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Contraceptive Effect of Ethanolic Extract of *Dioscorea villosa* Tuber on Reproductive Hormones of Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author DA designed the study, wrote the protocol and supervised the work. Authors EED and MDA carried out all laboratories work and performed the statistical analysis. Author JAT managed the analyses of the study. Author OOT wrote the first draft of the manuscript. Author LD managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The contraceptive effect of oral administration of ethanolic extract of *Dioscorea villosa* tuber for thirty days on reproductive hormones of female albino rats was investigated. Twenty four female albino rats weighing 150-220g were completely randomized into four groups (A-D) comprising six rats each. Animals in Group A (control) were administered 0.5ml of distilled water. Animals in groups B, C and D received 100, 200 and 400mg/kg body weight of ethanolic extract of *Dioscorea villosa* tuber respectively for 30 consecutive days. Preliminary phytochemical screening of ethanolic extract of

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Dioscorea villosa tuber revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides. The extract at all tested doses decreased serum luteinizing hormone (LH) levels, although this effect was statistically significant only at 100mg/kg body weight ($p<0.05$). The extract at all doses significantly ($p<0.05$) decreased the concentration of progesterone in the serum of the animals. A decrease in serum estradiol levels was observed only in animals that received 100 and 200mg/kg body weight of the extract while the 400mg/kg body weight of the extract did not significantly ($p>0.05$) affect the serum estradiol concentration when compared with the distilled water treated control animals. Serum levels of follicle stimulating hormone (FSH) increased following treatment in a dose-dependent manner, and this effect was statistically significant only at 400mg/kg body weight ($p<0.05$). These alterations in female rat reproductive hormones by the extract are hormonal imbalance and would have adverse effect on maturation and ovulation of follicles. Consequently, the extract may impair fertility and pregnancy in female rats. Therefore, the ethanolic extract of *Dioscorea villosa* tuber may be explored as a female contraceptive.

Keywords: Dioscorea villosa; contraceptive; fertility; progesterone; estradiol; luteinizing hormone; follicle stimulating hormone; pregnancy.

1. INTRODUCTION

Contraception refers to methods and devices used to prevent pregnancy [1]. Hormonal contraceptives inhibit ovulation and fertilization (conception) in females [2,3]. Female sexual function is characterized by desire, libido, female sexual arousal disorder (FSAD), pleasure, pain, sexual life, intercourse, orgasm, happiness and bother/emotion. An alteration in hormonal or enzymatic activity in any of these processes results in conception issues [4]. This is summed up as female sexual dysfunction (FSD) [5]. FSD is defined as a condition in which there are alterations in the processes involved in the female sexual response cycle or by pain associated with sexual intercourse [6]. FSD can be categorized into four main areas: (1) Sexual desire disorders, namely hypoactive sexual desire or sexual aversion disorder, (2) Sexual arousal disorders, (3) Orgasmic disorders, and

(4) Sexual pain disorders that include dyspaureunia and vaginismus [7]. Recent studies suggest that at least 30% [8] of women suffer with some form of difficulty.

A major subcategory of FSD is female sexual arousal disorder (FSAD). Female sexual arousal results in a series of vasocongestive and lubricative events resulting primarily from increased blood flow to clitoral, labial, and vaginal tissue [6,9]. This increased blood flow to the genital area is similar to the male sexual response of the erection. Peripheral innervation of the female genital tract occurs via central-

peripheral neural connections that are anatomically similar to those found in the male

[10]. At the level of the female genitalia it appears that the sympathetic, parasympathetic, and nonadrenergic noncholinergic (NANC) systems are responsible for the primary neural regulation of blood flow. Although implicated, the precise contribution (facilitatory/inhibitory) of the

cholinergic (parasympathetic), adrenergic (sympathetic), and NANC (nitric oxide (NO), vasoactive intestinal peptide, calcitonin gene-related peptide) systems to the vascular responses has not been elucidated [9,11,12].

There have been numerous suggestions regarding what are the most important etiologies of FSAD including vascular/endothelial [12-14] neurological [6] and hormonal disorders [15]. Unfortunately, addressing these problems therapeutically has met with less success than had been hoped. However, medicinal plants have been reported to mediate in this regard [16]. One of such medicinal plants is *Dioscorea villosa*.

