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Effects of purified stem bark extract of *Anogeissus leiocarpus* on Oestrous cycle dynamics, reproductive hormones, and ovarian histomorphology in female Wistar rats

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Abstract

The present study was designed to evaluate the oestrous cycle, ovarian hormone profiles and live weight changes of Wistar rats (*Rattus norvegicus*) orally administered optimized levels of *Anogeissus leiocarpus* purified extract. Twenty female Wistar rats weighing 194.05 ± 13.63 g were randomly placed into four groups comprising of five rats each. Group A served as control, while Groups B, C and D were orally administered 5mg/kg, 10mg/kg and 20mg/kg of the purified extract daily for twelve days respectively, feeds and water *ad libitum*. Vaginal smears were taken daily for twelve days to carry out vaginal cytology (to determine the stage of oestrous cycle). Serum samples were taken from day 0 till day 12 (at intervals of a day) for hormonal (oestrogen and progesterone) assays. The study showed that *Anogeissus leiocarpus* purified extract increased live weights in the treated group C (18.75 ± 0.00 grams) compared to the treated groups B (-3.5 ± 1.24 grams) and group D (-14.6 ± 0.14 grams) from day 1 to day 14. Wistar rats in group B (6.80 ± 1.48) and group D (6.20 ± 1.30) had significantly higher occurrence of estrus than Wistar rats in group C (3.50 ± 2.38) and group A (3.00 ± 0.00). Wistar rats in group B (1.00 ± 0.00) and group D (1.20 ± 0.45) had significantly lower occurrence of metoestrus than Wistar rats in group C (3.50 ± 2.38) and group A (3.00 ± 0.00). Wistar rats in group B (1.00 ± 0.00) and group D (1.60 ± 0.89) had significantly lower occurrence of dioestrus ($P < 0.05$) than Wistar rats in group A, control group (3.00 ± 0.00). There was higher occurrence of proestrus and estrus stages (follicular phase) and lower occurrence of metoestrus and dioestrus stages (luteal phase) of the oestrous cycle in the treatment groups, when compared with group A. The oestrogen levels were within the normal ranges in the treated groups B, C and D compared to A. The serum progesterone levels were significantly lower among the treatment groups B, C and D than A, though they were within the normal ranges on administration of the purified extract of *Anogeissus leiocarpus*. In conclusion, group C increased live weight, had higher occurrence of proestrus stages and shorter occurrence of dioestrus. Oestrogen levels increased in group C and progesterone levels decreased during estrus at day ten. Therefore, *Anogeissus leiocarpus* administered at 10mg/kg is a recommended safe dose for reproductive related studies.

Keywords: *Anogeissus leiocarpus*, oestrous cycle, oestrogen, progesterone and Wistar rats

Introduction

African Birch Tree (*Anogeissus leiocarpus*) is an evergreen tree that belongs to the Kingdom: Plantae; Division: Magnoliophyta; Class: Magnoliopsida, Family: Combretaceae, Genus: *Anogeissus* and Species: *leiocarpus* (Trevithick, 1927) [36]. Binomial names of *Anogeissus leiocarpus* include: *Cononcarpus leiocarpus* DC, *Anogeissus schimperi* Hochst and *Anogeissus leiocarpus* var *schimperi* Hochst (Hutchinson and Dalziel, 1954; Mattana *et al.*, 2018) [25].

The common and vernacular names reported include: African Birch Tree in English, *Kojoli* in Fulani, *Marike/Marke* in Hausa, *Abakiliki atarian* in Igbo, *Anum* in Kanuri, *Ayin or Pako dudu* in Yoruba (Ibrahim *et al.*, 2010) [16], *Maaki* in Tiv, *N'galama* in Bambara, *Siigi* in More',

Godoli in Peulh and *Sahib* in Shua Arab (Ssegawa and Kasenene., 2007) [34].

