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A Preliminary Study on the Effect of *Abrus precatorius* Linn on Reproductive Parameters in Female *Rattus norvegicus*, Wistar Strain

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IHO, OOE and AWO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IHO and OOE managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate the effect of methanolic extract of seeds and leaves of *Abrus precatorius* on implantation, hormonal profile, reproductive index and sexual behavior in adult female Wistar rats.
Study Design: Female wistar rats were treated with *A. precatorius* extracts (30 and 60 mg kg-1) for 7 estrous cycles and mated. Mid-gestation, number of implantation sites and embryos were determined in half of the pregnant females while reproductive parameters were analyzed for the remaining animals, post parturition. For the libido and hormonal assay, sexual behavioral parameters were evaluated and serum samples assayed.
Results: *A. precatorius* seeds caused a decline in the fertility index, numbers of uterine implants, corpora lutea and live fetuses in a dose dependent manner. Administration of seed extract (30 mg/kg and 60 mg/kg) resulted in a 30.5% and 66.7% post implantation loss respectively; however

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the effect of extract on libido was not significant even at the higher dose. The seed extract (60 mg/kg) significantly reduced levels of FSH, LH, PG and E_2 levels and increased prolactin level. Histology results of ovary showed significant reduction in folliculogenesis in all experimental groups, but external deformities in the pups were not observed. *A. precatorius* leaf extract promoted fertility by reducing Progesterone and E_2 levels in a manner that significantly boosted FSH and LH levels; it also caused 44%, 24% and 80% increases in the litter size for the 30 mg/kg, 60 mg/kg and clomiphene treated groups respectively. The mounting frequency and lordosis quotient were significantly increased; perceptive and copulatory behaviors were remarkably noticed amongst treated rats. Laparatomy and histology showed an enhanced folliculogenesis and ovulation in treated rats.

Conclusion: *A. precatorius* seeds have anti fertility effect on female rats while the leaf extracts were very effective as a fertility enhancing agent.

Keywords: Abrus precatorius; fertility; hormones; folliculogenesis; implantation; libido.

1. INTRODUCTION

The use of plant extracts as fertility enhancers is now on the increase because of the shifting of attention from synthetic drugs to natural products [1]. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [2]. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs [3]. Traditionally, natural aphrodisiacs are taken to enhance sexual performance, while other concoctions are prescribed as cures to reproductive disorders. The result of an ethnobotanical survey reveals that, *A. precatorious* is used in treating various female fertility and sexual health complaints in the southern part of Nigeria [Unpublished Data]. Unfortunately, there are limited scientific evidence regarding safety and efficacy to back up its therapeutic use. The rationale for their utilization has rested largely on personal experiences.

Abrus precatorius Linn is a slender, perennial climber that twines around trees, shrubs, and hedges. It is a legume with long, pinnately compound leaves of family name – fabaceae. Its flowers are arranged in violet or pink clusters. The seed pod curls back when it opens and reveals the seeds [4]. The seeds are truncate shaped, 1.5-2 cm long, with attractive scarlet - black color. The plant is native to India and Indonesia, however it now grows in tropical and subtropical areas of the world where it has been introduced [5,6].

A. precatorius seeds contain a number of chemical constituents including alkaloid, steroid, flavones, triterpenoides, proteins, amino acids etc., among which an albumotoxin, *abrin* (a highly toxic protein) considered to be primarily responsible for the poisonous effect of *A. precatorius*, with an estimated fatal dose of 0.1-1 µg/kg, in humans [7]. Literature confirms that heat-denatures abrin, causing it to lose its haemagglutinating toxicity and renders the seed harmless [4,8]. *A. precatorius* leaves are sweet tasting, and according to a human taste panel, the sweetness was 30-100 times greater than sucrose [9].

Previous studies have been carried out on the extracts made from seeds and leaves of *A. precatorious*. The seeds have been shown to have anti fertility effect in males and females [10-14], Sivakumar et al. [15] also demonstrated its cytotoxic and anti-tumor effect. Antimicrobial activities of the aqueous extract of the seed of *A. precatorius* have also been studied [16,17]. Georgewill et al. [18] has investigated the anti inflammatory effect of its seeds, the seeds are considered abortifacient [19] and useful in treating diabetes [20,21] and chronic nephritis.

