

Trends in Natural Products Research



Contraceptive Potentials of *Alstonia boonei* via Stimulation of Increased Prolactin Synthesis in Experimental Female Wistar Rats

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Abstract: This work aimed to evaluate the effect of methanol leaf extract of *Alstonia boonei* on the reproductive hormones of females. Adult female albino rats were randomly grouped into three groups of four rats each. Group 1 served as the control (feed and water only), group (2) received the standard drug Pregnancare (Vitabiotics) while group 3 was given the extract. The standard drug and extract used for this experiment were administered orally for three weeks. The hormones serum concentrations were determined by ELISA method. The results showed a non-significant ($p > 0.05$) increase in the serum concentrations of follicle stimulating hormone, estradiol and luteinizing hormone and a significant ($p < 0.05$) increase in the concentrations of progesterone and prolactin in the extract-treated group when compared with the control group. The results of this experiment show that this extract possesses positive effects on the female reproductive hormones and could be used in the management of primary ovarian failure, irregular menstruation and miscarriage.

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INTRODUCTION

All through the world many people still depends on herbal medicines because of culture, availability and economic hardship. Studies have also shown that some modern medicines are derived from plants that have been used by traditional medicine practitioners (Gragg and Newman, 2005). Plant components have been shown to have indirect action on the secretion of certain hormones by stimulating or inhibiting the hypothalamus and pituitary gland thereby controlling the functions of most other glands. For instance, quinolizidine alkaloids isolated from the roots of *Cnestis ferruginea* caused significant reduction in sperm counts, motility, viability, morphology and plasma levels of testosterone, luteinizing hormone and follicle stimulating hormone in male rats (Olayemi and Raji, 2011). Anti-fertility effects of alkylating agents and vinca alkaloids which is responsible for ovarian damage has also been reported (Cooke *et al.*, 1978; Lopes *et al.*, 2016)

The modern pharmaceutical industry itself also relies largely on diverse secondary metabolites in plants isolated through scientific findings (Mallikharjuna *et al.*, 2007). African countries such as Madagasca, Nigeria, Gambia etc are at a stage where traditional medicines are considered more for their capacity to generate other medicine (Rukangira, 2004; Van Wyk, 2008). Plants products hold great promise for discovery and development of new pharmaceuticals (McChesney *et al.*, 2007).

Fertility is the natural capacity to produce offspring. Fertility hormones are responsible for growth, development and reproduction (PCARM, 2008b). Female fertility hormones are commonly associated with oestrous cycle, menstruation/menstrual cycle etc. These processes are controlled by the interactions of several hormones (Gnoth *et al.*, 2003). Fertility problems in females are directly linked to hormonal imbalances and an ovulation. The endocrine system secrete and co-ordinate hormones thereby maintaining proper hormonal balance. Sometimes endocrine disorder especially under production of hormones occurs because of abnormal tissue response to hormones (PCARM, 2008a; Mascarenhas *et al.*, 2012).

Alstonia boonei is a native plant used locally for its medicinal purposes. It is a deciduous plant that grows in both tropics and subtropical regions of the world. It belongs to apocynacea family (Trease and Evans, 2002). In Nigeria; it is known as *Ahun* (Yoruba), *Egbu-ora* (Igbo) *Ukhu* (Edo) and *Ukpukunu* in Urhobo. It is distributed widely in the lowlands and rain forest areas of Nigeria. It is also known as Australian fever bush, Australian quinine, Devil tree, Dita bark, fever bark or palimara (Ayiku, 1992; Gosse *et al.*, 1997; Omoya and Oyebola,

2019) *A. boonei* extracts are extensively used in folk medicine especially in developing nations like Nigeria and other African countries to treat mental illness, malaria, inflammatory diseases and reproductive disorders, without scientific data about their efficacy and toxic effects, as well as measures of safety (Oze *et al.*, 2012). Phytochemical screening of *A. boonei* root extract showed the presence of medicinally active constituents such as terpenoids, alkaloids, flavonoids etc. (Nweze *et al.*, 2004). Alkaloids including: echitamine and echitamidine, voacangine and akuammidine, N-formylechitamidine and N-formyl-12-methoxyechitamidine have been isolated from the plant (Nweze *et al.*, 2004). This study was aimed at investigating the effect of the methanol extract of *A. boonei* on some female fertility hormones.

