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Effects of Aqueous Stem Bark Extract of *Anogeissus leiocarpus* on the Serum Levels of Progestin and Estradiol and the Estrous Cycle of Wistar Rat

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ABSTRACT

Background: The effect of aqueous stem bark extract of *Anogeissus leiocarpus* (AEAL) on the reproductive cycle of adult female Wistar rat and the serum levels of ovarian hormones (estradiol and progestin) was studied. **Methods:** Twenty-four (24) adult female Wistar rats were used. Rats were divided into groups (I - IV; n=6). Group I was control and received distilled water; groups II, III and IV received AEAL (200mg/kg, 400mg/kg, and 600mg/kg, oral, respectively) for the period of six (6) weeks. The estrous cycle changes were determined by daily observation of vaginal smear, while serum levels of estradiol and progestin was compared after the extract administration-using enzyme linked immunosorbent assay kits. **Results:** Result showed significant ($p< 0.05$) increase in the levels of serum progesterone and estradiol in groups II and III treatment; 19% higher when compared to the control. Treatment with AEAL at doses 200mg/kg and 400mg/kg prolonged di-estrus and estrus phases of the rats' estrous cycle. The increase in the duration of the two phases (diestrus and estrus) was dose dependent, significant ($p<0.05$) when compared to the control. **Conclusion:** AEAL has effect on the reproductive cycle and serum levels of ovarian hormones of Wistar rats, hence of potential use in fertility related studies.

Keywords: *Estrus cycle, Estradiol, Fertility, Progestin, Wistar rat.*

INTRODUCTION

Anogeissus leiocarpus (DC) Guill and Perr (family: *Combretaceae*) is a tall evergreen tree native to the savannas of West Africa.[1] It is the sole West African species of the genus *Anogeissus*, a genus otherwise distributed from Tropical, Central and East Africa through tropical Southeast Asia.[1]

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A. leiocarpus germinates in the soil produced by seasonal wetlands and grows at the edges of the rainforest, although not in the rainforest, in the savanna, and along riverbanks forming gallery forests. The tree flowers in the rainy season, from June to October. The seeds, winged samaras, are dispersed by ants.[1] The inner bark of the tree is used as a human and livestock anthelmintic for treating worms, and for treatment of a couple of protozoan diseases in animals, nagana an animal trypanosomiasis, and Babesiosis.[2] The inner bark used as a chewing stick in Nigeria and extracts of the bark show antibacterial properties.[3] The stem barks contains castalagin.[4] *A. leiocarpus* is also locally used for problems associated with reproduction and to enhance fertility in many parts of Northern Nigeria.The effect of *A. leiocarpus* on reproduction and fertility has not been investigated. Phytochemical studies conducted on *A. leiocarpus* revealed that the stem bark extracts has various chemical constitutions such as alkaloids, glycosides, steroid, anthraquinone, phenol, tannins, saponins, calcium, and fluorides.[3]

MATERIALS AND METHODS

Experimental Animals

Twenty-four (24) young adult Wistar rats weighing between 135 - 150g per group were obtained from the department of pharmacology of Ahmadu Bello University Zaria, Kaduna state, Nigeria. They were kept in plastic cages and maintained under laboratory condition at room temperature and humidity. Water and standard pellet diet were provided to the rats ad libitum. All animals received care in compliance with the guidelines of the ethical committee of medical research, ABU Zaria. Thereafter, the animals were divided into four groups of six animals each.

Plant Material

The fresh stem bark of *A. leiocarpus*, readily available within the Ahmadu Bello University Zaria, were collected and authenticated in the herbarium of Biological Sciences Department of ABU Zaria by a taxonomist U. S. Gallah with a voucher specimen number 1738 for future reference. Two kilogram of *A. leiocarpus* stem bark was air dried, minced and powdered using a clean, sterile laboratory mortar. 50g of the resulting powder was dissolved in 0.5 liters of distilled water using a soxhlet extractor. The solution was allowed to settle for twenty-four hours and filtered with Whatman's filter paper. The resultant solute was allowed to settle for one hour, and placed in water bath under reduced pressure at 35°C for evaporation. The extract was weighed and dissolved in distilled water to prepare the stock solution.

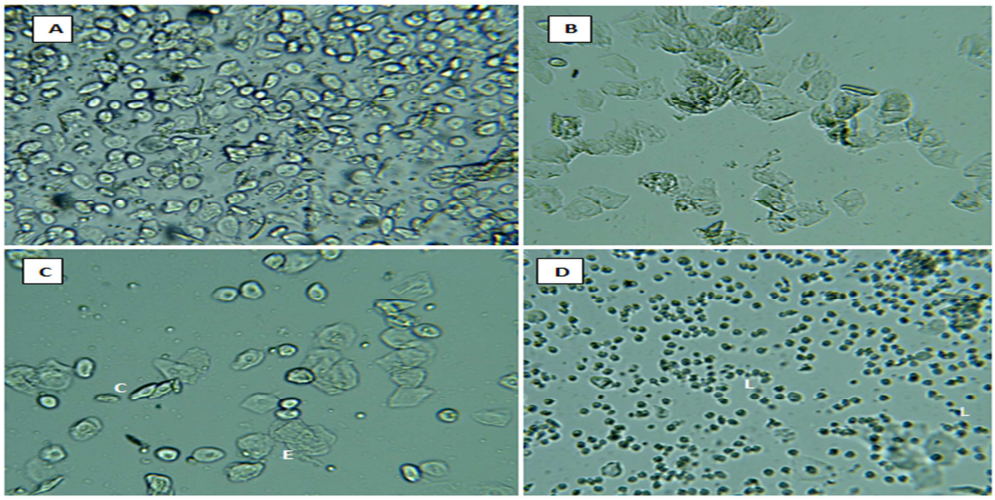
Hormonal Assay

Blood samples were collected from the rats on the last day of the experiment through cardiac puncture in a 5ml tube under deep anesthesia using chloroform. It is then centrifuged to obtain serum for the analysis of ovarian hormones (estradiol and progestin). The assay for the determination of estradiol and progesterone level in the serum was carried out using the Estradiol (E2), and Progestin ELISA kits.

