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Contraceptive Potential of the Methanol Seed Extract of *Piliostigma thonningii* (Schum) Milne-Redh in Female Wistar Rats

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Abstract

The investigation of contraceptive effects of *Piliostigma thonningii* on female Wistar rats offers. Fresh seeds of *Piliostigma thonningii* were collected from Kaduna State, Nigeria, shade-dried, and processed into methanol extract. A total of twenty-four healthy female Wistar rats, weighing 200-248 grams. The rats were divided into four groups (n=6), each receiving post-coital therapy; the control group was administered graded dosages of wild Bauhinia seed aqueous extract (200, 400, and 800 mg/kg). Prior to administration of the extract, the female rats were paired with male rats using 1:1 ratio for a period of 48 hours. During this period, the female rats were frequently monitored for the presence of vaginal sperm plug, indicative of imminent mating. The results indicated that graded doses (200, 400, and 800 mg/kg) of *Piliostigma thonningii* methanol seed extract showed a significant reduction in triglycerides (TAG), total cholesterol (TC), and low-density lipoprotein (LDL) within normal physiological ranges with slight elevation in high-density lipoprotein (HDL) levels when compared to the control group. The extract had a significant reduction in serum estrogen levels in comparison with the control. A marginally significant difference in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) was observed in the tested female rats compared with the control group. Additionally, a decrease in progesterone levels was observed when contrasted with the control. Histological analysis revealed no acute inflammatory cell infiltrate in the myometrium at the 200 mg/kg dose, which displayed a normal uterine wall, endometrium, and myometrium. Further research is imperative to establish consistent pharmacodynamics dosage regimens.

Keywords: Contraceptual; *Piliostigma thonningii;* Female; Wistar Rats.

INTRODUCTION

The plant belongs to the Fabaceae family of legumes, which is a subfamily of the Cercidoideae and contains about 133 genera. This tree is also known as "wild bauhina," camel's foot tree, and monkey bread. In the local dialect, the plant is known as "abefe" in Yoruba, "Kaloo" in Hausa, and "Okpoatu" in Igbo (Abubakar *et al*., 2018) "Omukpakpa ajalu" in Igala, "Nyihar" in Tiv, "Mchekeche" in Swahili, and "Kharub" in Arab. Numerous uses for humans have been documented for *Piliostigma thonningii* and other genus species, which provide animal and human sustenance to a variety of therapeutic applications. Among its many medical applications are the treatment of loose stool in teething children, wound dressing, ulcer therapy, worm infestation, stopping bleeding, inflammations, bacterial infections, gonorrhea, headaches, and stomachaches (Afolayan *et al*., 2018; Aina and Aina-pelemo, 2019). In Eastern Nigeria, the leaf extracts have been used to treat malaria, while locally, the roots and twigs have been used to cure fever, respiratory disorders, hookworm, skin diseases, snake bites, and dysentery (Ajayi *et al*., 2018). The stem and root are boiled in Zambia's southern province, and the resulting decoction is used to treat HIV-opportunistic illnesses like cough and STDs (Anywar *et al*., 2020). On the other hand, skin diseases are treated with the leaves and is made as an infusion (Ayenew *et al*., 2019). Similarly, in the eastern part of Namibia and in Lusaka, a decoction prepared from the root and bark is taken orally to cure syphilis, gonorrhea, and cough (Bala *et al*., 2014). The leaf, root, or bark is used to cure cough and diarrhea, or a mixture of the bark and *Elephantorrhiz agoetzei* (Harms) is used to treat cough and diarrhea, or the leaf is boiled and used as an incision on the sore leg or mixed with milk (Balde *et al*., 2016). Sexually transmitted infections, coughing, and inflammation in Guinea-Bissau Nevertheless, there was no mention of the preparation or administration method (Bhata and Das, 2018). For many years, herbal remedies have been utilized to cure it, and *P. thonningii* is one of the known medicinal herbs that are used. For example, in Mali, a leaf decoction is used to treat malaria, whereas in other countries, a leaf or stem bark decoction or maceration is used to treat malaria in Guinea and Mozambique (Bhakta *et al*., 2019). Although the parts,

