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Full Length Research Paper

THE EFFECTS OF AQUEOUS EXTRACT OF *XYLOPIA AETHIOPICA* FRUIT ON OESTROGEN LEVELS IN FEMALE WISTAR RATS

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ABSTRACT

Numerous plants and plant metabolites have proven to be very potent in treating diseases and modifying physiological processes. This study investigated the effect of aqueous extract of *Xylopia aethiopica* fruit on the reproductive system of mature virgin female Wistar rats. Aqueous extract of *Xylopia aethiopica* fruit was obtained by hot water maceration. Fifteen female Wistar rats were randomly divided into three groups(A, B, and C) of five animals per group, per cage. Group A served as the standard control group and was administered distilled water. In contrast, groups B and C served as the treatment groups and were administered 150and 300mg/kg body weight of the extract, respectively, for 15days orally. The effect of the plant extract on; reproductive hormonal profile (oestradiol, progesterone, and progesterone-estrogen ratio), reproductive organ and body weights, vaginal cytology, and reproductive histology were assessed. All the rats were at the follicular phase before and after treatment. All the measured parameters except oestradiol levels were statistically insignificant ($p > 0.05$). Serum oestradiol levels increased significantly ($p < 0.05$)in the 300mg/kg body weight group (68.67 ± 0.20 pg/ml) when compared with the control group (46.13 ± 5.07 pg/ml).The work thus showed that the plant could affect a female's reproductive cycle by enhancing oestradiol levels at the follicular phase.

Key words: *Xylopia aethiopica* fruit, oestradiol, vaginal cytology, reproductive histology

INTRODUCTION

Herbal remedies have been the most therapeutic approach to treat and manage ailments in man. Although there are numerous advantages in synthetic medicines, there has been an increase in demand for plant products in disease treatment, fertility improvements, and the management of some physiological disorders. The World Health Organization

estimated that about 75% of the world's population uses herbal remedies, primarily because it possesses fewer side effects than orthodox drugs (Liu *et al*., 2007). Hence, plant materials are continuously scrutinised and explored for their beneficial effects.

Many tropical plants and their extracts have shown beneficial therapeutic effects, including fertility enhancement and contraceptives (Raji *et al*., 2006). In Africa, *Xylopia aethiopica* (Dunal) A. Rich is a commonly used plant whose parts have high medicinal value (Burkhills, 1985). *Xylopia aethiopica* (Dunal) belongs to the Annonaceae family and is commonly called "Negro pepper", "African guinea pepper", or "Ethiopian pepper. In Nigeria, the fruits of *Xylopia aethiopica* is locally called "uda" in Igbo, "Peru Alamo" in Yoruba and "Chimba" in Hausa (Nnodim *et al*., 2013). Traditionally, it has been utilised for inducing placental discharge postpartum, management of rheumatism, asthma, headache, bronchitis, neuralgia, and colic pain (Woode *et al*., 2011). The seeds are traditionally used to enhance postpartum placental expulsion (Fetse *et al*., 2016). In addition, the fruit is applied in combination with other herbal remedies, like *Piper nigrum*, *Capsicum frutescence* and *Tetrepleura tetraptera* for newly delivered mothers; it helps in uterine contraction (Durugbo, 2013). Female infertility, haemorrhoids, uterine fibroid, and amenorrhoea are treated traditionally with *Xylopia aethiopica* (Nnodim *et al*., 2013).

There is global concern about the increasing incidence of reproductive dysfunction. Hence the need to scientifically assess plants traditionally used for reproductive purposes to evaluate their possible use as modulators of female reproductive physiology. Thus, this study was designed to assess the effect of the aqueous fruit extract of *Xylopia aethiopica* on the female reproductive system using Wistar rats as an experimental model. The female Wistar rat's reproductive histology, hormonal profile and reproductive organ weights were examined.

MATERIALS AND METHODS

Experimental Animal

Fifteen matured virgin female Wistar rats (10 - 12 weeks-old; 180 ± 20 g) were purchased and housed with good hygiene in sanitised cages. They were housed under standard environmental conditions (23 –25°C and 12 hrs per light/dark cycle) and were acclimatised for two weeks before the commencement of the research. The experimental animals had access to standard rat chow and tap water *ad libitum*. The rats were managed using the approved ethical guide for laboratory animals.

Identification and collection of plant material.

