

Spermicidal, Antifertility and Contraceptive Effect of *Azadirachta indica* A. Juss. Seed Extract in Female and Male Wistar Rats

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Abstract

This study investigates the spermicidal and contraceptive effect of *Azadirachta indica* seed aqueous extract in male and female Wistar rats. The animals were divided into four groups (n=6), graded doses (2.5, 5, and 10 mg/kg) of the extract. They were exposed to female rats in a ratio of 2:1 after 24 hours, 7, and 14 days of administration. The spermicidal properties were evaluated. The female contraceptive study involved 14 days of pre-coital and post-coital administration of the neem seed extract, and contraceptive indexes were investigated. The results obtained from the spermicidal activity showed a significant decrease in male hormonal levels at 2.5, 5, and 10 mg/kg of *A. indica* (0.73, 0.50, and 1.08 ng/ml); and the sperm cells (102.5, 111.5, and 97 counts) after 24 hours. Administration and mating, compared to the control. Also, the histopathology of the testes showed normal testicles. The contraceptive study elicited a significant decrease in estrogen (1.46 ng/ml), luteinizing hormone (0.094 mg/dl) and progesterone (1.82 ng/ml), at 5 mg/kg in day 14 post-coital study compared with the control. The histopathology of the treated uterus had no deteriorating effect compared with the control. The extract elicited spermicidal and contraceptive potential at a reduced dose, validating its folklore claim.

Keywords: Spermicidal; contraceptive; *Azadirachta indica*; Wistar rats.

INTRODUCTION

Azadirachta indica A. Juss. is a prevalent and highly wonderful tree (Alzohairy, 2016). It was known as neem a distinct tree associated closely with the socio-cultural and holistic aspects of the life of Indians since ancient times. It is a fast-growing tree with edible fruits and aromatic leaves. A mature tree can produce three hundred and fifty kilograms of leaves a year (Kelli, 2010). It can grow to a height of fifteen-twenty meters and seldom thirty-five meters, located in the subtropical and tropical world. Neem is in the mahogany family, meliaceae, native to the Indian subcontinent. Neem oil is usually produced from its fruits and seeds, the leaves and oil have antifertility effects (Ruchi *et al.*, 2014). INDRA the king of the gods, was on his way back to his kingdom with the pot of Amirthan (the divine nectar) which he had gotten from the devils after a tough-fought battle. Probably, he was fagged out, maybe careless or probably he did it intentionally, for little drops of the pot content poured over a neem tree, from that day henceforth, the neem tree had good qualities for healing ailments, of course, this is a folk tale. Meanwhile, the neem is a superb tree for millions of people and its parts

have several uses in healthcare, medicines, and agriculture, and this is no folklore (Ajayi, 2002). Neem is called Indian village pharmacy in the west, it is also called Arishta in the Sanskrit language which means relieving sickness. Africans usually call it Muarubaini which means forty uses or forty cures. Meanwhile, the Persians have given the most perfect name Azadirakht-hind which means a free tree of India, and perhaps from this its Latinized botanical name *A. indica* (Asiif, 2013). Almost all the parts of the wonderful tree have been used medicinally since ancient times. This is a rampant tree found in different countries including; India, Pakistan, Srilanka, Burma, Malaya, Indonesia, Japan, Australia (tropical region) even in the middle east. It is amongst the trees tagged holy by Hindus all over the world.

Contraception is defined as the inhibition of conception, but generally, it is taken to mean the prevention of pregnancy (Bansal *et al.*, 2010). Family planning has been encouraged through numerous means of contraception, but these contraceptives have several side effects produced by their steroid content (Chandra-Mouli *et al.*, 2014), such as contraceptive pills, Intra Uterine Devices, Tubectomy, Condoms, Diaphragm, and

coitus interruptus. These methods are also typically female-oriented. Contraceptive pills are generally female sex hormones such as estrogen, progesterone, or their derivatives either single or together. The concept of sterilization by female sex hormone is very old and it was initiated at the beginning of the twentieth century. Novid was the first "pill" permitted by the FDA for usage as a birth control agent (Chaudhuri, 2007). However, these pills lead to the development of some unwanted effects such as obesity, dysmenorrhea, vomiting, cardiovascular disorders, and carcinoma of the breast and uterus. These side effects from the use of the pills make them unsafe for long-term use. Countless measures have been taken to decrease the side effects of these pills but there is little success (Dash *et al.*, 2014). As a result of the serious adverse effects caused by synthetic steroidal contraceptives, the focus has been shifted to local plants for possible contraceptive effects. Although contraceptives comprising estrogen and progesterone are effective and common, the risks accompanying the drugs have prompted the need to synthesize newer molecules from medicinal plants. This study evaluates the antifertility properties of *Azadirachta indica* in male and female Wistar rats.

MATERIALS AND METHODS

Collection of plant material

Fresh seeds of the plants were collected from Kaduna State, in the northern part of Nigeria. It was identified and authenticated by Dr. Timothy Odaro in the herbarium unit of the Department of Plant Biology and Biotechnology, with the voucher number UBT-B184. It was further authenticated using theplantlist.org. Fresh seeds were harvested and sun-dried for two weeks. The dried seeds were ground to powder using a mechanical grinder.

Preparation of plant material

Two thousand (2000) grams of the sample, weighed using a scale was measured into a glass jar. 2L of n-hexane was poured into the glass jar containing the sample. The mixture was stirred, covered, and left for 36 hours. The mixture was then strained after 36 hours and a dark brown oily semi-solid extract was obtained. The extract was concentrated using a water bath at about 45°C. It was then kept in a sterile container in the refrigerator until needed for use.

Experimental animals

Sixty-eight (68) healthy whisker (albino) rats (male and female) weighed 180-250 g. The animals were acquired from Animal and Environmental Biology, University of Benin animal house. They were housed in well-ventilated woody cages in a normal laboratory state (12 hours light/dark cycle: 23 ± 2°C) and fed using a standard diet. Food and water were administered at free

choice (*ad libitum*) to the animals designed for experiments. The animals were properly handled using the ethics of Laboratory animals' approval from the ethical committee of the Faculty of Life Sciences with the ethical number LS20619.