preparation, and administration of the plant are unclear, it is also said to be used to treat malaria in Burkina Faso (Boualam *et al*., 2021). However, in Guinea, fever is treated with a decoction of the stem bark or leaf, while in Mozambique, fever is treated with a decoction or maceration of the leaf, root, and bark (Burkill, 1995). It was reported that a decoction is prepared from the bark of the plant and administered orally as a treatment for cancer in Sokoto state located in the north western part of Nigeria (Catarino *et al*., 2016). *P. thonningii's* bark and root were used to cure malignancies of the breast, face, neck, and throat. Typically, its components are dried, powdered into hot water after being ground into a fine powder. Two spoonfuls were then applied topically to the afflicted area twice a day (Catarino *et al*., 2016). Additionally, Guinea uses a leaf decoction to treat diabetes, which is comparable to Togo's approach (Chinsembu, 2016).

According to a recent study, the leaf decoction is used in northern Nigeria to treat a variety of viral illnesses, such as meningitis, smallpox, poliomyelitis, and hepatitis (Chipeta *et al*., 2010). Similarly, *P. thonningii* is said to be used in Burkina Faso to cure hepatitis, albeit it was not specified which portion or how it was utilized (Boualam *et al*., 2021). Additionally, it has been claimed that the stem bark is used to cure anemia in Ghana and Tanzania (Chukwuji *et al*., 2018; Codo-Toafode *et al*., 2022; Dali *et al*., 2019). The root powder has been used topically to treat wounds, sprains, and bone fractures, and the leaf has been made as a massage infusion. Other conditions that have been reported to be treated with various components of *P. thonningi*i include ulcers, inflammation, hemorrhoids, intestinal, respiratory, and cardiac issues (Dar *et al*., 2017), and gingivitis (Burkina Faso) (Boualam *et al*., 2021). It was discovered to be used in Mali to treat digestive issues, headaches, arthritis, hemorrhoids, backaches, abdominal flatulence, and child malnutrition.

MATERIALS AND METHODS

Collection of plant material

The fresh seeds of *Piliostigma thonningii* were collected from northern Nigerian of Kaduna State provided the fresh plant seeds. With voucher number UBT-B184, it was recognized and verified by Dr. Timothy Odaro in the Department of Plant Biology and Biotechnology's herbarium unit. Theplantlist.org was used for additional authentication. Harvested fresh seeds were allowed to dry in the sun for two weeks. A mechanical grinder was used to grind the dried seeds into a powder.

Preparation of plant material

A glass jar was filled with two thousand (2000) grams of the sample, which had been weighed on a scale. The glass jar with the sample was filled with 2 L of methanol. After stirring and covering, the mixture was left for thirty-six hours. After 36 hours, the mixture was filtered, yielding a dark brown, oily, semi-solid extract. A water bath set at roughly 45 degrees Celsius was used to concentrate the extract. After that, it was stored in the refrigerator in a sterile container until it was time to utilize it.

Experimental animals

Twelve (12) healthy female whisker (albino) rats weighing between 150 and 180 g were used. The animals were purchased from the University of Benin's Animal and Environmental Biology animal home. They were given a conventional meal and kept in well-ventilated hardwood cages in a typical laboratory setting with a 12-hour light/dark cycle (23 ± 2°C). The animals used in the tests were given food and drink at their discretion, or ad libitum. With the ethical number LS20619, the animals were handled in accordance with the guidelines for laboratory animals approved by the Faculty of Life Sciences' ethical committee.