Dioscorea villosa (Family- *Dioscoreaceae*) is commonly called ñcolic rootò or ñwild yamò in English. It is otherwise known locally in Nigeria as ñtsadarñ or ñdoya-dajiñ in Hausa, northern Nigeria; ñijikarañ or ñji-ofiañ in Igbo, southeastern Nigeria; ñisu-iyeyeñ in Yoruba, southwestern Nigeria. It is an herbaceous twining perennial plant. It is a species of tuberous vine twining over hedges, bushes and fences. The thin, woolly, reddish-brown stem grows up to 6m long

[17]. The slender, tuberous rootstock is crooked and laterally branched. Broadly ovate and cordate, the leaves are from 5-15cm long and 3-12cm wide, glabrous on top and finely hairy underneath. They are usually alternate, but the lower leaves sometimes grow in twos and fours. The small, greenish-yellow flowers bloom during June and July, the male flowers bloom in

drooping panicles, the female flowers bloom in drooping spicate racemes. The fruit is a three-winged capsule containing winged seeds. The rhizome of wild yam appears in slender contorted pieces and is oval, being flattened above and beneath as it creeps in a horizontal position beneath the surface of the ground. Its fame is based on its steriod content, like saponins which can be chemically converted to progesterone contraceptives and cortisone [18].

Previous studies by [18] have reported an assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (*Dioscorea spp.*) tubers of Nepal, [19] studied *in vitro* Ŭ-amylase and Ŭ-glucosidase inhibitory activities of the ethanolic extract of *Dioscorea villosa* tubers while [20] gave the final report on the amended safety assessment of *Dioscorea villosa* (wild yam) root extract. [21] discovered that *Dioscorea villosa* (wild yam) induces chronic kidney injury via pro-fibrotic pathways, [22] worked on the characterization of steroidal saponins from *Dioscorea villosa* while [23] isolated steroidal saponins and flavan-3-ol glycosides from *Dioscorea villosa*. Furthermore,

[24] studied the effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women while [25] worked on bioassay-guided evaluation of *Dioscorea villosa* ï an acute and subchronic toxicity, antinoceptive and anti-inflammatory approach. Despite all these studies and purported use of the tuber, there has not been any information in the open scientific literature that has specifically addressed its contraceptive activity in animal model. Again, with all the claims by traditional medical practitioners of the tuber as a female contraceptive, there has not been any scientific evidence to either substantiate or refute the purported claim. Therefore, the present study was designed to investigate the contraceptive effect of the ethanolic extract of *Dioscorea villosa* tuber on reproductive hormones of female albino rats.

2. MATERIALS AND

METHODS 2.1 MATERIALS

2.1.1 Plant materials and authentication

Tubers of *Dioscorea villosa* were collected in March 2014 at 8:00 am from Mallam Ibrahim farm in Zuru Local Government Area, Kebbi State, Nigeria and were authenticated by Dr. Djaja D. Soejarto of the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago where a voucher specimen number: BC630 was deposited at the Field Museum of Natural History Herbarium of the department.

2.1.2 Experimental animals

Female albino rats (*Rattus norvegicus*) weighing 150-220g were obtained from the Animal Facility Centre, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria.

2.1.3 Assay kits

ELISA kits for progesterone, estradiol, follicle stimulating and luteinizing hormones were products of Schiffgraben Diagnostic, Hannover, Germany. All other reagents used were of analytical grade, prepared with distilled water and stored in neat and airtight reagent bottles.

2.2 METHODS

2.2.1 Phytochemical screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones and cardiac glycosides were carried out by adopting the procedures described by [26-32] respectively.

2.2.2 Preparation of ethanolic extract of *Dioscorea villosa* tuber

Tubers of *Dioscorea villosa* were peeled, cut into slices, and air dried under shade until a constant weight was obtained. This was thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil). A known weight (200g) of the powder was extracted in 1000ml of ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator. The mixture was further transferred into steam bath where it was evaporated to give a brownish-black residue. This was then reconstituted in distilled water to give the required doses used in the present study.

2.2.3 Animal grouping and extract administration

A total of twenty four (24) female albino rats weighing 150-220g, housed in clean aluminum

cages contained in well ventilated standard housing conditions (temperature: 28-31°C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. The female albino rats were completely randomized into four groups (A-D) comprising six rats each. Animals in Group A (control) were administered 0.5 ml of distilled water. Animals in groups B, C and D received 100, 200 and 400mg/kg body weight of ethanolic extract of *Dioscorea villosa* tuber respectively for 30 consecutive days with the aid of metal oropharyngeal cannula. The study was conducted following approval from Departmental Ethical Committee on the care and use of experimental animals as well as guidelines of [33].