Experimental Design

This study involved two experimental protocols; male spermicidal activities and female contraceptive properties.

The male Wistar rats were randomly divided into 4 groups (n=9). Three of the groups were administered with graded doses (2.5, 5, and 10 mg/kg) of *A. indica* seed aqueous extract, the last group serves as the control group (Ogbuewu, 2011). They were administered with graded doses of the extract orally for 14 days. After 24 hours, 3 rats across the groups (making a total of 12 rats) were exposed to female rats using a ratio of 2 male to 1 female and then sacrificed. The same process was repeated for 7 and 14 days. Sperm, blood, and reproductive organs (testes, and epididymis) were collected from the sacrificed rats and were analyzed (Khillare and Shrivastava, 2003; Koresriem, 2013). The female contraceptive study was carried out in two (2) phases; the pre-coital and post-coital treatment with neem seed aqueous extract.

Phase 1: The pre-coital treatment involved 4 groups (n=4); the control group, graded doses (2.5, 5, and 10 mg/kg) of neem seed aqueous extract for 14 days, on the 14th day all the female animals were paired with male Wistar rats during their heat period for another 7 days using ratio 1:1, and the female rats were checked at every interval for possible signs of vaginal sperm plug as an indicator for mating (Gbotolorun *et al.*, 2008; Dreweke, 2014). After 7 days of pre-exposure to the male animals, the female rats were observed for traces of pregnancy for another 23 days, which is the gestational period of rats. The weight of the rats was obtained and sacrificed in mild anesthetics, and blood and female reproductive organs were isolated.

Phase 2: The post-coital treatment involved 4 groups (n=4); the control group, graded doses (2.5, 5, and 10 mg/kg) of neem seed aqueous extract (Harris, 2018; Hatcher *et al.*, 2007). Before the administration of the extract, the female rats were paired with male animals' using a ratio of 1:1 for 48 hours, the female rats were checked at every interval for possible signs of vaginal sperm plug as an indicator for mating (Nripendra *et al.*, 2019; Prakash *et al.*, 1988). The female animals were administered with graded doses of *A. indica* seed aqueous extract for 7 days of oral administration, the female rats were observed for traces of pregnancy for another 23 days, which is the gestational period of rats. The weight of the rats was obtained and sacrificed in mild anesthetics, and the number of resorption, Number of embryos, number of corpora, pre-implantation, and post-implantation mortality, and total prenatal mortality

were evaluated. Blood and female reproductive organs were isolated for analysis.

Sperm cell procedure

Sperm cells were collected from the vas deferens and placed in a sterile petri dish. To the petri dish, 6 µl of normal saline already adjusted to 37°C was added. A drop of the sperm cell suspension was taken from the petri dish and dispensed on a clean grease-free slide, further covered with a transparent cover slip. The slide was placed on the microscope and viewed with the 20x and 40x objective magnification lens. The motility was scored in percentage according to their nature of motility as, Progressive, Nonprogressive, and immotile sperm cells (Sethi *et al.*, 2017; Suryawanshi 2011; Ibeh *et al.*, 2018).

One volume of semen (a drop) was mixed into two volumes of eosin solution (1% diluted water). After 30 seconds three volumes of nigrosine solution (10% nigrosine) were added and the sample was homogenized. A thin smear was then made immediately and air dried. The stained slide using an improved Eosin and Leishman stain (Umadevi *et al.*, 2013; World Health Organization, 2010), was examined under the oil immersion objective lens (100x). Live spermatozoa were unstained (white) and the dead ones were red. The slide was viewed with at least 30 magnification fields, and the normal and abnormal sperm cells were spotted and scored in percentage (Suryawanshi 2011).

Determination of Serum Testosterone Concentration

Serum testosterone level of the tested animals' plasma was investigated using an established protocol from the manufacturer's manual. This was established on the standard competitive requisite between testosterone in the test plasma specimen (serum). This assay method involved the dispensing of 10 µL of testosterone reference standards at (0, 0.1, 0.5, 2.0, 6.0, and 18.0 ng/mL), serum (diluted ×5), and the controls of testosterone 1 and 2 into a Goat Anti-Rabbit IgG-coated microtitre wells (96 wells), 100 µL of testosterone-HRP conjugate reagent (blue color) and 50 µL of rabbit anti-testosterone reagent were distinctly distributed into each well. The resultant solution was systematically mixed for 30 seconds and allowed for incubation at 37°C for 90 minutes. This was left to a stable quantity of HRP-labelled testosterone to contend with endogenous testosterone of the standard, sample, or quality control serum for a constant number of binding spots of a detailed testosterone antibody (since the quantity of testosterone peroxidase conjugate with the immunological bound of the well gradually decreases as the testosterone concentration in the specimen increases) (Aversa *et al.*, 2000). The microwells were washed and skimmed 5 times in distilled water (to eliminate unbound testosterone peroxidase conjugate) previously dispensing 100 µL of TMB reagent into the well. The resultant solution was properly mixed mildly for 5

seconds. This was later incubated at room temperature for about 20 minutes to achieve blue coloration. The color change was ceased with an additional 100 µL of Stop Solution (1N HCl) to each well and when slightly mixed, the color altered from blue to yellow. The absorbance was recorded within 15 mins at 450 nm in a microtitre well reader. The greatness of the color made was comparative to the quantity of enzyme extant and was inversely relative to the total of unmarked testosterone in the sample (Gauthaman and Adaikan 2008). The testosterone level of the serum in the animals was considered from the calibration curve (plotting the concentration of the standard against the absorbance) using the expression:

$$\text{Testosterone concentration ng/mL} = C_s \times F, (1)$$

Where C_s is the Corresponding testosterone concentration from the calibration curve and F is the Dilution factor

Progesterone assay protocol

All reagents should be allowed to reach room temperature (18-25°C) before use. Pipette 50 µl of standards (ready to use) and diluted samples into appropriate wells within 5 minutes. Add 100 µl of progesterone Enzyme Conjugate Solution to each well (except those set for blanks). Mix well for 30 second. and incubate for 60 minutes at 37°C. You may use par film to cover the wells or use an appropriate zip-lock bag to store the plate during the incubation. Discard the contents of the wells and wash the plate 5 times with Wash Solution (250-300 µl) per well. Invert the plate, and tap firmly against absorbent paper to remove any residual moisture. Add 100 µl (TMB) Substrate solutions to all wells. Remember to follow the pipetting order. Incubate the plate at room temperature (18-28°C) for 10 minutes without shaking. Stop reaction by adding 50 µl of Stopping Solution to wells in the same sequence that the Substrate Solution was added and gently mixed. Read the absorbance at 450 nm with a microwell reader.