This study intends to corroborate the contraceptive and anti-oogenic effects of *A. precatorius* seed while investigating the suitability of its leaf extract as a fertility enhancer in female Wistar rats. Consequently, we studied the implantation, fertility and libido enhancing properties of the methanolic extracts of the seeds and leaves of *A. precatorius*.

2. MATERIALS AND METHODS

2.1 Plant Samples

2.1.1 Collection

Plant samples, *A. precatorius* seeds (APS) and *A. precatorius* leaves (APL) were collected from

a farmland in Urualla, Imo State, Nigeria, during dry season (December, 2012). They were identified and authenticated in the Department of Forestry, Faculty of Agriculture, University of Port Harcourt (UNIPORT), Nigeria. The voucher specimens were subsequently deposited in the departmental herbarium and were assigned specimen numbers, UPH/NO-P-052 and UPH/NO-P-053 for the leaves and seeds respectively.

2.1.2 Extraction

APS (100 g) and APL (680 g) were extracted with 70% v/v methanol in a laboratory, in the Department of Pharmacognosy, Faculty of Pharmacy, UNIPORT. Cold maceration method was used and the solvent was recovered at 60ºC, extract was dried in the fume cupboard, stored in sterile bottles and preserved in the refrigerator at -40ºC until needed.

2.1.3 Qualitative phytochemical screening

Qualitative phytochemical analyses of methanol leaf and seed extracts from *A. precatorius* were conducted following the standard procedures as follows [22].

2.1.4 Test for saponins

For this test, 15 ml of distilled water was added to 0.5 g of the extract in a test tube, and shaken for 15 mins. Frothing which persisted on warming was taken as a preliminary evidence for presence of saponins.

2.1.5 Test for tannins

For this test, 0.5 g of the extract was stirred with 1 ml of distilled water and filtered. 5% Ferric chloride solution was added to the filtrate. A blue-black or violet precipitate was taken as evidence for the presence of tannins.

2.1.6 Test for carotenoids

For this test, 0.5 g of each specimen sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% tetraoxosulphate (V1) acid was added. A blue colour at the interface showed the presence of carotenoids.

2.1.7 Test for alkaloids

For this test, 0.5 g of extract was stirred with 2 drops of dilute hydrochloric acid on a steam bath and filtered; 1 ml of the filtrate was treated with a few drops of the following reagents:

1. Mayer's reagent – A cream precipitate was taken as preliminary evidence for the presence of alkaloids.
2. Picric acid solution – A yellow precipitate was taken as preliminary evidence for the presence of alkaloids.
3. Dragendorff's reagent – An orange brown cream precipitate was taken as preliminary evidence for the presence of alkaloids.

2.1.8 Test for steroids (steroidal ring)

For this test, 0.5 g of extract was dissolved in 2 ml of chloroform; and concentrated tetraoxosulphate (V1) acid was carefully added to form a lower layer. A reddish brown color at the interphase is indicative of the presence of steroidal ring.

2.1.9 Test for flavonoids

For this test, 0.53 g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5 ml of dilute sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids.

2.1.10 Test for anthraquinones

For this test, 0.5 g of extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 mins. It was filtered and the filtrate shaken with equal volume of 100% ammonia solution. Pink, violet or red color in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones.

2.1.11 Test for glycosides (keller killiani test)

For this test, 0.1 g of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. A 1 ml of concentrated tetraoxosulphate (V1) acid was added gently by the side of the test tube. A brown ring obtained at the interphase indicates the presence of deoxy sugar characteristic of cardenolides.

2.1.12 Test for triterpenes (salkowski test)

For this test, 0.1 g of extract was dissolved in 1 ml of chloroform and filtered. The filtrates were

treated with few drops of concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

2.1.13 Test for proteins and aminoacids (ninhydrin test)

To 0.5 g of extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

2.1.14 Test for starch (molisch's test)

Two drops of alcoholic α-naphthol solution was added to 0.1 g of extract in a test tube. Formation of the violet ring at the junction indicates the presence of starch.

2.2 Experimental Animal

Wistar rats (6–8 weeks old) weighing between 170–200 g were housed in standard well ventilated cages in the rat control room. They were allowed free access to laboratory chow and distilled water *ad libitum*. The temperature range was between 26–28ºC; relative humidity 50–55% and animals were exposed to 12 h light and 12 h dark cycle. They were left to adapt to laboratory conditions for 2 weeks before initiation of the experiment and were re-weighed. Vaginal smears were taken daily between 8.00–9.00 am. Only 4 day cycling rats were used and those in the same estrus stage were grouped together for the experiment.