Experimental Design

The Wister rats were weighed both before and after the extracts were administered. To determine the precise rat weight and extract delivery dosage, the rats were marked. Group 1. After treatment the female Wistar rats were paired with males using a 1:1 ratio on the fifteenth day of treatment after attaining the heat phase across the females indicating vaginal sperm plug, which is a sign of mating (Dery *et al*., 2023; Drury and Wallinton, 2013). The female rats were monitored for signs of pregnancy for an additional 23 days, which is the gestational period of rats, following a 7-day pre-exposure to the male animals. Rats were weighed, slaughtered under light anaesthesia, and their blood and female reproductive organs were separated. Phase 2: Four groups (n=6) received post-coital therapy; the control group received graded dosages of wild bauhinia seed aqueous extract (200, 400 and 800 mg/kg) (Dunn *et al*., 2016; Eniojukan *et al*., 2015). The female rats were monitored for signs of pregnancy for an additional 6 months, which is the gestational period of rats. Rats were weighed, sacrificed under mild anesthesia, and tests were performed to determine the number of reabsorptions, embryos, corpora, pre-implantation and post-implantation mortality, and overall prenatal mortality. Female reproductive organs and blood were separated for examination.

Progesterone assay protocol

Prior to use, all reagents should be allowed to come to room temperature (18 – 25 degrees Celsius). Within five minutes, pipette 50 µl of ready-to-use standards and diluted samples into the proper wells. Each well (except from the blanks) should contain 100 ul of progesterone enzyme conjugate solution. Stir thoroughly for 30 seconds, then incubate at 37°C for 60 minutes. During

the incubation, you can store the plate in a suitable zip-lock bag or cover the wells with par film. After discarding the contents of the wells, wash the plate five times using 250 – 300 µl of Wash Solution per well. To get rid of any remaining liquid, invert the plate and tap firmly against absorbent paper (Ezebialu and Eke, 2013).

Fill each well with 100 µl of (TMB) substrate solutions. Do not forget to adhere to the pipetting order. Without shaking, incubate the plate for ten minutes at room temperature (18 – 28°C). 50 µl of Stopping Solution should be added to wells in the same order as the Substrate Solution and gently stirred to stop the reaction. Measure the absorbance using a microwell reader at 450 nm.

Luteinizing hormonal assay

Secure the desired number of coated wells in the holder as you want. Fill the proper wells with 50 µl of the standards, specimens, and controls. Fill each well with 100 µl of Enzyme Conjugate. Stir for 30 seconds. At this stage, having finished mixing is crucial. For two hours, incubate at ambient temperature (37°C). Flip the contents of the plate into a garbage receptacle to remove the incubation mixture. Use wash buffer to rinse and flip the microtiter wells five (5) times. To get rid of any last traces of water, swat the wells onto paper towels or absorbent paper. Fill each well with 100 µl of TMB solution. Mix gently for ten seconds. Incubate in the dark for 20 minutes at room temperature. To stop the reaction, add 50 µl 2N HCl (one drop) into every well. Mix slowly for 30 seconds. Seeing the color shift from blue to yellow is crucial. A microtiter well reader can be used to measure optical density at 450 nm.

Follicle-stimulating hormone

Before using, allow all samples and reagents to come to room temperature. Before the assay, defrost the sample and centrifuge it once more. It is advised to do duplicate analyses on all samples and standards. As instructed in the preceding sections, prepare all samples and reagents. Decide how many wells will be utilized, then return any extra wells and the desiccant to the pouch, seal the Ziploc, and keep any unused wells at 4°C. With no solution, set a blank well. Fill each well with 50 µl of the Standard or Sample. duplication of the standard need test. Before adding 50 µl of antibody to each well, add 50 µl of HRP-conjugate to each well (not the blank well). After thoroughly mixing, incubate at 37°C for 60 minutes. For a total of three washes, aspirate each well and then wash, doing this twice. Using a squirt bottle, multi-channel pipette, manifold dispenser, or auto-washer, fill each well with 200 µl of Wash Buffer. Then, let it stand for 10 seconds. Effective performance depends on the liquid being completely removed at each step. Aspirate or decant any leftover Wash Buffer after the last wash. Blot the dish with fresh paper towels after inverting it (Fanna, 2017). Fill each well with 50 µl of Substrate A and 50 µl of Substrate B, then thoroughly mix. At 37°C, incubate for 15 minutes. In the dark, the plate should be kept away from drafts and other temperature fluctuations. To guarantee complete mixing, add 50 µl of Stop Solution to each well and lightly tap the plate. Using a microplate reader set to 450 nm, find the optical density of each well in ten minutes.

