

Original Research Article

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

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 150-220 g 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 400 mg/kg body weight of ethanolic extract of *Dioscorea villosa* tuber respectively for 30 consecutive days. Preliminary phytochemical screening of ethanolic extract of *Dioscorea villosa* tuber revealed the presence of alkaloids, saponins, flavonoids and cardiac glycosides. 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. However, the extract at 200 and 400 mg/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. 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 200 mg/kg body weight of the extract while the 400 mg/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. 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. 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. These alterations in female rat reproductive hormones by the extract indicate the adverse effect on the 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

Comment [H1]: You better say: "The extract at all tested doses decreased serum luteinizing hormone (LH) levels, although this effect was statistically significant only at 100 mg/kg body weight ($p<0.05$)".

Comment [H2]: "Serum levels of FSH increased following treatment in a dose-dependent manner, and this effect was statistically significant only at 400 mg/kg body weight ($p<0.05$)".

up as female sexual dysfunction (FSD) [5]. FSD is classified 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 dyspareunia 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, 13, 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 “colic root” or “wild yam” in English. It is otherwise known locally in Nigeria as “tsadar” or “doya-daji” in Hausa, northern Nigeria; “ijikara” or “ji-ofia” in Igbo, southeastern Nigeria; “isu-iyeye” 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.

66 They are usually alternate, but the lower leaves sometimes grow in twos and fours. The small,
67 greenish-yellow flowers bloom during June and July, the male flowers bloom in drooping
68 panicles, the female flowers bloom in drooping spicate racemes. The fruit is a three-winged
69 capsule containing winged seeds. The rhizome of wild yam appears in slender contorted pieces
70 and is oval, being flattened above and beneath as it creeps in a horizontal position beneath the
71 surface of the ground. Its fame is based on its steroid content, like saponins which can be
72 chemically converted to progesterone contraceptives and cortisone [18].

73 Previous studies by [18] have reported an assessment of antinutritional factors and bioavailability
74 of calcium and zinc in wild yam (*Dioscorea spp.*) tubers of Nepal, [19] studied *in vitro* α -
75 amylase and α -glucosidase inhibitory activities of the ethanolic extract of *Dioscorea villosa*
76 tubers while [20] gave the final report on the amended safety assessment of *Dioscorea villosa*
77 (wild yam) root extract. [21] discovered that *Dioscorea villosa* (wild yam) induces chronic
78 kidney injury via pro-fibrotic pathways, [22] worked on the characterization of steroidal
79 saponins from *Dioscorea villosa* while [23] isolated steroidal saponins and flavan-3-ol
80 glycosides from *Dioscorea villosa*. Furthermore, [24] studied the effects of wild yam extract on
81 menopausal symptoms, lipids and sex hormones in healthy menopausal women while [25]
82 worked on bioassay-guided evaluation of *Dioscorea villosa* – an acute and subchronic toxicity,
83 antinoceptive and anti-inflammatory approach. Despite all these studies and the purported use of
84 the tuber, there has not been any information in the open scientific literature that has addressed
85 the contraceptive claim of the tuber. Therefore, the present study was designed to investigate the
86 contraceptive effect of the ethanolic extract of *Dioscorea villosa* tuber on reproductive hormones
87 of female albino rats.

88

89 2. MATERIALS AND METHODS

90 2.1 MATERIALS

91 2.1.1 Plant Materials and Authentication

92 Tubers of *Dioscorea villosa* were collected in March 2014 at 8:00 am from Mallam Ibrahim farm
93 in Zuru Local Government Area, Kebbi State, Nigeria and were authenticated by Dr. Djaja D.

94 Soejarto of the Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy,
95 University of Illinois at Chicago where a voucher specimen number: BC630 was deposited at the
96 Field Museum of Natural History Herbarium of the department.

97 2.1.2 Experimental Animals

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

101 2.1.3 Assay Kits

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

105 2.2 METHODS

106 2.2.1 Phytochemical Screening

107 Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins,
108 flavonoids, steroids, anthraquinones and cardiac glycosides were carried out by adopting the
109 procedures described by [26], [27], [28], [29], [30], [31] and [32] respectively.