The rats were administered oral plant extracts at doses, 30 and 60 mg/kg. The duration of treatment was for 28 days (7 cycles). The animals in the control group were given an equal volume of phosphate buffered saline (PBS) on the same days. The rats in the groups were weighed every three days during this period.

2.2.1 Implantation study

Extracts (APL and APS) were administered to experimental animals *per os* for 28 days (7 estrus cycles). The rats were randomly partitioned into 6 groups of 5 animals each and treated as follows: Group 1: PBS (0.2ml); Group 2: Clomiphene Citrate (Bruno Farmaceutici S.P.A. Roma) (10mg/kg); Group 3 - 4: methanolic extract of APL (30 and 60 mg/kg); Groups 5 - 6: methanolic extract of APS (30 and 60 mg/kg); During the last seven days of treatment, two untreated, but vigorous and sexually experienced males of proven fertility were introduced per cage overnight to allow for mating with the treated females. Successful mating was confirmed by the presence of sperm in the vaginal smear the following morning and this day was considered as day 1 of pregnancy (1 DoP). Only sperm-positive females were used for the study. On day 10 of pregnancy (mid-gestation period), half of all pregnant rats in the different groups were laparatomised under light urethane anaesthesia and the number of implantation sites were counted and the embryos, counted and weighed. The following parameters were thus deduced: Pre-implantation loss (%) = [(Total no. of corpora lutea – Total no. of implantation)/Total no. of corpora lutea] x 100 and Post-implantation loss (%) or Resorption rate (%) = [(Total no. of implantation – Total no. of viable fetuses) / Total no. of implantation] x 100.

2.2.2 Tissue collection

Rats were sacrificed and the ovaries, uteri and oviducts harvested for microscopic studies. The uterine horns of treated animals were also examined for the number of implantation sites and that of live or dead fetuses. Embryos that are clearly marginated and with bright reddish colour were considered to be normal while shrunk embryos, with blue or grayish colour, no clear margin were considered to be resorbing. The ovaries were excised and examined for the number of fresh corpora lutea using a microscope. The harvested organs from the sacrificed rats were carefully dissected out, trimmed of fat and connective tissue. The tissues were fixed in 10% formal saline, passed through a varying series of ethanol (50%, 70%, 90%, absolute alcohol), then cleared in xylene. The tissues were infiltrated in molten paraffin wax in the oven at 57ºC, thereafter the tissues were embedded in wax and made into blocks of wax. Microtome whose sectioning size knob was adjusted to six microns was used to section the block, fixed on clean slides and later stained with haematoxylin and eosin.

2.3 Reproductive Indices

All the remaining pregnant females from the implantation study group were allowed to give birth to their offspring. From day 19 of pregnancy, the animals' cages were inspected for births. As soon as possible after birth, the numbers of viable and dead newborns were recorded; the pups were weighed and inspected

for any deformity up to day 21 after birth. The following reproductive parameters were analyzed; Litter size and fertility index = (number of pregnant / number of mated) × 100. Other indices calculated are; viability index (number of live pups on day 4 of postnatal life/number of live offspring born x 100), lactation index (number of live pups on day 21 of postnatal life/number of live pups on day 4 of postnatal life x 100).

2.4 Libido Study and Hormonal Assay

Animals on the same estrous cycle stage were selected for this study. Extracts (APL and APS) were administered to experimental animals *per os* for 8 days (2 estrus cycles). The rats were randomly partitioned into 6 groups of 5 animals each and treated as follows: Group 1: PBS (0.2ml); Group 2: Clomiphene Citrate (Bruno Farmaceutici S.P.A. Roma) (10 mg/kg); Group 3 - 4: methanolic extract of APL (30 and 60 mg/kg); Groups 5 - 6: methanolic extract of APS (30 and 60 mg/kg). During the study period, the rats were weighed daily in order to monitor weight gain. Also, the following sexual behavioral parameters, were recorded for 30 min by a trained observer who was unaware of the treatment given to each group:

- Mount latency: Time duration (in minutes) from the introduction of the male into the cage till the first mount.
- Intromission latency: Time duration (in minutes) from the introduction of the male into the cage till the first intromission (vaginal penetration).
- Mount frequency: Total number of mounts preceding ejaculation.
- Lordosis quotient which is simply the ratio of the number of lordosis postures characterized by immobility by the female and arching of the back, also hind leg extension that elevates the rump and head, shown by a female in response to a 10 mounts, times 100.