Histopathological analysis

Neutral Bouin's fluid was used to repair the uterus. After completely dehydrating the attached organs with 99.9% ethanol, 70% ethanol, and 96% ethanol, they were cleaned with distilled water. Hematoxylin-eosin dye was used to stain the 4 µm slices that were produced. Stained tissues were examined at 400x magnification using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) (Gediya *et al*., 2011).

Statistical analysis

The results are displayed as Mean ± SEM. One-way analysis of variance (ANOVA) was used to assess the data, and the Dunnete test was used to compare the means. A difference was considered significant were measured at $p<0.05$. The statistical instrument utilized was Graph Pad Prism (Version 7, USA).

RESULTS AND DISCUSSION

The indexes used to evaluate toxicity studies are associated with lipidaemic properties such as Total cholesterol, Very Low-Density Lipoproteins, High density Lipoproteins Low density Lipoproteins and Triglycerides. *Piliostigma thonningii* methanol seed extract at Differentiated dosages (200, 400 and 800 mg/kg) maintained a physiological state of the TAG, TC and LDL concentrations within normal ranges, with a slight increase in HDL concentration of female rats when compared with the control (Table 1)

Table 1. Effects of *Piliostigma thonningii* extract on lipid profile in rats.

Treatment	Dose (mg/kg)	Total Cholesterol (mg/dl)	Total Triglyceride (mg/kg)	High Density Lipoprotein (mg/dl)	Low Density Lipoprotein (mg/dl)	Very Low-Density Lipoprotein (mg/dl)
Control	DW	63.20±0.25[a]	176.10±0.26[a]	12.73±0.52[a]	43.21±0.61[a]	42.71±2.55[a]
Extract	200	59.05±0.62[b]	216.70±0.50[b]	14.60±0.65[a]	29.48±0.42[b]	42.43±1.19[a]
Extract	400	65.12±0.21[b]	194.80±1.33[b]	13.14±0.25[a]	39.32±0.42[b]	38.66±0.39[b]
Extract	800	69.27±3.85[b]	212.80±1.24[b]	13.14±0.50[a]	41.59±1.88[a]	40.58±1.74[a]

Values were expressed as Mean ± SEM, *p-value <0.05 showed* the level of significant, DW---- distilled water.

In general, the utilization of contraception confers a multitude of health advantages, including the prevention of unplanned and unintended pregnancies, the facilitation of optimal and healthy intervals between births, the reduction of maternal and child mortality rates, the enhancement of developmental objectives, and the overall improvement of the quality of life (Halilu *et al*., 2017; Harris, 2018; Hatcher *et al*., 2007; Jimoh and Oladiji, 2005) for women and children. Women's reproductive health is still seriously threatened by the prevalence of unwanted pregnancies, especially in poor countries (Kpodar *et al*., 2015). According to Chipeta *et al*. (2010) and Ogboghodo *et al*. (2017), family planning protects women against high-risk pregnancies, unsafe abortion practices, infections of the reproductive tract, and sexually transmitted diseases including HIV/AIDS. It is currently irrefutable that contraception exerts a substantial positive influence on health and overall quality of life. The implementation of contraceptive methods can markedly mitigate the alarming rates of unplanned pregnancies and the acquisition of sexually transmitted diseases (MacDonald *et al*., 2021). As noted by Schwandt *et al*. (2015), in the context of Nigeria, the increasing adoption of contraception persists as a formidable obstacle amidst a high total fertility rate (5.7 births per woman) and a considerable unmet need for contraceptive methods (estimated at 20.2% of married women). The prevalence of modern contraceptive methods remains notably low at 10.5% among both married and unmarried sexually active women, with the male condom identified as the predominant contemporary method utilized (5% currently employing this method). Injectable contraceptives and oral pills (2%) constitute the next most prevalent methods, while lactational amenorrhea, intrauterine devices (IUDs), and sterilization account for the remaining method mix (2% collectively). Prolonged utilization of these commercially available orthodox contraceptive products can lead to infertility in users and increase their vulnerability to various life-threatening diseases. Therefore, in light of the adverse consequences associated with contraceptive pills and other synthetic products, it is imperative to shift our focus towards the production, research, and commercialization of herbal alternatives aimed at enhancing the well-being of humanity, irrespective of gender (Makosa *et al*., 2021).

