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Sperm boosting potentials of methanol extract of *Abrus precatorius* (Linn) leaves on some semen characteristics in Wistar rats

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Abstract

One of the major issues in the area of reproduction is male fertility. A wide range of plant-derived products are claimed to have fertility-boosting effects and are being used by a large number of people. Many of such claims however remain uninvestigated. The effect of methanol extract of *Abrus precatorius* linn leaves on sperm headcount and motility in Wistar rats was studied. Forty (40) rats were grouped into four groups (A, B, C and D) with 10 rats in each. The rats in groups B, C and D were subjected to repeated doses of the extract at 200 mg/kg, 400 mg/kg and 800 mg/kg b.w respectively orally for 28 days. Group A served as negative control and was administered distilled water. On days 14 and 28, three rats were randomly selected from each group and humanely sacrificed. Sections of the liver, kidneys, testes and epididymis were harvested and processed for histological study. The result on the sperm counts for twenty-eight days showed a significant ($P<0.05$) increase in the mean values of testicular sperm counts in groups treated with the extract. This is evident especially in groups treated with 800 mg/kg b.w with mean values 13.92±0.76, 16.00±0.66 and 17.00±0.25^3/mm^9 as compared with the control. The sperm motility was classified into three categories: progressive, in situ and immobile. Also, there was a significant increase in sperm progressive motility and viability at 14 – 28 days post-treatment. The result of the study revealed that methanol extract of *Abrus precatorious* leaves increased epididymal sperm count and motility in male Wistar rats.

Keywords: *Abrus precatorius* leaves, Methanolic, Semen, Headcount, Motility, Wistar Rats

Introduction

Male fertility is one of the most significant concerns in reproduction. Male fertility depends on normal structure and functions of the male reproductive organs which produces healthy and viable spermatozoa (Agarwal *et al.,* 2021). Spermatozoa are produced in the seminiferous tubules of the testes via a complex hormone regulated process of spermatogenesis, while complete maturation and motility occurs in the epididymis (Dalia *et al.,* 2019). The quality of Semen is a main indicator of male fertility. One of the key culprits in rising cases of male infertility is deteriorating semen quality. Any factor

that affects the quality of semen can invariably affects male fertility (Kumar & Singh, 2022). A lot of factors that predispose to infertility in male includes environmental pollution, hormonal, lifestyle, genetic, congenital malformations amongst others (Chiang *et al.,* 2017). From time immemorial, phytomedicine has been utilized in healing different ailments and currently provides a source of inspiration for novel drug compounds. An estimate of 75-90% of the rural population of the world still relies on herbs for their healthcare (Chukwuma *et al.,* 2015; Soni & Singh, 2019). So many phytochemical studies have steadily established that consumption of foods from plants rich in bioactive phytochemicals have healing and protective properties against different human ailments (Soni & Singh, 2019). This is because they contain secondary metabolites that are very important and beneficial clinically (Mikail *et al.,* 2022). *Abrus precatorius,* is an herbaceous, perennial seed propagated plant that is a woody twinning vine plant with characteristic toxic red seeds and black mark at the base (Chaudhari *et al.,* 2012). Commonly known as jecquirity bean, or "Crab's Eyes" or rosary pea in English. It has been identified as a plant with beautiful seeds that have been used in traditional treatments by many cultures for decades and it has a wide range of medicinal benefits (Attal *et al.,* 2010). It is native to India, at altitudes up to 1200 m on the outer Himalayas. It is now naturalized in all tropical countries (Attal *et al.,* 2010). In Nigeria, it is locally known as 'Idon zakara' in Hausa, 'Ewe ire yeye' in Yoruba and 'Otoberebere' in Igbo (Mahre *et al.,* 2017).

