metel* contains various alkaloids such as

scopolamine, hyoscyamine, and atropine. These alkaloids have been reported to have toxic effects on the reproductive system [9].

One of the main defense systems in mammals is the chain reaction between oxidative stress and inflammatory responses. Nevertheless, persistent inflammation or oxidative stress can start and advance a number of illnesses [10]. In several studies, it has been shown that *D. metel* and the compounds produced from it exhibit anti-inflammatory and antioxidant properties [11] [12]. Organs, including the nervous system, may be protected by a chemical with anti-inflammatory and/or antioxidant properties [13]. Moreso, *D. metel* has a higher level of antioxidant properties than other species like *D. stramonium*[14]. The aqueous extract of *D. metel's* seed and leaf is projected to have an antioxidant effect at concentrations of 1.5 and 2.5 mg/ml, respectively; researchers also discovered that it causes cardiac arrest to last for 35–37 minutes, compared to the heart's typical survival period of 14 minutes [15]. Datura species are highly toxic and psychoactive, particularly their seeds and flowers, and can result in delirium, arrhythmias, fever, hallucinations, respiratory depression, anticholinergic syndrome, psychosis, and even death. Throughout history, different groups have occasionally employed Datura species as hallucinogens in addition to poisons and medicines due to their impacts and symptoms [16]. The narcotic property of *Datura metel* has increased its consumption in our society today, thereby making it important to carry out this study which is targeted to provide scientific evidence on the effect of *Datura metel* seed extracts on the reproductive parameters of albino rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty-five (25) Sprague dawley rats, weighing 180-240g were used for this study.

These animals were purchased from the Animal house of the Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State. They were grouped into five (5) groupsof five (5) rats each and maintained under normal laboratory conditions. They were allowed access to standard feed (finisher) and water *ad libitum*. Their beddings were changed and cages cleaned daily. The albino rats were allowed to acclimatize for two weeks with 12 hours light/dark exposure.

2.2 Plant Identification

The plant was identified and authenticated and voucher specimen deposited with voucher number NDUP/24/02 at the Herbarium of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.3 Ethical approval

Ethical approval was obtained from the College Ethics Committee, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State.

2.3*Datura Metel* Extracts

The LD50 of the *Datura metel* seed extracts was performed using Fixed Dose method[17]. Briefly, nine (9) female rats were divided into three subgroups of A as follows: A1, A2, A3. Group A1, A2 and A3, were given 300, 500 and 1000mg/kg of the seed extract of *D. Metel*. The animals were observed for 48 hours for signs of toxicity. There were no signs of toxicity using the dosages of the different extracts used for this study. Therefore, this study adopted 150, 300 and 600mg/kg of *D. Metel* extracts for use.

2.4 Dosage Calculation

The dosages were determined using the formula:

Dose = [Weight of Rat/1000] x Dosage = [238/1000] x 150mg/kg = 35.7mg

This amount of the extract was weighed and dissolved in 2.4ml of distilled water and given to each rat in group 3 (seed extract) according to the OECD guidelines (2001).

2.5 Experimental Design

Twenty-five animals were placed into five (5) groups with five (5) albino rats in each group.

Group 1 rats served as the negative control receiving just the commercial feed and water.

Group 2 rats were administered0.5ml of 300mg/kg of a known neurotoxic agent, lead acetate.

Groups 3 rats received 150 mg/kg body weight of *Datura metel*hydroethanoic seed extract.

Group 4 rats received 300 mg/kg body weight of *Datura metel*hydroethanoic seed extract.

Group 5 rats received of 600 mg/kg body weight of *Datura metel*hydroethanoic seed extract.

Throughout the investigation, all of the animals had access to food and water.

2.6 Study Duration

This study spanned for ‘duration of five (5) weeks (May 2023 to July 2023). Two weeks for acclimatization, 1 week for acute study and two weeks for administration of *Datura metel* seed extract for chronic study [18].

2.7 Route of administration

Datura metel seed extracts were administered orally by means of gavage intubation.