Hormonal assay:

This study investigated in the effects of differentiated doses (200, 400, and 800 mg/kg) of *Piliogstima thonningii* methanol seeds extract, showed a significant decrease across the doses in serum oestrogen compare to the control as shown in Figure 1.

According to Bhakta and Das (2018), the majority of the constituents present in any herbal preparation typically exert their influence on the essential organs of the body in accordance with their specific affinity. The herbal formulations associated with contraceptive effects primarily target the genitalia, regardless of sex, to regulate both morphological characteristics and the hormonal milieu within the bloodstream. The herbal extracts manifest their pharmacological effects through various mechanisms. In the case of females, they predominantly influence the ovaries concerning folliculogenesis and also modulate the levels of female hormones in circulation. Should the administration of these herbal extracts be terminated abruptly, conception may resume without inflicting any detrimental effects on the vital organs of the body. In the male context, the herbal extracts affect the testes with regard to spermatogenesis and also regulate the levels of male hormones in the bloodstream. According to Bhakta *et al*. (2019), medicinal plants may induce infertility through diverse mechanisms. They may impact the ovary, uterus, hormone synthesis, inhibition of hormonal action; interfere with implantation, and sperm production. Certain herbal substances prevent fertilization by creating a protective barrier around an ovum. Hyperlipidaemia is characterized by an abnormal elevation in blood lipid levels (detrimental fats), instigated by specific phytochemicals derived from plants. The categories of these lipids encompass cholesterol, triglycerides, lipoproteins, and phospholipids (Ogbiko, 2021; Maroyi, 2013).

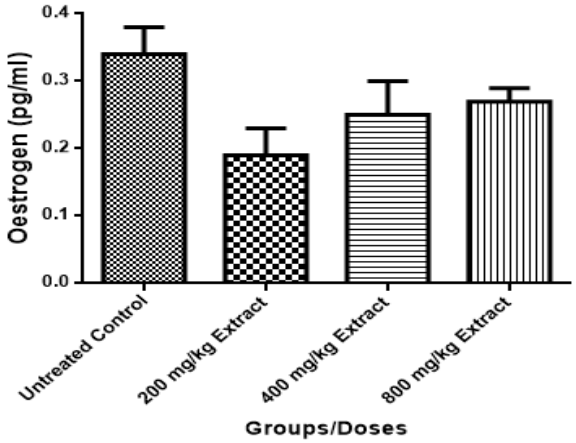

Figure 1. Effects of *Piliostigma thonningii* extract on estrogens level in rats

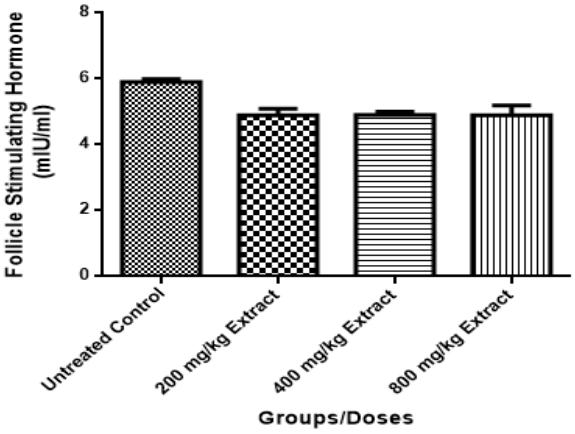

Figure 2. Effects of *Piliostigma thonningii* extract on follicle stimulating hormone level in rats.