Anogeissus leiocarpus reaches up to 70 feet (21.336 meters) in height (averagely 15-18 meters) with light green foliage (Trevithick, 1927) [36]. The tree possesses a trunk with a wider base and dense crown having drooping branches. The bark of the tree is beige to grey in color which becomes black and fibrous with thin scales as it gets older. The stems are finely pubescent while the leaves are elliptic to ovate-lanceolate in shape and are arranged adjacently. In dry areas, the individual tree grows with smaller leaves and much hairy flowers (Dalziel, 1937). The flowers are yellow-green and scented with white hairs. The fruits are somewhat orange and brown, and are shaped like a trapezium. Earlier report showed that each fruit was broadly winged, beaked by a persistent tubular portion of the receptacle and contained one seed small in size. The fruits are produced in many numbers (Ssegawa and Kasenene, 2007) [34].

Traditionally, the plant had been used for the treatment of different ailments including the diabetic ulcers, general body pain, blood clots, asthma, coughing, jaundice, pile and tuberculosis (El Ghazali *et al.*, 2003; Victor, 2013; Abubakar *et al.*, 2017; Namadina *et al.*, 2019) [28]. The inner bark of the tree had served as an anthelmintic in humans and livestock. It has also been used to treat protozoan diseases in animals such as animal trypanosomosis (nagana) and Babesiosis (Bizimana, 1994) [6]. Other uses of the inner bark include chewing stick in Nigeria and its extracts have been shown to possess antibacterial properties (Mann *et al.*, 2008) [23]. *Anogeissus leiocarpus* showed its usefulness for problems associated with reproduction and enhancement of fertility in many parts of Northern Nigeria (Jibril *et al.*, 2016). The serum levels of ovarian hormones: progesterone and oestrogen of the Wistar rats were increased following administration with *Anogeissus leiocarpus* aqueous extract (Jibril *et al.*, 2016).

The sexual maturation of the female Wistar rat was identified by vaginal opening (Lewis *et al.*, 2002) [22] with four phases in the oestrous cycle, namely proestrus, estrus, metoestrus and dioestrus and lasts for 4 to 5 days (Auta and Hassan, 2016) [4]. In the proestrus stage, the vaginal opening appears full, swollen and moist, its tissues are pink, with striations in both the dorsal and ventral lips of the vulva. During estrus, these appearances are similar but less in intensity with the exception of more striations than in proestrus stage. In the metoestrus stage, the vaginal opening is pale. Dioestrus is characterized by a wet, closed and unswollen vagina opening. Any disruptions to the regular functioning of the reproductive organs produced an impact on the animal's ability to reproduce (Knapp *et al.*, 2012) [19].

Materials and Methods

Study Location

This research was conducted at the Department of Theriogenology and Production, Ahmadu Bello University Zaria. The female Wistar rats were housed at the Animal House of the Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, located in the Northern Guinea Savannah zone of Nigeria on Latitude 11°5'8" N and Longitude 7°43'12" E at an elevation of 650m above sea level. Zaria has two seasons: dry season (November to April) and rainy season (May to October). The average maximum and minimum temperature are 33±0.67 °C and 18.74±63 °C, respectively (Abbas and Arigbede, 2012) [1]. The average

monthly rainfall is 176±31.79mm while the mean monthly relative humidity is 46.19%.

Ethical Clearance

Ethical approval for the study was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number: ABUCAUC/2023/111.

Plant Collection and Preparation

Fresh stem barks were harvested from African birch tree at Areas C and BZ Staff Quarters of Ahmadu Bello University, Zaria, Kaduna State. The stem bark samples were identified and authenticated in the Herbarium unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria with a voucher number, ABU01756. The samples were kept under shade for seven days at room temperature, dried and pulverized by grinding in a mortar in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The hydroethanolic extraction and purification was at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Phytochemical Screening

The *Anogeissus leiocarpus* stem bark was tested for various classes of phytoconstituents using the methods described by Trease and Evans (1996) [35]. All biological activities of these tests on the animal tissues.

Preliminary Phytochemical screening

The phytochemical evaluation of the *Anogeissus leiocarpus* purified extract showed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids and terpenoids as seen in Table 1.

