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Research Article

Effects of the Methanolic Leaf Extract of *Abrus precatorius* Linn on Body weight, Sperm Morphology and Viability in Wistar Rats

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ABSTRACT
Male factor infertility is a critical component in animal reproduction that is often caused by low sperm count or poor sperm motility. Because treatments are expensive and invasive, there is growing interest in phytotherapy. While many plant products are claimed to boost fertility, most of these claims have not yet been scientifically proven or reported. The effect of methanolic extract of *Abrus precatorius* linn leaf on body weight, sperm morphology and viability in male Wistar rats was evaluated in this study. Fifty-two (52) male Wistar rats were divided equally into 4 groups (10 rats per group), and weighed on day 0, 7, 14, 21 and 28. Group A were administered distilled water (Control group) while Group B, C and D received 200, 400 and 800 mg/kg of the extract respectively. Rats were randomly selected and sacrificed on days 14 and 28 for histological analyses of their testes and epididymis tissue. Results revealed that all extract-treated groups (200, 400, and 800 mg/kg) experienced a significant decrease ($P < 0.05$) in body weight from day 7 through day 28 lower than the control group, but showed a significant increase ($P < 0.05$) in normal sperm morphology (healthier structure) on both days 14 and 28. Similarly, sperm viability showed a significant increase ($P < 0.05$) in live, viable sperm cells on both days 14 and 28. While the methanolic extract of *Abrus precatorius* leaves causes weight loss, it appears to improve overall sperm quality by preserving both morphology and viability in male rats.

Keywords: *Abrus precatorius;* Body weight; Methanol extract; Sperm Morphology; Sperm Viability; Wistar Rats

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INTRODUCTION

Infertility is clinically defined by the World Health Organization (WHO) as the inability of a couple to achieve pregnancy after 12 months of regular, unprotected sexual intercourse (WHO, 2025). While historical and cultural narratives often disproportionately placed the burden of reproductive failure on women, modern reproductive medicine recognizes that male-associated factors contribute to approximately 50% of all infertility cases worldwide (Okonofua *et al.,* 2022). Biological foundation of male fertility relies on spermatogenesis, a highly organized dynamic process occurring within the seminiferous tubules of the testes (Gül *et al*., 2024). This complex transformation of spermatogonial stem cells into mature spermatozoa take roughly 74 days and is highly governed by the Hypothalamic-Pituitary-Gonadal (HPG) axis. Any disruption in this endocrine crosstalk-whether via primary testicular failure or secondary hypothalamic-pituitary disorders leads to endocrinopathies like male hypogonadism, severe spermatogenic arrest, and subsequent infertility

(Durairajanayagam, 2018; Bold and Swinburne, 2022; Gül *et al*., 2024). Fortunately, behavioral modifications such as transitioning to antioxidant rich herbs have shown considerable efficacy in improving sperm parameters (Bold and Swinburne, 2022).

Abrus precatorius, commonly known as Saga-saga, Rosary pea, Jequirity bean, Gunja and Crab's eye in English is a slender, perennial twining deciduous multi-branched climbing shrub that can reach heights of 10 to 20 feet when supported by surrounding vegetation (Attal *et al*., 2010; Garaniya and Bapodra, 2014). The leaves are alternate pinnately compound resembling tamarind leaves featuring 20 to 30 small oblong leaflets. The seeds are highly distinctively glossy, ovoid pods that are scarlet red in colour with a prominent black spot around the hilum. The seeds contain the protein toxin, abrin which is deadly when ingested even at a small dose (Mondal *et al*., 2017). In Nigeria, it is locally called 'Idon Zakara' in Hausa, 'Ewe ire yeye' in Yoruba and 'Otoberebere' in Igbo (Mahre *et al*., 2017).