Luteinizing hormonal assay

Secure the desired number of coated wells in the holder. Dispense 50 µl of standards, specimens, and controls into appropriate wells. Dispense 100 µl of Enzyme Conjugate into each well. Mix for 30 seconds. It is very important to have completed mixing at this step. Incubate at room temperature (37°C) for 2 hours. Remove the incubation mixture by flicking the plate contents into a waste container. Rinse and flick the microtiter wells five (5) times with wash buffer. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets. Dispense 100 µl of TMB solution into each well. Gently mix for 10 seconds. Incubate at room temperature for 20 minutes, in the dark. Stop reaction by adding 50 µl (one drop) of 2N HCl to each well. Gently mix for 30 seconds. It is

important to observe a color change from blue to yellow. Read optical density at 450 nm with a microtiter well reader.

Follicle-stimulating hormone

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate. Prepare all reagents and samples as directed in the previous sections. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the Ziploc, store unused wells at 4°C. Set a Blank well without any solution. Add 50 µl of Standard or Sample per well. Standard need test in duplicate. Add 50 µl of HRP-conjugate to each well (not to Blank well), then 50 µl Antibody to each well. Mix well and then incubate for 60 minutes at 37°C. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or auto-washer, and let it stand for 10 seconds, complete removal of the liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels (Neychev and Mitev 2016). Add 50 µl of Substrate A and 50 µl of Substrate B to each well, and mix well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Add 50 µl of Stop Solution to each well, and gently tap the plate to ensure thorough mixing. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

Histopathological analysis

Testes and uterus were fixed in neutral Bouin's fluid. Affixed organs were utterly dehydrated with 99.9 % ethanol along with 70 % ethanol, and 96 % ethanol and washed using distilled water. 4 µm sections were prepared and stained in hematoxylin-eosin dye. Stained tissues were subjected to optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and

viewed at 400x magnification (Drury and Wallinton, 2013).

Statistical analysis

Results obtained are presented as Mean ± SEM. Data were analyzed via one-way analysis of variance (ANOVA) and means were compared using the Dunnett test. Significant differences were measured at $p < 0.05$. Graph pad prism (Version 7, USA) was the statistical tool used.

RESULTS AND DISCUSSION

Spermicidal property

The body and organs weight of the treatment groups elicited a slight significant increase when compared with the control at 24 hours. 7 and 14 days of the study (Tables 1 and 2). Sperm cells in the graded doses of the extract (2.5, 5.0, and 10 mg) at 24 hrs. 7 and 14 days elicited a significant decrease in sperm count, progressive motility with an increase in non-progress motility, and immobility when compared with the untreated control as shown in Figure 1. The testosterone level, follicle-stimulating hormone, luteinizing hormone, and progesterone had a significant reduction in the treatment groups when compared with the control, eliciting spermicidal and antifertility properties of the extract as shown in Figure 2. The abnormal weight loss or gain serves as an indication of toxicity possibly due to structural protein breakdown or muscle wastage (Kumar and Mishra, 2010). This study present showed that *A. indica* seed aqueous extract at graded doses maintains the level of a significant increase in the body weight with the spermicidal effect for 24 hours. 7 and 14 days (Table 1-3). This result consented with Singh *et al.* (2018) report on grapefruit components to ameliorate reproductive toxicological organs' weight loss. Similarly, the visceral organs such as the testes, cowpea gland, and epididymis, elicited a slight significant increase with no damaging effect across the treatment groups compared with the control at 24 hours, 7, and 14 days.

Table 1. Effect of *Azadirachta indica* seed aqueous extract on the body and reproductive organs weight after 24 hours.

Treatment	Dose mg/kg	Initial body weight (g)	Final body weight (g)	Weight of testes (g)	Weight of cowpea gland (g)	Weight of epididymis (g)
<i>A. indica</i>	2.5	193.50±0.50	185.50±5.50	1.20±0.00	0.40±0.04	1.05±0.15
<i>A. indica</i>	5.0	207.50±1.50	187.00±2.00	1.25±0.05	0.60±0.10	0.80±0.05
<i>A. indica</i>	10	182.00±1.80	178.00±1.70	1.25±0.05	0.35±0.05	0.90±0.05
Control	0.5 ml/kg	204.00±3.40	204.50±3.40	1.35±0.15	0.30±0.00	0.70±0.04

The results were expressed in mean±SEM, $p > 0.05$.

Table 2. Effect of *Azadirachta indica* seed aqueous extract on the body and reproductive organs weight after 7 days.

Treatment	Dose mg/kg	Initial body weight (g)	Final body weight (g)	Weight of testes (g)	Weight of cowpea gland (g)	Weight of epididymis (g)
<i>A. indica</i>	2.5	212.50±1.70	223.50±1.50	1.50±0.10	0.60±0.02	0.85±0.05
<i>A. indica</i>	5.0	182.00±1.50	185.00±3.00	0.75±0.07	0.40±0.01	0.55±0.05
<i>A. indica</i>	10	186.50±1.50	191.50±2.50	1.25±0.15	0.55±0.02	0.55±0.05
Control	0.5 ml/kg	205.00±3.60	207.50±3.50	0.60±0.05	0.20±0.01	0.65±0.05

The results were expressed in mean±SEM, p>0.05.