These were determined using on-site observation, stopwatch and videotape review and were taken as an index of libido.

On the last (day 8) of treatment, animals were fasted and opened up under anaesthesia. Blood samples were taken from abdominal aorta using a syringe and needle, then centrifuged at 2500 rpm for 15 min at 4ºC, using Uniscope Laboratory Centrifuge (Model M800B, Surgifriend Medicals and Essex, England) to extract the serum for hormonal profile analysis. The sera were aspirated with Pasteur pipettes into clean, dry, sample bottles and used within 12 h of preparation for the hormonal assay. Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Progesterone (PG) and 17 β-estradiol (E_2) concentrations were estimated by the enzyme-linked immunosorbent assay (ELISA) using assay kits (Fortress Diagnostics Ltd., UK).

2.5 Statistical Analysis

Data were analyzed using SPSS 17.0 software. Comparisons between groups were performed with the two-tailed analysis of variance and thereafter Duncan's Multiple Range Test was employed. The results were considered significant at $p<0.05$. All results are expressed as Mean ± Standard Error of Mean (S.E.M).

2.6 Ethical Approval

Ethical approval was obtained from the Research Ethics Committee, of the College of Health Sciences, University of Port Harcourt. Also, animal handling and test procedures were in accordance to the guiding principles on animal use in experiments.

3. RESULTS

3.1 Qualitative Phytochemical Screening

3.1.1 Implantation study

The effects of administration of methanolic extract of *A. precatorius* leaves (APL) and seeds (APS) at 30 and 60 mg kg-1for 7 estrous cycles on implantation in female Wistar rats are depicted in Table 2.

3.1.2 Reproductive study

The effects of administration of methanolic extract of *A. precatorius* leaves (APL) and seeds (APS) at 30 and 60 mg kg-1 for 7 estrous cycles on reproductive indices is depicted in Tables 3 and 4.

3.1.3 Hormone assay

The effects of administration of methanolic extract of *A. precatorius* leaves (APL) and seeds (APS) at 30 and 60 mgkg-1 for 2 estrous cycles, on the concentration of serum reproductive hormones in the female rats are depicted in Table 6.

Table 1. Phytochemical constituents of methanolic extract of *A. precatorius* plant samples

A. precatorius leaves	Present/absent	*A. precatorius* seeds	Present/absent
Alkaloids	+	Alkaloids	+
Glycosides	+	Glycosides	-
Tannins	-	Tannins	+
Flavonoids	+	Flavonoids	+
Saponins	+	Saponins	-
Triterpenes	+	Triterpenes	+
Steroids	+	Steroids	+
Gums and mucilage	-	Gums and mucilage	-
Proteins	+	Proteins	-
Starch	+	Starch	-
Fats and fixed oils		Fats and fixed oils	-

Present = + Absent = -

Table 2. Effect of methanolic extract of *A. precatorius* on implantation in female rats

Treatment groups	Implantation sites	Reabsorbing fetuses	Viable fetuses	Corpora lutea	Pre-implantation loss (%)	Post-implantation loss (%)
Control	82 ±1.0	0±0.0	82±2.0	96 ±2.0	14.6±2.0	0±0.0
CC (10 mg/kg)	94±3.0*	22±1.0*‡	72±1.0	105±4.0*	10.5±2.1	23.4±1.3*
APL – 30 mg/kg	86±2.0	2±0.0†	84±1.0	98±3.0	12.3±1.2	2.4±0.3†
AP L– 60 mg/kg	92±1.0*	3±0.0†	89±1.0	101±3.0	9.0±1.0*	3.3±0.4†
APS – 30 mg/kg	23±2.0*†‡	7±1.0*‡	16±1.0*‡	85±2.0†	73.0±3.8*‡	30.5±1.6*
APS– 60 mg/kg	9±1.0*†‡	7±1.0*‡	3±0.0*‡	34±2.0*‡	73.6±2.4*‡	66.7±3.7*‡

