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The effect of oral administration of aqueous extract of *Newbouldia laevis* leaves on fertility hormones of male albino rats.

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Abstract: *The continuous use of plants in folklore for reproductive health has prompted the need to search for plants with fertility enhancement or anti-fertility potentials. Hence, the effect of oral administration of aqueous extract of Newbouldia laevis leaves for twenty one (21) days on reproductive hormones of albino male rats was investigated. Twelve male albino rats were grouped into three (A, B and C) of four (4) each. Group C (the control) received orally distilled water on daily basis for 21days. Groups A and B were treated like the control except that they received 200 and 400 mg/kg body weight of the extract respectively. After administration of the extract for twenty one days, the results of serum concentrations of testosterone, follicle stimulating and luteinizing hormones showed no significant ($p<0.05$) differences in groups A and B when compared to the control. The result reveals that Newbouldia laevis extract could act as an adjunct that can inhibit or promote hormonal imbalances in males at certain dosages as exemplified in the experimental animal models.*
Keywords*: Newbouldia laevis, testosterone, prolactin, estradiol, follicle stimulating, luteinizing hormones*

I. Introduction

In Africa and indeed Nigeria, a wide variety of plants are of great medicinal and nutritional importance. It has been documented that from time immemorial, plants have been used medicinally (Ogbe *et al.,* 2009). Many plants/plants extracts have been used as fertility agents in folklore and traditional medicines without producing apparent toxic effects (Sharma *et al.,* 2007; Vasudeva and Sharma, 2007; Singh and Singh, 2009). Infertility is a major clinical problem affecting people medically and psychologically (Akomolafe, 2012). The management options available for the treatment of infertility in males include the use of drugs and a variety of surgical procedures (Purvis and Christiansen, 2008). Thus, having a balanced level of hormone is essential to proper fertility in the reproductive health of both men and women. On this accounts, many plant derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible benefits as well as adverse effects (Gamache and Acworth, 1998).

In Nigeria, the bark is chewed and swallowed for stomach pains, diarrhea and toothache (Iwu, 2000). The plant is useful for ear ache, sore feet, chest pain, epilepsy and convulsion in children (Akunyili, 2000). Sedative effects of the methanol leaf extract of *N. laevis* in mice and rats have also been studied and reported (Amos *et al.,* 2002). The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, pile, toothache and as a vermifuge to round worms (Okeke, 2003). The stem bark with clay and red pepper is used against pneumonia, fever, cold and cough (Idu *et al.*, 2003). An extract (decoction) made from the leaves and used as mouthwash was shown to arrest bacterial action in dental caries (Arbonnier, 2004). The antimalarial potency of the root extract has been documented (Gbeassor *et al.,* 2006). The antimicrobial potential of the methanol leaf extract has been reported (Kutete *et al.*, 2007; Usman and Osuji, 2007; Ejele *et al.*, 2012). The anti-inflammatory activities of the extract have been documented (Usman *et al.*, 2008). In South-Eastern and part of Mid-Western Nigeria, the plant is used for the treatment of septic wounds and eye problems (Akerele *et al.*, 2011). Also, the anti-diabetic activity of the leaf extract has been reported by Owolabi *et al.*, (2011) and Anaduaka *et al.,* (2013a). Literature have also reported the phytochemicals constituents present in *Newbouldia laevis* (Akaneme,2008; Ogbe *et al.,* 2009; Anaduaka *et al.,* 2013b).

Additionally, some of these plants are known to possess anti-fertility effect through their action on the hypothalomopituitory- gonodal axis or direct hormonal effects on reproductive organs. Therefore, this work is a preliminary study aimed at evaluating the effect of oral administration of aqueous extracts of *Newbouldia laevis* leaves on selected fertility hormones (testosterone, progesterone, prolactin, estrogen, follicle stimulating hormone FSH and leutinizing hormone LH) in albino rats and this will verify its use in folklore as therapeutic component for reproductive disorder.

II. Materials And Methods

Materials

Collection and identification of plant materials

The leaves of *Newbouldia laevis* was used for this study. The plant samples were collected from a single population within the premises of the main campus of Michael Okpara University of Agriculture, Umudike, Abia, Nigeria, and were authenticated by a botanist at the Department of Plant Science and Biotechnology, College of Natural Sciences; Michael Okpara University of Agriculture , Umudike. The fresh leaves of *Newbouldia laevis* were first washed with distilled water and subsequently, normal saline to remove dirt and possible mycotoxins. The samples were dried under shade for several days and then pulverized into fine powder using Blender/Miller III, (model MS - 223, China).

Extraction of plant materials

A quantity, Thirty (30) grams of the powder was extracted in 300ml of cold distilled water and left to stand for 48 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper and the resulting filtrate was concentrated on steam bath until a semisolid residue (brownish black slurry) which weighed 6.0g was obtained. The percentage yield was calculated and equivalent amount of the residue was separately reconstituted in 100 ml of distilled water to give the required doses of 200 and 400 mg kg-1 body weight respectively.