Table 3. Effect of *Azadirachta indica* seed aqueous extract on the body and reproductive organs weight after 14 days.

Treatment	Dose mg/kg	Initial body weight (g)	Final body weight (g)	Weight of testes (g)	Weight of cowpea gland (g)	Weight of epididymis (g)
<i>A. indica</i>	2.5	177.50±1.60	199.00±1.00	1.15±0.05	0.35±0.02	0.45±0.05
<i>A. indica</i>	5.0	174.50±1.20	199.00±1.30	1.10±0.00	0.50±0.03	0.45±0.05
<i>A. indica</i>	10	184.50±6.50	199.50±2.50	1.15±0.05	0.15±0.00	0.50±0.02
Control	0.5 ml/kg	179.50±4.50	187.00±4.00	0.90±0.00	0.55±0.05	0.55±0.05

The results were expressed in mean±SEM, p>0.05.

The semen analysis serves as the onset of selection for fertility evaluation and is frequently utilized in the definition of semen quality (morphology, viability, and sperm motility) and quantity (sperm count) (Khan, 2008). The findings from this study elicited a significant decrease in sperm count, progressive motility, and a significant increase in non-progressive, and immotile sperm cells across 2.5, 5.0, and 10 mg/kg of *A. indica* seed aqueous extract displaying the spermicidal effect of the extract when compared with the control in 24 hrs., 7

and 14 days of the exposure as shown in Figure 1. The semen analysis serves as an onset target for spermicidal activities utilized in the definition of an effective sperm killer. This agreed with the report of Asiif (2013) on a review of spermicidal activities on *Azadirachta indica*. Studies had shown that certain constituents in several plant materials possess spermicidal properties, which aid in controlling overpopulation (Umadevi *et al.*, 2013; Suryawanshi, 2011). *A. indica* seed aqueous extract serves as a potent spermicidal.

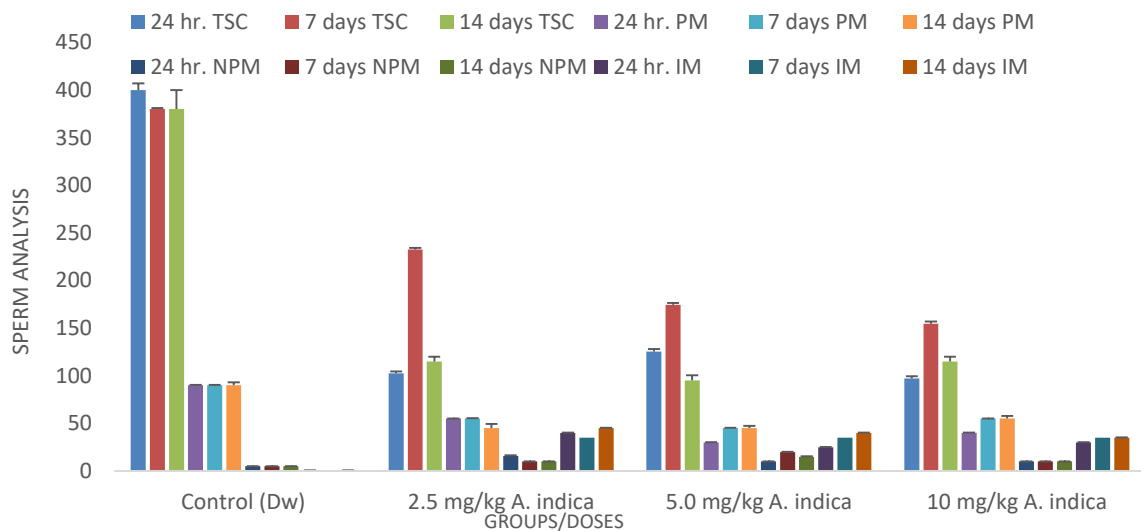


Figure 1. Spermicidal effect of *Azadirachta indica* seed aqueous extract on the sperm analysis after 14 days. Keywords: Total sperm cell count (TSC), Progressive motility (PM), Nonprogressive motility (NPM), immotile (IM).

The extract promotes a significant decrease in the level of testosterone which further triggers reduced libido activity as shown in Figure 2. This is in line with the report of Khan, M. A. (2008). *A. indica* seed aqueous extract, also are linked with the androgen inhibitory

function, which could be responsible for the detraction of male sexual performance as shown in this study (Deshpande *et al.*, 1980). Certain phytoconstituent stimulate sexual urges with spermicidal properties (Chauhan, 2008). Enhancement in the spermicidal effect

in this research study could be a result of the active constituents in *A. indica* seed aqueous extract. An increase in the level of androgen enhanced Leydig cells to stimulate luteinizing hormone resulting in an increased in spermatogenesis and increase epididymal sperm, but for the associated mechanism of action of *A. indica* seed aqueous extract the reverse is the case due to its spermicidal property. Findings by Chaudhuri, (2007) Practice of fertility control in weight, size and secretory role of the epididymis, testes and auxiliary organs. Modifications in the circulating level of androgen may be distorted by *A. indica* seed aqueous extract leading to interference during spermatogenesis (Jensen, 2002). Variation in the reproductive organ can be used as a marker to improve androgen levels in reproductive

glands, in the case of this study, the extract possibly impedes the feedback action involved in sperm formation. Meanwhile, the androgenic property is associated with serum testosterone concentration (Harris, 2018), the extract interferes with the function of testosterone discharge consenting by blocking the secretion of the hormone in gonads as displayed in Figure 2. A contradictory study by Gbotolorun *et al.* (2008) reported on *Mondia whitei* hexane extract on male sexual reproductive in rats.

Testosterone is the main androgenic hormone responsible for the sexual application to display a vital role in spermatogenesis. Findings showed that sexual zeal is triggered and maintained by the penis tissues that facilitated erection (Aversa *et al.*, 2000).