MATERIALS AND METHODS

Collection and Extraction of Plant Materials

The leaves *A. boonei* was harvested from matured plant and authenticated by Dr. Garuba Omasum of Plant Science and Biotechnology (PSB), Michael Okpara University of Agriculture, Umudike (MOUUAU), Abia State, Nigeria. The leaves were thereafter air dried and milled into fine powder. A hundred grams of the powder obtained, was soaked in 700 ml of methanol for 72 hours. The mixture obtained was filtered and the filtrate evaporated to dryness using a water bath at temperature of 35-40 °C.

Experimental Animals

Twelve (12) female adult Wistar rats (90-100 g) were acquired from the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were fed with standard feed (Vital feeds, Nidea) and water *ad libitum* in a well ventilated environment with 12 hours light/darkcycle. They were kept in aluminum cages and allowed to acclimatize for period of two weeks before the onset of the experiment.

Experimental Design

The rats were randomly divided into three groups consisting of 4 rats each. Group 1 (normal control) was given feed and water. Group 2 served as standard group and was administered the standard drug (Pregnanicare (Vitabiotics)). Group 3 received 400 mg/kg body weight of plant extract. The oral administration lasted for a period of 21 days. At the end of the experimental period, the rats were fasted overnight and sacrifice under cervical dislocation and blood samples collected through cardiac puncture in plain sample bottles. The blood was allowed to stand for 30 minutes to clot and then

centrifuged at 3000 rpm for 10 minutes using laboratory centrifuge. The sera were into clean dry, sample bottles and used for the biochemical assay.

Hormonal Assays

Determination of serum concentration of the following hormones: estradiol, prolactin, progesterone, luteinizing and follicle stimulating hormones were done using enzyme-linked immunosorbent assay (ELISA) techniques with the method of Until *et al.*, (1981).

Statistical Analysis

Analysis of variance (ANOVA) was used for statistical analysis of the collected data with Statistical Package and Service Solutions (SPSS) version 21. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of the Extract on Reproductive Hormones

There was a non-significant ($p > 0.05$) increase in the serum FSH concentration in *A. boonei* treated group relative to the normal control group (Figure 1). The serum concentration of estradiol was not significantly ($p > 0.05$) affected by the extract (Figure 2). Treatment with the extract significantly ($p < 0.05$) increased the serum prolactin level (Figure 3). Similarly the progesterone level was significantly ($p < 0.05$) elevated (Figure 4), while the level of LH was not significantly ($p > 0.05$) increased (figure 5).