Humans have known for ages that certain plants could treat particular illnesses, as a result, empirical data was crucial to the use of therapeutic plants (Abdallah, 2011). An empirical framework gave way to a more evidence-based approach when the fundamental causes of some medicinal plants' efficacy in treating particular illnesses began to become clear (Petrovska, 2012). The oldest type of medicine is derived from medicinal plants, which have been utilized for thousands of years in traditional medicine throughout many nations (Khan, 2014). Over the millennia, human groups have passed down empirical information about their positive impacts. Natural products are an essential source of pharmaceutical chemicals, and many contemporary medications that are derived from traditional herbal medicine are presently employed in pharmacotherapy (Khan, 2014). *Abrus precatorius* Linn (AP) is a traditional medicinal plant with a long history of use around the world. It is widely used to treat a variety of conditions, including bronchitis, jaundice, hepatitis, contraception, tumors, abortion, malaria, and more (Qian *et al*., 2022). In addition to its chemical makeup and biological activity, which are of increasing interest to the scientific community, AP is regarded as a plant with dual values of medicine and economy (Qian *et al.,* 2022). Many people employ a variety of plant-derived products that are said to have fertility-boosting properties. Such potential is said to exist in the leaf of *Abrus precatorius*. The aim of this study was to examine how methanolic extract of *Abrus precatorius* leaves affected certain semen characteristics in male Wistar rats.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh *Abrus precatorius* leaves were collected in Maiduguri, Borno State, Nigeria. It was identified by a botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria, and assigned the specimen voucher number UMM/FVM/VPB/F1/03.

Plant Extraction

The fresh leaves were collected in Maiduguri. They were rinsed with tap water and allowed to air dry at room temperature. By doing this, moisture is eliminated, microbiological growth is hindered, and shelf life is maintained. Using a wooden mortar and pestle, the dry material was ground into 231 g of powder, which was then extracted using methanol in a soxhlet extractor at a ratio of 1:5 (w/v). Simple distillation and evaporation were used to concentrate the extracted material. After obtaining the extract yields (61.6 g), the dried extract was labeled and kept in an airtight container at 4^{o}C in the refrigerator until needed.

Experimental animals

For this investigation, 52 adult male albino rats were used. 40 for the primary study and 12 for acute toxicity. The National Veterinary Research Institute (NVRI) in Jos, Plateau state, is where the rats were purchased from a private breeder. They were housed in the University of Maiduguri's Faculty of Veterinary Medicine's animal house and given 2 weeks to acclimate. Water and standard feed were freely available. Throughout the course of the study, their initial body weights were measured and then every week after that.

Ethical Approval

The University of Maiduguri's Faculty of Veterinary Medicine Animal Utilisation Protocol and Ethical Committee granted ethical clearance and permission (AUP-R001/2023).

Acute Toxicity (LD_{50}) Study

Lorke's approach (Lorke's, 1983) was used for the acute toxicity test (LD50). This is divided into two stages. Phase 1: The animals were split up into three groups (I, II, and III) of three rats each, and they were given oral methanolic leaf extracts of *Abrus precatorius* at single test dosages of 10, 100, and 1000 mg/kg body weight. After then, the rats were watched for behavioral changes and general toxicity signs and mortality. The time gap between Phase 1 and Phase 2 was 24 hours. Phase 2: In this phase, the rats were also grouped into three (IV, V and VI) with

one rat in each group and a graded dose of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg body weight was administered orally to each group respectively.

Semen Analysis

The rats were splitted up into four groups (A, B, C, and D), each consisting of ten rats. For 28 days, the rats were given oral doses of the extract at 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight, respectively. Group A was administered distilled water orally and acted as a control. For the first day of the experiment, the rats were closely monitored every hour. After that, they were monitored every day for any obvious indications of weakening or inactivity. Three rats were randomly chosen from each group on days 14 and 28, and they were humanely slaughtered to assess the viability and morphology of the sperm. Sections of testes and epididymis were harvested and fixed in 10 % formalin for histological study.