110 2.2.2 Preparation of Ethanolic Extract of *Dioscorea villosa* Tuber

111 Tubers of *Dioscorea villosa* were peeled, cut into slices, and air dried under shade until a
112 constant weight was obtained. This was thereafter pulverized in a blender (PHILIPS, Model HR-
113 1724, Brazil). A known weight (200 g) of the powder was extracted in 1000 ml of ethanol for 72
114 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone,
115 UK) and the resulting filtrate concentrated in a Rotary Evaporator. The mixture was further
116 transferred into steam bath where it was evaporated to give a brownish-black residue. This was
117 then reconstituted in distilled water to give the required doses used in the present study.

118 2.2.3 Animal Grouping and Extract Administration

119 A total of twenty four (24) female albino rats weighing 150-220 g, housed in clean aluminum
120 cages contained in well ventilated standard housing conditions (temperature: 28-31°C;

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

131 2.2.4 Preparation of Serum

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

140

141 2.2.5 Determination of Serum Hormone Concentration

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

145 2.2.6 Statistical Analysis

146 Results were expressed as the mean $\pm$ SEM of six determinations. Means were analyzed using
147 Duncan's Multiple Range Test and complemented with Student's t-test. The differences were
148 considered statistically significant at $p < 0.05$. All these analyses were done using SPSS 16.0
149 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

150 **3. RESULTS**

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

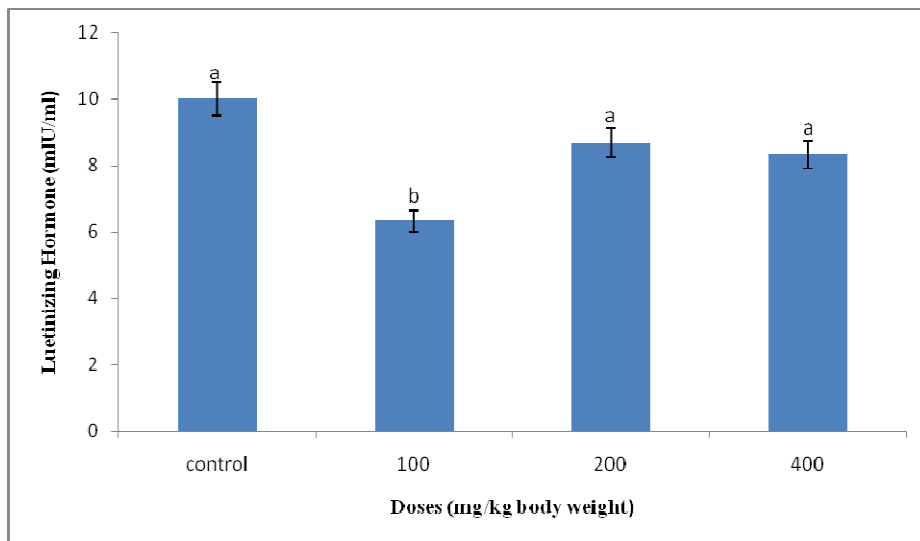
153 **Table 1: Phytochemical Constituents of Ethanolic Extract of *Dioscorea villosa* Tuber**

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

154 **Key:** (+) = Detected
155 (-) = Not detected
156

157
158 The effects of administration of ethanolic extract of *Dioscorea villosa* tuber at 100, 200 and 400
159 mg/kg body weight for 30 days on the concentration of serum reproductive hormones in the
160 female rats are depicted in Figures 1-4.

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

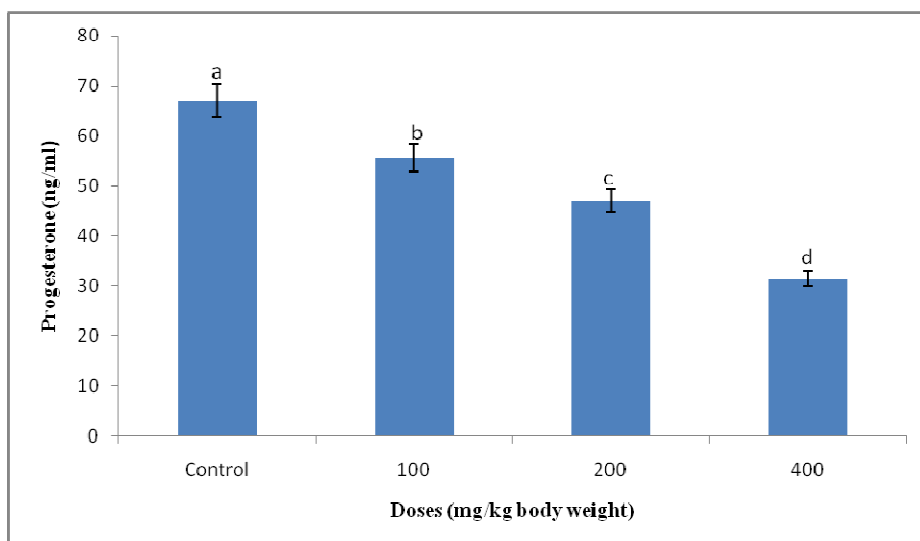


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167 **Fig. 1: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum luteinizing**
 168 **hormone concentration**