Data represents the Mean ± S.E.M for each group of rats, n = 5.
**p<0.05 = significant difference with respect to the control group.*
†p<0.05 = significant difference with respect to the CC (10mg/kg) group.
‡p<0.05 = significant difference with respect to all treated groups.
Pre-implantation loss (%) = [(Total no. of corpora lutea – Total no. of implantation)/Total no. of corpora lutea] x 100
Post-implantation loss (%) or Resorption rate (%) = [(Total no. of implantation – Total no. of viable fetuses)/Total no. of implantation] x 100

Table 3. Effect of methanolic extract of *Abrus precatorius* on body and reproductive organ weight of female rats

Treatment groups	Initial body weight (g)	Final body weight (g)	Uterine weight (g)
Control	186±4.0	200±2.0	0.05±0.002
CC (10 mg/kg)	190±1.0	238±1.0*	0.25±0.02
APL – 30 mg/kg	186±2.0	210±1.0	0.14±0.01*
AP L– 60 mg/kg	184±1.0	223±2.0	0.19±0.02*
APS – 30 mg/kg	188±3.0	210±3.0	0.03±0.17*†
APS– 60 mg/kg	190±4.0	211±2.0	0.02±0.09*†

Data represents the Mean ± S.E.M for each group of rats, n = 5.
**p<0.05 = significant difference with respect to the control group.*
†p<0.05 = significant difference with respect to the CC (10mg/kg) group.

Table 4. Effect of methanolic extract of *Abrus precatorius* on reproductive index of female wistar rats

Treatment groups	Fertility Index (%)	No of pups per litter	Pups body weight (g)	Viability Index (%)	Lactation index (%)
Control (PBS, 0.2 ml)	4/5 (90%)	0.05±0.02	6.24±0.04	100	100
CC (10 mg/kg)	5/5 (100%)	0.25±0.02*	5.35±0.17	100	100
APL – 30 mg/kg	5/5 (100%)	0.14±0.01*	5.86±0.20	100	100
APL – 60 mg/kg	5/5 (100%)	0.19±0.02*	6.11±0.34	100	100
APS – 30 mg/kg	2/5 (40%)*‡	0.03±0.17†	6.17±0.09	50*‡	30*‡
APS – 60 mg/kg	0/5 (0%)*‡	0.02±0.09†	5.48±0.143	0*‡	0*‡

Data represents the Mean ± S.E.M for each group of rats, n = 5
**p<0.05 = significant difference with respect to the control group*
†p<0.05 = significant difference with respect to the CC (10mg/kg) group
‡p<0.05 = significant difference with respect to all treated groups
Fertility index (%) = (No of pregnant animals/ No of mated animals) x100
Viability index = number of live pups on day 4 of postnatal life/number of live offspring born × 100
Lactation index = number of live pups on day 21 of postnatal life/number of live pups on day 4 of postnatal life ×100

Table 5. Effect of methanolic extract of *Abrus precatorius* on sexual behaviour of female wistar rats

Treatment groups	Mount latency (min)	Mount frequency	Intromission latency (min)	Lordosis quotient
Control (PBS, 0.2 ml)	3.88±0.40	16.20±0.71	4.22±0.18	85.42±2.41
CC (10 mg/kg)	3.86±0.15	15.85±0.56	4.20±0.21	77.39±1.83*
APL – 30 mg/kg	2.43±0.40*†	18.14±0.32	3.26±0.23*	88.17±1.87
APL – 60 mg/kg	2.05±0.16*†	18.48±0.19†	3.05±0.28*†	93.25±1.29*†
APS – 30 mg/kg	3.71±0.11	16.23±0.17	3.94±0.30	79.55±2.09
APS – 60 mg/kg	3.89±0.30	16.12±0.35	4.20±0.13	81.25±1.29*

Data represents the Mean ± S.E.M for each group of rats, n = 5
**p<0.05 = significant difference with respect to the control group*
†p<0.05 = significant difference with respect to the CC (10mg/kg) group
‡p<0.05 = significant difference with respect to all treated groups

Table 6. Effect of methanolic extract of *Abrus precatorius* on female reproductive hormone levels in female wistar rats