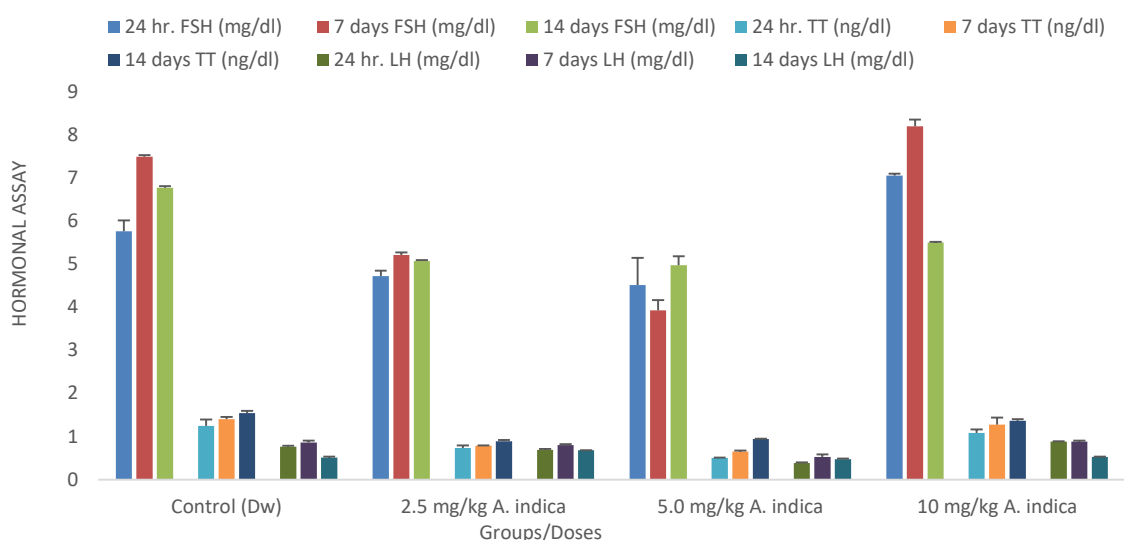


Figure 2. Spermicidal effect of *Azadirachta indica* seed aqueous extract on the hormonal level after 14 days. Keywords: Follicle stimulating hormone (FSH), Testosterone (TT), Luteinizing hormone (LH).

Studies from Gauthaman and Adaikan (2008) showed that male sexual dysfunction is related to several factors such as; androgen insufficiency. Testosterone agents (hormonal replace therapy) are exhibited to develop sexual roles and libido. Some plant extracts such as *A. indica* seed aqueous extract inhibit the synthesis or secretion of testosterone and serves as potent spermicidal or antifertility properties (Bansal *et al.*, 2010). A significant decrease in the level of testosterone is effective at a reduced dose as shown in *A. indica* seed aqueous extract when compared with the control (Figure 2), also a decrease in the follicle-stimulating hormone, luteinizing hormone, and progesterone inhibits the release of testosterone thereby eliciting antifertility effect of the extract (Figure 2).

The histopathological study of the testes exposed the standard architectural framework when compared with the control as shown in Plate 1. The treated organs at 2.5, 5.0, and 10 mg/kg of the *A. indica* seed aqueous extract showed an enhanced architecture structure of the testes. However, across the treated groups, an absence of spermatogenic action in the lumen of the seminiferous tubule was observed. This stimulated cellular activity occurrence from the cellar membrane all through to the lumen in seminiferous tubules found in the testes inhibits the secretion of the primary spermatogonia as proof for this study. It is agreed with Gbotolorun *et al.* (2008) reported results.

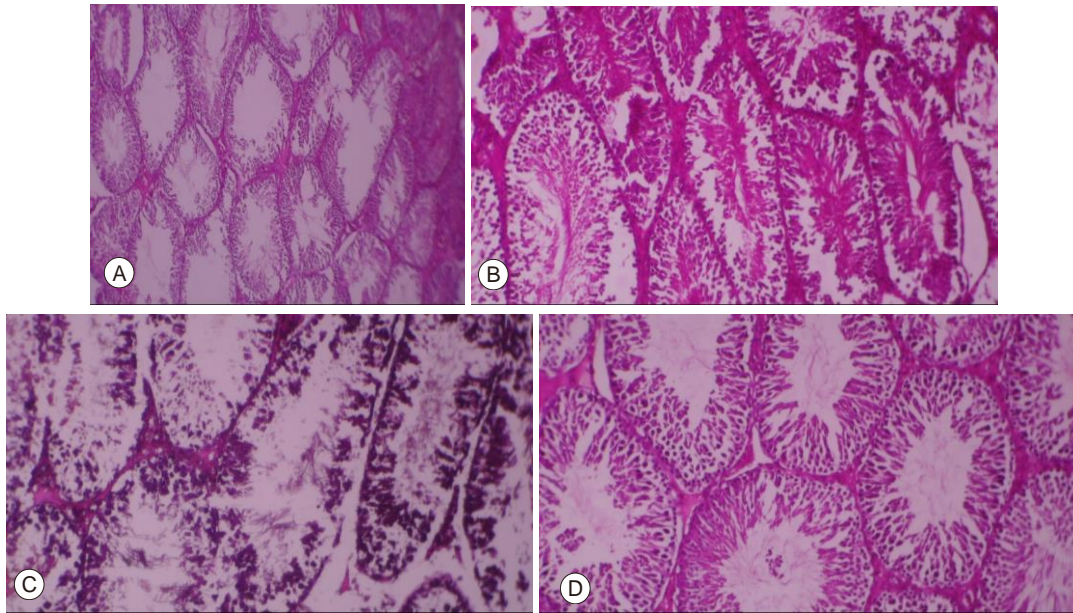


Plate 1. Effect of *Azadirachta indica* seed aqueous extract on the histopathology of testes after 14 days. (A. Control Testis: Seminiferous tubules with spermatocytes, interstitial space, and Sertoli cells. B. 2.5 mg/kg *A. indica* seed aqueous extract: Seminiferous tubules with normal sequential maturation of spermatocytes. C. 5.0 mg/kg *A. indica* seed aqueous extract: Seminiferous tubules with normal sequential maturation of spermatocytes. D. 10 mg/kg *A. indica* seed aqueous extract: Seminiferous tubules with normal sequential maturation and mild spermatogenic arrest (H&E x 1).)