Figure 2 showed slightly significant difference in follicle stimulating hormone of the female animals at graded doses (200, 400 and 800 mg/kg) of *Piliostigma thonningii* methanol seeds extract, when compared with the control.

Cholesterol functions as a metabolic steroid hormone in humans, facilitating the secretion of aldosterone, cortisol, progesterone, and androgens, which collectively govern numerous physiological functions. A cholesterol concentration exceeding 200 mg/dl poses a significant risk for the onset of coronary heart diseases. Consequently, it incites arterial alterations, resulting in vasoconstriction, which in turn precipitates hypertension or vascular obstruction, culminating in myocardial infarction. The data presented in Table 1 elucidate the impact of *Piliostigma thonningii* seed extract administered at varying dosages (200, 400, and 800 mg/kg) on the regulation of triglyceride (TAG), total cholesterol (TC), and low-density lipoprotein (LDL) levels, alongside a marginal elevation in high-density lipoprotein (HDL) concentration in comparison to the control group (Table 1). The maintenance of TC, TAG, and LDL concentrations within normative ranges, coupled with the increase in HDL, indicates a potential therapeutic effect of the extract in the context of hyperlipidaemia, a finding that is consistent with the observations made by Ayenew *et al*. (2019), Marquardt *et al*. (2020), and Ndikom *et al*. (2018). The observed activities can be attributed to an augmented suppression of intestinal cholesterol absorption, interference with lipoprotein synthesis, enhanced hepatic expression of low-density lipoprotein (LDL) receptors along with their protective functions, culminating in a heightened removal of LDL from the bloodstream to facilitate catabolism and cholesterol degradation within the organism (Ozolua *et al*., 2009).

The effect of *Piliostigma thonningii* methanol seeds extract had a significant effect on the 200 and 400 mg/kg with a slight decrease at 800 mg/kg of the extract in luteinizing hormone tested in the female animals in contrast to the control, as depicted in Figure 3.

The data presented in the preceding table (Table 1) indicate that the administration of a 200 mg/kg extract resulted in a statistically significant reduction in total cholesterol levels when juxtaposed with the control group. This finding may be linked to a decrease in female reproductive hormones, as total cholesterol plays a pivotal role in the biosynthesis of hormones within the organism (Oppong *et al*., 2020). The results demonstrate that there was an insufficient quantity of total cholesterol to support the synthesis of hormones essential for conception. This observation can be attributed to the influence of the extract, which significantly lowered total cholesterol levels in comparison to the control. The concentration of high-density lipoprotein present in the extracts exhibited a notable elevation in comparison to the control group; this indicates that the extract exerted a significant influence on cholesterol levels by surpassing the control, thereby diminishing the circulation of hormones within the bloodstream in the arteries and resulting in contraception. This phenomenon occurs due to the absence of the hormone necessary for conception, thereby ensuring the occurrence of contraception (Neychev and Mitev, 2016; Nripondra *et al*., 2019). Conversely, the levels of low-density lipoprotein and very low-density lipoprotein in the extracts were markedly reduced relative to the control group, which suggests a potential decrease in the synthesis of conception hormones, thereby enhancing the contraceptive efficacy of the extracts. The presence of low-density lipoprotein and very low-density lipoprotein is instrumental in hormone accumulation; thus, when their levels are subnormal, hormone synthesis is impaired, resulting in a reduction in conception (Ogbiko, 2021). The overall concentration of Triglycerides in the experimental rats exhibited a marked elevation in

