Journal of Morphological Sciences
J Morphol Sci
Brazilian Society of Anatomy

Original Article
J Morphol Sci 2024;42:26-33

# The Effect of *Azadirachta Indica* a Juss (Neem) Extract on Some Female Reproductive Organs in Albino Wistar Rats: a Histologic and Morphometric Study

**Hauwa Danladi Malgwi[1], Ruth O. Samuel[1], Martha Orendu Oche Attah[1,2], Nathan Isaac Dibal[1]**

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno State
[2]Faculty of Medicine, Cyprus International University, Nicosia, Cyprus

**Disclose and conflicts of interest: none to be declared by all authors**

## ABSTRACT

**Introduction:** medicinal plants are commonly and widely used in developing countries as an alternative to orthodox therapy for several reasons including cost, availability and affordability. Neem (*Azadirachta indica*) is one of the most important medicinal plants used worldwide with applications in Ayurvedic medicine. In Nigeria, women use it mainly for its anti-malarial effect. The aim of the present study is to determine the effect of oral ingestion of the aqueous extract of Azadirichta indica leaf on the histomorphology of female reproductive organs.

**Methodology:** Thirty rats of were sorted in 5 groups (A-E) receiving *Azadirachta indica* at different concentrations (100mg/kg - 400mg/kg). The first group was control and the treatment was administered orally for a period of 28 days for the rats in groups A-D and 48 days for rats in group E. The rats were sacrificed and the female reproductive organs harvested and processed to obtain histological sections which were stained, mounted and observed. Morphological measurement of several parameters were obtained and the results analyzed.

**Results:** *Azadirachta indica* caused general weight loss, decrease in number of ovarian follicles, distorted the epithelial lining of the uterus, caused accumulation of inflammatory cells in the lamina propria of the uterus and reduced the epithelial thickness of the vagina.

**Conclusion:** The results suggests that the extract has an adverse effect on female reproductive organs and could be explored as a potential prospect for development of reversible herbal contraceptive for the control of female fertility in mammals.

**Keywords:** Endometrium; Epithelium; *Azadirachta indica*; Uterus.

## Introduction

*Azadirachta indica* (Neem) has been considered as one of the most important medicinal plants used worldwide with many applications in Ayurvedic medicine in many parts of the world[1,2]. It is a large evergreen tree growing 10-11 m tall. The leaves are divided into numerous leaflets each resembling a full-grown leaf[1,3,4]. The plant contains different alkaloids which include: nimbitin, azadirachtin and salanin[4].

In Ayurvedic medicine, a decoction made from the bark, leaf, root, fruit, and flower of the plant is used in the treatment of blood morbidity, biliary afflictions, itching, skin diseases and peptic ulcers[5]. The bitter astringent bark is applied as a decoction for haemorrhoids. The leaves are steeped in water and used as treatment for malaria[1]. Neem leaf juice, infusion, or paste is applied externally to wounds and carbuncles[6,7].

*Azadirachta indica* seed oil is strongly antifungal[6], antiviral and active against spermatozoa[8]. Neem seed oil in experimental studies appeared to induce a pre-implantation block in fertility; having possible anti-fertility effects[5], it has been used to treat leprosy[6,7]. The seed oil of Azadirachta indica A. Juss (Neem) is also used in traditional medicine for its anti-diabetic, spermicidal, anti-fertility, anti-bacterial, and wound healing properties[5]. *Azadirachta indica* leaves serve as antibacterial, antifungal, anti-inflammatory, immunomodulatory, antihyperglycemic, antiulcer, antifungal, antibacterial, antimutagenic, anticancer, antimalarial, antiviral, antioxidant[9], antifertility[5,9]. *Azadirachta indica* leaves have been found to cause vacuolization of sertoli cells, diminished cytoplasmic inclusions in Leydig cells, as well as defects in the mitochondrial sheaths in late spermatids in the testes. Thus, *Azadirachta indica* leaves may affect spermatogenesis through antispermatogenic and antiandrogenic properties[1,10] with no notable side effects[9].

Women in many parts of the world consume different parts of the *Azadirachta indica* plant for several reasons. In Northwest Madagascar, women consume neem leaves to prevent pregnancy, In Gambia and Ghana, *Azadirachta indica* leaves are made as tea


DOI: 10.51929/jms.42.26.2025
ISSN 2177-0298

drink to stop pregnancy at the age of the first 2-3[14]. In Nigeria, many women in several stages of pregnancy and lactation use the plant mainly for its anti-malarial effect[1,2]. Anecdotal reports from these women suggest that they consume the extract because of the belief that it is potentially harmless, hematopoietic and protects them from malaria[11]. Few studies suggest that *Azadirachta indica* oil reduces number of developing follicles in the rat ovary and induces degeneration of oocyte in vitro[12,13,14].

The aim of the present study is to determine the effect of oral ingestion of several dosages of the aqueous extract of *Azadirichta indica* leaf on the histomorphology of the ovary, uterus and vagina.