According to ethno-botanical literature, the genus *Abrus* is widely used for a range of conditions in African traditional medicine. The plant is now considered as a valuable source of unique natural products for the development of medicines against various diseases and for the development of industrial products (Anant & Maitreyi, 2012; Sonali & Shonkor, 2020). A wide range of plant-derived products are claimed to have fertility-boosting effects and are being used by a large number of people. *Abrus precatorius* leaf is alleged to possess such potential. This study aimed to investigate the effects of methanolic extract of *Abrus precatorius* leaves on some semen characteristics in male Wistar rats.

Materials and Methods

Experimental animals and management

Fifty-two adult male albino rats were used for this study; 12 for acute toxicity and 40 for the main study. The Rats were acquired from a private breeder in National Veterinary Research Institute (NVRI) in Jos, Plateau state. They were kept at the animal house of the Faculty of Veterinary Medicine, University of Maiduguri and allowed to acclimatize for 14 days. Standard feed and water were provided *ad libitum*. Their initial body weights were measured, and thereafter, on weekly basis throughout the period of the study.

Ethical approval

Ethical clearance and approval (AUP-R001/2023) was issued by the Faculty of Veterinary Medicine Animal Utilization Protocol and Ethical Committee, University of Maiduguri.

Plant collection and identification

Fresh leaves of *Abrus precatorius* were collected at London Ciki area in Maiduguri, Borno State, Nigeria. The leaves were identified and authenticated by a Botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria with specimen voucher number UMM/FVM/VPB/F1/03.

Plant extraction

The fresh leaves were cleaned, washed with tap water and air - dried at room temperature. This is to remove moisture, prevent microbial growth and preserve shelf life. The dried material was pulverized using wooden mortar and pestle to get 231g powder for extraction using methanol in a soxhlet extractor at 1:5 (w/v). The extract obtained was concentrated by simple distillation and evaporation. The yields (61.6g) of the extract were obtained and the dried extract was labeled and stored in an airtight container in the refrigerator at 4^{o}C until further use.

Phytochemical analysis

Phytochemistry was done to detect the presence or absence of such constituents as alkaloids, flavonoids, glycosides, tannins, terpenoids and carbohydrates using the standard procedures (Ncube *et al.,* 2008; Tiwari *et al.,* 2011).

Acute toxicity (LD_{50}) study

The Acute toxicity test (LD_{50}) was done using Lorke's method (Lorke's, 1983). which consists of two phases. Phase 1: In this phase, the animals were divided into three groups (I, II and III) of three rats each and administered respective single test doses of 10, 100 and 1000 mg/kg body weight of the methanol leaf's extracts of *Abrus precatorius* by the oral route. The rats were then observed for 24 hours for behavioural

changes, 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 divided into four groups (A, B, C and D) with 10 rats each and were subjected to repeated doses of the extract at 200 mg/kg, 400 mg/kg and 800 mg/kg body weight respectively for 28 days. Group A served as a control and was given distilled water through the oral route. The rats were keenly observed on an hourly basis for the first day and afterwards daily throughout the experiment for any visible signs of weakness or inactivity. On days 14 and 28, three rats were randomly selected from each group and humanely sacrificed for the determination of sperm volume (Head Count) and sperm characteristics (Motility). Sections of testes and epididymis were harvested and fixed in 10 % formalin for histological study.

Sperm headcount

The testes and epididymis from the sacrificed rats were dissected out. The tunica albuginea was removed from each testis before its homogenization in 5 ml of normal saline. The head, body and tail of each epididymis were separately homogenized in 2 ml of normal saline. The sperm head count per milliliter of the homogenate was done using a haemocytometer (Almquist & Amann, 1961; Amann & Lambiase, 1969). The total sperm head count per homogenate was determined using the formula:

Total sperm = (volume of homogenate) × (count in 5 squares) × (0.05×10^6) (WHO, 2020).