The fruit of *Xylopia aethiopica* was obtained from the central market at Gwagwalada, Abuja, Nigeria and authenticated at the Herbarium Unit, National Institute for Pharmaceutical Research and Development, Idu Industrial Layout, Abuja- Nigeria. A sample of the plant was deposited at the herbarium and assigned a specimen voucher number, NIPRD/H/7215.

Aqueous Extraction of *Xylopia aethiopica* fruit

One thousand grams (1000 g) of the fruit was coarsely ground. The material was then immersed in hot water at a

temperature of 80 ºC for 24 hours. The suspension was filtered through a muslin cloth and a cotton wool plug in a funnel. The filtrate was dried at 70 - 80 ºC in a water bath with a shaker within four days to yield aqueous *Xylopia aethiopica* fruit (XAF) extract.

Qualitative phytochemical screening of XAF extract.

The extract was qualitatively screened for the presence of tannins, saponins, triterpenes, flavonoids, sterols, and carbohydrates using standard methods described by Evans (1996) and Silva *et al*. (1998).

Experimental Design

The 15 female Wistar rats were randomly distributed into three groups (groups 1, 2, and 3) of five rats per group. Group 1 served as the control group and received distilled water, while Groups 2 and 3 received 150 mg/kg b.w and 300 mg/kg b.w of aqueous *X. aethiopica* extract, respectively. The extract was administered orally using an oral cannula for 15 days. During treatment, vaginal cytology of all the female Wistar rats were performed on day-1 before treatment and on day-15 of treatment using Cora *et al*. (2015) as a guide. The smears were stained with Giemsa stain. The body weights of the Wistar rats were taken and recorded on days 1, 5, and 15.

On day 15, blood samples were collected from all the rats by ocular puncture into labelled plain vacutainer tubes and processed for the serum content. The serum samples were then stored at -20 0C until they were profiled for progesterone, estradiol, and follicle-stimulating hormone (FSH) levels using a chemiluminescence immunoassay analyzer (Maglumi 800, made in China). The exsanguinated rats were euthanised by cervical dislocation, and their reproductive organs (ovary, uterus, cervix-vagina) were harvested. The organs' weights were assessed before the organs were fixed in 10 % formalin and processed for histology. The ovary and cervix-vagina were longitudinally cut, while the uterine horn was transversely cut and embedded in paraffin wax. Thereafter the tissues were processed using routine histology procedures described by Baker and Silverton (1985).

Statistical Analysis

The data were analysed with SPSS 20 software package, using one way ANOVA. When P was < 0.05, it was considered statistically significant. Means were separated by Turkey post hoc test and presented as Mean ±S D.

RESULTS

Plant Extraction Yield.

The plant material yielded 33.46 g of XAF, and the percentage yield was calculated to be 3.35 %. The extract obtained was brown and slightly oily with a sticky texture.

Phytochemical Content.

The XAF extract contained tannins, saponins, triterpenes, sterols, and carbohydrates. Flavonoids and alkaloids were absent.

Effect of XAF on Oestrous Cycle

The vaginal cytology of the Wistar rats observed during the experiment is presented in Figure 1. The proestrus, oestrus, and diestrus stages were identified by the dominant presence of nucleated cells, cornified cells and leucocytes,

respectively. Metestrus was identified by the equal presence of the three cell types.

The effects of XAF treatment on the oestrous cycle stages are presented in Figure 2. There seemed to be a dose-dependent increase in the progression of the oestrous cycle stage. However, this increase was not significant (P-value > 0.05)