Qualitative Phytochemical Screening

S/No	Phytoconstituents	Test	Remarks (+/-)
	Alkaloids	Dragendorff test	+
	Cardiac Glycosides	Keller-Kiliani test	+
	Saponins	Frothing test	+
	Phenolic compounds	Lead acetate test	+
	Tannins	Ferric Chloride test	+
	Steroids	Salkowski test	+
	Carbohydrates	Molisch test	+
	Flavonoids	Shinoda test	+
	Terpenoids	Liebermann Burchard test	+
	Anthraquinones	Bontrager's test	-

KEYS + = Present, - = Absent

Partition of Ethanol Extracts into Fractions

The ethanol extract was partitioned with ethyl acetate as described by Ebada *et al.* (2008) [8]. About 100mL of water was placed into a separating funnel in order to form a crude suspension. This was followed by the addition of 500 mL of ethyl acetate into the separating funnel after which the mixture was vigorously shaken 4 times at 3 minutes interval. The funnel was kept standing and undisturbed for 1 hour. Following the separation of the mixture into layers, the upper layer (ethyl acetate) was removed with caution. This was repeated severally so as to ensure that all ethyl acetate soluble compounds were released. The ethyl acetate fraction was concentrated with the aid of the rotary evaporator, while the aqueous fraction was concentrated on water bath before being stored at 20 °C.

Column Chromatography

The ethyl acetate fraction was subjected to column chromatography packed with silica gel (70-230 mesh). Briefly, silica gel (500 g) was mixed with 95% n-hexane and 5% ethyl acetate to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The sample to load on the column was prepared by dissolving 20 g of the ethyl acetate fraction in 100 mL of 9:1 of n-hexane and ethyl acetate. To the solution, 30 g of silica was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature. The dried silica extract mixture was layered on the column layer bed. The column was first eluted with n-hexane as the mobile phase with the polarity increasing by 5% increments of ethyl acetate. After getting to 100% ethyl acetate, the polarity was further increased by 5% increments of methanol. For each eluent system, the fractions were collected in vials. The fractions collected were concentrated to dryness by evaporation at 40 °C (Harvey, 2000) [14].

Thin Layer Chromatography

Thin layer chromatography (TLC) of the concentrated fractions collected from the column chromatography was carried out. Briefly, a spot of each fraction was carefully applied with capillary tube onto a thin layer chromatographic plate (coated with silica) and left to dry. After about five minutes, the plate was dipped in a suitable solvent which allowed the compounds in the spot to move upwards by capillary attraction. The plate was then removed from the solvent and left to dry. The positions of different compounds were observed by fluorescence under UV-light and was sprayed with sulfuric acid for the presence of specific compounds. Fractions with similar TLC profiles were pooled (Mbaveng *et al.*, 2008) [26]. The partition of the ethanolic extract into fractions, the column chromatography and the thin-layer chromatography were detailed elsewhere (Esievo *et al.*, 2023) [10]. This was carried out to purify the *Anogeissus leiocarpus*.

Determination of median lethal dose

The median lethal dose (LD₅₀) for the purified extract of *Anogeissus leiocarpus* was estimated using the limit test under the Organization for Economic Cooperation and Development (OECD) Guidelines. The dose was found to be 2000mg/kg (Esievo *et al.*, 2023) [10]. Therefore, optimized doses of 5mg/kg, 10mg/kg and 20mg/kg of the purified extract was used in this study.

Study Animals

Twenty (20) sexually mature apparently healthy, female Wistar rats (*Rattus norvegicus*), 12 weeks old, with body weight of 194.05 ± 13. 63g were purchased from the Laboratory Animal House of the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

The female Wistar rats were kept in standard plastic shoebox type cages, covered with wire mesh and wood shavings were used as litter materials. The rats were allowed to acclimatize to the experimental environment for two weeks (National Research Council, 1996) [29]. The rats were fed with commercially sourced grower mash (Top feeds, Nigeria Limited Sapele) and clean drinking tap water. Feed and water were given *ad libitum* throughout the period of the study.

Study Design

The twenty (20) female Wistar rats were divided at random into four groups A, B, C, D, comprising of five rats each as follows:

Group A was administered feed and plain water (serving as control).

Group B was administered 5 mg/kg of the purified extract.

Group C was administered 10 mg/kg of the purified extract.

Group D was administered 20 mg/kg of the purified extract.

The female Wistar rats were dosed orally at 9:00am daily for twelve (12) days using 20ml graduated syringe. Vaginal smears were collected from day 0 till day twelve (12) for vaginal cytology. Serum was harvested from day 0 till day twelve (12), (at intervals of one day) for hormonal analysis. Live weights were taken on day 0, 7 and 14.