Sperm motility

Ten microliters of the extended semen from the rats were pipetted onto a clean, pre-warmed microscope slide. A cover slip was lowered onto the sample, making sure no air bubbles formed, and the sperm motility in the slide was examined using a microscope with a 40 X objective. The sperm motility of the examined spermatozoa was classified in three categories. These classifications are based on their motility: progressive, in situ and immobile. The spermatozoa with progressive motility are those with forward linear movements; *in situ*, motility refers to local or circular movements, and immobile sperm are spermatozoa without movements. At least ten widely-spaced fields were examined to provide an estimate of the percentages of motile cells obtained in each category (WHO, 2010).

Histological evaluation

Sections of the testes and epididymis were harvested and fixed in 10 % formalin and processed for histological study as described by (Carleton & Drury, 1967)) and modified by (Banks, 1986). The tissues were dehydrated and treated with ascending graded alcohols (70 %, 90 % and 100 %), cleared in xylene and embedded in paraffin wax in an oven at 63 ℃. Tissues were serially sectioned at 4 µm thickness using standard rotatory microtome blades. The tissue sections were then floated on warm water at 45 °C and were placed on glass slides smeared with egg albumin and dried in the oven at 45 °C. 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).

Statistical analysis

Data was expressed as the Means ± S.D and statistical analysis was performed using One-way analysis of variance (ANOVA) for parametric multiple comparisons between control and the treatment groups. Statistical software package, GraphPad Instat, 3.0 (GraphPad Instat, 2003). was used for the analysis. Differences were considered significant when the P value was less than 0.05 ($p < 0.05$).

Results

The phytochemical analysis of the *Abrus precatorius* leaves revealed the presence of flavonoids, cardenolides, terpenoids, tannins, carbohydrates, saponin and glycosides, while anthraquinone and alkaloids were absent as presented in Table 1. Acute toxicity study of methanolic extract of *Abrus precatorius* was evaluated using Lorke's method (Lorkes, 1983). The extract was administered orally. There was no sign of toxicity and mortality in the first phase. But, signs such as weakness, inactivity and reduced feeding were observed in the second phase following the administration of 5000 mg/kg of the extract. The LD_{50} therefore is greater than 5000 mg/kg. (Table 2). The results of the methanolic extract of *Abrus precatorius* for 14 days are presented in Table 3. The results showed no significant ($P<0.05$) increase in the testicular count as compared to the control group. There was a marked significance ($P<0.05$) increase in the epididymal

Table 1: Qualitative phytochemical constituents of methanolic extract of *Abrus precatorius* linn leaves

Phytochemical constituents	Type of test	Results
Alkaloids	Dragendroff's rest	-
	Mayer's test	-
Flavornoids	Sodium hydroxide	-
	Ferric chloride	+
	Shinoda's test	+
Cardenolides	Keller-Killani test	+
Terpenoids	Test for terpenoids	+
Cardiac glycoside	Salkwaski's test	-
	Lieberman-Burchard	+
Tannin	Ferric chloride	+
	Lead acetate	-
Carbohydrates	Molisch's test	+
	Test for Monosaccharide's	-
	Test for free reducing sugar (Fehling)	+
	Test for combined sugar	+
Anthraquinone	Test for free anthraquinone	-
Test for saponin glycoside	Frothing Test	+

Key: - (Absent)
+ (Present)

Table 2: 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 behaviour 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 behaviour 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 3: Effects of 14-day treatment of methanolic extract of *Abrus precatorius* linn leaves on the sperm counts in Albino rats

Group Dose (mg/kg)	Epididymis Sperm Count (mg/kg)			
	Testis	Head	Body	Tail
Control	11.67±0.08	10.00±0.66	8.00±0.25	11.92±0.76
200	12.08±0.63	21.43±1.48	9.40±0.17	13.33±1.13
400	15.17±0.95	13.08±0.38	11.58±0.95	15.42±0.38
800	16.08±0.52	14.67±0.88**	13.17±0.80**	16.42±0.80**

(Means ± SD)
Key: ** = moderately significant ($p<0.05$) increase as compared with the control

sperm count especially in the treatment group given 800 mg/kg body weight as compared with the control group. The mean values of the epididymis sperm count of the groups treated with 800 mg/kg body weight of the extract were 14.67±0.88, 13.17±0.80, 16.42 ± 0.80.