2.8 Sample Collection

At the end of the period of administration with the *Datura metel*seed extracts, the albino rats were sacrificed after an overnight fast on the 15th day. They were anaesthesized using chloroform. The ovaries and testes of the animals were harvested, rinsed and fixed in 10% formal saline for histological analysis. Blood sample was obtained by cardiac puncture from each animal into plain bottles for laboratory analysis.

2.9 Histological Analysis

An automatic tissue processor model:Leica TP 1020was used to process the tissue, after which tissue was sectioned using a rotary microtome to provide thin sections.Haematoxylin and eosin staining technique [19]was used to stain the tissues at the Niger Delta University Teaching Hospital, (NDUTH) Okolobiri.

2.10 Biochemical Analysis

Enzyme linked immunosorbent assay (ELISA) method [20]was used in the estimation of the hormone and antioxidant parameters. Accu Bind Elisa kit with lot number EIA- 12K1C4 was used.

2.11 Statistical Analysis

Data was analyzed with Statistical Package for Social Sciences (SPSS) program (SPSS Inc., Chicago, IL, USA; Version 23) and expressed as mean ±SD. One Way Analysis of Variance (ANOVA) was also used for comparison within the groups. Data is presented using mean ± standard deviation (Mean ± SD) for all quantitative values with $p \leq 0.05$ being considered statistically significant.

3 RESULTS AND DISCUSSION

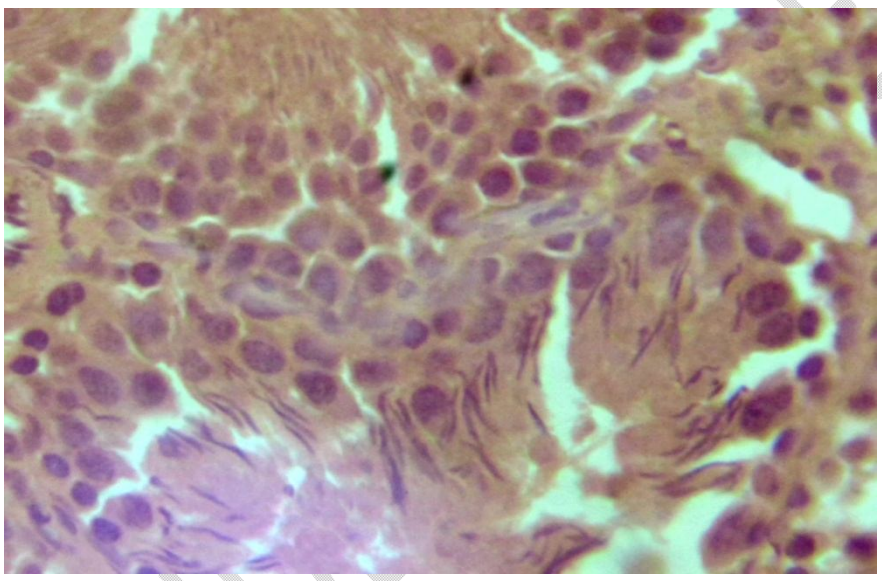

PLATE 1: Photomicrograph of Negative Control (Group 1) albino rats showing seminiferous tubules with sertoli cells (SC), spermatogonia (SG), primary spermatocytes (PS) and spermatids (ST). H&E Stained Section X400.