Vaginal Cytology

Vaginal swabs were collected every other day post daily administration of the extract for twelve days with the use of cotton balls to determine the phases of the oestrus cycle. Each rat and their forepaws were restrained with the tail elevated to visualize the vagina. A vaginal swab was obtained using cotton-tipped swab wetted with ambient temperature physiological saline and introduced into the vagina of the restrained female Wistar rat for twelve (12) days. The swab was gently turned and rolled against the vaginal wall and then removed. Cells collected were then transferred to a dry glass slide by moving the swab across the slide. The slide was air-dried, stained with Giemsa Stain (10 ml of Giemsa stain with 90 ml of Buffer A and B) (Auta and Hassan, 2016) [4]. The slide was then overlaid with a coverslip and examined under a light microscope immediately at lowest to highest magnification. Generally, the vaginal secretion contained three types of cells, the leucocytes, cornified epithelial cells and nucleated epithelial cells. Estimation of the phase of estrous cycle was based on the proportion of these cells in the vaginal secretion (Auta and Hassan, 2016) [4]. The number of occurrences of each stage of the oestrous cycle was recorded, the oestrous cycle index and estrous cycle ratio were calculated.

Determination of estrous cycle

The cell types were used to determine the stages of the oestrous as described by Mahuta *et al* (2026).

Hormone assay

Blood obtained from anaesthetized rats was centrifuged at 808.5 g for 5 min; the serum was harvested and stored at -20 °C until use. The serum was used to assay for estradiol and progesterone using an Accu Bind® ELISA kit manufactured by Monobind Inc. The assay was performed by following the manufacturer's instructions as previously described by Mahuta *et al.* (2026).

Live Weight Changes

The live weights of all female Wistar rats were measured in grams using the Mettler Toledo (a Swiss Company founded in United States of America) weighing balance with 0.1 mg sensitivity. The live weights were taken on day 0, 7 and 14 during the period of the study in order to evaluate the effect of *Anogeissus leiocarpus* purified extract on live weight changes.

Statistical Analysis

The data of stages, index and ratio of the oestrous cycle were subjected to descriptive statistics using the GraphPad Prism® statistical package and ivivostat (version 8.0) for analysis with Tukey's post-hoc tests to analyze the difference between groups. The data of the hormonal (oestrogen and progesterone) levels and live weight changes were subjected to one-way ANOVA using the GraphPad Prism® statistical package and ivivostat (version 8.0) for analysis with Tukey's post-hoc tests to analyze the difference between groups. Values of $P < 0.05$ were considered significant. The results obtained from this study were presented in charts and tables of means $\pm$ standard deviation (SD).

Results

Stages of the Oestrous Cycle

The stages of the oestrous cycle of Wistar rats administered *Anogeissus leiocarpus* purified extracts is presented in Table 4.1. Wistar rats in group B (3.20 ± 1.48 days) and group D (3.00 ± 0.71 days) had insignificantly higher occurrence of proestrus ($P > 0.05$) than Wistar rats in group A (3.00 ± 0.00 days). Wistar rats in group C (5.00 ± 0.82) had significantly higher occurrence of proestrus ($P < 0.05$) than Wistar rats in group A, control group (3.00 ± 0.00 days). However, Wistar

rats in group B (6.80 ± 1.48) and group D (6.20 ± 1.30) had significantly higher occurrence of estrus ($P < 0.05$) than Wistar rats in group C (3.50 ± 2.38 days) and group A (3.00 ± 0.00 days). Wistar rats in group B (1.00 ± 0.00 day) and group D (1.20 ± 0.45) had significantly lower occurrence of metoestrus ($P < 0.05$) than Wistar rats in group C (3.50 ± 2.38 days) and group A (3.00 ± 0.00 days). Wistar rats in group B (1.00 ± 0.00 day) and group D (1.60 ± 0.89 day) had significantly lower occurrence of dioestrus ($P < 0.05$) than Wistar rats in group A (3.00 ± 0.00 days). However, the Wistar rats in group C (1.50 ± 0.58) had a significantly lower occurrence of diestrus stage than Wistar rats in group A (3.00 ± 0.00 days). Therefore, Wistar rats in group B (1.00 ± 0.00 day) and group D (1.20 ± 0.45 days) had significantly lower occurrence of the metoestrus stages ($P < 0.05$), while Wistar rats in group C, 10 mg/kg (2.00 ± 1.41 days) also had insignificantly lower occurrence of the metoestrus stages ($P < 0.05$); Wistar rats in groups B (1.00 ± 0.00), C (1.50 ± 0.58 day) and D, 20mg/kg (1.60 ± 0.89 day) had significantly lower occurrence ($P < 0.05$) of the diestrus stages than Wistar rats in group A, control (3.00 ± 0.00 days). In summary, the results were indicating higher occurrence of follicular phase and lower occurrence of luteal phase in the treated groups B, C and D compared to group A, the control.