The results of the methanolic extract of *Abrus precatorius leaves* on the sperm counts for 28 days are presented in Table 4. The results showed that there was marked significant ($P<0.05$) increase in the mean values of testicular count in groups treated with 200 mg/kg, 400 mg/kg and 800 mg/kg of the extract compared to the control. The mean values of

the testicular sperm count groups were 13.92±0.76, 16.00±0.66 and 17.00±0.25^3/mm^9 while the control had 11.50±0.66^3/mm^9. In the head of the epididymis sperm counts, there was high significant (P<0.05) increase in the mean values of all the groups treated with the extract as compared with the control groups. The values were 14.37±0.15, 15.27±0.21, 16.47±0.25 as compared to the control group 9.03±0.21 (Table 4). These patterns of a marked increase in epididymal sperm counts were dose dependent with the highest increase in the group treated with 800 mg/kg body weight.

The effect of the methanolic extract of *Abrus precatorius* on sperm motility is presented in Table 5. There was an increase in progressive sperm motility but it was not significant in all the treatment groups

Table 4: Effects of 28-day treatment of methanolic extract of *Abrus precatorius* leaves on the sperm counts in Albino rats

Group Dose (mg/kg)	Epididymis Sperm Count (mg/kg)			
	Testis	Head	Body	Tail
Control	11.50±0.66	9.03±0.21	8.27±0.21	9.83±0.35
200	13.92±0.76**	14.37±0.15***	10.27±0.021	16.47±0.38
400	16.00±0.66***	15.27±0.21***	12.50±0.30**	17.67±0.15
800	17.00±0.25***	16.47±0.25***	13.00±0.36***	18.30±0.20

(Means ± SD)