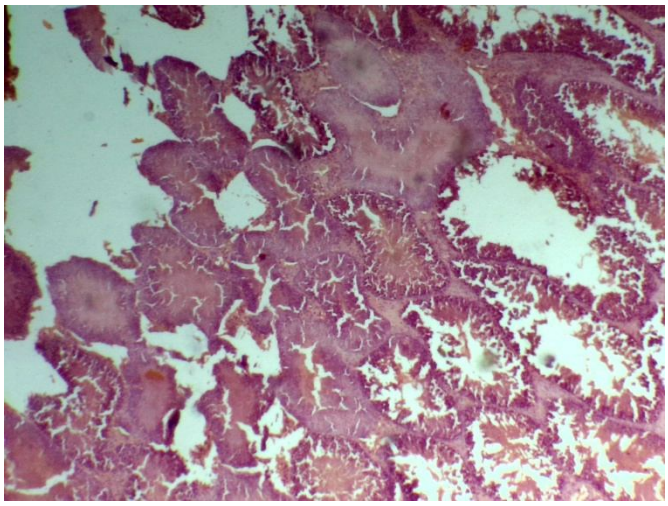

Plate 2: **Photomicrograph of positive control (Group 2) albino rats** showing seminiferous tubules (S), sloughing of germ cell (SGC) elements into tubular lumen and necrosis (N) in the interstitial region. H&E stained section X100.

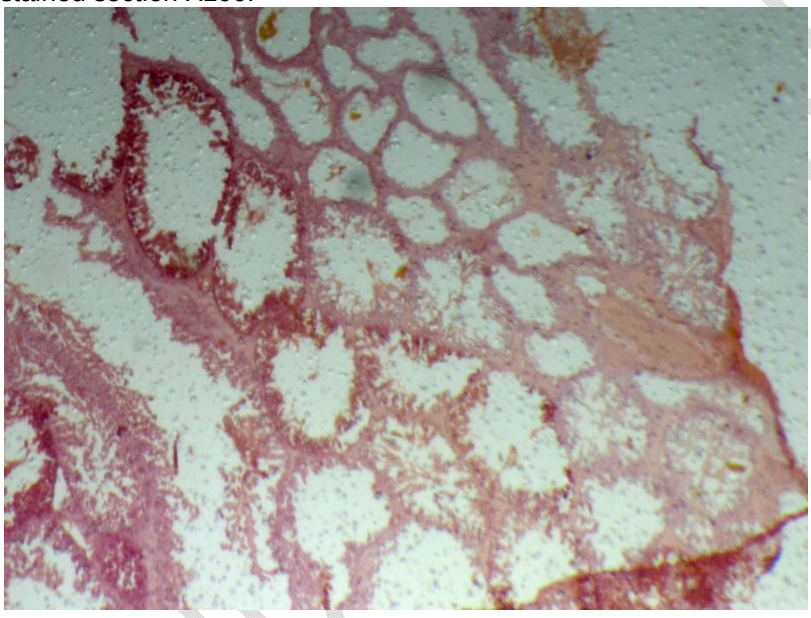

Plate 3: **Low power photomicrograph of Group 3 Albino Rats** that were administered 150mg/kg *Datura metel* seed extract. Section showed loss of spermatogenic cells and tubular atrophy. H&E stained section X100.

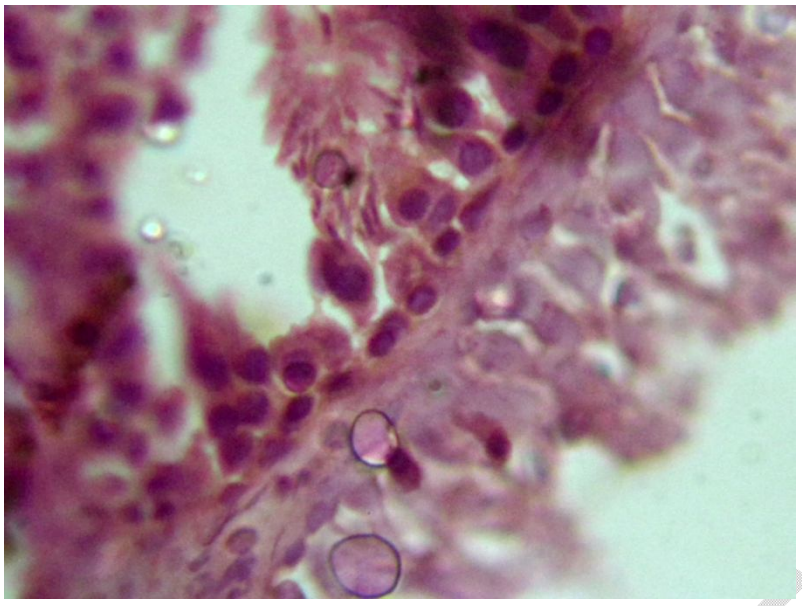

Plate 4:High power photomicrograph of Albino Rats in Group 4 (administered 300mg/kg of *Datura metel* seed extract). Section shows fibroblasts (F), spermatogonia (SG) and necrotic area (N). H&E stained section X400.