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

171



172

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

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

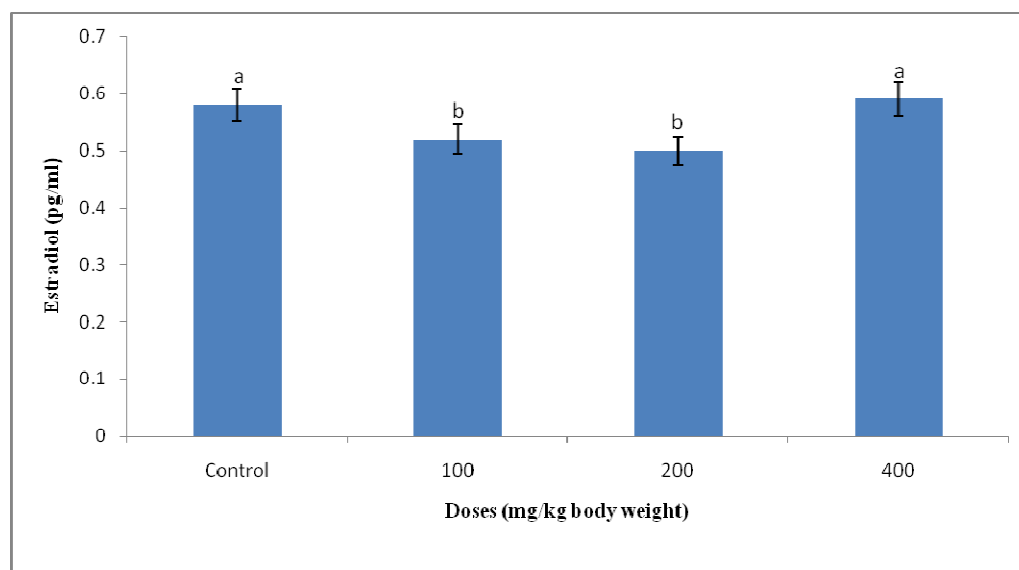


Fig. 3: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum estradiol concentration

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 (Figure 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 (Figure 4).

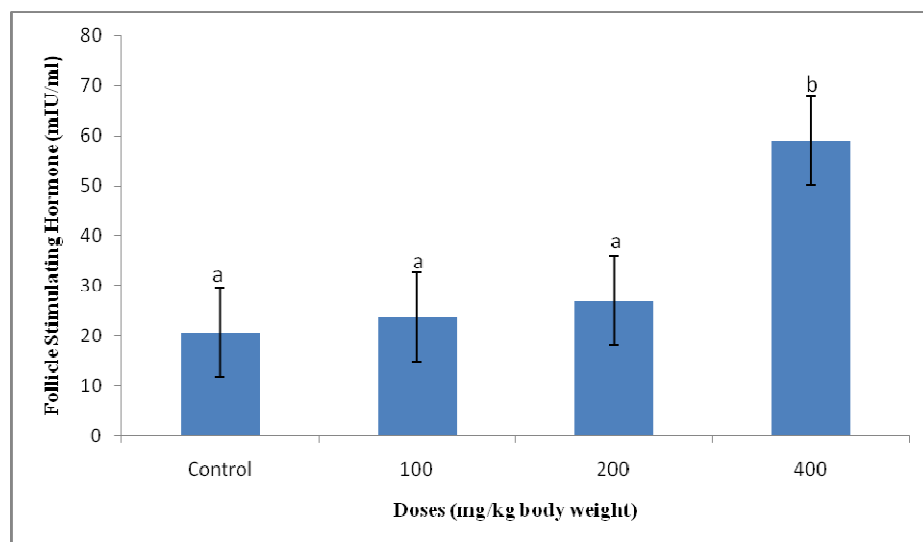


Fig. 4: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum follicle stimulating hormone concentration