2.2.4 Preparation of serum

The rats were anaethesized in a glass jar containing cotton wool soaked in diethyl ether. The unconscious rats were quickly removed and the neck area cleared of fur. The jugular vein which was slightly displaced (to avoid contamination of the blood with interstitial fluid) was cut with a sterile scapel blade and an aliquot of the blood was collected into a sample bottle. The blood was then left undisturbed for 10 minutes at room temperature to clot. The blood was thereafter centrifuged at 224xg for 10 minutes using Uniscope Laboratory Centrifuge (Model 800D, New Life Medical Instrument, England). The sera were later aspirated with Pasteur pipette into dry, sample bottles and used within 12 hours of preparation for the hormonal assays.

2.2.5 Determination of serum hormone concentration

The serum hormone concentrations were quantitatively determined following the procedures outlined in the manufacturer manual contained in the ELISA kits. The serum hormone concentrations were then interpolated from their respective calibration curves.

2.2.6 Statistical analysis

Results were expressed as the mean ± SEM of six determinations. Means were analyzed using Duncanôs Multiple Range Test and complemented with Studentôs t-test. The differences were considered statistically significant at $p<0.05$. All these analyses were done using SPSS 16.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

3. RESULTS

Phytochemical screening of the extract revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides while tannins, steroids and anthraquinones were not detected (Table 1).

Table 1. Phytochemical constituents of ethanolic extract of *Dioscorea villosa* tuber

Constituents	Inferences
Alkaloids	+
Saponins	+
Anthraquinones	-
Flavonoids	+
Steroids	-
Tannins	-
Cardiac glycosides	-

Key: (+) = Detected, (-) = Not detected

The effects of administration of ethanolic extract of *Dioscorea villosa* tuber at 100, 200 and 400mg/kg body weight for 30 days on the concentration of serum reproductive hormones in the female rats are depicted in Figs. 1-4.

Compared with the distilled water treated control animals, the extract at 100 mg/kg body weight significantly ($p<0.05$) decreased the concentration of luteinizing hormone (LH) in the serum of the animals (Fig. 1). The extract at 200 and 400mg/kg body weight did not significantly ($p>0.05$) alter the concentration of LH in the serum of the animals when compared with the control (Fig. 1).

Administration of the extract at all doses significantly ($p<0.05$) decreased the concentration of progesterone in the serum of the animals (Fig. 2).

There was significant ($p<0.05$) decrease in serum estradiol concentrations only in animals that received 100 and 200mg/kg body weight of the extract while the 400mg/kg body weight of the extract did not significantly ($p>0.05$) alter the serum estradiol concentration when compared with the control (Fig. 3).

Compared with the control, the extract at 400 mg/kg body weight significantly ($p<0.05$)

increased the follicle stimulating hormone (FSH) concentration in the serum of the animals (Fig. 4). Administration of the extract at 100 and 200 mg/kg body weight did not significantly ($p>0.05$) alter the concentration of FSH in the serum of the animals when compared with the control (Fig. 4).