## Materials and Methods

### Procurement of Plant Material and Extraction

The plant was obtained from a *Azadirachta indica* plant that was more than twenty years of age. The plant leaves were collected at sunrise and were carefully air dried in a well-ventilated room. The leaves of the plant were mechanically ground into powder which weighed 495g. This powder was subjected to extraction to obtain an aqueous extract. The powder was dissolved in 1.5 liters of distilled water and left to stand without agitation for 24 hours. This solution was filtered, sieved and the filtrate was allowed to evaporate overnight at a temperature of 40$^{o}$C.

### Animal Husbandry

Thirty (30) female adult albino rats were purchased and accommodated in the Animal House, Department of Human Anatomy, Faculty of Basic Medical Sciences. They were grouped into a control group (Group A) and treatment groups (Groups B, C, D, and E as indicated below). The weight of the rats were recorded at the start of the experimental study and then weekly during the experimental period. The rats were allowed to acclimatize for two weeks before the start of the experimental study. The rats were properly fed with standard rat chow and water ad libitum. They were housed in plastic cages with stainless steel mesh covers. Adequate cross ventilation was provided in the animal house.

### Treatment Protocol

The treatment protocol of the grouped rats is described as follows:

Group A: Distilled Water

Group B: 100mg/kg of *Azadirachta indica* for 28 days

Group C: 200mg/kg of *Azadirachta indica* for 28 days

Group D: 400mg/kg of *Azadirachta indica* for 28 days

Group E: 400mg/kg of *Azadirachta indica* and Recovery for 28 days

The extract was administered orally at the same time daily to rats in the treatment groups as determined by treatment protocol. This was continued for a period of 28 days for the rats in groups A-D and 48 days for rats in group E. The last group was sacrificed after another 28 days to determine if there was any reversal in the effects of the administered extract.

After the animal sacrifice, the uterus, ovary and vagina were quickly removed from a laparoscopic incision on the anterior abdominal wall. The ovaries were weighed and preserved in Bouin's fluid in preparation for tissue processing as well as to preserve the tissue and prevent autolysis and putrefaction. The tissues were subjected further stages of tissue preparation to obtain micrographs for observation using a microscope.

### Morphometric Analysis

The images of the histological sections were observed and photographed using an Amscope light microscope (MBJX-ISCOPE, Los Angeles) fitted with a digital camera (M500, X 64, version 3.7) under X100 and X400 magnifications. Photographic images of the histological sections were taken using 10X objective lens and were also analyzed using an Amscope Image and ImageJ software. An ocular micrometer, which was standardized with a stage micrometer, was also used to measure parameters of interest in the histological slides. The results of the measurements were analyzed using GraphPad Prism Software (Version 8.0.2).

The histological section of the uterus, and vagina in all groups were morphometrically analyzed to observe the following parameters:

a) The length of the epithelial lining of the uterus (µm) which is measured as the distance of the line drawn from the basement membrane of the simple columnar epithelial cells of the uterus to its apical surface

b) The thickness of the non-keratinizing stratified epithelial lining of the vagina (µm) which is measured as the length of the line drawn from the stratum basale of the vagina to the apical cells of stratum corneum.

c) Width (µm) of the endometrial glands which is the measurement of the thickness of the glands found in the lamina propria of the uterus.

### Ethical Considerations

During the research work, the Authors adhered to the ethical guidelines outlined by the Declaration of Helsinki: Statement of Ethical Principles for Medical Research and University of Maiduguri Research and Ethical Committee, and the National Institutes of Health (NIH) guide for the CARE and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and conformed to Directive 2010/63/EU. The present research was approved by the Ethical Committee of the Department of Human Anatomy, University of Maiduguri, with code number UM/HA/UGP 19.20073/085.

## Results

### Effect of *Azadirachta indica* on the Weight of the Rats

Figure 1 presents the effect of the extract on the weights of the rats by presenting the initial and final weight of the rats in all groups. The control group showed a slight weigh increase (5g) at the end of the experimental study. In the treated groups however, there were increments in the weights in groups B (20g), D (7g) and E (15g). There was weight loss (24g) recorded in group C at the end of the experimental period.

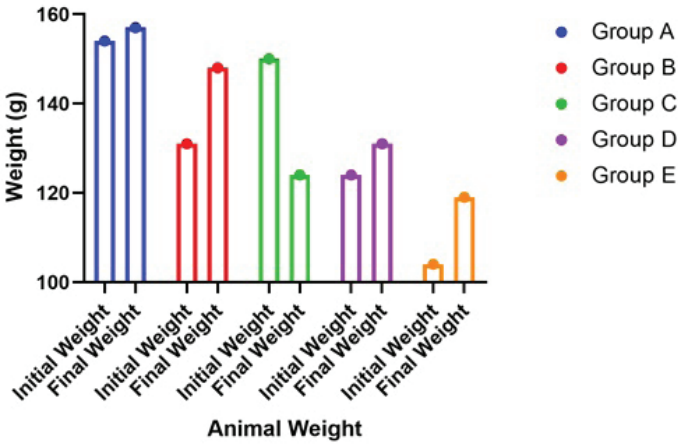


**Figure 1.** Showing the difference between the initial and final weight.

### Effect of *Azadirachta indica* on the Weight of the Ovaries

*Azadirachta indica* caused both an increase and decrease in the weights of the ovaries when compared to the control group. In groups B and E, the weights of the ovaries were highly increased when compared to the control group and in groups C and D, there was a decrease in the weight of the ovaries when compared to the control group (Figure 2).