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 [37,38,39,40,41]. Saponins contain sapogenin known as diosgenin. **Diosgenin**

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

210 Luteinizing hormone (LH) is secreted from cells in the anterior pituitary called gonadotrophs.
211 LH is responsible for final follicular growth and ovulation. It activates secretion of progesterone
212 by converting granulosa and theca cells to progesterone-secreting cells [43]. In females,
213 ovulation of mature follicles in the ovary is induced by a large surge of LH secretion during the
214 pre-ovulatory periods. The non-significant effect of the extract at 200 and 400 mg/kg body
215 weight indicated that, at these doses, the extract will not affect serum LH concentration. The
216 reduction in the serum LH levels at 100 mg/kg body weight may be explained by an inhibitory
217 effect of the extract on the release of LH which may trigger disruption of ovulation. This may
218 result in impairment of oestrous cycle; hamper conception and normal reproduction in the
219 females [44]. The ethanolic extract of *Dioscorea villosa* tuber decreased serum levels of LH
220 most likely by suppressing the release of gonadotropic-releasing hormones (GnRH) by the
221 hypothalamus. This shows that the extract has the ability to inhibit ovulation and this will
222 prevent conception/pregnancy.

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

228 Estradiol stimulates the growth of the uterine lining, causing it to thicken during the pre-
229 ovulatory phase of the cycle. It is well established that estradiol is directly responsible for the
230 growth and development of reproductive organs. In synergy with FSH, estradiol stimulates
231 granulosa cell proliferation during follicular development [48, 49]. The reduction in the serum
232 estradiol concentration at 100 and 200 mg/kg body weight may be attributed to a decreased
233 aromatase activity or substrate supplementation during estrogen synthesis [50]. Consequently,
234 such decrease in serum estradiol concentration may hamper ovulation and preparation of the
235 reproductive tract for zygote implantation [51]. Administration of the extract at 400 mg/kg body
236 weight, which compared well with the control, indicates that this dose will not affect the serum
237 estradiol concentration of the animals.

238 Follicle stimulating hormone (FSH) is the central hormone of mammalian reproduction, essential
239 for gonadal development and maturation at puberty as well as gamete production during the
240 fertile phase of life [52]. FSH is known to induce the proliferation and maturation of ovarian
241 follicle cells which produce estrogens and particularly estradiol as they are growing. FSH
242 stimulates the early growth of primary follicle up to the antral stage. During this antral stage, the
243 antral fluid produced and secreted by the granulosa cells have high estrogen concentration [53].
244 Administration of the extract at 400 mg/kg body weight which significantly increased the serum
245 FSH in the rats might have caused the slight increase in serum estradiol (estrogen) level which
246 was observed at 400 mg/kg body weight. The increase in serum FSH level at 400 mg/kg body
247 weight was only specific for the 400 mg/kg body weight which caused corresponding increase in
248 serum estradiol level. This agrees with the fact that FSH is known to stimulate ovarian follicle
249 cell proliferation and therefore to stimulate estradiol synthesis.

250 The decreased serum estradiol concentration at 100 and 200 mg/kg body weight might point to
251 the fact that *Dioscorea villosa* extract will only have an effect on serum estradiol concentration
252 when administered at a high dose (400 mg/kg body weight). ~~The extract increased the serum~~
253 ~~FSH levels at 400 mg/kg body weight by stimulating the hypothalamus to release high quantities~~
254 ~~of GnRH.~~ The ethanolic extract of *Dioscorea villosa* tuber has properties that qualify it to be
255 used as an potential oral contraceptive because it has the ability to increase or decrease female
256 reproductive hormones. This hormonal imbalance (alteration) might have consequential effect on
257 ovulation which would hinder conception, therefore suggesting that the extract “may” act as a
258 contraceptive.

259 5. CONCLUSION

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

264 RECOMMENDATION

265 It is therefore recommended that further studies should be conducted to isolate and characterize
266 the contraceptive bioactive agent of the ethanolic extract of *Dioscorea villosa* tuber. The possible

267 molecular mechanism of action of its bioactive agent should be elucidated. Its contraceptive
268 effect could also be compared with that of a reference contraceptive drug that would serve as
269 positive control.

270 **COMPETING INTERESTS**

271 **Authors have declared that no competing interests exist.**

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