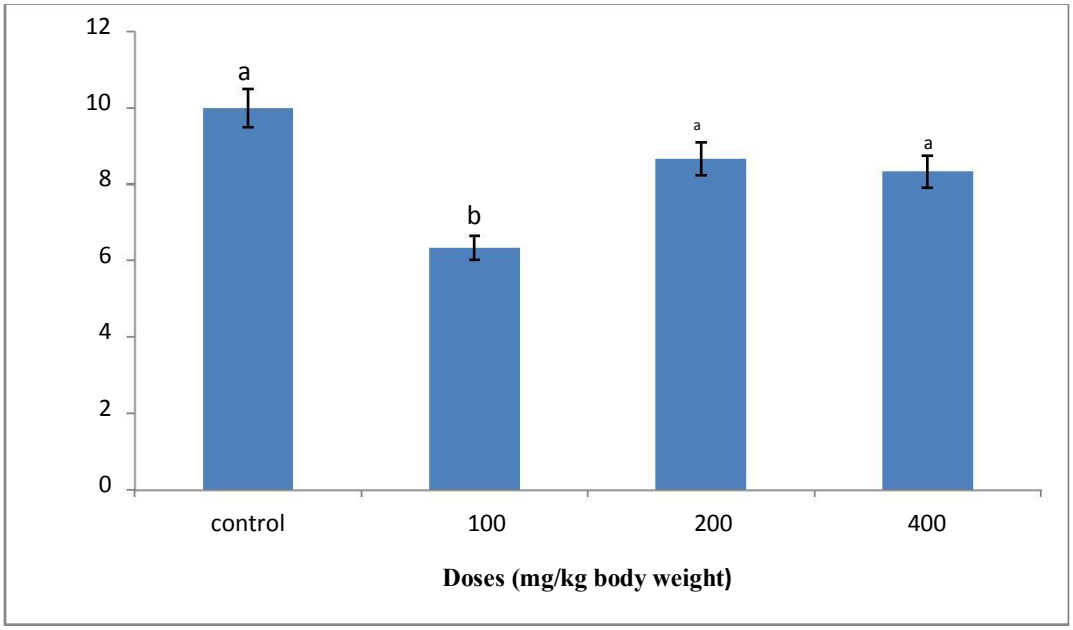

Fig. 1. Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum luteinizing hormone concentration

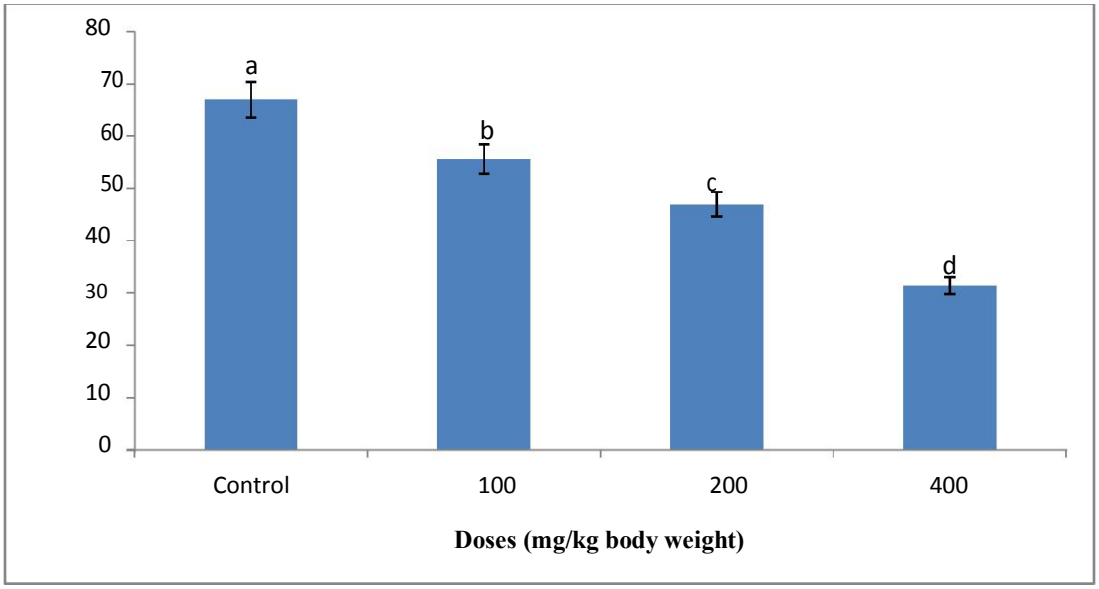

Fig. 2. Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum progesterone concentration

