

Original Research Article

Contraceptive Effect of Ethanol 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. The extract at all doses significantly ($p < 0.05$) decreased the levels of serum luteinizing hormone (LH). 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. The extract at all doses significantly ($p < 0.05$) increased the serum follicle stimulating hormone (FSH) concentration in the treatment groups. 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 are indications of adverse effect on the maturation and ovulation of follicles. Consequently, the extract may impair fertility and conception 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, Conception.

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 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 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* α -amylase and α -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* – an acute and subchronic toxicity, antinoceptive and anti-inflammatory approach. Despite all these studies and the purported use of the tuber, there has not been any information in the open scientific literature that has addressed the contraceptive claim of the tuber. 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 from Mallam Ibrahim farm in Zuru Local Government Area, Kebbi State, Nigeria and were authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Jos, Nigeria.

2.1.2 Experimental Animals

Female albino rats (*Rattus norvegicus*) weighing 150-220 g 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], [27], [28], [29], [30], [31] and [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 a shade until a constant weight was obtained. This was thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil). A known weight (200 g) of the powder was extracted in 1000 ml 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 the required 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 150-220 g, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31⁰C; 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 400 mg/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 anaesthetized 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 224x g 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 $\pm$ SEM of six determinations. Means were analyzed using Duncan's Multiple Range Test and complemented with Student's 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).

149

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

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

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 all doses significantly
162 ($p < 0.05$) decreased the concentration of luteinizing hormone (LH) in the serum of the animals
163 (Figure 1). The extract at 200 and 400 mg/kg body weight did not significantly ($p > 0.05$) alter the
164 concentration of LH in the serum of the animals (Figure 1).

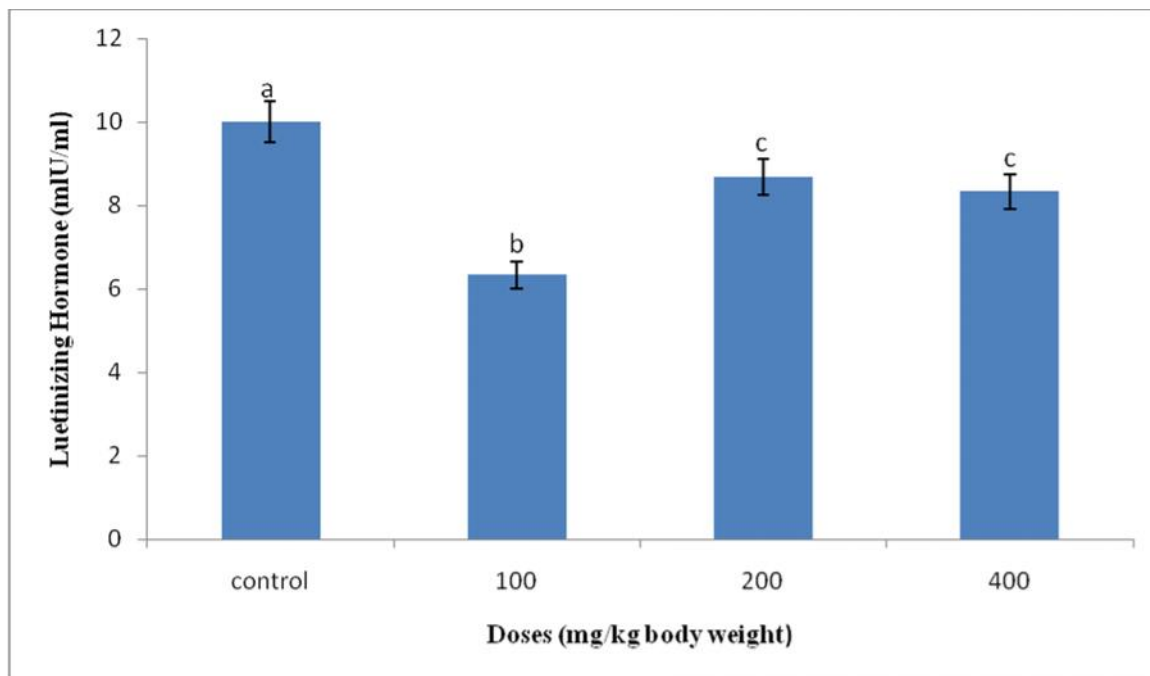


Fig. 1: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum luteinizing hormone concentration