Table 1: Mean $\pm$ SD of the Stages of the oestrous cycle of Wistar Rats Administered Purified Extracts of *Anogeissus leiocarpus* for Three Cycles

Stage of Oestrous Cycle (Days)	Groups			
	A (Control)	B (5 mg/kg)	C (10 mg/kg)	D (20mg/kg)
Proestrus	3.00 ± 0.00^a	3.20 ± 1.48^a	5.00 ± 0.82^b	3.00 ± 0.71^a
Estrus	3.00 ± 0.00^a	6.80 ± 1.48^b	3.50 ± 2.38^a	6.20 ± 1.30^b
Metestrus	3.00 ± 0.00^a	1.00 ± 0.00^b	2.00 ± 1.41^a	1.20 ± 0.45^b
Dioestrus	3.00 ± 0.00^a	1.00 ± 0.00^b	1.50 ± 0.58^b	1.60 ± 0.89^b

Values with different superscripts ^a^b in the same rows are significantly ($P < 0.05$) different.

Oestrous Cycle Index and Ratio

The oestrous cycle indices were presented in table 4.2. The Wistar rats in group C (41.67) had higher proestrus index than group A (25.00). The Wistar rats in group B (56.67) and group D (51.67) had higher estrus indices than group A (25.00) and was significant. The Wistar rats in group C (29.16) had higher estrus index than group A (25.00). Wistar rats in group B (8.33) and group D (10.00) had significantly

lower metoestrus indices than group A (25.00). Wistar rats in group B (8.33) and group D (13.33) had significantly lower dioestrus indices than group A (25.00). However, the Wistar rats in group C (16.67) had significantly lower dioestrus index than group A (25.00).

The oestrous cycle ratio is also presented in Table 4.2. The oestrous cycle ratio was significantly higher ($P < 0.05$) in the treatment groups compared to the control group.

Table 2: Mean $\pm$ SD of Oestrous Cycle Index and Ratio of Wistar Rats Administered Purified Extracts of *Anogeissus leiocarpus* for Three Cycles

	Groups			
	A (Control)	B (5 mg/kg)	C (10mg/kg)	D (20mg/kg)
Proestrus index	25.00	26.67	41.67	25.00
Estrus index	25.00	56.67	29.16	51.67
Metoestrus index	25.00	8.33	16.67	10.00
Dioestrus index	25.00	8.33	12.50	13.33
Oestrous cycle ratio	1.00 ± 0.00^a	5.01 ± 1.49^b	2.43 ± 1.79^b	3.29 ± 1.62^b

Values with different superscripts ^a^b in columns are significantly ($P < 0.05$) different.

Oestrogen Levels (pg/ml) of Wistar rats Administered *Anogeissus leiocarpus* Purified Extracts

The effects of purified extracts of *Anogeissus leiocarpus* on oestrogen level is presented in Table 4.3. The levels of oestrogen in rats in group A on day 0 was 8.47 ± 0.40 pg/ml. The levels of oestrogen in rats in group C (5.45 ± 0.17 pg/ml) were significantly ($P < 0.05$) low at day 0. It became significantly ($P < 0.05$) higher at day 2, which is estrus in group B (5.25 ± 0.73 pg/ml) and group C (5.95 ± 0.91 pg/ml), also significantly ($P < 0.05$) lower at day 6, which is estrus in group B (6.66 ± 0.51 pg/ml) and group D (3.24 ± 0.45 pg/ml)

treatment groups than group A (4.26 ± 0.86 pg/ml). Therefore, it indicated, the oestrogen level was low at day 0, which is proestrus in group C (5.45 ± 0.17 pg/ml) than group A. The oestrogen level was lower at day 2, which is estrus in group B (5.25 ± 0.73 pg/ml) and group C (5.95 ± 0.91 pg/ml) than group A (8.35 ± 0.32 pg/ml). The oestrogen level was lower at day 6, which is estrus in group B, 5mg/kg (6.66 ± 0.51 pg/ml) and group D, 20mg/kg (3.24 ± 0.45 pg/ml) group than group A, control (4.26 ± 0.86 pg/ml). Oestrogen levels were normal in the treated groups compared to the control.