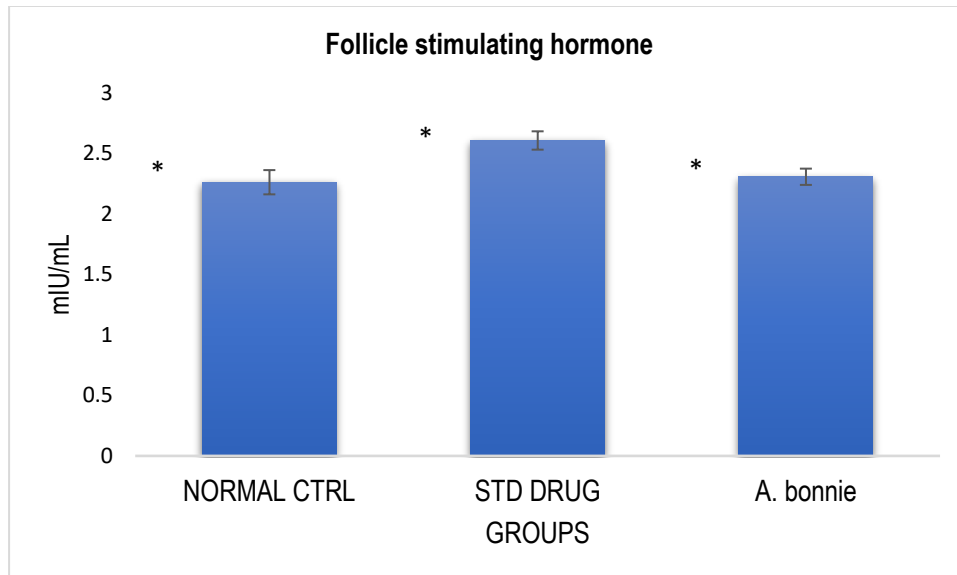


Figure 1: Effect of the Extract on Serum Follicle Stimulating Hormone concentration

*Non-significant difference when compared against normal control

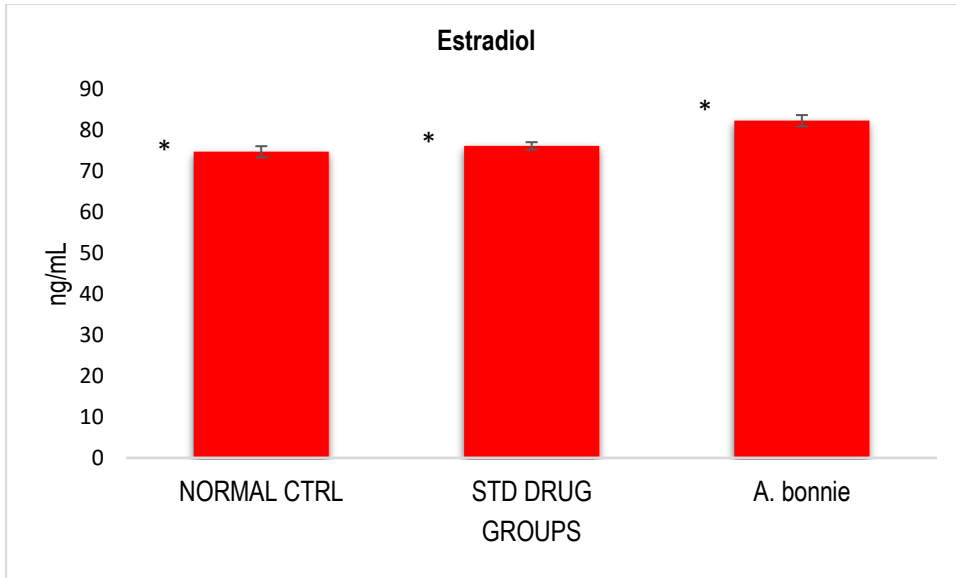


Figure 2: Effect the Extract on serum Estradiol Concentration
*Non-significant difference when compared against normal control

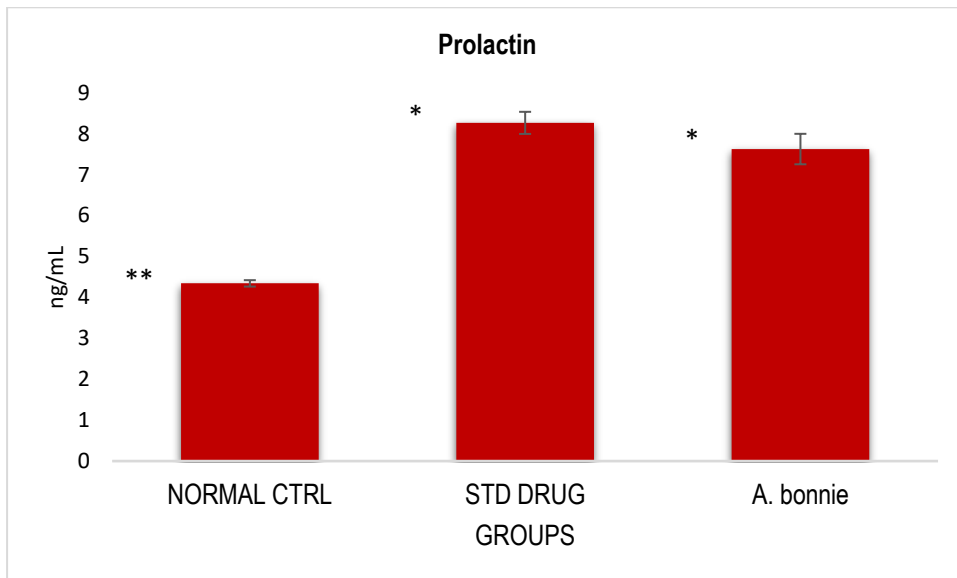


Figure 3: Effect of Extract on Serum Prolactin Concentration
*Significant difference when compared against the normal control