	Prolactin conc. (ng/ml)	Estradiol conc. (ng/ml)	Progesterone conc. (ng/ml)	Follicle stimulating hormone conc. (mIU/ml)	Luteinizing hormone conc. (mIU/ml)
Control	155±0.14	620±0.92	55±0.10	3±0.96	2.7±0.16
CC(10 mg/kg)	147±0.26	616±0.17	52±0.15	5.1±0.89	3.2±0.95
APL(30 mg/kg)	145±0.28	602±0.11	53±0.13	4.4±0.13	3.5±0.41
APL(60 mg/kg)	141±0.11*	594±0.13	31±0.17*‡	4.6±0.17	3.7±0.21
APS(30 mg/kg)	152±0.19	623±0.40	62±0.15	2.2±0.11	2.5±0.03
APS(60 mg/kg)	160±0.13*‡	631±0.73*†	66±0.11*	1.8±0.19**	2.1±0.99†

Data represents the Mean ± S.E.M for each group of rats, n = 5
**p<0.05 = significant difference with respect to the control group*
*** p<0.05 = significant difference with respect to the CC (10mg/kg) group*
†p<0.05 = significant difference with respect to APL (30mg/kg) and APL (60mg/kg) group
‡p<0.05 = significant difference with respect to all treated groups

3.1.4 Gestation length

The gestation length was not significantly different from any of the groups and controls. For the PBS (0.2 ml), CC (10 mg/kg), APL-30 mg/kg, APL-60 mg/kg, APS-30 mg/kg and APS-60 mg/kg, the mean gestation lengths (in days) were 22.1±0.3, 22.0±0.1, 22.3±0.2, 21.8±0.2, 22.4±0.04 and 22.0±0.3 respectively.

3.1.5 Deformity

On inspection of the pups at birth and daily up to the 21th day post delivery, there were no noticeable external deformities in all the treatment groups.

4. DISCUSSION AND CONCLUSION

Preliminary phytochemical investigation of *A. precatorius* extracts revealed that the seed contains alkaloids, tannins, flavonoids and triterpenes while the leaves contain alkaloids, glycosides, flavonoids, saponins, steroids, protein and starch (Table 1 above). The results of our LD 50 study of *A. precatorius* leaf and seed extracts showed that extract were safe up to the dose of 2400 mg/kg and 1800 mg/kg respectively [23].

Female Wistar rats were used in this study because rats have been shown to have a well-defined reproductive system and are recognized as the preeminent model for mammalian system in numerous fields, including endocrine pharmacology [24].

Implantation is a crucial event in mammalian embryonic growth and development which occurs 4–6 days after fertilization in human and rodents. It is regulated by a timely interplay of the ovarian hormones (estrogens and progesterone) and any disturbance in the equilibrium level of these hormones may lead to loss of implantation and may cause infertility [25,26]. Post-coital administration of APS extract, for seven consecutive days to sperm-positive female rats caused marked reduction in the number of implantation sites. On the other hand, the number of uterine implantation sites showed a dose-dependent increase with the administration of the APL extracts and CC. Unlike in *A. precatorius* seed extract treated groups; those that received the leaf extracts had more viable fetuses and a number of healthy corpora lutea (Table 2). This result implies that while the plant seed has contraceptive and anti-implantation properties, the leaves induce a favourable milieu for zygote implantation and development. It could also mean that *A. precatorius* may have a protective effect on the number of resorptions or helps to prevent abortion. With this result, we lend support to Okoko et al. [27] findings with regards to the anti-implantation activity of *A. precatorius* seeds while demonstrating the dose dependent positive effect of the leaf extracts on implantation.

The fertility index of CC and APL treated groups were similar and significantly higher than all other test groups. The significant increase in the number of the pups per litter of the rats by the clomiphene citrate group is not surprising, given its follicle stimulating effect [28,29]; however, the high number of the pups per litter of the rats after exposure of the reproductive system to 28 days administration of *A. precatorius* leaf extracts – 30 mg/kg and 60 mg/kg when compared to the PBS treated control is worthy of note and it further denotes its fertility enhancing effect. The pup body weight of all groups was similar (Table 4).

Uterine weight in pregnant rats is an index of uterine decidualization [30]. Decrease in uterine weight in APS treated rats is an evidence of suppression of uterine decidual changes; however, there was a significant ($p<0.05$) increase in the in uterine weight in APL treated rats. Maternal weight of all but Clomiphene citrate treated group showed a normal weight gain pattern. Clomiphene citrate has been shown to cause weight gain [28] and this was evidenced from our results (Table 3).

There were similarities in the number of corpora lutea and graffian follicles found in the CC and APL-60 mg/kg treated groups. While there was a decline in the number of graffian follicles and increase in number of atretic follicles in especially the APS -60 mg/kg treated group indicating the antiovulatory effect of APS.