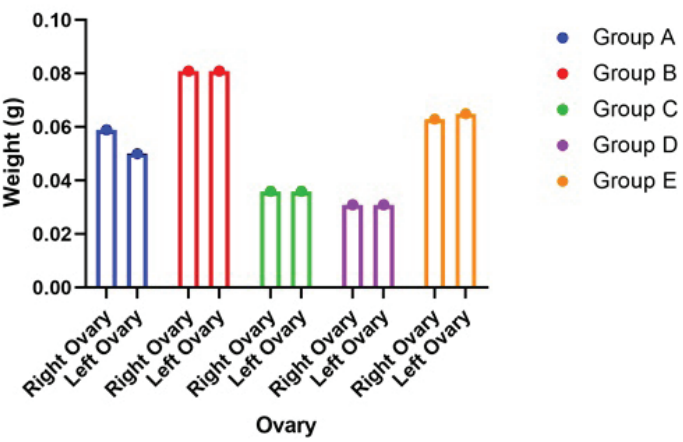


**Figure 2.** Showing the weights of the ovaries in the experimental groups.

### Effect of *Azadirachta indica* on the Histomorphology the Uterus and Vagina

The epithelial lining of the uterus was thickest in group B as the thickness was statistically significant ($p<0.05$) when compared to Groups A, C and E (Figure 3).

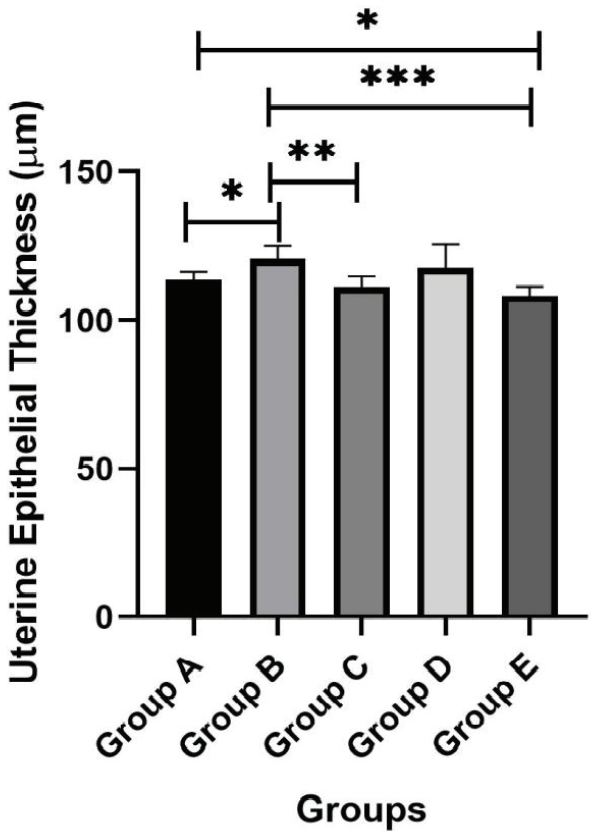


**Figure 3.** Showing the measurement of uterine epithelial thickness in rats in all groups.

The vaginal epithelial lining was thickest in group C as it was statistically significant ($p<0.05$) when compared to the lining in groups D and E (Figure 4).

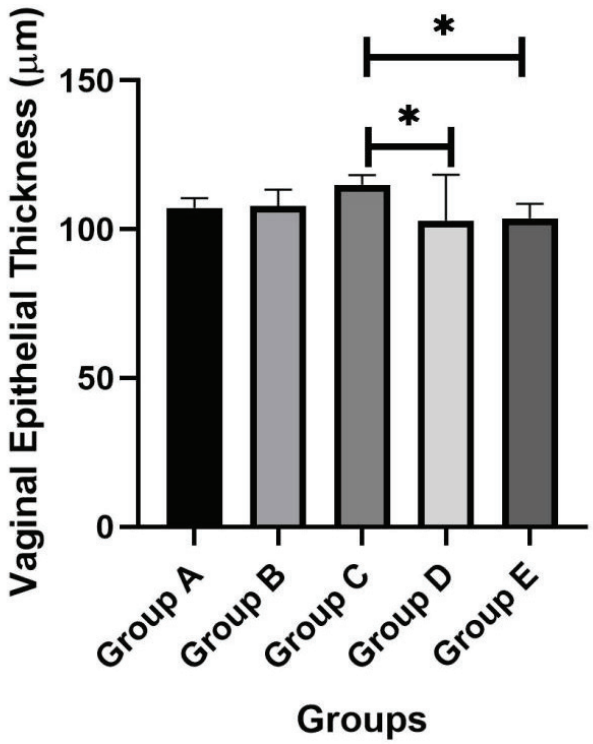


**Figure 4.** Showing the vaginal epithelial thickness in rats in all groups.

The uterine glands were wider in groups B and E and this was statistically significant ($p<0.05$) when compared to groups C, D and A (Figure 5).