comparison to the control group. An escalation in Triglyceride levels is associated with the pathophysiological process of arteriosclerosis, characterized by the stiffening of arterial walls, which subsequently leads to inflammatory responses. As indicated in the preceding table (Table 1), the extract resulted in a statistically significant augmentation of Total Triglycerides relative to the control condition. This phenomenon may have adversely impacted the synthesis of pregnancy-associated hormones, as the elevation in total triglycerides contributes to the rigidity of the arterial supply to the uterus, thereby diminishing the hormonal milieu necessary for uterine preparation for conception in the rats. It is well-established that a reduction in serum estrogen levels reflects the adverse effects of the extracts on fertility, with a consequential response that promotes infertility (Saalu, 2016). In the current investigation, the assessment of female reproductive parameters in female specimens administered graded doses of 200, 400, and 800 mg/kg of *Piliostigma thonningii* methanol seed extract revealed a significant decline in serum estrogen levels when juxtaposed with the untreated control group. This proposed product has the ability to reduce oestrogen level significantly acting as a strong contraceptive agent by either reducing its synthesis or causing metabolic impairment, as Figure 1 illustrates. Female animals showed a decrease in follicle-stimulating hormone and luteinizing hormone in response to consumption of *P. thonningii* methanol seed extract. Results from related studies have been published to shed light on the possible relationship between female sex hormone and a reduction in luteinizing hormone and follicle stimulating hormone impairment brought on by increased toxic substance dosages, which impact these hormones' production and secretion (Schwandt *et al*., 2015; Solanke, 2017). Thus, this study demonstrated that, in comparison to the control group, luteinizing and follicle stimulating hormone significantly decreased in the treatment groups as seen in Figure 2 and 3. it suggested that the product significantly decrease the secretion of sex hormones with fertility properties. Also, prolong intake of *P. thonningii* methanol seed extract rather depress plasma progesterone concentration in females. This result consent with Osonuga *et al*. (2014) report on *Momordica charantia* to reduce the reproductive hormones of adult female Wistar rats. This study showed a significant decrease in serum progesterone level across the graded dose of 200, 400 and 800 mg/kg of the treatment groups (Figure 4). A decrease in progesterone concentration as recorded from this study impairs fertility with little or mild adverse effect on reproductive morphological state evaluated. Hence, it was observed from the hormonal assay that a decrease in these hormones as observed in this study denotes a potent contraceptive via inhibiting the secretion of vita hormones triggered to cause antifertility.

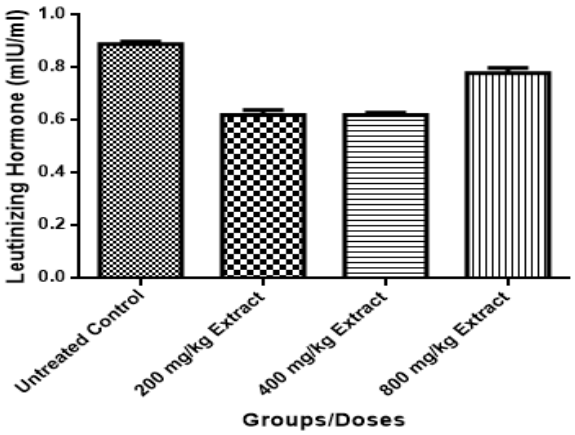

Figure 3. Effects of Piliostigma *thgonningii* extract on luteinizing hormone level in rats.

Result obtained from Figure 4 had a drastic decrease at 200, 400 and 800 ng/kg. The extract dropped the progesterone level of the female rats in contrast to the control (Figure 4).