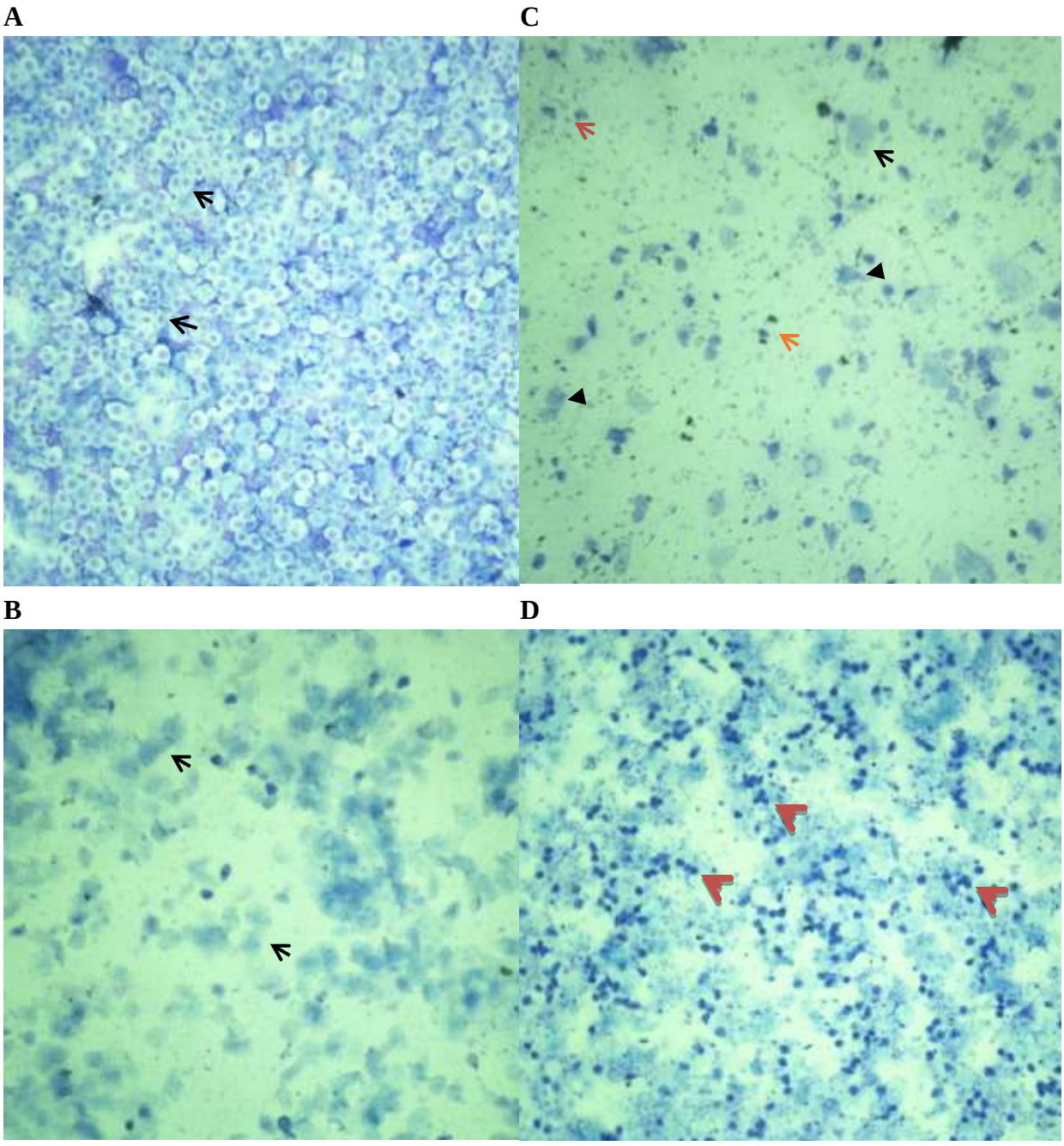


Fig1:The four stages of the rat's oestrous cycle (Giemsa stain X100)

The proestrus stage (A): Characterised by a dominance of nucleated epithelial cells (arrow). The oestrus stage (B): Characterised by the presence of predominantly cornified epithelial cells (arrows). The metestrus stage (C): had an equal presence of nucleated cells (black arrow), cornified cells (arrowhead) and leucocytes (red arrows). The diestrus stage (D): leucocytes (red arrow heads) are the predominant cells in the vaginal smear.