Table 3: Mean $\pm$ SD of Oestrogen Levels (pg/ml) of Wistar Rats Administered *Anogeissus leiocarpus* Purified Extracts

Stages of Estrous Cycle (Days) / Estrogen levels(pg/ml)	Group A Control	Group B 5 mg/kg	Group C 10 mg/kg	Group D 20 mg/kg
Proestrus 0	8.47 $\pm$ 0.40 ^a	8.18 $\pm$ 0.28 ^a	5.45 $\pm$ 0.17 ^b	8.13 $\pm$ 0.16 ^a
Estrus 2	8.35 $\pm$ 0.32 ^a	5.25 $\pm$ 0.73 ^b	5.95 $\pm$ 0.91 ^{ab}	8.46 $\pm$ 0.20 ^a
Met/Dioestrus 4	3.54 $\pm$ 0.70 ^a	4.30 $\pm$ 0.60 ^a	6.30 $\pm$ 0.20 ^a	11.54 $\pm$ 0.62 ^a
Estrus 6	4.26 $\pm$ 0.86 ^a	6.66 $\pm$ 0.51 ^{ab}	5.12 $\pm$ 0.73 ^a	3.24 $\pm$ 0.45 ^{ac}
Met/Dioestrus 8	6.18 $\pm$ 1.30 ^a	5.48 $\pm$ 0.74 ^a	4.81 $\pm$ 0.15 ^a	7.72 $\pm$ 0.48 ^a
Estrus 10	4.71 $\pm$ 0.14 ^a	6.63 $\pm$ 2.39 ^a	5.68 $\pm$ 0.56 ^a	6.61 $\pm$ 0.20 ^a

Values with different superscripts ^{aabac} in columns are significantly ($P < 0.05$) different.

Progesterone Levels (ng/ml) of Wistar rats Administered *Anogeissus leiocarpus* Purified Extracts.

The effect of purified extracts of *Anogeissus leiocarpus* on progesterone level is presented in Table 4.4. The levels of progesterone in the Wistar rats in group A on day 0, which is proestrus was 5.96 $\pm$ 0.82 ng/ml. The levels of progesterone in the Wistar rats in group A on day 2, which is estrus was 5.78 $\pm$ 1.77 ng/ml. The levels of progesterone in the Wistar rats in group A, control (8.97 $\pm$ 1.58 ng/ml) on day 4, which is diestrus was significantly higher ($P < 0.05$). The levels of progesterone in the Wistar rats in group A (4.54 $\pm$ 0.80 ng/ml) became significantly lower ($P < 0.05$) on day 6, which is estrus. The levels of progesterone in the Wistar rats in group C on day 0, which is proestrus was 3.10 $\pm$ 0.78 ng/ml. The levels of progesterone were significantly low ($P < 0.05$) during

proestrus at day 0 in Wistar rats in group C (3.10 $\pm$ 0.78 ng/ml). Progesterone levels were significantly lower ($P < 0.05$) during estrus at day 2 in group B (4.32 $\pm$ 1.51 ng/ml), also significantly lower ($P < 0.05$) during dioestrus at day 4 in group B (5.37 $\pm$ 0.73 ng/ml), group C (7.61 $\pm$ 0.55 ng/ml) and group D (6.71 $\pm$ 1.20 ng/ml) treatment groups than group A (8.97 $\pm$ 1.58 ng/ml). The levels of progesterone also were significantly lower ($P < 0.05$) during estrus, at day 10 in group B (3.86 $\pm$ 0.73 ng/ml), group C (2.97 $\pm$ 1.43 ng/ml) and group D (3.84 $\pm$ 1.35 ng/ml) treatment groups than group A (6.22 $\pm$ 2.30 ng/ml). The progesterone levels were significantly lower ($P < 0.05$), when compared with the control. Progesterone levels were normal in the treated groups compared to the control.