Sperm Viability

The viability and sperm cell morphology examination was done using a colorant constituted by nigrosin, eosin dissolved in distilled water. Using a Pasteur pipette, 0.1 ml of semen from the rats and 0.1 ml of the colorant were dropped on a microscope slide and mixed with a toothpick. Another slide was used to make a smear and then allowed to dry, after which one hundred randomly chosen spermatozoa per field was evaluated under the microscope objective at x100 using oil immersion to view the sperm cells using the battlement method of counting (WHO, 2010).

Sperm Morphology

The spermatozoa from the rats in the treated groups were scored normal or abnormal, according to the strict sperm morphology criteria as described by WHO, 2010 Assessment of morphological abnormalities was classified into head, mid-piece and tail defects. Finally, the percentages of normal and abnormal shaped sperms were calculated. The cells that appear pink were counted as dead due to loss of msembrane integrity, while the white cells were counted as live. The live-dead ratio was noted and recorded. The percentage abnormalities were recorded (WHO, 2010).

Histological Evaluation

According to Drury and Wallington's (1979) and Banks's (1986) modifications, sections of the liver, kidneys, testes, and epididymis were removed, fixed in 10% formalin, and prepared for histological examination. After being dehydrated, the tissues were treated with increasingly graded alcohols (70%, 90%, and 100%), cleaned in xylene, and then embedded in paraffin wax in a 63°C oven. Standard rotatory microtome blades were used to serially segment the tissues at a thickness of 4 µm. After floating on warm water at 45°C, the tissue sections were put on egg albumin-smeared glass slides and dried at 45°C in an oven. The sections were then stained with Haemotoxylin and Eosin (H&E) for histological examination using light microscope DESC-LN-0100-MG001, Vamed Engineering, UK). Microphotographs were taken using Canon IXUS Camera, pixel: 16.5 (China).

Data Analysis

One-way analysis of variance (ANOVA) was used for parametric multiple comparisons between the control and treatment groups. The statistical software program GraphPad Instat, 3.0 (2003) was utilized for the analysis; differences were deemed significant when the p value was less than 0.05 ($p < 0.05$). The data was expressed as the Means ± S.D.

RESULTS

Acute Toxicity

There were no evidence of toxicity or mortality in the first phase but there were signs of weakness, inactivity and reduced feeding in the second phase following the administration of 5000 mg/kg of the extract. The LD50 consequently is more than 5000 mg/kg (Table 1).

Effects of Methanolic Extract of *Abrus precatorius* Linn Leaves on Body Weights

The results of the mean body weight in grams were presented in Table 2. The results showed that there were significant ($P<0.05$) decrease in body weight after one week of extract administration in groups treated with 200, 400 and 800 mg/kg compared with the control. These decreases were also observed in week two of extract administration of 400 and 800 mg/kg body weight much lower than the control. In groups treated for three weeks, there was significant ($P<0.05$) decrease in rats treated with the highest dose of the extract 800 mg/kg body weight. In the fourth week of extract administration, there was slight significant ($P<0.05$) decrease in body weight in group treated with the highest dose of 800 mg/kg compared with the control group.

Effects of Methanolic Extract of *Abrus precatorius* Linn Leaves on Sperm Morphology

Effect of methanolic extract of *Abrus precatorius* leaves on Sperm Morphology showed that there was significant increase ($p<0.05$) in normal morphology (normal appearance of head, mid-piece and tail) of sperm cells in all the treatment groups given 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of the extract as higher than the control groups. These

increases were seen in both Day 14 as well as Day 28 of the extract administration (Table 3).

Effects of Methanolic Extract of *Abrus precatorius* Linn Leaves on the Sperm Viability

The Effect of Methanolic extract of *Abrus precatorius* leaves on the sperm viability as shown in Table 4 showed significant decrease ($p<0.05$) in non-viable treatment groups (groups with weak non motile spermatozoa) on both Day 14 and Day 28 as compared to control group. There was increase in sperm viability in both Day 14 and 28.