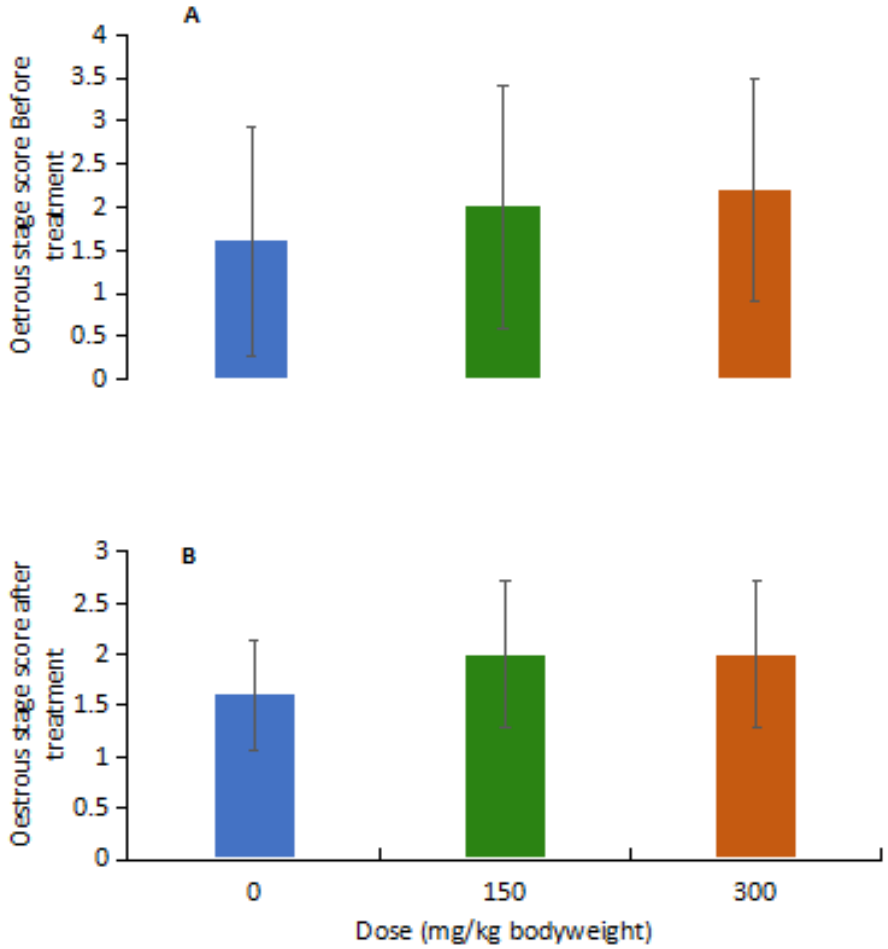


Fig 2: Effect of Xylopia aethiopica aqueous fruit extract on the oestrous cycle of female Wistar rats (A) Before treatment, (B) After treatment. Key: 1=Proestrus, 2= Oestrus, 3= Metestrus, 4= Diestrus.There was no significant difference in the oestrous stage score beforeand after treatment ($p > 0.05$).

Effect of *Xylopia aethiopica*Aqueousfruit extract on bodyweight andreproductive organ weight

In this study, no significant difference (p-value > 0.05)was observed in the weight of the ovaries and reproductive tract relative to the body weight. There was also no significant difference (p-value > 0.05) in body weight changes between groups on days-5, 10, and 15 of treatment (Fig. 3).

Hormonal Profile.

The effect of aqueous extracts of the fruits of *Xylopia aethiopica*on serum oestrogen, progesterone, and progesterone-oestrogen ratio is shown in Figure 4. Serum oestrogen level differed significantly ($p < 0.05$) between groups. The serum oestrogen level was higher in the group given 300 mg/kg body weight of XAF than the other groups. Serum progesterone levels and serum progesterone-oestrogen ratio did not vary significantly ($p > 0.05$) between the experimental groups. Although there was a dose dependent decrease in progesterone-oestrogen ratio, the difference was not statistically significant