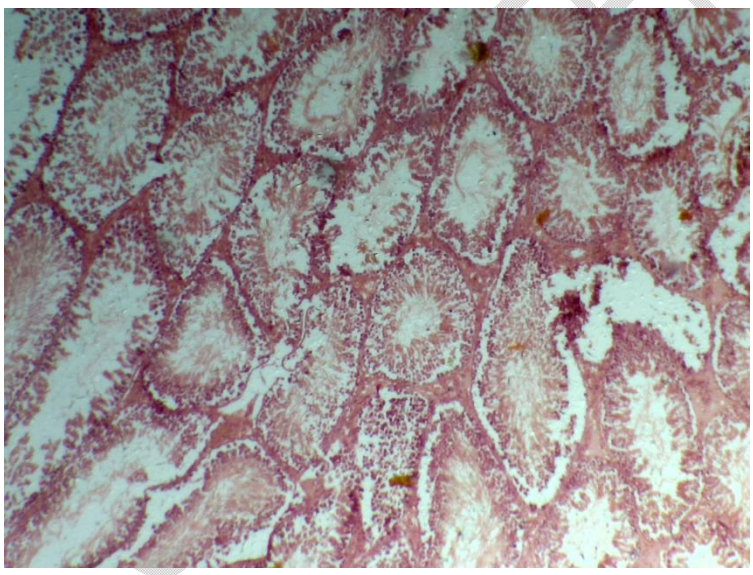

Plate 5: **Photomicrograph of Albino Rats in Group 5** that were administered 600mg/kg of *Datura metel* seed extract. Section shows seminiferous tubules (S) with some cells detached from membrane. Distorted epithelium is evident (DE). H&E stained section X 100.

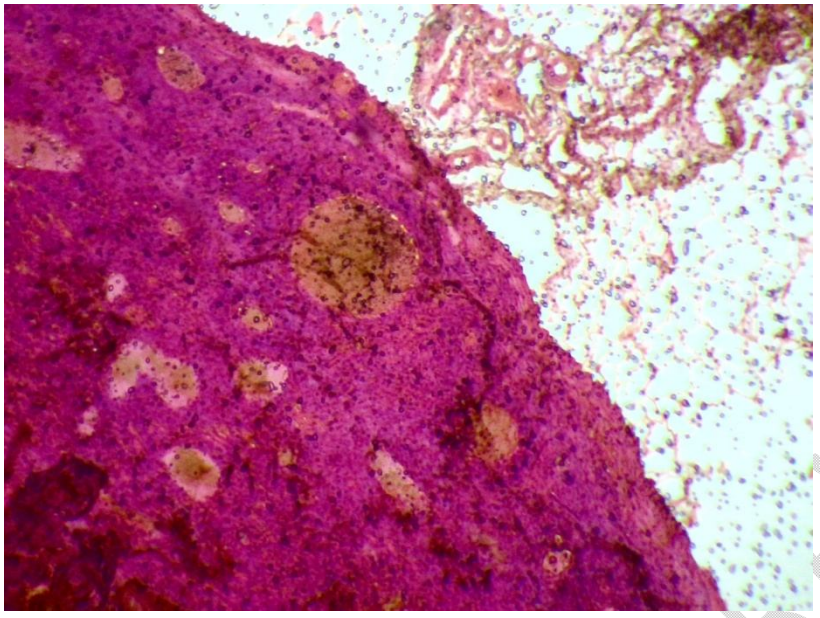

Plate 6:Photomicrograph of an ovary from Negative Control (Group 1) Albino Rats. Section shows unilaminar primary (UF), multilaminar primary (MF) follicles, oocytes (O), granulose (follicular) cells (G), germinal epithelium (E) and Tunica albuginea (A). H&E Stained Section X100.