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

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

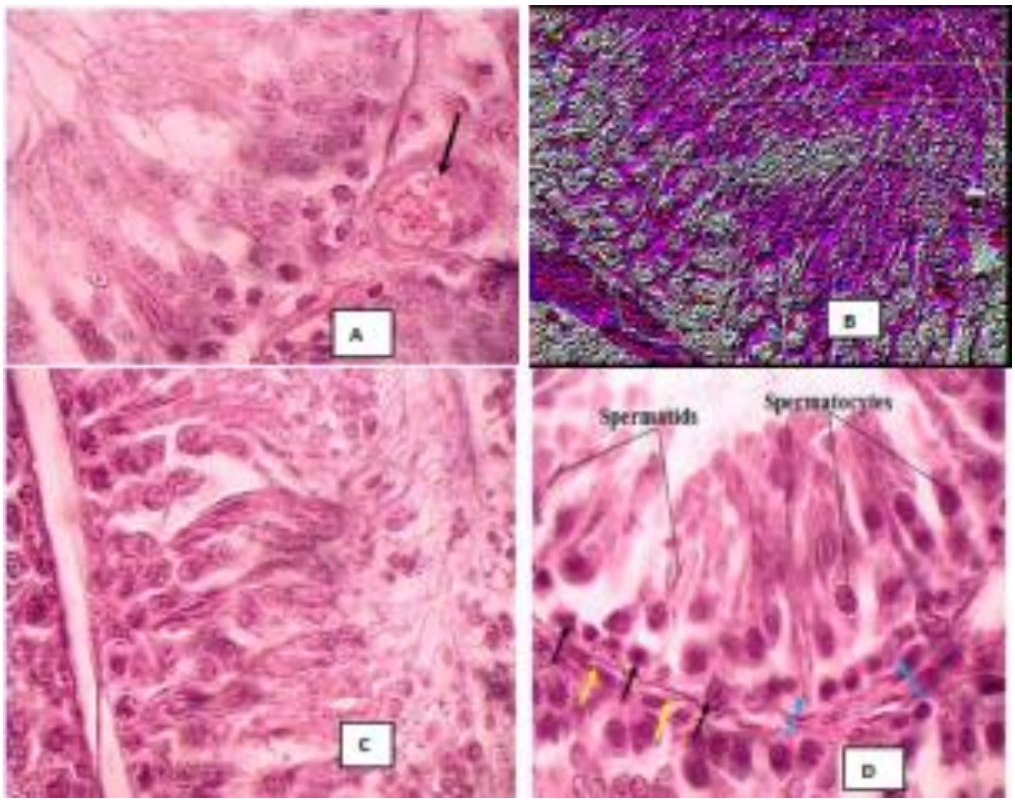

Plate I: Photomicrograph of rat testis of control and treated groups showing normal features. There was no adverse effect on the spermatogenic cells series. H&E x1000

SC = Spermatocytes

TBX = Seminiferous tubules

Black arrow = Immature spermatozoa

Blue arrow = Spermatids

Yellow arrow = Wall of the seminiferous tubules

Table 5: Effect of methanolic extract of *Abrus precatorius* linn leaves on sperm motility in Albino rats

Group dose (mg/kg)	14 Days		Means ± SD (mg/kg)	28 Days	
	Progressive	Non-Progressive		Progressive	Non-Progressive
Control	56.33±1.53	43.67±1.53		53.33±2.08	44.67±2.08
200	59.32±0.58	40.67±0.58		59.00±1.00	41.00±1.00*
400	59.33±0.58	40.67±0.58		58.33±0.58	41.67±0.58*
800	60.33±0.58	39.67±0.58		61.00±1.00	39.00±1.00**