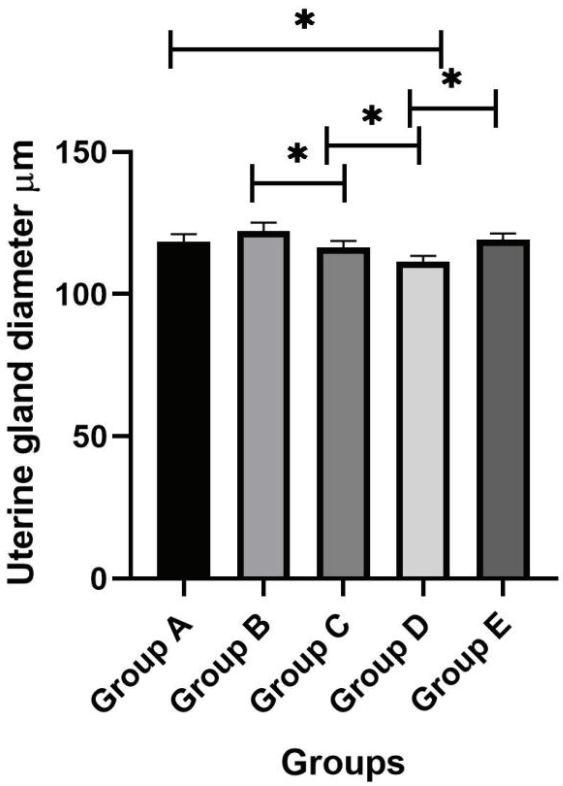


**Figure 5.** Showing uterine gland diameter in all groups.

**Effect of *Azadirachta indica* on the Histology the Ovaries, Uterus and Vagina**

The vaginal epithelium of the animals in all groups are represented in Figure 6 A-E showing the normal histology of the vagina with the areas arranged into an epidermal and dermal regions. The basal epithelium was easily separated from the underlying dermis and consisted of low columnar epithelial cells resting on a basement membrane. The cells in this lining had eosinophilic cytoplasm and nuclei which had a prominent nucleoli. The layer above consisted of polyhedral cells with a less eosinophilic cytoplasm than in the basal lining. The nuclei were also centrally located and were observed in the micograph as rounded circular structures. The nucleoli was prominent in most of the nuclei. Stratum granulosum and corneum were not easily separated but the apical cells of stratum corneum were observed to exfoliate from the surface of the epithelial lining. The cells observed in this layer were flattened into sheets and had lost their nuclei (Figure 6A). The dermis of the vagina was separated into the papillary and reticular regions with the papillary region located just below stratum germinativum. the reticular layer of the dermis was denser and had more connective tissue elements arranged in that region. The dermis was highly vascularized and numerous cells were also found in this area.

The vaginal epithelial lining in the treated groups showed a similar arrangement with the epithelia found