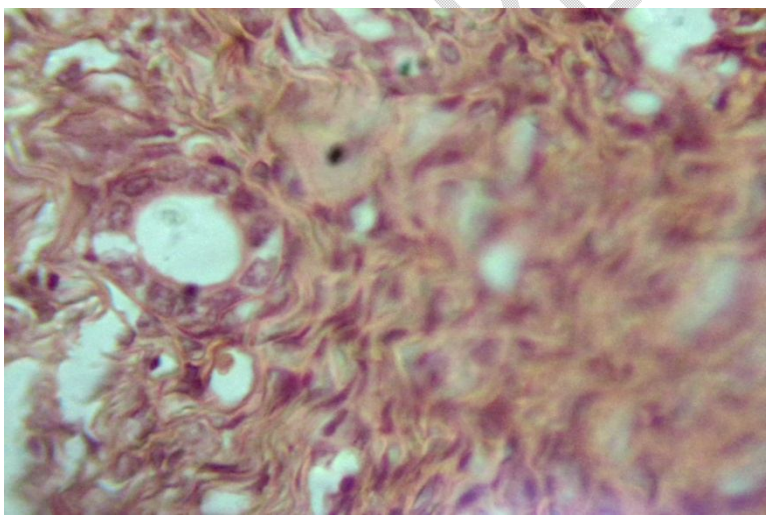

Plate 7:Photomicrograph of an Ovary of Group 2 Albino Rats (positive control). Section Shows Granulosa Cell Layer (G), Unilaminar primary (UF), Follicular Atrophy and Fibrous Connective Tissue. H&E Stained Section X400

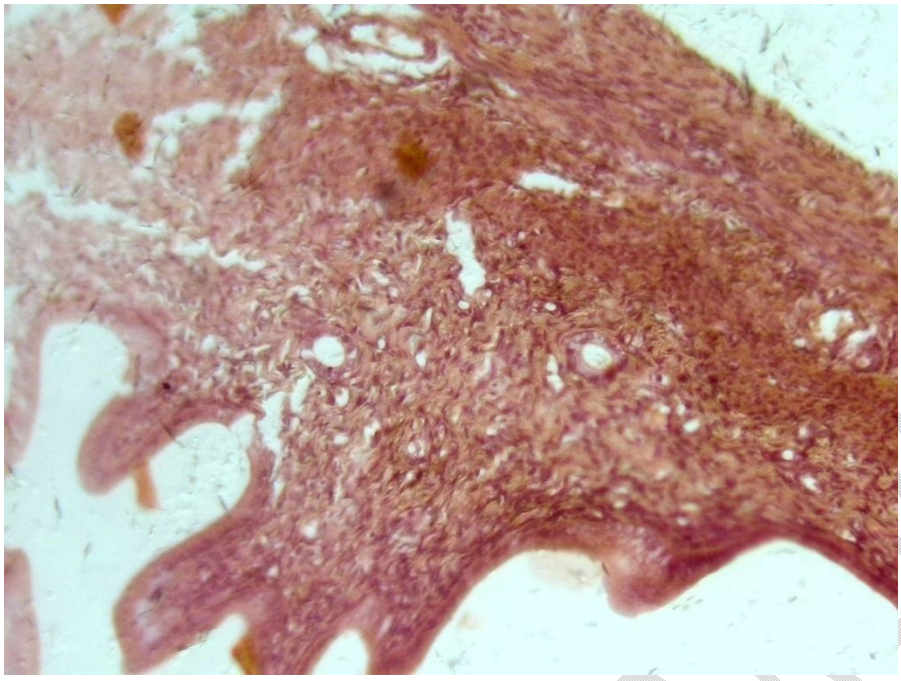

Plate 8:Photomicrograph of an ovary of Albino Rats in group 3 that received 150mg/kg *Daturametel* Seed Extract. Section is Illustrating the Germinal Epithelium (E), Unilaminar Primary (UF) Follicles and Fibrotic Connective Tissue (CT) with Fibroblast all around. H&E Stained Section X100.