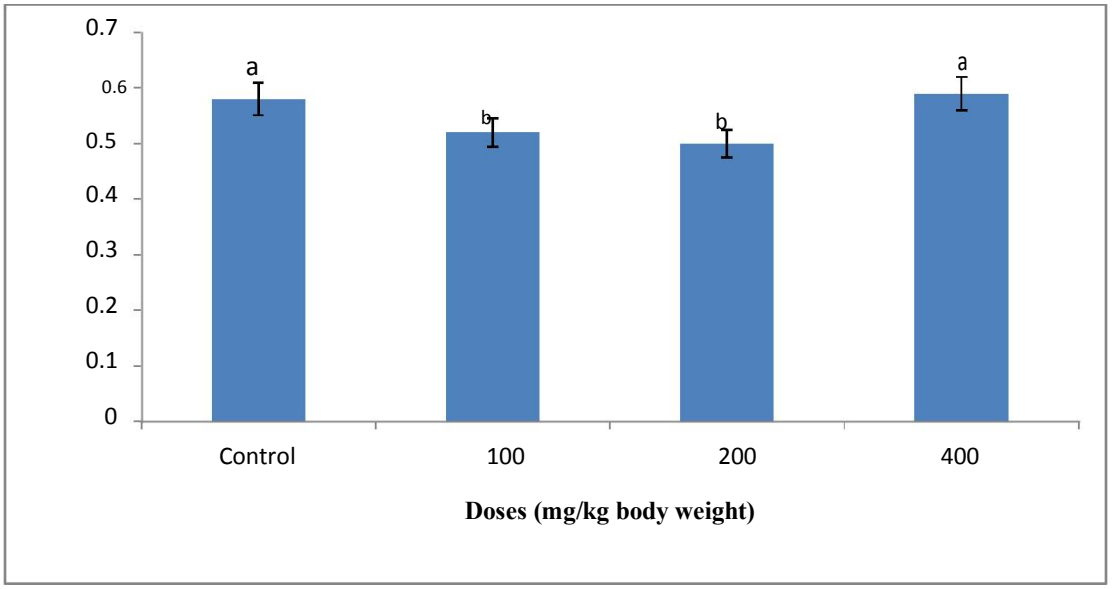

Fig. 3. Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum estradiol concentration

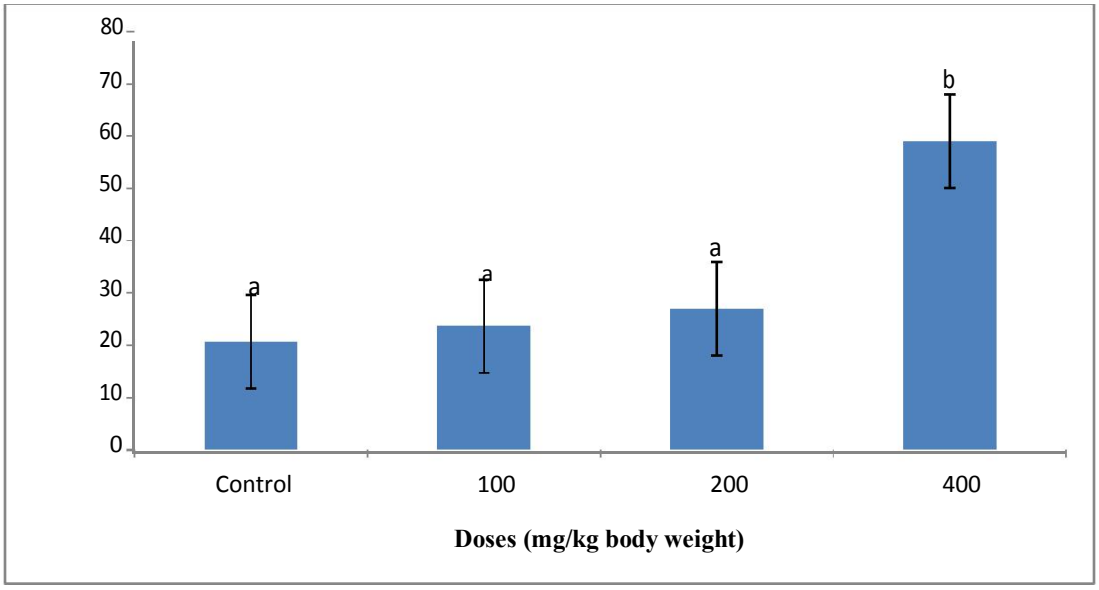

Fig. 4. Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum follicle stimulating hormone concentration

4. DISCUSSION

The female hormonal system consists of three hierarchies of hormones which control the menstrual cycle, ovulation, implantation and conception. These hormones are; 1) Gonadotropin-releasing hormone (GnRH) released from the hypothalamus. 2) anterior pituitary sex hormones, Follicle stimulating hormones (FSH) and luteinizing hormone (LH), both of which are secreted in response to the release of GnRH. 3) ovarian hormones, estrogen and progesterone, which are secreted in response to the two female anterior pituitary sex hormones [34,35]. Ovarian hormones prepare the uterus for pregnancy and the withdrawal of these hormones towards the end of the cycle leads to menstrual bleeding [36].

Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides. The contraceptive activity observed in female rats treated with ethanolic extract of *D. villosa* tuber may be attributed to the presence of any of these compounds; alkaloids, saponins, flavonoids or cardiac glycosides, which may exert its effect either singly or in synergy with other phytoconstituents [37-41]. Saponins contain sapogenin known as diosgenin.

Diosgenin helps women to regulate menstrual cycles, relieve cramping as well as make pregnancy and child birth (labour) less painful [42].