In the reproductive index, both the APS, APL treatment and the control groups showed no external malformations in their pups at all dose levels. This again implies that APS extracts may not be teratogenic but is clearly abortifascient in a greater population of treated rats; however more studies are needed to establish the mechanism through which this extract acts.

The most significant result in the libido study was seen with the rats which received APL – 60 mg/kg, as their lordosis quotient and mounting frequency were significantly higher than all other experimental groups (Table 5 above). Also

darting, wiggling of the ear, solicitation sounds by the female were remarkably noted amongst APL treated rats. These are the indicators of enhanced sexual behavior, thus we conclude that *A. precatorius* is a potent stimulator of sexual arousal in female rats. It may be that the methanolic leaf extract of *A. precatorius* acts by exerting a relaxing effect on the clitoral cavernosal muscle and vaginal smooth muscle or by improving blood flow to the genitals. However, it does seem that APS treatment did not have a significant ($p>0.05$) effect on the libido of the treated rats since no marked difference was recorded in their MF and LQ when compared to PBS control group.

Administration of the *A. precatorius* seed extracts (30 mg/kg and 60 mg/kg) produced a slight increase in the serum prolactin concentration while significantly reducing those of estradiol, progesterone, follicle stimulating and luteinizing hormones (Table 6). This is an indication of adverse effect on the maturation and ovulation of follicles and consequently suggests that the extract can be used as a contraceptive. Also, scientific studies have shown that prolactin reduces libido [31], this may be the cause of the low libido recorded amongst the APS treated groups. Prolactin levels can suppress the secretion of FSH and GnRH, leading to hypogonadism, and sexual dysfunction in both sexes [32]. Also, high prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropic-releasing hormones (GnRH), which are necessary for ovulation. Such increase in prolactin may inhibit ovulation and promote the loss of menstrual periods which will hinder conception. Thus, reduced level of prolactin in the AP leaves treated rats suggests an ovulation induction property of the leaf extract, while the elevated level of prolactin in the AP seed treated rats reaffirms the possible contraceptive property of the seeds.

Administration of *A. precatorius* leaf extracts (30 mg/kg and 60 mg/kg) caused a dose dependent rise in FSH and LH levels, but did not cause a significant variation in the estradiol levels of PBS control and treated rats. Therefore, *A. precatorius* leaf extracts did not show estrogenic effect. Estrogen acts in a feedback mechanism, influencing the production of follicle stimulating hormones (FSH) from the pituitary gland; FSH in turn promotes the development of the immature ovarian follicles, which increases the production of estrogen from the ovary [33]. Follicle stimulating hormone is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life [34]. In females, ovulation of mature follicles in the ovary is induced by a large surge of LH secretion during the pre-ovulatory periods. However, when FSH is sufficiently suppressed by the estrogen, follicular growth will be minimum and the occurrence of ovulation would be unlikely. Present findings indicate that the administration of the seed extracts showed significant increase in the estrogen level and induce inhibitory effect on FSH and LH resulting in failure to ovulate. In view of the discussion above, we postulate that APL might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone secreted by the hypothalamus.

Progesterone which is produced in the ovaries, placenta, and adrenal glands, helps to regulate the monthly menstrual cycle, preparing the body for conception and pregnancy as well as stimulate sexual desire [35]. The feedback inhibition of GnRH secretion by estrogens and progesterone provides the basis for the most widely-used form of contraception. Such feedback inhibition of GnRH prevents the midcycle surge of LH and ovulation. The reduction in the levels of serum progesterone by *A. precatorius* seed extract may also have been responsible for the implantation failure in the treated rats. Alkaloids have equally been reported to inhibit the synthesis of cellular progesterone [36] and it was found in large quantity in *A. precatorius* seeds. Therefore, the reduced level of progesterone by *A. precatorius* seed extract may not be unconnected with the alkaloidal component of the extract.

In conclusion, treatment of female rats with the crude methanolic extract of *A. precatorius* seeds and leaves showed obvious effects on the reproductive parameters studied. The results obtained from these studies portend the fertility enhancing property of the leaf extract while confirming the anti-implantation and contraceptive effect of the seeds, hence the extracts can be useful in female fertility management. It is remarkable though that different part of a plant could exert directly opposite pharmacological functions. Detailed chromatographic and characterization studies of the plant extract are underway.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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