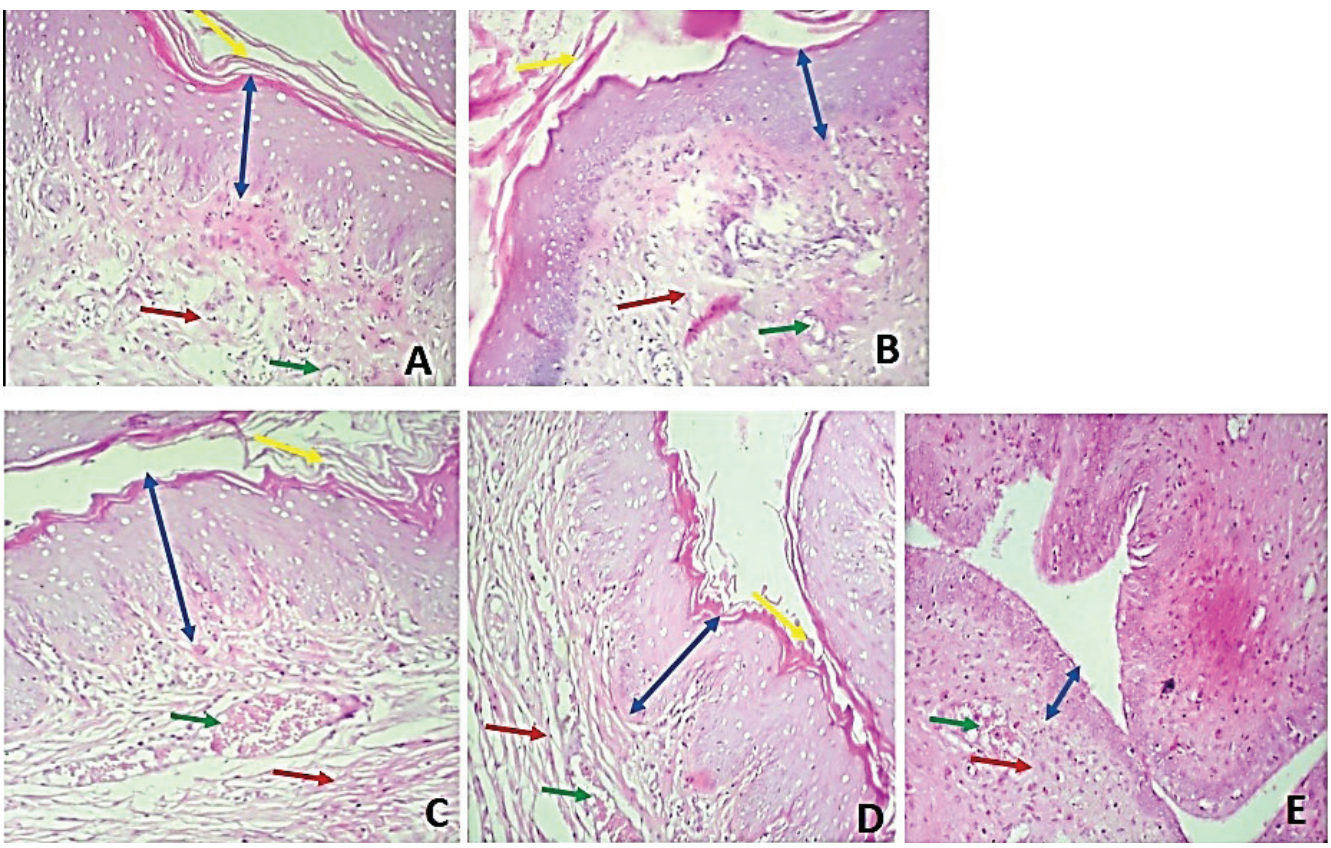


**Figure 6.** Showing the epithelial lining of the vagina in all groups (A-E). Blue arrow represents the epithelial lining of the vagina which was thinnest in group E. The yellow arrow represents stratum corneum of the epidermis and green arrow representing blood vessels in the lamina propria (red arrow). H and E X100.

in the control group with varying thickness with the that in group E being the thinnest (Figure 6E). the epithelium in this group was also lacking an overlying stratum corneum. The dermis in all the groups were vascularized but the blood vessels were more dilated in the dermis of rats in groups C and D (Figure 6C and D). The dermis of the rats in group E were denser and numerous inflammatory cells were located and easily identified by their dark staining, rounded nuclei and pale staining cytoplasm. These cells were more in number in this group than in any other group (Figure 6E).

The histology of the ovaries showed follicles at different stages of maturation in the control group, however, majority of the follicles observed were primary follicles with surrounding granulosa cells. These were distributed in the cortical ovarian stroma along with numerous primordial follicles (Figure 7A). The histology of the ovary in the other groups showed fewer ovarian follicles in the cortical stroma when compared to the control group. The ovary of the rats in group C showed the highest number of corpora lutea and had few primordial and primary follicles (Figure 7C). They were fewer primordial follicles in the ovarian stroma of rats groups C, D and E when compared to the control group (Figures 7C,D and E). The histology of the ovaries in groups D and E had several maturing follicles in the ovarian cortex which were surrounded by granulosa and thecal cells (Figure 7D and E).

The micrograph representing the uterus is represented in Figures 8A-E. The endometrium consisted of simple columnar epithelial cells resting on a basement membrane. The endometrial lining was thrown into folds and underlying this layer was a connective tissue lining which extended into the endometrial folds. The lamina propria was densely packed with connective tissue and was highly vascular. Few uterine glands were located in the connective tissue lining (Figure 8A). The endometrium in group B had similar cellular arrangement as observed in group 8A. There was a marked infiltration of cells into the lamina propria of the treated groups and these were also present in group E where treatment was discontinued for 28 days. More uterine glands were observed in this group compared to the control group. The endometrial epithelium in group C had cells that had pale-staining nuclei and were disorganized. The columnar arrangement was not observed in this group as also was seen in the epithelium of the uterus in groups D and E (Figure 8C, D and E). The dermis of the uterus in groups D and E had numerous uterine glands in the lamina propria.