Method of Taking Vaginal Smear

Vaginal secretion from the experimental animals was collected according to the method described by Marcondes *et al.*, 2001.[5] Every morning between 8:00 and 9:00 a.m. rats were allowed to remain undisturbed for an hour before sampling. The genital area of each rat was cleaned with cotton wool soaked in methylated spirit. The tip of a pasture pipette filled with 10µls of normal saline (Nacl 0.9%) was inserted into the rat vagina, but not deeply. Vaginal fluid obtained was used to make a smear on a clean glass slides.

One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope with 10 and 40× objective lenses. Estrous stage was then evaluated according to the method of Long and Evans, 1922; Mandl, 1995.[6,7]



Graph 1: Photomicrograph showing the vaginal smear of the experimental rats at different estrous stages. (A) Proestrus: round and nucleated ones are epithelial cells. (B) Estrus: irregular ones without nucleus are the cornified cells. (C) Metestrus: the same proportion of cells in proestrus, estrus and diestrus. (D)Diestrus: and the little round ones are the leukocytes. (x 200).

Statistical Analysis

The results obtained were analyzed using Statistics 5.0 software and Microsoft Excel 2007. All results were expressed a mean value ± SD. The variance of the data and the confidence interval were determined using the analysis of variance (ANOVA) and a value of $P<0.05$ was considered as statistically significant. Dunnet's post hoc tests were used to determine where the level of significance lies.

RESULTS

Table 1: The mean ± S.D of the percentage of rats at different phases of Estrus Cycle on different doses of the extract at the last day of experiment.

Group	Percentage (Mean ± S.D) of rats at different Phases of Estrus Cycle			
	Proestrus	**Estrus**	**Metestrus**	**Diestrus**
I - Control	15.46±3.2	11.73±1.8	51.07±3.2	23.38±2.8
II - 200mg/kg	5.30±1.8	35.03±4.7	21.30±4.7	39.05±3.7
III - 400mg/kg	15.26±3.2	33.43±3.6	5.35±4.6	47.31±5.4
IV - 600mg/kg	23.32±3.6	40.03±5.4	31.06±3.2	6.83±3.6

S.D = Standard deviation

Table 2: Mean serum level of Progesterone at the end of administration of *Anogeissus leiocarpus* extract by 42 days.

Group	Progesterone Mean(pg/ml) ± SD
I- Control	5.31± 0.13
II- 200mg/kg	5.61± 0.09*
III- 400mg/kg	6.20± 0.10*
IV- 600mg/kg	4.46± 0.14

P <0.05
SD = Standard deviation
*= Statistically Significant

Table 3: Mean serum level of Estradiol at the end of administration of *Anogeissus leiocarpus* extract by 42 days.

Group	Estradiol Mean(pg/ml) ± SD
I- Control	5.91 ± 0.13
II- 200mg/kg	6.41 ± 0.14*
III- 400mg/kg	6.70 ± 0.13*
IV- 600mg/kg	6.76 ± 0.14*

P <0.05
SD = Standard deviation
*= Statistically Significant

DISCUSSION

There was a gradual change of estrus cycle pattern observed in each group. The variation in the phases of the estrus cycle in the treated rats has shown a dose dependent pattern with a prolonged diestrus in the group that received 400mg/kg, and prolonged estrus in the group that received 600mg/kg. The prolongation of the same phase of estrus cycle is considered to be irregular.[5] The presence of saponins and alkaloids in the extract of *A. leiocarpus*, as indicated from the phytochemical analysis may be responsible for the disruption of regular cyclicity in the rats. Singh *et al*., 2007, suggested that when female rats are exposed to plant steroidal saponins, the elevation of circulating estrogen is resulted in the female rats.[8] This prolonged the estrus and proestrus phases, as seen in the high dose group (600mg/kg) of this study.

There was an increase in the concentration of both progesterone in groups II, and III; also estradiol in groups II, III and IV following the extract administration. The progesterone level in the orally treated group was higher and statistically significant ($p<0.05$). Dichloromethane fraction from *A. leiocarpus* stem bark has been identified as the most active inhibitor on cyclic nucleotide phosphor-diesterases (PDEs), and preferentially inhibits calmoduline-dependent phosphodiesterase PDE1.[9] Phosphodiesterases (PDE) are responsible for the breakdown and concomitant inactivation of the cyclic nucleotides cAMP and cGMP and are implicated in the regulation of oocyte meiotic maturation. Selective inhibitors of phosphor-diesterase type 3 (PDE3) prevent meiotic resumption of mammalian oocytes.[9] It was suggested that the use of arresters of meiosis could improve cytoplasmic maturation of immature oocytes by controlling the period of prophase I.[9] The inhibitory effect of AEAL to phosphodiesterase as reported by the studies conducted by Lazare *et al*.,

2008, has probably resulted in the increase in mean serum concentration of progesterone in the experimental group 1 and II, through negative feedback effect, where the decreasing level of the steroid hormone from the ovary result in excess release of progesterone.[9] Also the hormone level in the control in the control group of the pre administration phase is higher when compared to the post administration phase in the animals. This is in agreement with the normal reproductive physiological mechanism.

CONCLUSION

AEAL has effect on the reproductive cycle and serum levels of ovarian hormones of Wistar rats, hence of potential use in fertility related studies.

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