Histological Evaluation

Histologically, the rat testis showed control and treated groups depicting normal structure without adverse effects on the testicular architecture as seen in the tunica albuginea and seminiferous tubules. The rat epididymis of the treated groups showed tubules filled with spermatozoa with epithelial cells stretched due to accumulation of spermatozoa and connective tissues Figures 2, 3 and 4).

Table 1: Acute Toxicity (LD_{50}) of Methanolic Extract of *Abrus precatorius* linn leaves by Oral Route (phase I and II) in Albino rats

Group Dose (mg/kg)	Observation	Number/percentage mortality
	First phase (n=3) per group)	
10	Rats' behavior and activities were normal	0(0) %
100	Same as group one	0(0) %
1000	Same as group one	0(0) %
	Second phase (n=1 per group)	
1600	Rats' behavior and activities were normal	0(0) %
2900	Same as group one	0(0)%
5000	Weakness, reduced motility and appetite	0(0) %

Key: n = number of rat(s)

Table 2: Effects of Methanolic Extract of *Abrus precatorius* linn leaves on the Body Weight (Mean ± SD) in Albino rats

Group Dose (mg/kg)	0 Days	7 Days	14 Days	21 Days	28 Days
Control	188.22±32.80	247±40.79	216.23±34.18	210.06±36.27	220.23±43.65
200	188.38±20.45	199.09±21.59**	176.78±16.93	186.60±26.21	189.99±33.63
400	176.11±36.40	186.06±38.37***	168.28±35.97*	169.80±25.14	187.99±23.35
800	181.40±16.47	148.44±16.32***	145.15±17.50***	147.16±11.93**	162.80±17.91*

Key: * = slightly significant ($p<0.05$) decrease as compared with control; ** = moderately significant ($p<0.05$) decrease as compared with the control; *** = highly significant ($p<0.05$) decrease as compared with the control

Table 3: Effects of Methanolic Extract of *Abrus precatorius* linn leaves on the Spermatozoan Morphology in Albino rats

Group Dose (mg/kg)	14 Days		Means ±SD (mg/kg)	28 Days	
	Abnormal	Normal		Abnormal	Normal
Control	26.00±1.00	74.00±1.00		26.67±1.53	73.33±1.53
200	19.67±1.53	80.33±1.53***		16.00±2.65	84.00±2.65***
400	16.00±1.00	84.00±1.00***		13.33±2.31	86.67±2.31***
800	15.00±1.00	85.00±1.00***		14.67±2.31	85.33±2.31***

Key: *** = highly significant ($p<0.05$) increase as compared with the control

Table 4 Effect of Methanolic Extract of *Abrus precatorius* linn leaves on the Sperm Viability in Albino rats

Group Dose (mg/kg)	14 Days		Means ±SD (mg/kg)	28 Days	
	Viable	Non-viable		Viable	Non-viable
Control	81.00±4.58	19.00±4.58		80.33±0.58	19.67±0.58
200	88.67±2.31	11.33±2.31***		84.67±2.52	15.33±2.52**
400	92.00±1.00	8.00±1.00***		85.33±1.53	14.00±1.00***
800	91.67±1.53	8.33±1.53***		92.33±0.58	7.67±0.58***

Key: ** = moderately significant ($p<0.05$) decrease as compared with the control
*** = highly significant ($p<0.05$) decrease as compared with control