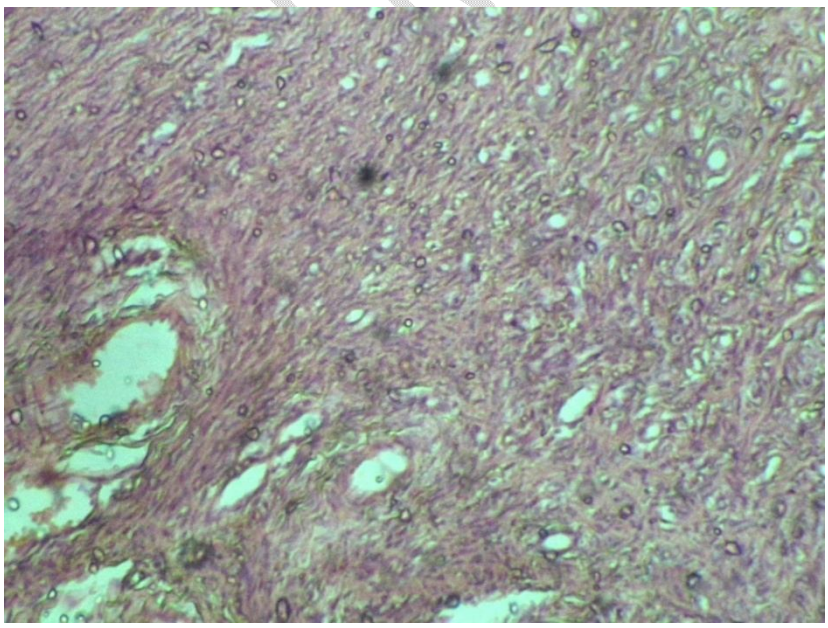

Plate 9: **Photomicrograph of the Ovary of Group 4 Albino Rats** that received 300mg/kg *Datura metel* Seed Extract. Section Illustrated Unilaminar Primary (UF) Follicles and the Multilaminar Primary (MF) Follicles. Cellular and Follicular Degeneration are noted. H&E Stained Section X100.

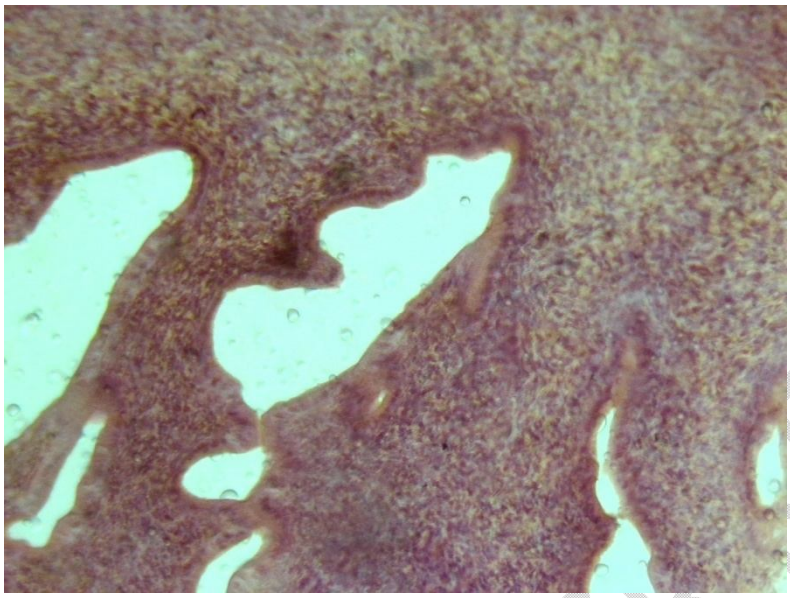

PLATE 10:Photomicrograph Section of an Ovary of Group 5 Albino Rats that received 600mg/kg *Datura metel* Seed Extract. Section illustrates Fibrotic Connective Tissue and Follicular Atrophy. H&E Stained Section X40.