Contraceptive study

Oestrogen is secreted during the menstrual cycle. The serum fuels of estrogen are usually low during every follicular phase rising gradually until about 1 day before ovulation. This present study in pre-coital treatment as shown in Table 3 elicited no significant increase in estrogen level, with a slight increase in follicle-stimulating and luteinizing hormone concentration across the treatment groups (2.5, 5.0, and 10 mg/kg *A. indica* seed aqueous extract) when compared with untreated control. While Progesterone controls ovulation and investigated luteal functions at 0.2-0.8mg/ml (follicular phase) and 4.0-20.0mg/ml (luteal phase), it is the hormone responsible for pregnancy. This study showed a significant decrease in the progesterone level across graded doses of the extract when compared with an increase elicited contraceptive property of the extract Tables 4 and 5.

Since antiquity, traditional medicines and several formulations from the herbal plant are known to be

effective as a natural contraceptives (Umadevi *et al.*, 2013). It has been established that a decrease in serum estrogen, showed the defect of the extracts against fertility property with the magnitude response which stimulates antifertility known as a contraceptive (Sedgh *et al.*, 2014). In this present study, the female contraceptive activities in female animals treated groups with graded doses of 2.5, 5.0, and 10 mg/kg of *A. indica* seed aqueous extract, elicited no significant difference in serum estrogen level in the pre-coital treatment but a slight significant increase in the post-coital phase of the contraceptive study when compared with the control. This concurred with Sharma *et al.* (2013) work on antifertility. This proposed product cannot stimulate estrogen levels serving to be a potent contraceptive agent either by decreasing its production or via metabolic impairment as shown in Tables 4 and 5 (Rajandeep *et al.*, 2011).

Table 4. Contraceptive effect of *Azadirachta indica* seed aqueous extract on pre-coital treatment after 14 days in Female animals.

Treatment	Dose (mg/kg)	LH (mg/dl)	PG (ng/dl)	EST (ng/dl)	FSH (mg/dl)
Control (Dw)	0.5 ml/kg	0.68±0.01 ^a	0.24±0.01 ^a	0.21±0.02 ^a	3.82±0.07 ^a
<i>A. indica</i>	2.5	0.75±0.01 ^b	0.19±0.01 ^a	0.23±0.02 ^a	4.05±0.17 ^b
<i>A. indica</i>	5.0	0.74±0.02 ^b	0.31±0.03 ^b	0.22±0.04 ^a	4.28±0.17 ^b
<i>A. indica</i>	10.0	0.71±0.04 ^a	0.34±0.03 ^b	0.25±0.02 ^a	4.41±0.17 ^b

The results were expressed in mean±SEM, p>0.05; Keywords: Follicle stimulating hormone (FSH), Progesterone (PG), Luteinizing hormone (LH), and Estrogen (EST).

Table 5. Contraceptive effect of *Azadirachta indica* seed aqueous extract on post-coital treatment after 14 days in Female animals.

Treatment	Dose (mg/kg)	LH (mg/dl)	PG (ng/dl)	EST (ng/dl)	FSH (mg/dl)
Control (Dw)	0.5 ml/kg	0.64±0.01 ^a	0.44±0.11 ^a	0.17±0.02 ^a	4.84±0.09 ^a
<i>A. indica</i>	2.5	0.79±0.01 ^b	0.77±0.04 ^b	0.32±0.01 ^b	4.84±0.08 ^a
<i>A. indica</i>	5.0	0.82±0.01 ^b	0.77±0.08 ^b	0.39±0.03 ^b	4.99±0.12 ^a
<i>A. indica</i>	10	0.88±0.01 ^b	0.82±0.08 ^b	0.33±0.01 ^b	5.70±0.02 ^b

The results were expressed in mean±SEM, $p>0.05$; Keywords: Follicle stimulating hormone (FSH), Progesterone (PG), Luteinizing hormone (LH), and Estrogen (EST).

In regards to *A. indica* seed aqueous extract intake, an increase in the luteinizing hormone and follicle-stimulating hormone in female rats was recorded. This finding is similar to previous studies reported by Pathak *et al.* (2005) to clarify the potential effect of female sex hormones with a decrease in luteinizing hormone and follicle-stimulating hormone impairment due to higher doses of toxic substances, affecting the production and secretion of this hormones. Hence, this study showed that luteinizing and follicle-stimulating hormones showed a slight significant increase in the treated groups

when compared with the control (Tables 4 and 5). Also, prolonged intake of *A. indica* seed aqueous extract rather reduced plasma progesterone concentration in females (Shaikh *et al.*, 2009). The results from this study showed a significant decrease in serum progesterone level across a graded dose of 2.5, 5.0, and 10 mg/kg of the treatment when compared with the control in the pre-coital phase with a slight increase in the post-coital phase (Table 4 and 5). A decrease in progesterone concentration as recorded in this study enhances the contraceptive effect in a dose-dependent manner (Neychev and Mitev, 2016).