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

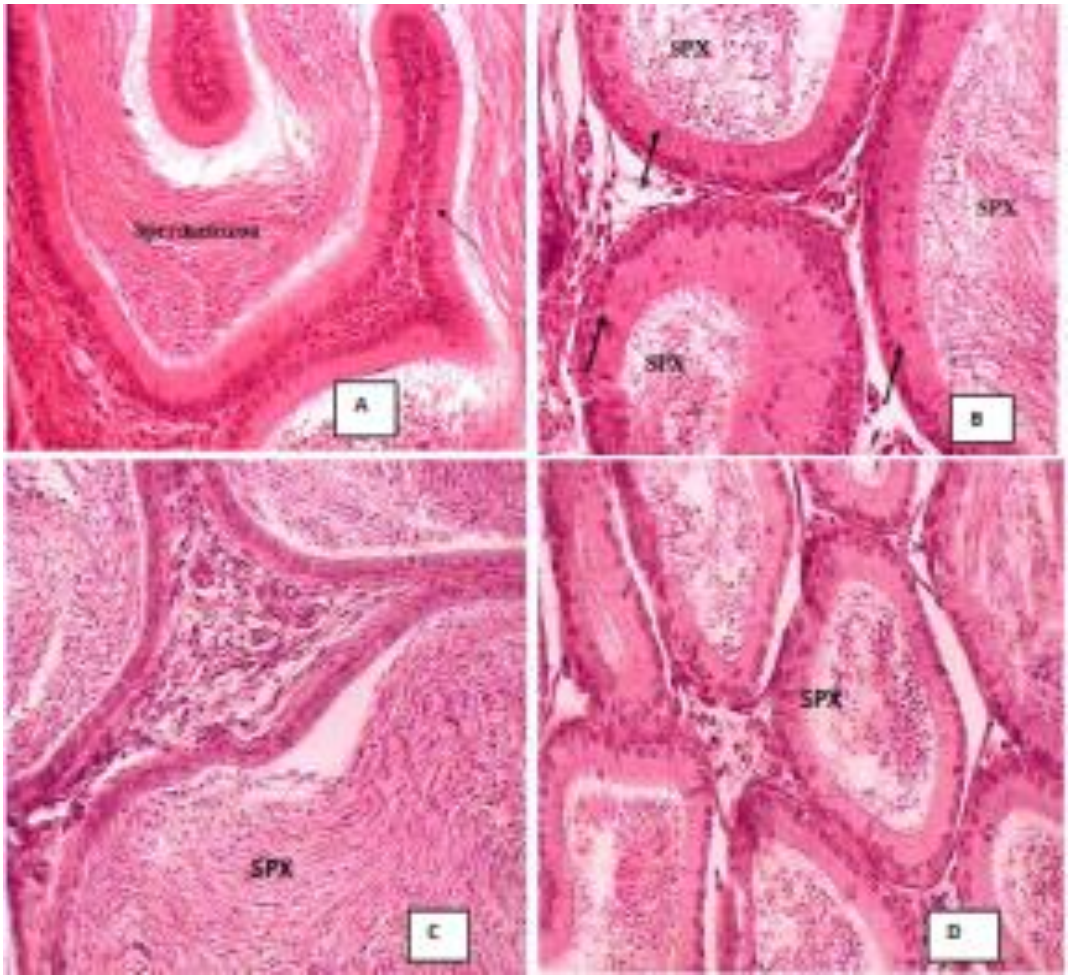

Plate II: Photomicrograph of rat epididymis of control and treated groups showing tubules filled with spermatozoa (SPX), epithelial cells (black arrows). H&E x400

as compared to the control group on Day 14. On day 28, there was a significant decrease in the non-progressive sperm motility groups given 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of extract as compared with the control.

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 (Plate I). The rat epididymis of the treated groups showed tubules filled with spermatozoa with epithelial cells stretched due to the accumulation of spermatozoa and connective tissues (Plates II and III).

Discussion

The results of the phytochemical screening of *Abrus precatorius* leaves revealed the presence of cardenolides, cardiac glycosides, carbohydrates, flavonoids, saponins, tannins and terpenoids with alkaloids being absent. The presence of these phytochemicals is similar to what was reported by Dafar *et al.* (2023) who reported the presence of