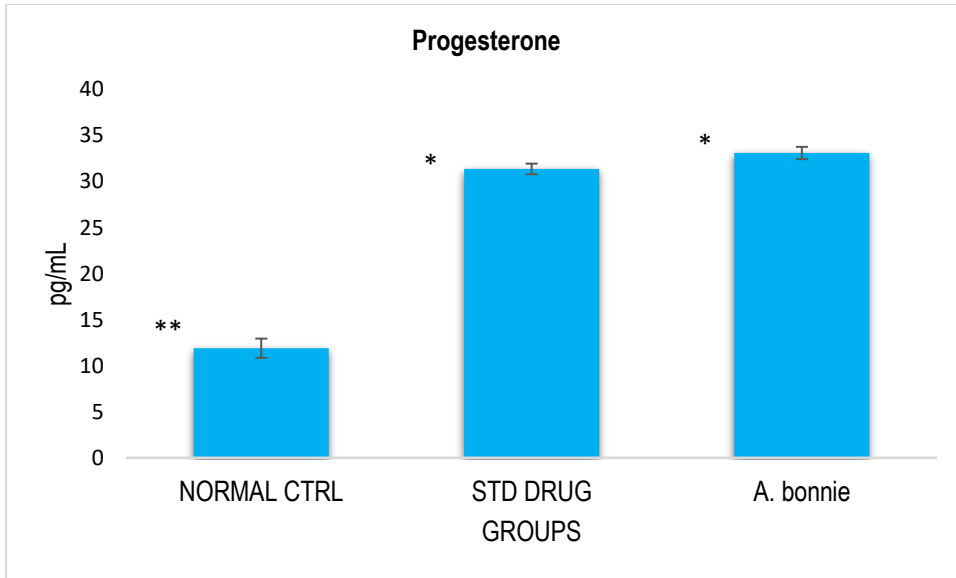


Figure 4: Effect of the Extract on Serum Progesterone Concentration

*Significant difference when compared against the normal control

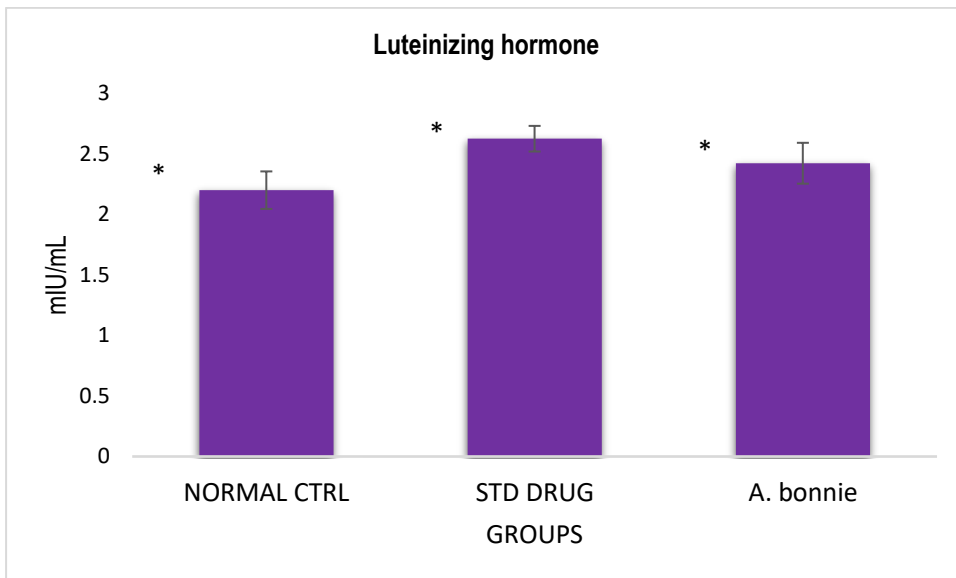


Figure 5: Effect of the Extract on Serum Luteinizing Hormone Concentration

*Non-significant difference when compared against the normal control

DISCUSSION

Results from this study revealed that *A boonei* caused significant increase in prolactin and progesterone concentrations while causing a non-significant increase in luteinizing hormone, follicle stimulating hormone and estradiol concentrations in female rats. High serum concentration of prolactin is associated with suppression of ovulation and by extension conception in females. This is the reason lactating females have suppressed or lack of ovulation and naturally cannot conceive. In humans lactating mothers consider it “safe” during this period and are usually free from pregnancy related phobia. Prolactin again plays a role in stimulating lactogenesis and mammary gland development. It is thought to achieve this role especially by acting as dopamine antagonist (Fitzgerald and Dinan, 2008). The extract appears to have stimulated increased production of prolactin thus making it a candidate for contraceptive use. High prolactin concentration in serum has the capacity to inhibit other hormones such as follicle stimulating hormone whose actions promote and enhance ovulation (Mohamed *et al.*, 2018). When this happen ovulation and conception cannot take place. In recent times family planning and child spacing have taken the front burner in many discuss bringing to the fore the need to explore more options in contraceptive use.

Progesterone is produced in the ovaries and provides many hormonal actions leading to the establishment and maintenance of pregnancy (Montaserti *et al.*, 2007). The increased serum level of progesterone is in agreement with the work of Oze *et al.* (2012). Progesterone with other hormones provides negative feedback inhibition that result in inhibition of pregnancy. This is the basis of action of many contraceptives (Raji *et al.*, 2005).

The contraceptive property of *A boonei* may be due to the presence phytochemicals such as alkaloids and flavonoids (Benie *et al.*, 2003; Yakubu *et al.*, 2005; Opoku and Akoto, 2015; Omoya and Oyebola, 2019).