Table 1: Comparison of Antioxidant Parameters in rats that received *D.metel* Seed Extract

	SOD	CAT	MDA	GST
Group 1 (n=5)(Negative Control)	9.43±0.43[a]	8.43±0.50[a]	15.32±4.45[a]	2.28±0.37[a]
Group 2 (n=5)(Positive Control)	5.49±0.45[b]	2.32±0.30[b]	28.01±0.46[b]	1.91±0.12[b]
Group 3 (n=5)(150mg/kg *D. metel* seed extracts)	7.51±0.72[c]	5.41±0.43[c]	22.97±0.27[c]	1.98±0.26[b]
Group 4	7.82±0.29[c]	5.92±0.28[d]	22.15±0.22[d]	1.96±0.14[b]

(n=5)(300mg/kg *D. metel* seed extracts)				
Group 5 (n=5)(600mg/kg *D. metel* seed extracs)	6.40±0.39[d]	4.98±0.21[e]	24.47±0.28[e]	1.96±0.99[b]
p-value	<0.001	<0.001	<0.001	<0.001
F-value	56.193	99.727	17.237	38.219

Values with different superscripts are significantly different from each other (p≤0.05)

Table 2 Comparison of Reproductive Hormones in Rats that received Seed Extract

	FSH	**E2**	**LH**	**TESTO**
Group1 (n=5)(Negative Control)	1.55±0.17[a]	5.17±1.80[a]	0.20±0.07[a]	0.44±0.01[a]
Group 2 (n=5)(Positive Control)	1.10±0.03[b]	4.01±0.86[b]	0.12±0.10[b]	0.22±0.01[b]
Group 3 (n=5)(150mg/kg *D. metel*seed extract)	1.20±0.14[c]	4.39±0.42[c]	0.16±0.04[c]	0.34±0.04[c]
Group 4 (n=5)(300mg/kg *D. metel*seed extract)	1.30±0.26[c]	4.39±042[c]	0.15±0.01[c]	0.35±0.03[c]
Group 5 (n=5)(600mg/kg *D. metel* seed extract)	1.23±0.27[c]	4.44±0.42[c]	0.19±0.34[c]	0.31±0.08[d]

p-value	0.032	0.023	0.036	0.014
F-value	1.668	0.288	1.288	97.760

Values with different superscripts are significantly different from each other ($p \leq 0.05$)

Superoxide Dismutase (SOD)'s primary role is to catalyze the superoxide anion's disproportionation into hydrogen peroxide (H_2O_2), thereby minimizing the harmful consequences of this free radical. In contrast, the enzyme catalase transforms the hydrogen peroxide that SOD normally produces into water and molecular oxygen [9]. The data from this study show that there was a significant decrease in SOD level of rats exposed to lead acetate (positive control) when compared to the negative control group. The mean levels of (SOD) in the groups administered with *Datura metel* seed extracts were significantly higher than the SOD levels in the group administered with lead acetate (positive control), and significantly lower than the SOD level of the negative control group. More so, the catalase (CAT) enzyme levels of the test groups were significantly higher than the positive control and significantly decreased when compared CAT level of the negative control group.

There was no significant difference with the Glutathione S-transferase (GST) levels of groups that were exposed to *Datura metel* seed extracts when compared to the positive control group. However, the test groups showed a significant decrease in GST levels when compared to the negative control group. This finding does not corroborate with the research work of [15] that showed a significant increase in the SOD and CAT activity of hearts of frogs treated with aqueous extract of leaves and seeds of *Datura metel* when compared to the normal heart.