Animals: A total of 12 matured male rats weighing between 180-220g were housed in clean metabolic cages contained in well ventilated standard housing conditions (temperature: 28–31°C; photoperiod: 12 hours natural light and 12 hours dark; humidity: 50–55%). The animals were allowed free access to water and rat pellets obtained from Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria. They were acclimatized for two weeks before the commencement of the experiment. The rats were completely randomized into three groups of 4 each as follows:

Group A: treatment, received 200 mg/kg body weight of the extract.

Group B: treatment, received 400 mg/kg body weight of the extract.

Group C: Control, received 5 ml/kg body weight of distilled water.

The various groups were orally administered with 1 ml each of distilled water and the extract (200 and 400 mg/kg body weight) once daily for 21 days respectively using orogastric tube. All rats from each group were sacrificed after 21 days. This study was carried out following approval from the Departmental Ethical Committee on the Care and Use of Experimental Animals for Research.

At the end of the experimental period the rats were starved for 12 h and then sacrificed under ether anaesthetized. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged for 10 minutes at 3000 r.p.m to separate serum. Serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20^0C) until estimation of some biochemical parameters.

HORMONAL ASSAY

The levels of hormones were measured in serum by ELISA testosterone, FSH and LH standard kits (Biocheck, Inc. Foster City CA, USA). The procedure described in the hormone assay kits was used according to the principle highlighted by Tietz (1995) for testosterone while the method of Uotila *et al.,* (1981) was used for luteinizing and follicle stimulating hormones.

STATISTICAL ANALYSIS

The data obtained from this study were expressed as the mean of three replicates ± standard error of mean (Mean ± SEM) and were analyzed using a one-way analysis of variance (ANOVA) with multiple comparisons and values at $p<0.05$ (5% level) were considered statistically significant. In all the Figures, bars carrying letters different from the control are significantly different ($p<0.05$).

III. Results

EFFECT ON SERUM TESTOSTERONE

The 200mg/kg dose of the extract (group 1) showed a non significant ($p > 0.05$) decrease in the concentration level when compared to the control. Also the 400mg/kg (group 2) showed a non-significant decrease in the concentration level when compared to the control as shown in Table 1.

EFFECT ON FSH

The 200mg/kg dose showed a non significant ($p > 0.05$) increase in the concentration level when compared to the control. The 400mg/kg also showed a non-significant increase in the concentration level when compared to the normal control as shown in Table 1.

EFFECT ON LIEUTENIZING HORMONE (LH)

The 200mg/kg and 400mg/kg dose showed a non significant ($p > 0.05$) effect in the concentration level when compared to the control as shown in Table 1.

Table 1. Effects of Aqueous Extract on testosterone concentration in Rats

TREATMENT	TESTOSTERONE (ng/ml)	FSH (miu/ml)	LH (miu/ml)
200 mg/kg aqs *N. laevis*	25.90±10.68^{ns}	8.07±1.09^{ns}	9.28±0.52^{ns}
400 mg/kg aqs *N. laevis*	24.80 ± 4.82^{ns}	8.19±0.42^{ns}	9.20 ±0.36^{ns}
Control	27.37 ± 4.89^{a}	7.74±0.06^{a}	9.19 ±0.09^{a}

Values are mean **± SEM.**

Values are statistically significant compared to control group at: $P< 0.05$, ns = not significant

FSH Follicle stimulating hormones, LH Lieutenizing hormones

IV. Discussion

The use of plant extracts as fertility enhancer in animals is now on the increase because of the shifting attention from synthetic drugs to natural plant products (Dada and Ajilore, 2009). Hormones play a vital role in semen production and men's fertility (Browning *et al.*, 1998). Hormonal imbalances may be caused by numerous chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive as well as toxic agents of plant extracts that can affect the regulation of conception and reproduction (Edeoga *et al.*, 2005 and Yakubu *et al.*, 2005). Alkaloids and flavonoids have been shown to reduce plasma concentrations of some fertility hormones (Browning *et al.*, 1998 and Bianco *et al.*, 2006). Therefore, the presence of these phytochemicals may account for the alterations in the levels of the circulating hormones observed in this study.

Testosterone is a male hormone that has significant impact on spermatogenesis (Lee *et al.*, 2001). It is secreted by the leydig cells of the testicles, the adrenals and the ovaries, and is the most important androgen secreted into the blood. A low sperm count may indicate a problem with testosterone levels. In this study, a non-significant decrease in the level of serum testosterone was observed and thus indicates no harm to the host.

Follicle stimulating hormone regulates the growth of seminiferous tubules and maintenance of spermatogenesis in males. FSH is also critical for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation. Diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In the male, LH acts upon the Leydig cells of the testis and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intra-testicular activity on spermatogenesis (Glazener *et al.*, 1987; Colao *et al.*, 2004). The result shows that the aqueous extract of *Newbouldia laevis* boosted the FSH and LH levels in the experimental animals as represented in Table 1.

V. Conclusion

This study reveals that *Newbouldia laevis* leaves on modulates some of the fertility hormones assayed for. However, its consumption should be taken with caution because of it reduction in the level of testosterone although not significant. Further experimental studies are necessary to explain such interations.

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