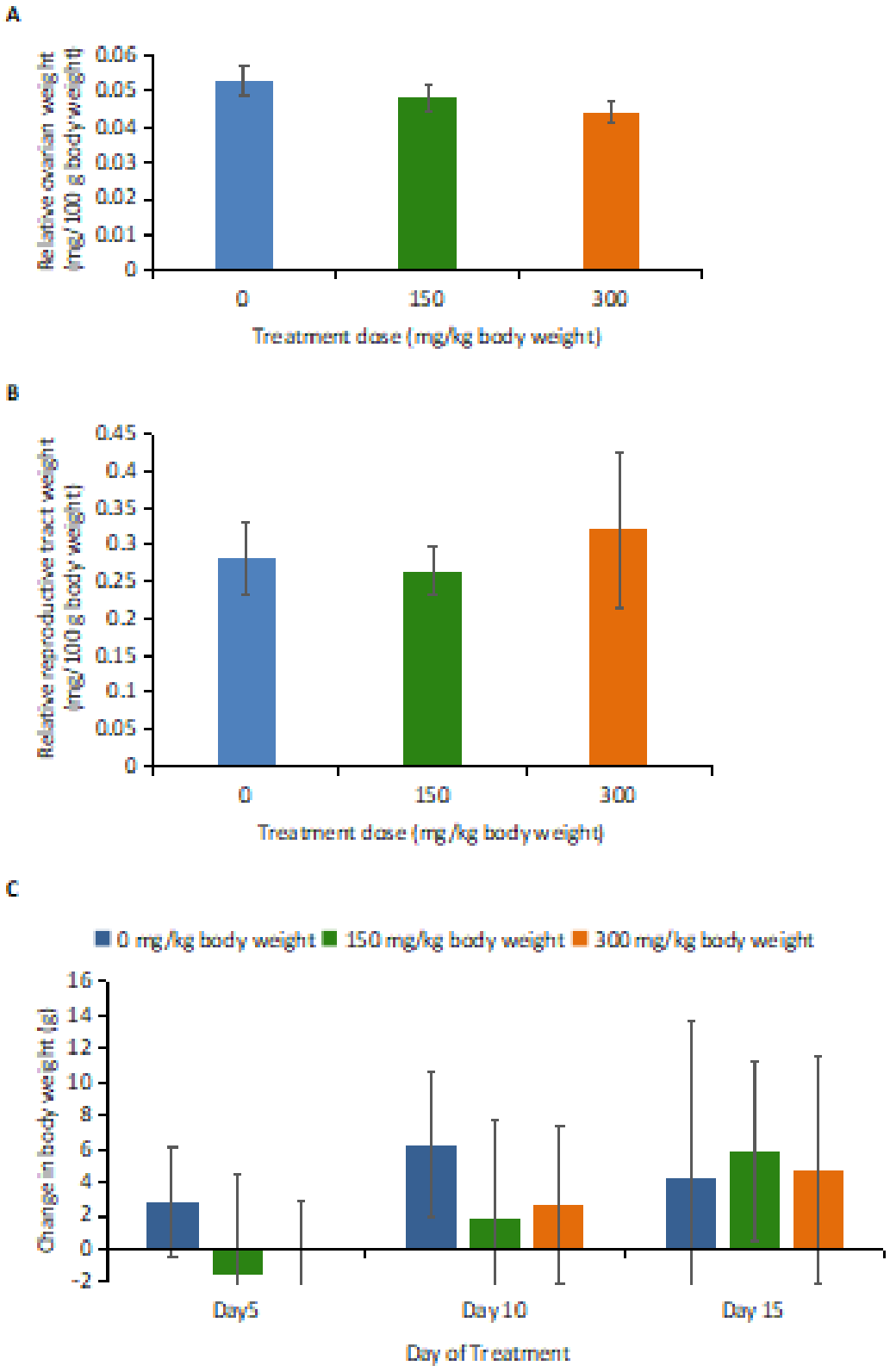


Fig 3: Reproductive organ and changes in body weights of female Wistar rats given *Xylopia aethiopica* aqueous fruit extracts. There was no significant difference between the treatment groups ($p < 0.05$**)**in the relative ovarian weights (A), relative reproductive tract weights (B), and changes in body weight on days 5, 10, and 15 of treatment(C).