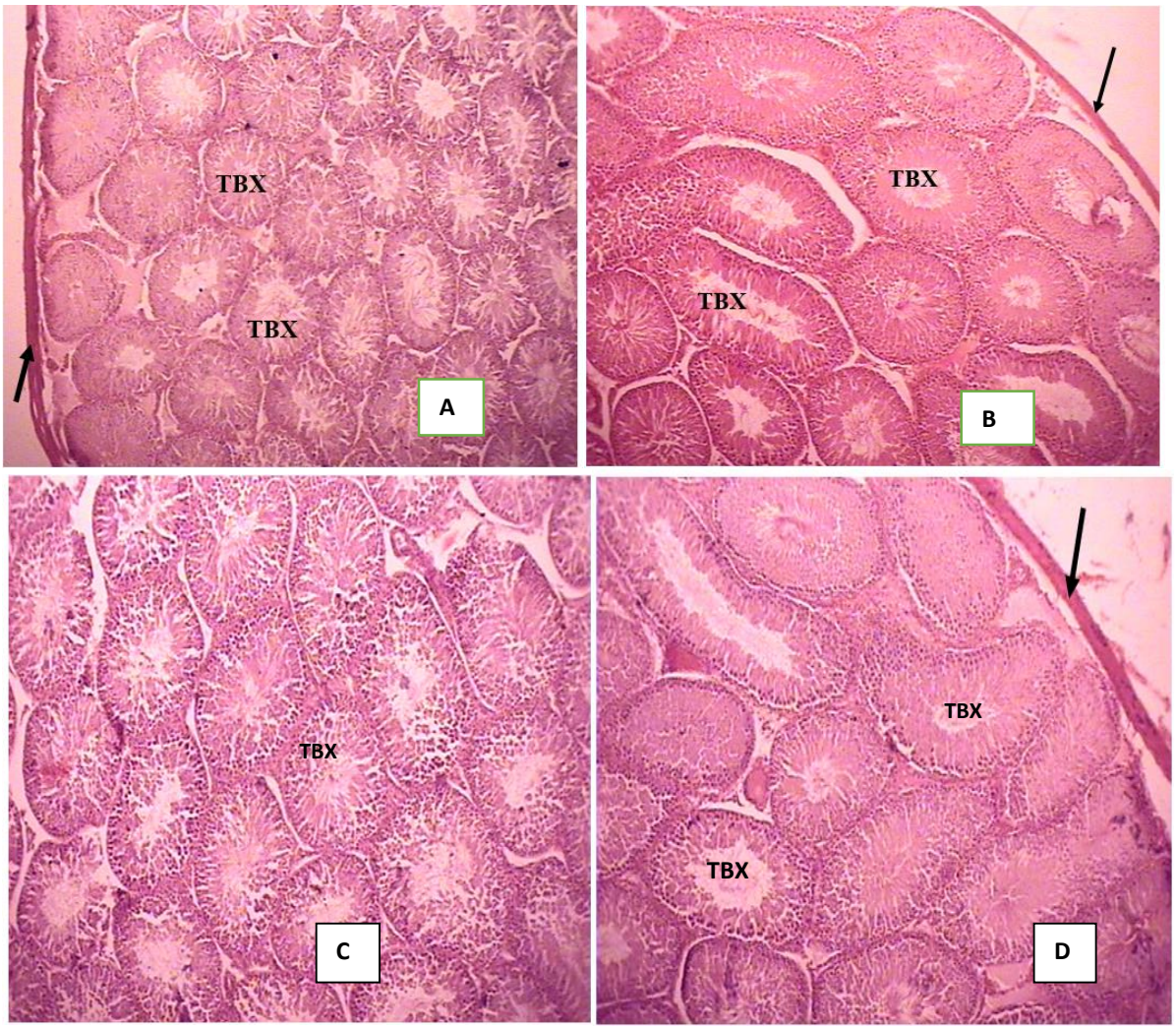

Figure 2: Photomicrograph of rat testis on day 14 showing control and treated groups depicting normal structure without adverse effect on the testis (black arrows tunica Albuginea), TBX (seminiferous tubules) H&E x100.
A=Control, **B**=200 mg/kg, **C**=400 mg/kg, **D**=800 mg/kg