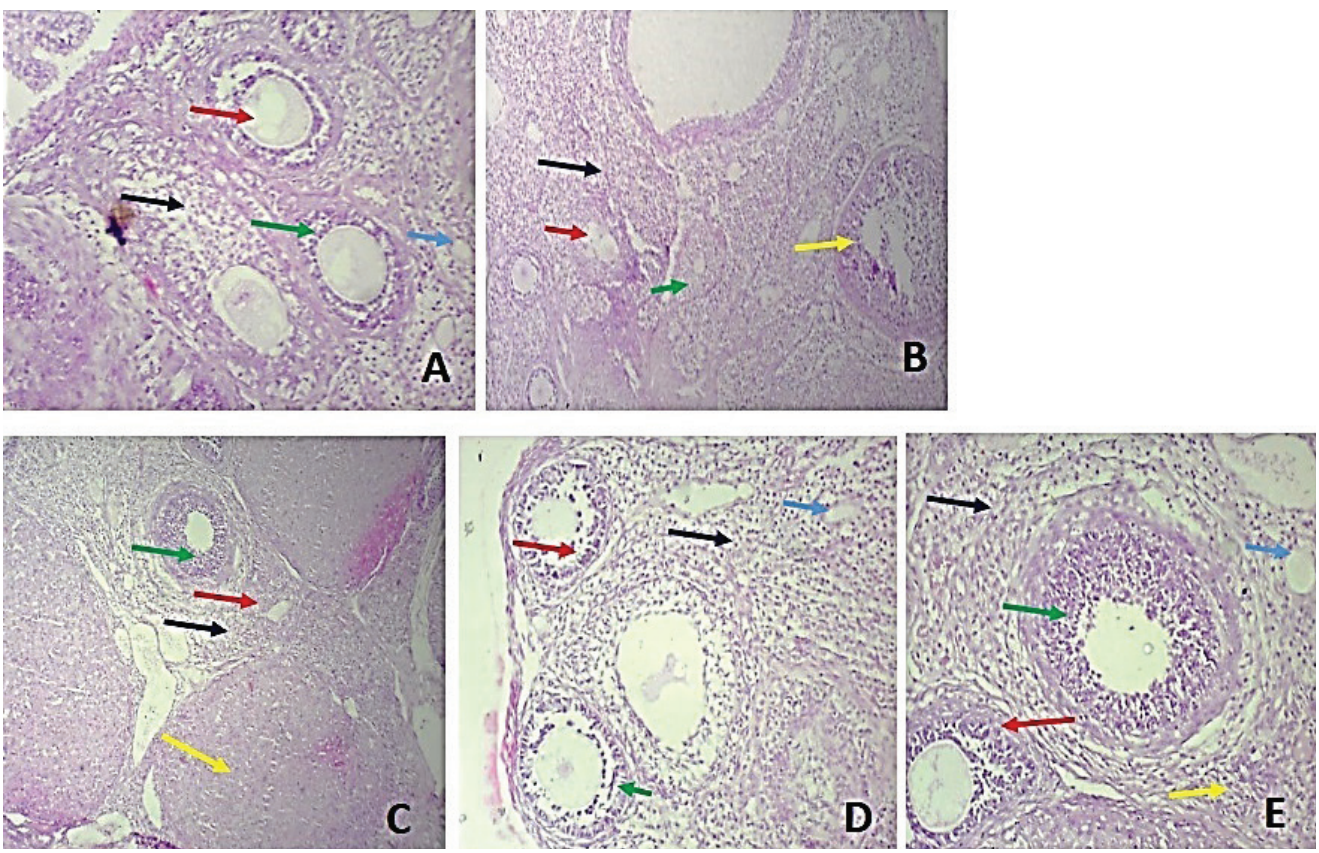


**Figure 7.** showing the micrograph of the ovaries of rats in all groups with the red arrow pointed at the ovarian follicles (yellow arrow) which were surrounded with granulosa cells (green arrow). These were most numerous at several stages of development. Primordial follicles (blue arrow) were abundant in the cortical stroma (dark blue arrow) in group B. Several corpora lutea (yellow arrow) were present in the ovary of rats in groups B, C and E. H and E X100.