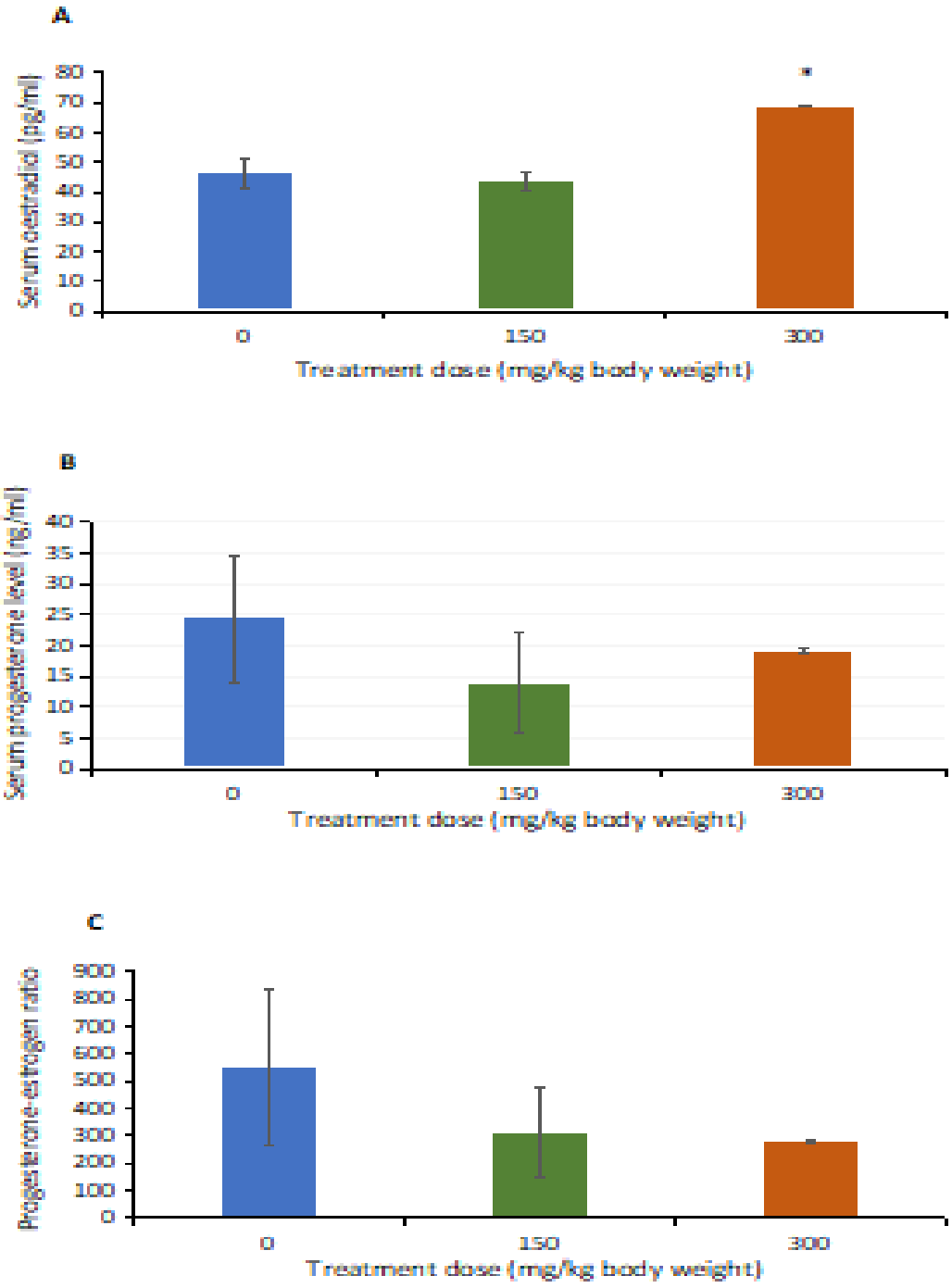


Fig 4: Serum hormonal levels of female Wistar rats treated with *xylopia aethiopica* aqueous fruit extract.*****Statistically significant at $p < 0.05$**.** Serum oestradiol levels (A): the group given 300 mg/kg body weight of the extract had significantly higher oestradiol levels than other groups, $p < 0.05$. Serum progesterone levels (B) and Progesterone-oestradiol ratio (C) were not significantly different between groups, $p > 0.05$.

Histology Findings of the Reproductive System

The histology features of the reproductive organs of the experimental rats showed that most of the rats in the control group were in late proestrus/early oestrus characterised by four vaginal epithelial cell layers intact or with the fourth layer (stratum muceum) sloughing off, minimal uterine epithelial cell necrosis, and a current corpus luteum with infiltrating fibrinous cells and signs of vacuolar degeneration. The rats in the 150 and 300 mg/kg body weight groups were in late oestrus or mid oestrus characterized by only the stratum germinativum and granulosum intact while the stratum cornuem had varying levels of sloughing. The uterine epithelium had numerous necrotic cells while the ovaries had new forming corpus lutei with basophilic robust cells and blood sinusoids formation (Fig. 5).