Follicle stimulating hormone (FSH) plays key role in the female reproductive cycle. A reduction in the normal serum concentration of FSH affects folliculogenesis adversely and by extension hampers pregnancy (Yakubu *et al.*, 2005). In this study extract of *A boonei* did not stimulate increased production of FSH in contrasts the report of Jashni *et al.*, (2016)

Estradiol is another key hormone in the female reproductive system. It works with FSH to ensure maturation of follicles (Telefo, *et al.*, 1998). The inability of the extract to stimulate increased production of estradiol confirms its anti-fertility

property. Plant extracts have been reported to elevate and decrease serum estrogen levels depending on the nature of the plant and the prevailing environmental circumstances (Osonuga *et al.*, 2014; Olanipekun *et al.*, 2016).

Luteinizing hormone (LH) plays important roles in the female reproductive cycle by stimulating the release of mature follicles from the ovary, thus enhancing fertilization given the presence of other necessary factors. An inhibition of LH action in vivo will likely impede conception and pregnancy. The extract did not elevate LH concentration and by extension LH action thus discouraging fertilization and pregnancy (Gallo, 1981; Hashimoto *et al.*, 1987).

Plant extracts have been reported to decrease serum estradiol concentrations in experimental animals (Semik-Orzech *et al.*, 2017). In such situations, the actions of LH are hampered leading to disruption in the female reproductive cycle and thus infertility (Yakubu *et al.*, 2008; Onyegeme-Okerenta and Essien, 2015) and secondary metabolites in these plants employ different mechanisms to exert their hormone modulatory actions (Wink and Van-Wyk, 2010; Michael, 2015).

CONCLUSION

These results reveal that the methanol leaf extract of *A. boonei* significantly increases the serum concentration of some female fertility hormones such as prolactin and progesterone. This suggests that the extract may have an inhibitory effect on female fertility and reproduction. It could thus be exploited in the development of a natural contraceptive free from the usual adverse complications associated with synthetic hormonal contraceptives.

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