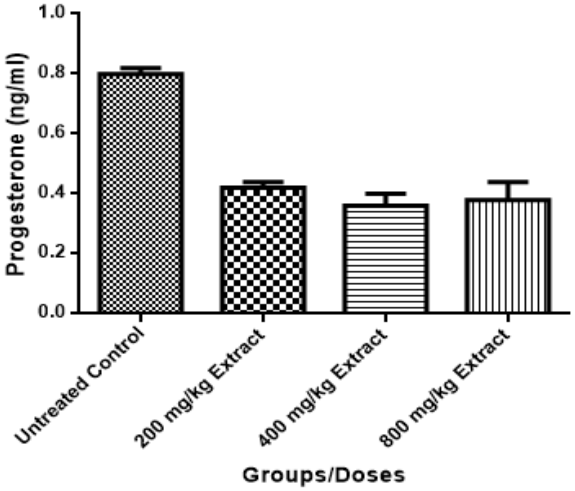

Figure 4. Effects of *Piliostigma thonningii* extract on progesterone level in rats.

The uterus is a stimulating organ that promotes the release of vita hormone during and after child birth. Hence, the result obtained from plate 1 showed that the female animals in the treatment groups elicited normal function of the uterus when compared with the control.

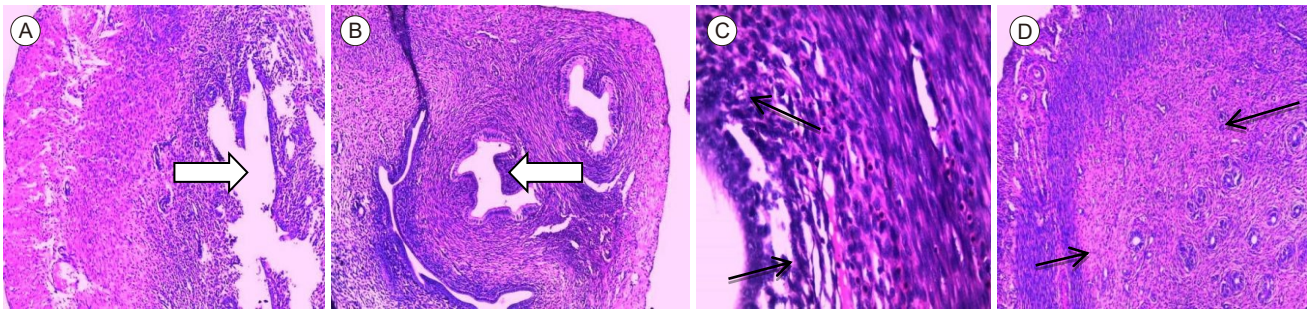

Plate 1. Effect of *Piliostigma thonningii* extract in the uterus.

A. : Control uterus showing normal uterine wall endometrium (blue arrow) and myometrium (white arrow) lined by columnar epithelial cells.
B. : **200 mg/kg** uterus showing normal uterine wall endometrium (blue arrow) and myometrium (white arrow) lined by columnar epithelial cells. No abnormality seen.
C. : **400 mg/kg:** uterus showing acute inflammatory cell infiltrate (black arrow) in the myometrium.
D. : **800 mg/kg** uterus showing acute inflammatory cell infiltrate (black arrow) in the myometrium. Myometrial inflammation is present.

Histopathological examination of visceral organs like the ovary and uterus administered with graded doses of 200 mg/kg of *Piliostigma thonningii* methanol seed extract reveals normal renal cell with its functional unit, the 400 and 800 mg/kg had mild acute inflammatory cells when compared with the control (Osonuga *et al*., 2014; Zongo *et al*., 2023). This suggested that the 200 mg/kg extract possess protective effect of the uterus and ovary against complications, hence 200 mg/kg is the guide. These observations are in line with the antioxidant property shown in a previous study, which concurred with the report of Makosa *et al*. (2021) that showed proanthocyanidins with strongest protective mediating against oxidative stress in visceral organ.

CONCLUSION

In conclusion, this scientific study carried out validated the seeds of *Piliostigma thonningii* of its contraceptive properties and reproductive safety at 200 mg/kg, thus adhering to the ethnomedicinal report, thereby requiring further studies to authenticate claims.

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