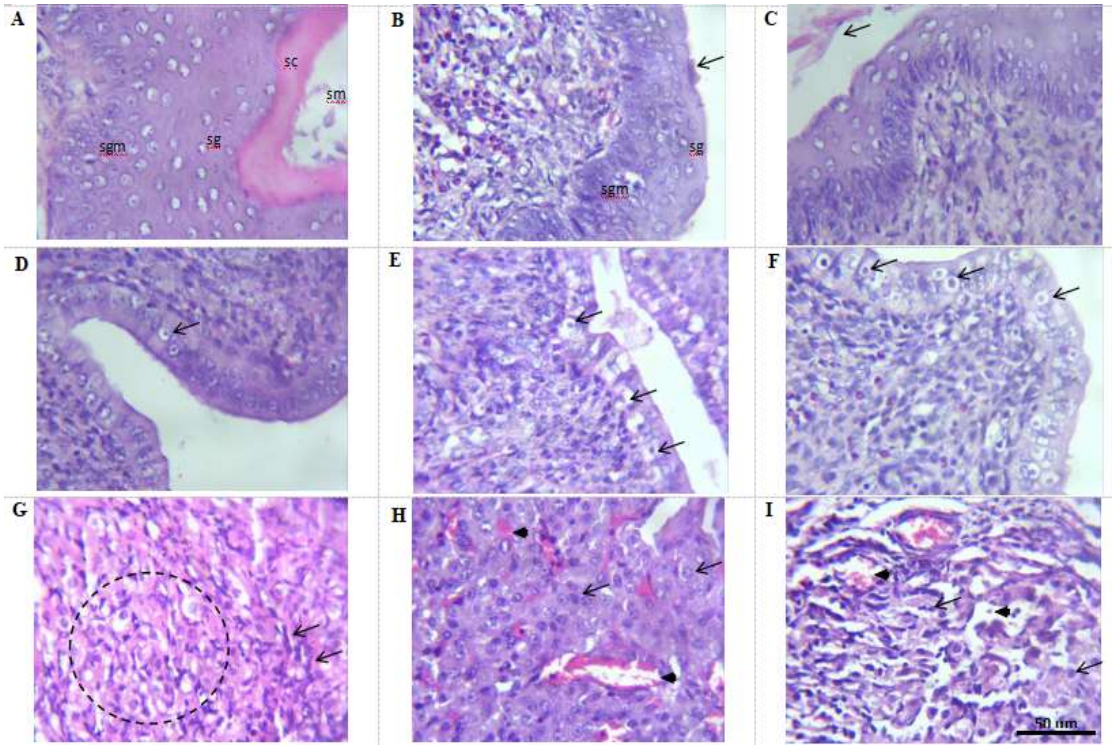


Fig 5: Reproductive microstructure of Wistar rats treated with different doses of Xylopia aethiopica aqueous fruit extract. **Control (0 mg/kg body weight) group**: The vagina (A) shows intact stratum germinativum (sgm), stratum granulosum (sg) and stratum corneum (sc) while sloughing off of the stratum muceum is in progress. The uterus (D) has columnar epithelial cells with a few cells undergoing necrosis (arrows), a sign of approaching oestrus. A current corpus luteum of the ovary (G) has a concentrated region of vacuolated cells (encircled region) and some fibrinous cells (arrows). **150 mg/kg body weight group:** The vagina (B) is characterised by an intact stratum germinativum (sgm) and stratum granulosum (sg). In contrast, only a small patch of the stratum corneum (arrow) is left, indicating late estrus. The uterus (E) has numerous epithelial cells necroses (arrows), a typical sign of estrus. A new current corpus luteum (H) shows blood sinusoids (arrowheads) and infiltrating basophilic corpus luteum cells (arrows). **300 mg/kg body weight group:** The vagina (C) shows typical sloughing of an oestral rat's stratum corneum (arrow) epithelial layer. The stratum germinativum (sgm) and granulosum (sg) are intact. The uterus (F) is characterised by numerous necrotic epithelial cells (arrow) of an oestrus rat. A new current corpus luteum (I) with infiltrating basophilic cells (arrows) amidst sinusoids (arrowheads). H & E stain, X 400 (bar = 50µm).

DISCUSSION

Hot aqueous extraction was similar to the method of preparing common traditional decoctions of the plant. The presence of carbohydrates, tannins, saponins, sterols, and triterpenes in *Xylopia aethiopica* extract is in tandem with the findings of Egharevba *et al*. (2015), who found, in addition, pseudo tannins, saponin glycosides, and basalms in the hexane, acetyl acetate, and methanol extracts of *X. aethiopica*. These secondary metabolites have rich pharmacological uses.