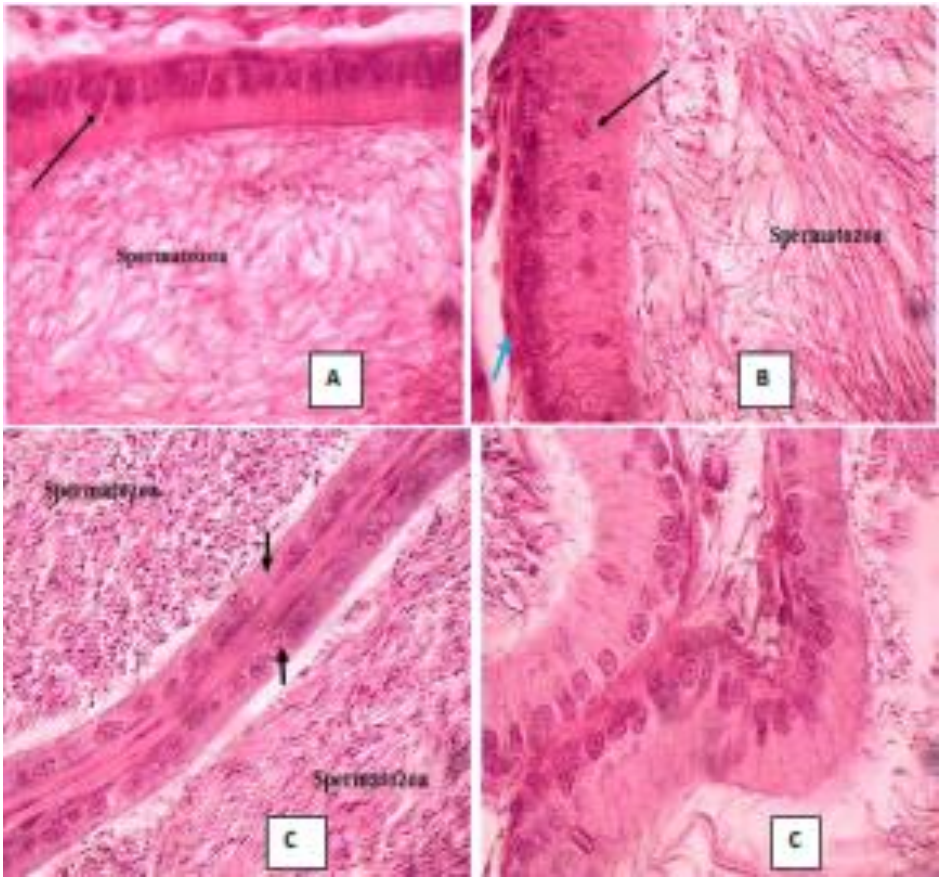

Plate III: Photomicrograph of rat epididymis of control and treated groups showing tubules filled with spermatozoa (SPX), epithelial cells (black arrows), notice the epithelial cells stretched due to accumulation of spermatozoa (short black arrows) and connective tissues (blue arrow). H&E x1000

same metabolites. However, the absence of alkaloids in this study contrasts with the one conducted by Ogbueghi *et al*. (2015) which revealed the presence of alkaloids, glycosides, flavonoids, saponins, steroids, protein and carbohydrates in *Abrus precatorius* leaves. This slight difference might be due to geographical location, climate variation, soil type, as well as year and season of sample collection between the two places where the plant is being cultivated (Kumar *et al.,* 2017).

With regards to sperm counts, there were significant increases in the mean values of the testicular sperm count in groups treated with the extract in ascending order of graded doses as compared with the control. The epididymal sperm count of the head shows a significant increase in groups treated with a dose of 800 mg/kg body weight of the extract. Similarly, there was a significant increase in the mean epididymal sperm count of the body and tail after 14 – 28 days post-treatment. These observed patterns of increase in the epididymal sperm count were dose-dependent with the highest increase seen in groups treated with the highest dose of the extract (800 mg/kg).

This finding might be attributed to the rich antioxidant properties of the *Abrus precatorius* leaf extract (Pavithra *et al*., 2020). Medicinal plants rich in antioxidants have fertility-enhancing properties by preventing the formation of free radicals and lipid peroxidation and reducing oxidative stress, preventing damage to sperm cells (Moher *et al.,* 2009). Correspondingly, Ajibo *et al.* (2018) reported that the methanol extract of *Abrus precatorius* leaf demonstrated a significant hormone-boosting effect in female rats and could offer a candidate drug in the management of infertility arising from hormonal deficiency.

Additionally, it was observed that there was significant increase in progressive sperm motility at 14 and 28 days post treatment. These marked changes were dose dependent from 200 to 800

mg/kg body weight of administered extract. Methanolic leaf extract of *Abrus precatorious* was observed to increase epididymal sperm concentration and motility in Wistar rats. On the contrary, results of previous works done on ethanolic, methanolic and aqueous extracts of *Abrus precatorius* seed extract showed antifertility effect in male and female rats at varying degrees (Jahan *et al.*, 2009; Abu *et al.,* 2012; Saranika *et al.,* 2014; Mahre *et al.,* 2017). These variations show that seed extract of *Abrus precatorius* suppresses fertility, unlike leaf extract.

In conclusion, the methanolic extract of *Abrus precatorius* leaves is relatively safe in male Wistar rats and shown to enhance male fertility by increasing epididymal sperm count and sperm motility in groups treated with 800 mg/kg body weight of the extract at day 28. It is recommended that further studies be done on individual fractions to unravel the most effective fractions and doses with minimal toxicity so that the active compound can be isolated.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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