Table 4: Mean $\pm$ SD of Progesterone Levels (ng/ml) of Wistar rats Administered *Anogeissus leiocarpus* Purified Extracts

Progesterone level (ng/ml)/Stages of Estrous Cycle (Days)	Group A Control	Group B 5 mg/kg	Group C 10 mg/kg	Group D 20 mg/kg
Proestrus 0	5.96 $\pm$ 0.82 ^a	6.84 $\pm$ 0.95 ^a	3.10 $\pm$ 0.78 ^b	6.72 $\pm$ 2.06 ^a
Estrus 2	5.78 $\pm$ 1.77 ^a	4.32 $\pm$ 1.51 ^b	5.70 $\pm$ 1.19 ^a	5.89 $\pm$ 1.00 ^a
Met/Diestrus 4	8.97 $\pm$ 1.58 ^a	5.37 $\pm$ 0.73 ^b	7.61 $\pm$ 0.55 ^{ab}	6.71 $\pm$ 1.20 ^a
Estrus 6	4.54 $\pm$ 0.80 ^a	4.54 $\pm$ 1.13 ^a	4.89 $\pm$ 1.39 ^a	5.42 $\pm$ 0.69 ^a
Met/Diestrus 8	5.70 $\pm$ 2.06 ^a	4.92 $\pm$ 1.24 ^a	5.85 $\pm$ 0.55 ^a	4.27 $\pm$ 1.49 ^a
Estrus 10	6.22 $\pm$ 2.30 ^a	3.86 $\pm$ 0.73 ^b	2.97 $\pm$ 1.43 ^{bc}	3.84 $\pm$ 1.35 ^b

Values with different superscripts ^{aabbc} in columns are significantly ($P < 0.05$) different.

Live Weight Changes of Wistar rats Administered *Anogeissus leiocarpus* Purified Extract.

Live weights significantly increased in group C (18.75 $\pm$ 0.00 grams) compared to groups B (-3.5 $\pm$ 1.24 grams) and D (-14.6 $\pm$ 0.14 grams) in this study. Live weights slightly decreased in group B (-3.5 $\pm$ 1.24 grams) and markedly decreased in group D (-14.6 $\pm$ 0.14 grams) from day 1 till day 14. (Table 4.5).

Table 5: Mean $\pm$ SD of Live Weight (grams) Changes of Wistar Rats Administered *Anogeissus leiocarpus* Purified Extract from day 0 to day 14

Days	0	7	14
Group A (Control)	209.6 $\pm$ 8.3 ^a	217.2 $\pm$ 16.1 ^a	217.8 $\pm$ 12.9 ^a
Group B (5 mg/kg)	215.0 $\pm$ 14.7 ^a	216.0 $\pm$ 21.1 ^b	210.8 $\pm$ 19.2 ^b
Group C (10 mg/kg)	178.5 $\pm$ 15.7 ^a	193.0 $\pm$ 14.0 ^a	197.3 $\pm$ 17.2 ^a
Group D (20 mg/kg)	174.2 $\pm$ 15.8 ^a	167.8 $\pm$ 10.9 ^b	165.2 $\pm$ 2.6 ^{ab}

Values with different superscripts ^{abab} in columns are significantly ($P < 0.05$) different across the group on each day

Discussion

The phytochemical analysis in this study revealed the presence of alkaloids, cardiac glycosides, saponins, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids in the purified stem bark extract of *Anogeissus leiocarpus*. These findings are consistent with the reports of Mann *et al.* (2010) [23] and Ogundiya *et al.* (2006) [30], who identified similar phytoconstituents in the stem bark extract,

although carbohydrates and phenolic compounds were not reported in their studies. In addition, Esievo *et al.* (2025) [9] similarly reported the presence of these phytochemicals, except anthraquinones. The presence of these bioactive compounds may account for the observed physiological and reproductive effects of the extract in the present study, considering that flavonoids, tannins, saponins, alkaloids, and terpenoids are known to possess endocrine-modulating, antioxidant, and growth-regulating properties.