Luteinizing hormone (LH) is secreted from cells in the anterior pituitary called gonadotrophs. LH is responsible for final follicular growth and ovulation. It activates secretion of progesterone by converting granulosa and theca cells to progesterone-secreting cells [43]. In females, ovulation of mature follicles in the ovary is induced by a large surge of LH secretion during the pre-ovulatory periods. The non-significant effect of the extract at 200 and 400 mg/kg body weight indicated that, at these doses, the extract will not affect serum LH concentration. The reduction in the serum LH levels at 100mg/kg body weight may be explained by an inhibitory effect of the extract on the release of LH which may trigger disruption of ovulation. This may result in impairment of oestrous cycle; hamper conception and normal reproduction in the females [44]. The ethanolic extract of *Dioscorea villosa* tuber decreased serum levels of LH most

likely by suppressing the release of gonadotropic-releasing hormones (GnRH) by the hypothalamus. This shows that the extract has the ability to inhibit oestrous/menstrual cycle
(ovulation) and this will prevent conception/pregnancy.

Progesterone which is produced in the ovaries, placenta, and adrenal glands, helps to regulate the monthly menstrual cycle, prepare the body for conception and pregnancy [45,46] as well as stimulate sexual desire. The significant reduction in serum progesterone concentration by the *D. villosa* ethanolic extract at all doses may have consequential effect on conception in females; impede ovulation which may result in anovulation and sequelae [47].

Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the cycle. It is well established

that estradiol is directly responsible for the growth and development of reproductive organs. In synergy with FSH, estradiol stimulates granulosa cell proliferation during follicular development [48,49]. The reduction in the serum estradiol concentration at 100 and 200mg/kg body weight may be attributed to a decreased aromatase activity or substrate supplementation during estrogen synthesis [50]. Consequently, such decrease in serum estradiol concentration may hamper ovulation and preparation of the reproductive tract for zygote implantation [51]. Again, such decrease in serum estradiol concentration at 100 and 200 mg/kg body weight might point to the fact that *Dioscorea villosa* extract will only have an effect on serum estradiol concentration when administered at a high dose (400mg/kg body weight). Administration of the extract at 400mg/kg body weight, which compared well with the control, indicates that this dose will not affect the serum estradiol concentration of the animals.

Follicle stimulating hormone (FSH) is the central hormone of mammalian reproduction, essential for gonadal development and maturation at puberty as well as gamete production during the fertile phase of life [52]. FSH is known to induce the proliferation and maturation of ovarian follicle cells which produce estrogens and particularly estradiol as they are growing. FSH stimulates the early growth of primary follicle up to the antral stage. During this antral stage, the antral fluid produced and secreted by the granulosa cells

have high estrogen concentration [53]. Administration of the extract at 400mg/kg body weight which significantly increased the serum FSH in the rats might have caused the slight increase in serum estradiol (estrogen) level which was observed at 400mg/kg body weight. The increase in serum FSH level at 400mg/kg body weight was only specific for the 400mg/kg body weight which caused corresponding increase in serum estradiol level. This agrees with the fact that FSH is known to stimulate ovarian follicle cell proliferation and therefore to stimulate estradiol synthesis. It could also be that the extract increased ovarian cell resistance towards gonadotropines (FSH) stimulation. This resistance may prevent ovulation. This may be the possible mechanism whereby the extract induces its contraceptive effect. With all these, the ethanolic extract of *Dioscorea villosa* tuber has properties that qualify it to be used as a ñpotentialò oral contraceptive because of its ability to either increase or decrease female

reproductive hormones. This hormonal

imbalance (alteration) might have consequential effect on ovulation which would hinder conception, therefore suggesting that the extract ñmayò act as a contraceptive.

5. CONCLUSION

Available evidence from the present study indicated that the ethanolic extract of *Dioscorea villosa* tuber caused alteration of hormone levels in female rats. This may inhibit maturation and ovulation of follicles, which will prevent fertility and pregnancy. Therefore, the extract may be employed as a potent female contraceptive.

6. RECOMMENDATION

It is therefore recommended that further studies should be conducted to isolate and characterize the contraceptive bioactive agent of the ethanolic extract of *Dioscorea villosa* tuber. The possible molecular mechanism of action of its bioactive agent as it relates to ovarian expression of FSH receptors should be elucidated. Its contraceptive effect could also be compared with that of a reference contraceptive drug that would serve as positive control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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