The oestrous cycle stages before and after treatment did not differ between groups. Most of the rats were at the follicular phase (proestrus and oestrus stages) of the oestrous cycle before treatment and after treatment. This finding shows that some sortof oestrous synchronisation had occurred before treatment began and the oestrous synchrony observed after treatment may not have been due to the treatment given. McClintock (1978) reported that the oestrous cycles of female rats living together become synchronised as the period of living together progressed to the third and fourth oestrous cycles, unlike the oestrous cycles of female rats living in isolation. In the current study, the rats were kept together for about two weeks (approximately three oestrous cycles) of acclimatisation before the experiment commenced and after 15 days (three cycles) of treatment. There could have been a natural synchronisation of the oestrous cycle. Natural synchronisation of the oestrous cycle in female Wistar rats is associated with olfactory response to airborne chemical signals (pheromones) emanating from female rats within cohorts or cohorts in proxy (McClintock, 1983). The experimental design was limited and failed to investigate the transition of each stage of the oestrous cycle during the 15 days of treatment. This investigation would have revealed if there was an effect on the oestrous cycle during the treatment.

Xylopia aethiopica aqueous fruit extract neither had a detrimental effect on body weight gain nor effect on the reproductive organ weights. Godam *et al*.(2021) also reported no difference in body weight between female Wistar rats given distilled water and females given 100 mg/kg body weight of *Xylopia aethiopica* for 14 days. *Xylopia aethiopica* may be safe as a component in food. No wonder it is usually incorporated in special traditional meals of Eastern Nigerian.

The current study showed a significant increase in oestradiol and no effect on serum progesterone levels. However, Onuka *et al*. (2017) reported that the 300 mg/kg body weight of *Xylopia aethiopica* fruit extract decreases FSH, LH, progesterone, and estrogen compared to the control, irrespective of oestrous stage. Onyebuagu *et al*. (2013) also reported decreased oestradiol and progesterone plasma levels when *X. aethiopica* was included in female Wistar rat's feed. Okonkwo *et al*. (2021) reported increased progesterone levels in pregnant rats given 100 - 200 mg/kg body weight of the extract prior to conception, while it decreased when given at a lower dose. Nnodim *et al*.

(2013) reported that 80 mg/kg body weight of *X. aethiopica* fruit lowered oestrogen, testosterone, FSH, LH and prolactin serum levels in male Wistar rats. Onuka *et al*. (2017), Onyebuagu *et al*. (2013), and Okonkwo *et al*. (2021) did not work with synchronised rats. They did not determine the oestrous stage of the female rats when treatment began and when parameters were collected. Perhaps, the effect of *X aethiopica* on reproductive hormones may be oestrous stage dependent.

In the current study, the histology features of the reproductive organs were typical of the corresponding oestrous cycle stage that was determined by vaginal smear analysis. The histology features as described by Westwood (2008) and Sato *et al*. (2014) showed that rats in the control, 150 and 300 mg/kg body weight groups were mostly at late proestrus, early oestrus and mid oestrus, respectively. The histology features did not correspond to the hormonal profile in this study. In the rat, estrogen peaks at proestrus and is minimal at oestrus (Tabatabaei *et al*., 2014, Nilsson *et al*., 2015). The oestradiol level in the treated groups should have been lower than the level in the control group who were at proestrus. The ovary and reproductive tract respond to increase oestrogen or progesterone levels or estrogen/progesterone imbalance by hyperplasia, neoplasia, atrophy, or asynchronous features of oestrous cycle changes (Andersson *et al*., 2013). However, the histology features of the treated rats were normal. This misnomer could not be investigated in the current study design because single point histology and serum hormone sampling were done on the last day of treatment. Thus, the treated rats' duration in the various oestrous cycle stages, progressive changes in reproductive histology, and possibly sustenance of high estrogen levels were not assessed. The aqueous extract of *Xylopiaaethiopica* fruit significantly affected the female reproductive system by increasing oestradiol levels of female Wistar rats at the follicular phase. It may play a significant role in regulating the female reproductive cycle.

Conclusion

Xylopia aethiopica fruit aqueous extract affects the female reproductive system by enhancing serum oestradiol levels, an important hormone regulating the female reproductive cycle.

Recommendations

More studies should be conducted on the effect of the *Xylopia aethiopica* fruit on the reproductive system under strict environmental control to guard against the natural oestrus synchrony of female rats by female pheromone. Furthermore, multiple parameters sampling should be done within the investigation period to understand the progressive effects of treatment on the female reproductive system.

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