The administration of purified *Anogeissus leiocarpus* extract significantly altered the oestrous cycle characteristics of the experimental Wistar rats. Group C showed a prolonged proestrus phase, while groups B and D showed prolonged estrus phases and shortened metestrus and dioestrus phases compared to the control group. Overall, the treatment groups confirmed a prolonged follicular phase and a reduction in the luteal phase of the oestrous cycle. These findings are in agreement with those reported by Idris *et al.* (2023) [17], Mustapha *et al.* (2011) [27], Abu and Uchendu (2011) [2], and Essiet *et al.* (2018) [11], who observed prolonged proestrus and estrus stages with shortened metestrus and diestrus stages in rats exposed to glyphosate-based herbicides and various plant extracts. However, the findings differ from those of Rahman *et al.* (2017) [32], Baligar and Kaliwal (2002) [5], and Auta and Hassan (2016) [4], who reported prolonged proestrus, estrus, and metestrus stages with shortened diestrus stages following administration of *Eurycoma longifolia*, carbofuran, and

Azadirachta indica respectively.

The prolonged proestrus and estrus phases observed in this study may be attributed to the estrogenic activity of *Anogeissus leiocarpus*. The extract may possess phytoestrogenic compounds capable of binding to oestrogen receptors or mimicking endogenous oestrogen activity, as suggested by Benachour and Seralim (2009), Gasnier *et al.* (2009) [13], and Cobb and Reade (2011) [7]. Sustained oestrogenic stimulation is known to prolong the follicular phase and maintain animals in persistent estrus (Westwood, 2008) [37]. Additionally, the shortened luteal phases observed in this study may be associated with reduced progesterone secretion, since progesterone plays a critical role in the maintenance and regulation of the luteal phase and acts synergistically with oestrogen in controlling the oestrous cycle (Olurode, 2017) [31].

The hormonal profile obtained in this study further supports the observed alterations in oestrous cyclicity. Serum oestrogen concentrations fluctuated across treatment groups and experimental days, with increased oestrogen levels during estrus in groups B and C relative to the control group, suggesting enhanced follicular activity. Conversely, progesterone concentrations were consistently reduced among the treatment groups throughout the experimental period. These findings agree with Mahuta *et al.*, who reported increased oestrogen and decreased progesterone concentrations in rats exposed to dichlorvos. Sustained oestrogen dominance with reduced progesterone may induce persistent estrus and prolonged follicular activity, while insufficient oestrogen elevation may result in persistent proestrus (Feldmann and Nelson, 2004) [12]. The hormonal imbalance observed in this study indicates that the purified extract of *Anogeissus leiocarpus* possesses endocrine-modulating effects capable of disrupting normal reproductive cyclicity.

The effects of the extract on body weight revealed dose-dependent variations among the treatment groups. Group C demonstrated significant weight gain, whereas groups B and D showed reductions in body weight during the experimental period. The increased live weight observed in group C agrees with the findings of Esievo *et al.* (2025) [9], who reported increased body weight in diabetic rats administered crude ethanolic stem bark extracts of *Anogeissus leiocarpus*. Similar improvements in growth performance and carcass characteristics have also been reported following supplementation with extracts of *Thonningia sanguinea*, *Eugenia caryophyllus*, and *Anogeissus leiocarpus* in poultry (Yao *et al.*, 2017; Afrah *et al.*, 2018; Konan *et al.*, 2020) [38, 20]. The growth-promoting effect observed at moderate doses may be linked to the antibacterial, antiparasitic, and antioxidant properties of *Anogeissus leiocarpus*.

In contrast, the reduction in body weight observed in group D at the highest dose may be associated with reduced feed intake and impaired nutrient utilization. This finding is in line with the report of Fahey and Jung (1989), who attributed growth depression in rats fed tannin-rich diets to reduced feed consumption. High tannin concentrations have previously been reported in *Anogeissus leiocarpus* extracts (Agaie *et al.*, 2007) [3], and tannins are known to reduce palatability and interfere with nutrient digestion and absorption. Consequently, the decrease in body weight at higher doses may reflect the adverse nutritional effects of excessive tannin intake. Overall, the findings of this study suggest that purified *Anogeissus leiocarpus* extract exerts dose-dependent effects on reproductive physiology and growth performance in

female Wistar rats, likely mediated through its diverse phytochemical constituents and endocrine-modulating activities.

Conflict of Interest

Not available

Financial Support

Not available

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