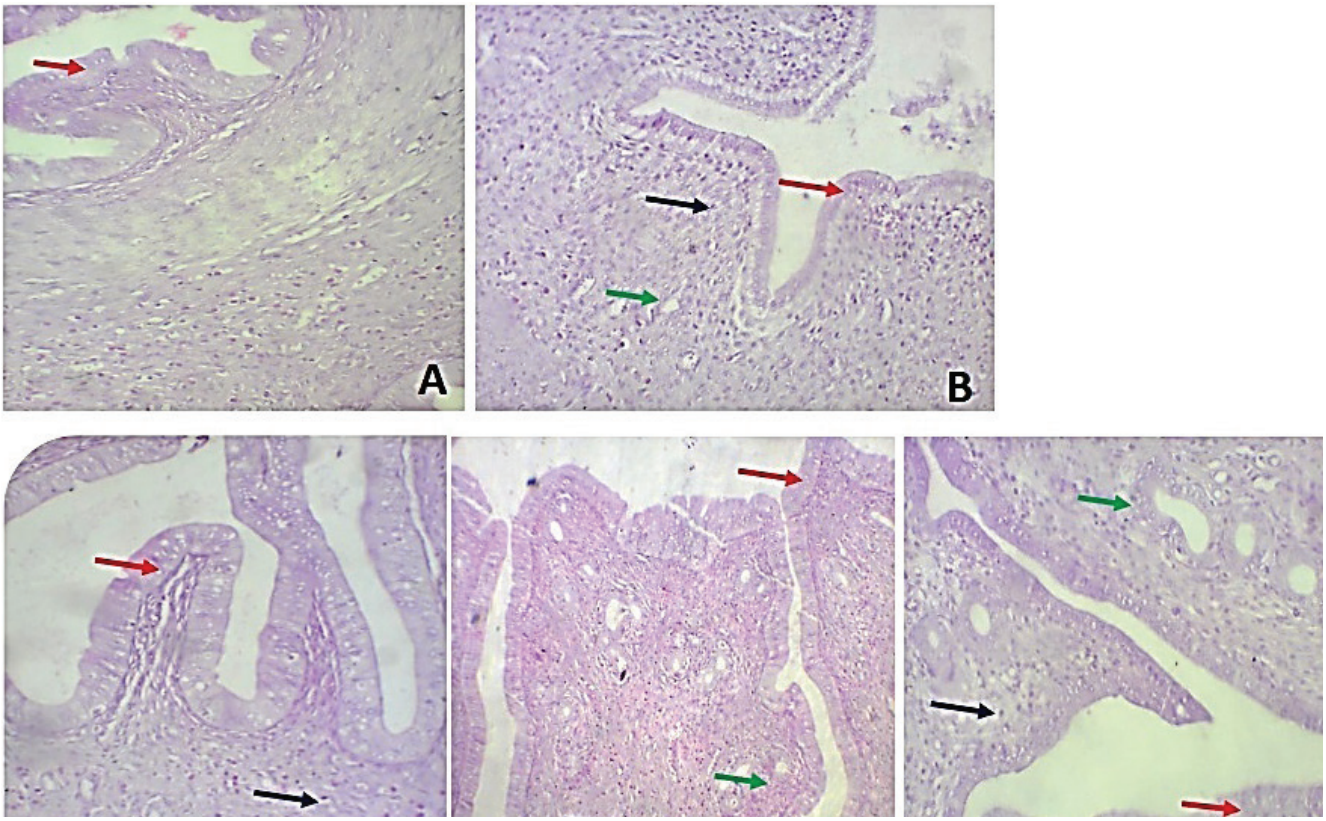


**Figure 8.** Showing the micrograph of the uterus in groups A-E with the red arrow showing the uterine epithelial lining. Uterine glands (green arrow) were numerous in the lamina propria with infiltration of inflammatory cells (black arrow) of rats in groups D and E. H and E X 100.

## Discussion

Medicinal plants are commonly and widely used in developing countries as an alternative to orthodox therapy for several reasons including cost, availability and because knowledge of these medicinal plants was passed down through word of the mouth down many generations. In Africa alone, hundreds of plants are used traditionally for the management and/or control of many medicinal disorders. Regrettably, only a few of such African medicinal plants have received scientific examination[15].

*Azadirachta indica* plant extract serves a lot of medicinal purposes and is consumed in many parts of the world with little or no knowledge of its effect on the reproductive tract by the local populace that ingest the extract for medicinal purposes. The present investigation was therefore undertaken to determine the effect of aqueous leaf extract of *Azadirachta indica* L on antifertility parameters specifically on the epithelial lining of the uterus, vagina and histology of the ovaries in female Wistar rats as a model to reduce the risk of infertility resulting from the use of the plant as malaria therapy.

The results of the present study determined that *Azadirachta indica* plant extract caused a decrease in total body weight when compared to the control group and this was inconsistent with studies carried out by Researchers who pointed out that aqueous leaf extract of Neem had no adverse effect on growth and body weight of the rats by showing that there was a general increase in body weight of all rats in the treatment group[1] however, this increase was not statistically significant. In another experimental study, it was determined that *Azadirachta indica* caused a non-significant increase in weight in the treated animals[16].

The extract caused an increase in the weight of the ovaries in the rats that received the lowest dosage and this could be seen to be greater than the weight of the ovaries in the control group. There was then a subsequent decrease in the weight of the ovaries in groups that received increasing dosages of the extract and this could be attributed to the anti-fertility effect of the extract which caused the extract to reduce the amount of viable follicular cells in the ovary as collaborated by researchers who determined that *Azadirachta indica* induced apoptosis in follicular granulosa cells as well as in the ovary as it was found to deteriorate oocyte quality after ovulation[16]. The weight of the ovaries increased again after the extract was withheld for 28 days suggesting that the effect of the extract was reversed after treatment stopped. Studies by scientists however reported that Neem seed oil has been reported to have no role on ovarian function[5,13]. Further studies revealed that alcoholic extract of flowers from *Azadirachta indica* harmed the ovulation of adult Sprague-Dawley rats, suggesting its viability as a contraceptive[15].

In the current study, the uterine epithelium was thickest in the group that received the lowest concentration of the extract and it could be deduced

that at this concentration, there was no disorganization of the columnar epithelial cells as was observed in the other treatment groups. It was discovered that the uterine histology of the horn treated with neem oil was typical of the organ at diestrous stage and no pathological changes were noted[5]. The vagina of rats treated with 200mg/kg of the extract had the thickest epithelial lining and also, the glands in the dermis was thickest in the group that received the lowest dose and in the group that received no treatment for 28 days prior to sacrifice, suggesting that when the extract administration was was discontinued, there was proliferation in vaginal epithelial lining and uterine glands.

Histological observations in the present study determined that *Azadirachta indica* caused localized bleeding in the dermal region of rats that received treatments of the etract but this was absent in the group where the extract was discontinued. Cellular infiltration was also observed in the lamina propria of the uterus as evidenced by the presence of more nuclei in the connective tissue layer which was not evident in the control group. It was also reported epithelial growth factor receptor (EGFR) localization in the luminal and glandular epithelium, by causing massive leukocytes infiltration into the uteri[17]. Histological observations of the uterine epithelium in studies carried out confirmed that application of neem oil showed cellular infiltration consisting mainly of macrophages and neutrophils while control animals, in contrast, had no such cellular infiltration in the uterine epithelium[5]. In the male reproductive organs, *Azadirachta indica* extract was found to reduce the rats' weight; decreased both the weight and dimension of the testes; reduced the number of germinal epithelial lining cells in the seminiferous tubules of the testes, the epididymal and prostatic secretory epithelial cells[19].

Studies have shown show that treatment with *Azadirachta indica* leaves and seeds extract can decrease the pregnancy viability on the mice, although not statistically significant when compared with control group treated[18].