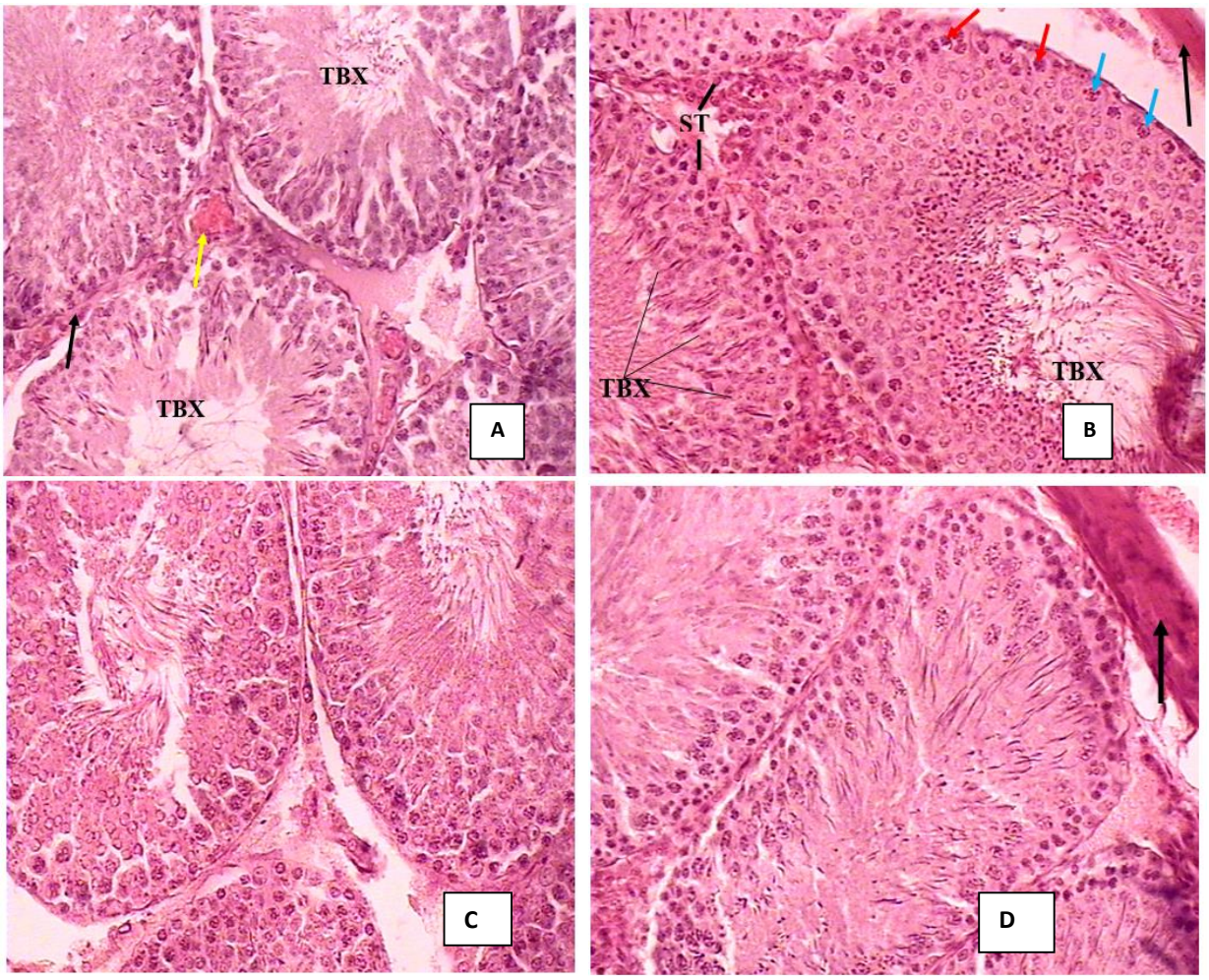

Figure 3: Photomicrograph of rat testis on day 28 of control and treated groups with extract showing normal structures of the seminiferous tubules. H&E x400
A=Control, **B**=200 mg/kg, **C**=400 mg/kg, **D**=800 mg/kg