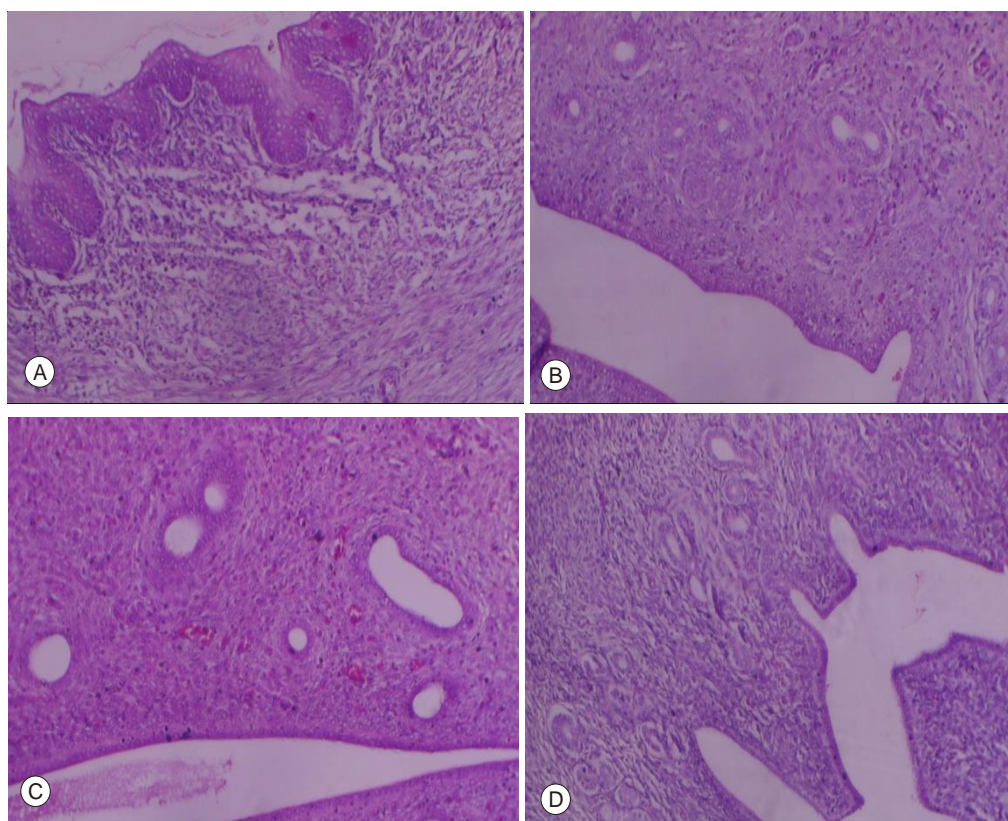


Plate 2. Contraceptive effect of *Azadirachta indica* seed aqueous extract on the Uterus in rats (A. Control. Rat uterus: ecto cervical epithelium, and subepithelial stroma. B. 2.5 mg/kg *A. indica* seed aqueous extract: Normal endometrial lining, stromal infiltrates of inflammatory cells and endometrial glands. C. 5.0 mg/kg *A. indica* seed aqueous extract: Stromal congestion, infiltrates of inflammatory cells, and glandular epitheliosis. D. 10 mg/kg *A. indica* seed aqueous extract: Stromal infiltrates of inflammatory cells (H&E x 100).)

Histopathological examination of the uterus administered with graded doses of 2.5, 5.0, and 10 mg/kg of *A. indica* seed aqueous extract reveals a

normal structure of the uterus muscles when compared with the untreated control (Drury and Wallinton 2013) (Plate 2). This suggested that the extract possesses a

protective effect on the uterus by inhibiting the stroma and other cells from secreting progesterone responsible for the occurrence of pregnancy. This concurred with the report of Dosaa *et al.* (2011) that showed proanthocyanidins with the strongest protective mediating against oxidative stress in the visceral organ.

CONCLUSION

This study elicited the herbal spermicidal and contraceptive properties of *Azadirachta indica* seed specifically at a reduced dose. This study provided anti-fertility properties of the extract, hence validating the folklore benefits. Therefore, further study is needed for compound isolation and elucidation.

Competing interests: The authors declare that there are no competing interests.

REFERENCES

- Ajayi, F.A. (2002). Formulation and promotion of neem—seed derived Biopesticides as an alternative to persistent organic pollutants (POPs) for the control of vegetable crop pests. In: Ukwe CN, Folorunso AO, Ibe AC, Z Z, N.E.S NES, Sieghart L (eds) Sustainable industrial utilization of neem tree (*Azadirachta indica*) in Nigeria. UNIDO Regional Development Centre, Lagos, pp 83–90.
- Alzohairy, M. A. (2016). Therapeutic role of *Azadirachta indica* and the active component in disease prevention and treatment. [Accessed; April 24th 2019] [<http://dx.doi.org/10.1155/2016/7382506>].
- Asiif, M. (2013). A review of spermicidal activities on *Azadirachta indica*. *Journal of Pharmacognosy and phytochemistry*. 1(5) 1-19.
- Aversa A, Andrea MI, Fabrini E, De-Martin M (2000) Androgen and penile erection: Evidence for a direct relationship between free testosterone and cavernous vasodilation in men with erectile dysfunction. *Clinical Endocrinology*, 53: 517-522.
- Bansal, P., Bansal, R. and Gupta, V (2010). Antifertility effects of *Azadirachta indica* (Neem) – A Review. *Annals of Biological Research* 1(2): 108-113.
- Chandra-Mouli V., McCarragher, D.R., Phillips, S.J., Williamson, N.E. and Hainsworth, G. (2014). Contraception for adolescents in low and middle-income countries: Needs, barriers, and access. *Reproduction Health*: 11:1.
- Chaudhuri, S.K. (2007). Practice of fertility control, [7th ed] Elsevier, New Delhi, 2: pp18.
- Chauhan, A., Agarwal, M., Kushwaha, S., and Mutreja, A. (2008). Antifertility studies of Aegle marmelos Corr., an Indian medicinal plant on male albino rats. *Egyptian Journal of Biology* 10:28-35.
- Dash, S. P., Singh, S. and Hussain, R. (2014). Intended and unintended pregnancy worldwide and recent trends. 45(3) 301-314.
- Deshpande, V.Y., Mendulkar, K.N. and Sadre, N.L. (1980). Male antifertility activity of *Azadirachta indica* in mice. *Journal of Postgraduate Medicine* 26:167-70.
- Dosaa E. D., Gabuj, M. S., Abdel, R. F. and Ahmed E. A (2011). Physiological and Histological impact of *Azadirachta indica* (neem) leaves extract in a rat model of cisplatin-induced hepatic and nephrotoxicity. *Journal of Medicinal Plant Research* 5(23):55-62.
- Dreweke, J. (2014). Contraception is not abortion; The strategic campaign of Antiabortion groups is to persuade the public otherwise. 17(4): 36-48.
- Drury RAB, Wallinton EA (2013) Carletons Histological Technique, 16th Edn, Oxford University Press, London, Pp124-136.
- Garg S, Taluja V, Upadhyay SN, Talwar GP (1993) Studies on the contraceptive efficacy of Praneem polyherbal cream. *Contraception* 48: 591-596.
- Gauthaman K, Adaikan PG (2008) The hormonal effect of *Tribulus Terrestris* and role its role in the management of erectile dysfunction- an evaluation using primates rabbit and rats. *Phytomedicine*, 15(1): 44- 54.
- Gbotolorun, S.C, Osinubi, A.A., Noronha, C.C. and Okanlawon, A.O (2008). The antifertility potential of neem female Sprague-Dawley rats. *African Health Science* 8:168-173.
- Harris, H. G., (2018). The future of birth control in India could be the needle. [<https://92.com/india/1425325/indian-scientists-make-breakthrough-in-birth-control>] [Accessed 2nd August, 2019].
- Hatcher, R.A., Trussell, J. and Nelson, A.L. (2007). Contraceptive Technology. [19th rev.ed.] New York: Ardent Media: 120: 148-759.
- Ibeh, N.I., Otabor, F.E., Oyedeji, J.T., Emili, A. and Omorodion, N.T. (2018). Improvised Eosin and Leishman as a morphological stain for sperm cell analysis, Adult Wistar rats and rabbits as a model of study. *Acta Scientifica Microbiology*. 1(1):18-20
- Jensen, J.T. (2002). Male contraception. *Current Women's Health Reports* 2(5), 338-45.
- Kelli, M. (2010). Neem tree Assessment for social economic empowerment in Rural Burkina Faso, pp: 4-5.
- Khan, M. A. (2008). An ethnomedicinal inventory of plants used for family planning and sex diseases in Samahni valley, Pakistan. *Indian J Tradit Know*. 7:277–83.
- Khillare B, Shrivastav TG (2003). The spermicidal activity of *Azadirachta indica* (neem) leaf extract. *Contraception* 68: 225-229.
- Khillare, B. and Shrivastava, T.G. (2003). Spermicidal Activity of *Azadirachta indica* (neem) leaf extract. *Contraception Sep.*, 68(3)79: 131-136.
- Koresriem, K.M.M. (2013). Review on pharmacological and toxicological effects of oleum *Azadirachta* oil. *Asian Pacific Journal of Tropical Biomedicine*. 3(10): 834-840.
- Kumar, D., Mishra P.K. (2010). Plant-based contraceptive popular among Tribals of Jharkhand. *Bioscience Discovery*, 2(1): 11-4.
- Neychev V, Mitev V (2016). Pro-sexual and androgen enhancing effects of *Tribulus terrestris* L.: fact or fiction. *Journal of Ethnopharmacology* 179:345–355.
- Nripendra, S., Ritu, S., Surenda, S., and Puja, A. (2019). A study on contraceptive action. 2(5). 26-44.