## Conclusion

In the present study, *Azadirachta indica* caused general weight loss, decrease of ovarian follicles, distorted the epithelial lining of the uterus, caused accumulation of inflammatory cells in the lamina propria of the uterus and reduced the epithelial thickness of the vagina. Based on the current results obtained and apoptotic-inducing quality of the extract on ovarian follicles and uterine epithelial cells, there is adequate evidence to suggest that the extract could be explored as a potential prospect for the development of reversible herbal contraceptive for the control of female fertility in mammals. However, further studies are recommended to determine the exact mechanism by which this activity is achievable.

## Acknowledgements

The Authors wish to acknowledge the assistance of the laboratory staff of the Gross and Histology Laboratories of the Department of Human Anatomy, Faculty of Basic Medical Sciences for their assistance during the current research work.

## References

1. Azekhumen GN, Osayande OE and Jonathan MO. Effect of Aqueous Leaf Extract of Azadirachta Indica (Neem) on the Reproductive Hormones of Male Albino Wistar Rats African Scientist 2017; 18(4) 227-233
2. Akpantah AO, Ekong MB, Uruakpa KC, Akpaso M, Eluwa MA, Ekanem TB. Gonadalhisto-morphologies and serum hormonal milieu in female rats treated with Azadirachta indica leaf extract. Indian J Reprod Med 2010; 8(4): 185-190.
3. Kausik BI and Ranajet KB: Biological activities and medicinal properties of Neem plant. Curr Sci 2002;82: 1336-1345.2002.
4. Koul O, Isman MB, Ketkar CM: Properties and uses of Neem, Azadirachta indica. Can J Bot 1995; 68:1-11. 1995.
5. Mitra A. Evaluation of Contraceptive Properties of Neem Oil - A Prospective Study Sci. and Cult. 2018; 84 (1–2) 67-70
6. Sankarnarayan D. Antifungal Activity of Neem Oil and its Constituents, Mediscope 1965:8. Pg. 322-26.
7. Sinha KC, Riar SS and Tiwary RS. Neem oil as a Vaginal Contraceptive, Ind. J. Med. Res.1984;79, 131136
8. Upadhyay SN, Kaushic C and Talwar GP Antifertility Effects of Neem (Azadirachta indica) Oil by Single Intrauterine Administration: A Novel Method for Contraception Biological Sciences ,1990; 242 (1305). pp. 175-179
9. Seriana I, Akmal M , Darusman and Wahyuni S, Neem leaves extract (Azadirachta indica A. Juss) on male reproductive system: a mini-review, IOP Conf. Series: Earth and Environmental Science 2019;399: 012106
10. Kasturi M, Manivannan B, Ahamed RN, Shaikh PD, Pathan KM: Changes in epididymal structure and function of albino rat treated with Azadirachta indica leaves. J Ethnopharmacol, 1988;23: 53-59. 1988.
11. Iyare E and Obaji N. Effects of Aqueous Leaf Extract of Azadirachta Indica on some Haematological Parameters and Blood Glucose Level in Female Rats. Niger J Exp Clin Biosci 2014; 2:54-58.
12. Chaube SK, Prasad PV, Khillare B, Shrivastav TG. Extract of Azadirachta indica (Neem) leaf induces apoptosis in rat oocytes cultured in vitro. Fertil Steril 2006; 85:1223–1231
13. Chaube SK, Shrivastav TG, Tiwari M , Prasad S, Tripathi A and Pandey AK. Neem (Azadirachta indica L.) leaf extract deteriorates oocyte quality by inducing ROS- mediated apoptosis in mammals; SpringerPlus 2014, 3:464
14. Tripathi A, Shrivastav TG, Chaube SK. An increase of granulosa cell apoptosis mediates aqueous neem (Azadirachta indica) leaf extract induced oocyte apoptosis in rat. Int J Appl Basic Med Res 2013;3:27–36
15. Santiago M & Salvo L. Effects of the neem extract (Azadirachta indica) on mammalian reproduction. Journal of Analytical & Pharmaceutical Research. 2018; 7: 8(2) 10.15406/japlr.2018.07.00227.
16. Aigbiremolen AA & Odigie MO Modulations of Neem Leaf Extract on Reproductive Hormones of Male Wistar Rats. J Bioengineer & Biomedical Sci 2018; 8: 248. doi:10.4172/2155-9538.1000248
17. Juneja SC, Williams RS, Farooq A, Chegini N. Contraception potential of neem oil: effect on pregnancy success in the mouse. J

Assist Reprod Genet. 1996;13(7) : 578-85.
18. Lisanti E. Effect of Ethanolic Extract of Neem (Azadirachta Indica A.Juss) Leave and Seed to The Female Mice Reproduction (Mus Musculus Albinus) Biology, 2013;10(2);88-93
19. Zadva AK, Mshelia GK, Enoch F, Attah MOO. Morphometrical and Histological Analyses of the Epithelial Lining of Male Reproductive System in Wistar Rats Following Administration of Neem Leaves Extract. IJT 2022; 16 (4) :247-258

Received: September 1, 2024
Accepted: February 6, 2025

Corresponding author
Martha Orendu Oche Attah
E-mail: marthaorendua@unimaid.edu.ng, mattah@ciu.edu.tr