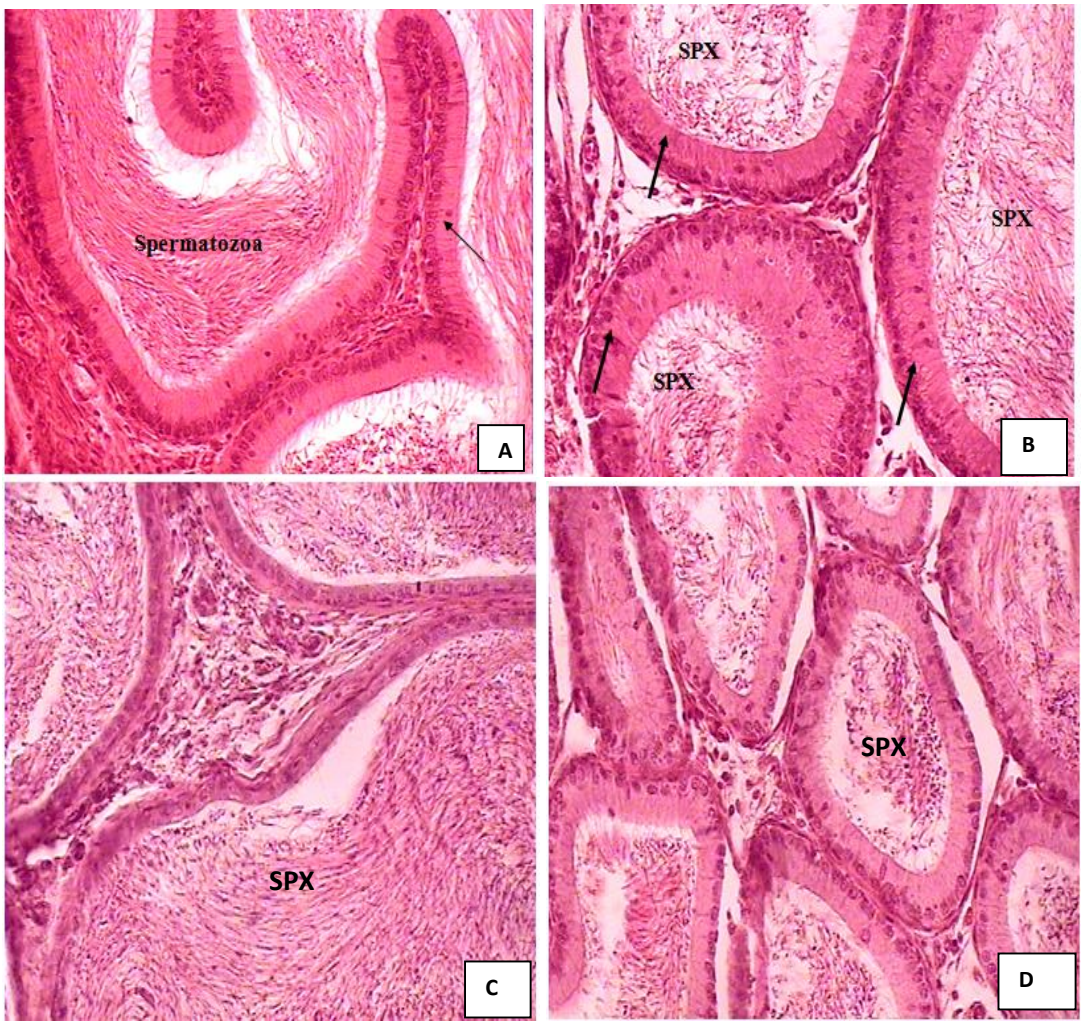

Figure 4: Photomicrograph of rat epididymis on day 28 of control and treated groups showing tubules filled with spermatozoa (SPX), epithelial cells (black arrows) H&E x400.
A=Control, **B**=200 mg/kg, **C**=400 mg/kg, **D**=800 mg/kg