- Ogbuewu, I. P. (2011). The growing importance of Neem (*Azadirachta indica* A Juss) in the agriculture industry, medicine, and environment. *5*(3) Pp: 230 - 245.
- Pathak, A.K., Mallurwar, V.R., Kondalkar, A.K. and Soni S. (2005). A review of plants with anti-fertility activity. *Nigerian Journal of Natural Products and Medicine* **0**(9): 4-10.
- Prakash, A.O., Tewari, R.K. and Mathur, R. (1988). Non-hormonal post-coital contraceptive action of neem oil in rats. *Journal of Ethnopharmacology* *23*: 53-59.
- Rajandeep, K., Anil, S., Ravinder, K. and Rajeev, K. (2011). Rising trends towards herbal contraceptives. *Journal of Natural Products and Plant Resources*, **1**(4): **5-12**
- Raghuvanshi, P., Bagga, R., Malhotra, D., Gopalan, S., Talwar, G. P (2001). Spermicidal & contraceptive properties of Praneem polyherbal pessary. *Indian J Med Res* *113*: 135-141.
- Ruchi, T. Amit, K. V., Sandip, C., Kuldeep, D. and Shoor, V. S. (2014). Neem (*Azadirachta indica* A juss) is the potential for safeguarding the health of animals and humans. *14*(2): 110-123.
- Sedgh, G., Singh, S. and Hussain, R. (2014). Intended and unintended pregnancies worldwide in 2012 and recent trends. *Studies in Family Planning* *45*:301-14.
- Sethi, J., Singh, J., Gurung, N., and Aggraval, A. (2017). Medicinal plants as a potential source of male contraceptive agents. *1*(11) 7-12.
- Shaikh, M. A., Naqvi, S. N. and Khani, Z. A. (2009). Effect of Neem oil on the structure and function of the mature female albino rat ovaries. *7*: 28-34.
- Sharma, R.K., Goyal, A.K. and Bhat, R.A. (2013). Antifertility of plant extracts on female Reproduction: a review. *IJBS*. **3**(3): 493-514.
- Singh, R., Kakar, S., Shah, M., and Jain, R. (2018). Some medicinal plants with antifertility potential; A current Status. [[http; doi/10.413/2278960x.194512](http://doi/10.413/2278960x.194512)] [Accessed; 13th September, 2019].
- Suryawanshi, J. A. S. 2011. Neem-natural contraceptive for male and female– an overview. *International Journal of Biometrics and Bioinformatics* **1**(2):1-6.
- Suryawanshi, J. A. S. (2011). Neem-natural contraceptive for male and female– an overview. *International Journal of Biometrics and Bioinformatics* **1**(2):1-6.
- Umadevi, K., SampathKhumar, P.K., Bhowmik., D, and Duraivel, S (2013). Medicinal Plants with Potential Antifertility Activities. *Journal of Medicinal Plants Studies*. *1*(1): 26-33.
- Upadhyay, S.N., Dhawan, S., Garg, S., and Talwar, G.P. (1992). Immunomodulatory effects of neem (*Azadirachta indica*) oil. *International Journal of Immunopharmacology*. **14**(7): 1187–1193.
- World Health Organization. (2010). *Laboratory Manual for the Examination and processing of human semen* 5th edition.Pp 1-260