Malondialdehyde (MDA) on the other hand, is a known end result of lipid degradation, and because of its strong correlation with the breakdown of cell membranes, it is regarded as the primary indicator of oxidative stress[21].This study showed that the level of malondialdehyde (MDA) in groups that received *Datura metel*hydroethanoic seed extracts were significantly raised than the MDA levels of the negative control group and significantly lower when compared to the positive control group. This is not in line with the work of [15] who revealed that the reduction in MDA content suggests that the aqueous extract of the leaves and seeds of the tested plant may have a positive effect on the lipid peroxidation induced by

hydrogen peroxide. It also agrees with [21] were they observed similar outcome with the aqueous extracts of *Nigella sativa* seeds and *Allium sativum* as well as that of the leaves of *Rosmarinus officinalis*. This decrease in the activity of SOD, GST and CAT activity recorded in this study could partly explain the increase in the level of lipid peroxidation (MDA) observed upon exposure of the albino rats to *Datura metel* extracts.

One of the indicators of toxicity is weight loss. In this research, a significant reduction in the weight of the animals was observed among the lead (positive) control groups and across the groups that received *Datura metel* seed hydroethanoic extract when compared to the negative control group. This reduction in weight is in line with the work of [22] who recorded a significant decrease in weight of epididymis of rats exposed to *Datura stramonium* extract. It also agrees with [23] that reported remarkable weight loss in the final body weights of lead-intoxicated rats when compared to the healthy controls.

The histological analysis done in this study signify that *Datura metel* seed extract had a deleterious effect on the histoarchitecture of the ovaries and testes of animals exposed to the extract when compared to the negative control. The histomorphological changes observed in the ovary include, fibrotic connective tissues, cellular degeneration and follicular atrophy. Consequently, the testicular tissue of rats exposed to the seed extract showed the following histomorphological aberrations, loss of spermatogenic cells, tubular atrophy, necrosis and distorted epithelium. Moreso, this present work vividly demonstrates the significant damage caused by *Datura metel*hydroethanoic seed extract through reactive oxygen species (ROS) to the ovary and testicular tissue. The increased MDA levels observed in rats exposed to this extract sufficiently indicates the significant degree of lipid peroxidation that occurred in the tissues. It is already established that MDA is an end point product of lipid peroxidation hence considered a key biomarker of oxidative stress [21]. Furthermore, the specific mechanism through which the alkaloids present in the seed extracts promote cellular degeneration correlates with the involvement of the increased activities of MDA and reduction in SOD, CAT and GST levels of groups administered *Datura metel* seed extracts. These findings are in concordance with the work of [24] who noted the degeneration of brain cells as well as hepatocytes in mice.

The reproductive hormone results of this research revealed that *Datura metel* seed extract administration led to decreased levels of Follicle Stimulating Hormone (FSH), Estradiol (E2), Luitenizing Hormone (LH) and Testosterone (Testo). This is in agreement with the work of [22] that noted a decreased FSH, Testosterone and LH levels in rats upon administration of *Datura stramonium* methanolic seed extracts. This reduction in production of hormones can be tied to the cellular degeneration, tubular and follicular atrophy as observed on histological sections.

4. CONCLUSION

Over the years, there has been increased concern on the possible adverse effects of environmental factors including exposure to different plant derived substances on the reproductive parameters, one of such plants being *Datura metel*. This study was therefore carried out to evaluate the effect of *Datura metel* seed extracts on reproductive parameters. The outcome showed that there were alterations in the histoarchitecture of the ovary and testes of albino rats exposed to seed hydroethanoic extracts of *Datura metel*compared to those not exposed.

Exposure of albino rats to *Datura metel*seed hydroethanoic extracts impacted the Follicle Stimulating Hormone, Luteinizing Hormone, testosterone and estradiol levels negatively as compared to the negative control groups. There was also asignificant decreasein Superoxide Dismutase, catalase and Glutathione-S-Transferase (GST) activity followed by an increase in malondialdehyde level in albinoratswhen compared to the negative control groups consisting of animals not exposed to *Datura metel*seedhydroethanoic extracts.

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