DISCUSSION

The Lorke's method for testing acute toxicity in this research is in two phases. The highest dose required to be administered in Phase I (one) is 1000 mg/kg did not cause any issue. Phase II further expands the relative safety of the extract at a dose higher than 1000 mg/kg. Therefore, the methanol extract of *Abrus precatorius* leaves showed no signs of toxicity or death recorded even at highest dose of 5000 mg/kg of the extract. This correlates with the reports of Ogbueghi *et al*. (2015) and that of Okoko *et al*. (2010). It is also in agreement with the study conducted by Umamahesh and Veeresham (2016) who reported no toxicity of the leaf extract in albino rats. However, this study contrasts with the one conducted by Adedapo *et al*. (2007) who reported that aqueous extract of *Abrus precatorius* leaves is toxic in rats when administered orally. This disparity might be due to environmental and geographical variations.

There was slight decrease in body weight of animals treated with various graded doses of the extract as compared with the control group. Marked significant decreases were seen in ascending order of graded doses administered. The group that was administered 800 mg/kg dose showed substantial decrease in weight than the preceding groups. This might probably be due to reduced feeding pattern exhibited by the treated groups as compared with the control group which was keenly observed during the experiment. This drop in feeding could be a side effect of the extract which manifested as appetite suppression. Changes in body weight are pointers of adverse effects of drugs or chemicals especially if the body weight loss is more than 10% from the initial weight (Teo *et al*., 2002). This however contrasts with

the study conducted by Ogbueghi *et al*. (2015) which showed no increase or loss in body weight of the rats treated with 70% Methanolic extract of *Abrus precatorious* within the test period.

For the sperm morphology (% of normal and abnormal shaped sperm) it was observed that there was significant increase in normal sperm morphology at 14 and 28 days post treatment. These marked changes were dose dependent from 200 to 800 mg/kg body weight of administered extract which is higher than the control. Similarly, there was significant increase in sperm viability (% of live and structurally intact spermatozoa) as the dose increases. The highest dose of 800 mg/kg recorded the highest percentage of viable sperm cells. There was significant decrease in non-viable sperm cells compared to the control which implies that the extract prevented death or damage of the sperm cells. Medicinal plants contain dense matrices of alkaloids, saponins, tannins and flavonoids which serves as superb free radical scavengers that protects sperm structures from oxidative stress (Alahmadi, 2020). This finding agrees with the one conducted by Okwute *et al*. (2023) who reported that high dose extract of *Abrus precatorious* increased the serum testosterone levels significantly while the low dose extract significantly reduce the level of testosterone when compared with the control. The sperm boosting potentials of *Abrus precatorious* leaf extract was also reported by Sanya *et al*. (2024) who stated that the methanolic leaf extract of *Abrus precatorious* increased epididymal sperm count and motility in wistar rats. Similarly, Bhakta and Das (2019) studied the herbal contraceptive effect of *Abrus precatorious, Ricinus communis* and *Syzygium aromaticum* on anatomy of the testis of male Swiss Albino mice and reported that the mixture effectively worked on the male gonad (testis). On the contrary, the seed extract of *Abrus precatorious* was reported to cause reproductive disorders by many researchers. Bhatia *et al*. (2013) experimented sperm production with seed extract of *Abrus precatorius*. The intraperitoneal administration of 20 and 60 mg/kg of ethanol seed extract of *Abrus precatorius* caused a highly significant ($p< 0.001$) decrease in daily sperm production and increase in sperm production was observed in all the treated animals after 20 days of withdrawal of treatment. In the same vein, oral administration of both organic (methanol soluble at 50 and 75 mg/kg bw) and aqueous soluble *A. precatorius* seed extracts caused infertility in studied subjects at varying degrees as reported by Talukder *et al*. (2013).

CONCLUSION

In conclusion, the methanol leaf extract of *Abrus precatorius* leaves boosted the reproductive architecture (sperm morphology and viability) and reduced the body weight of male Wistar rats. While the potential for improving semen characteristics is noted, the risk of reproductive dysfunction at high doses cannot be overlooked.

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Conflict of interest

The authors declare that there is no conflict of interest whatsoever with this work.

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