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

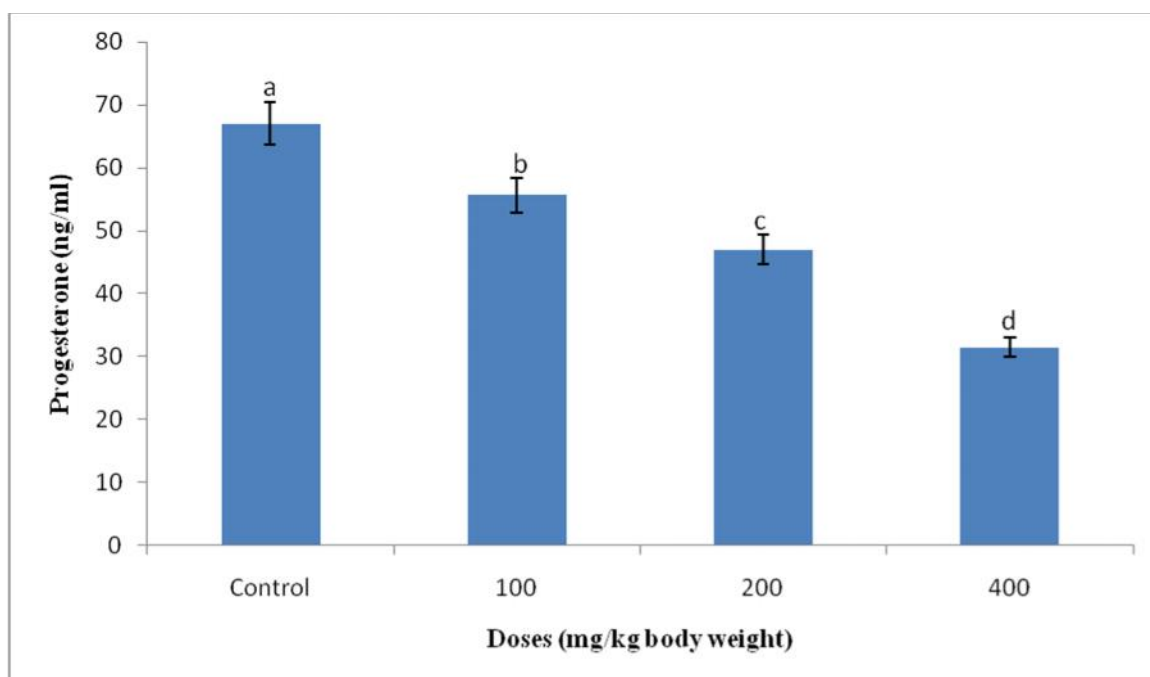


Fig. 2: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum progesterone concentration

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).

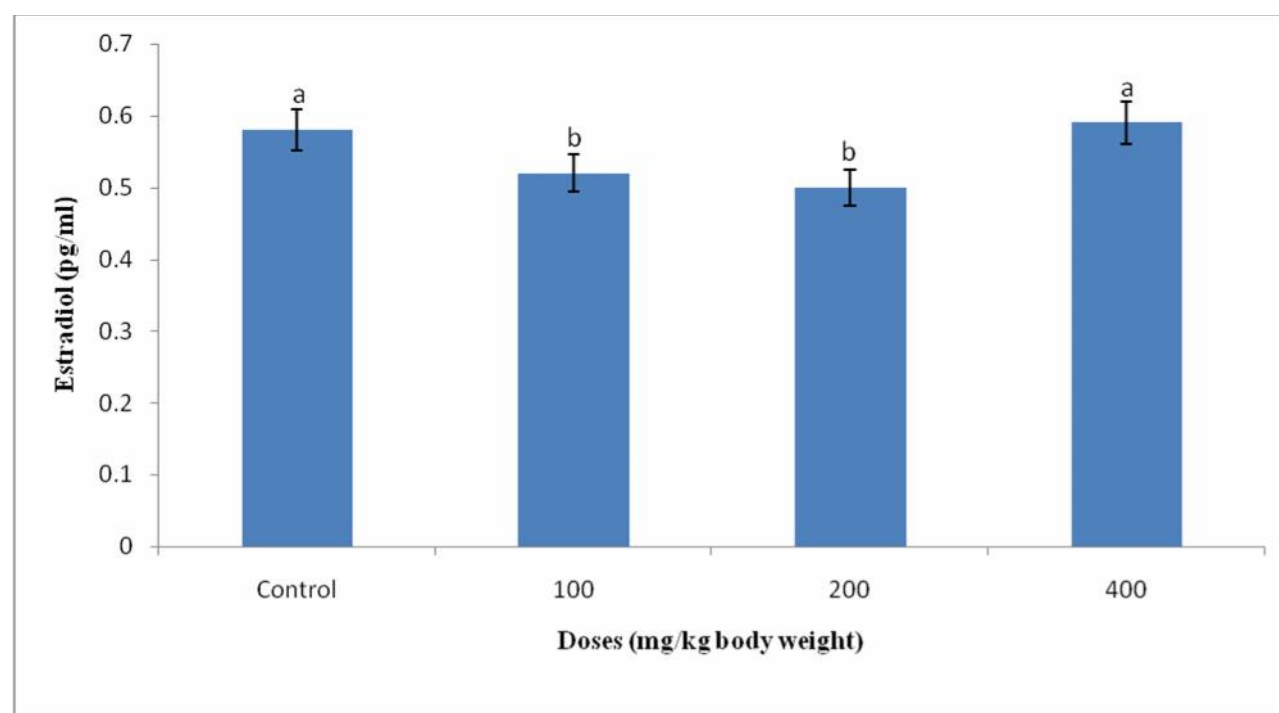


Fig. 3: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum estradiol concentration

The extract at all doses significantly ($p<0.05$) increased the serum follicle stimulating hormone (FSH) concentration in the treatment groups (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).

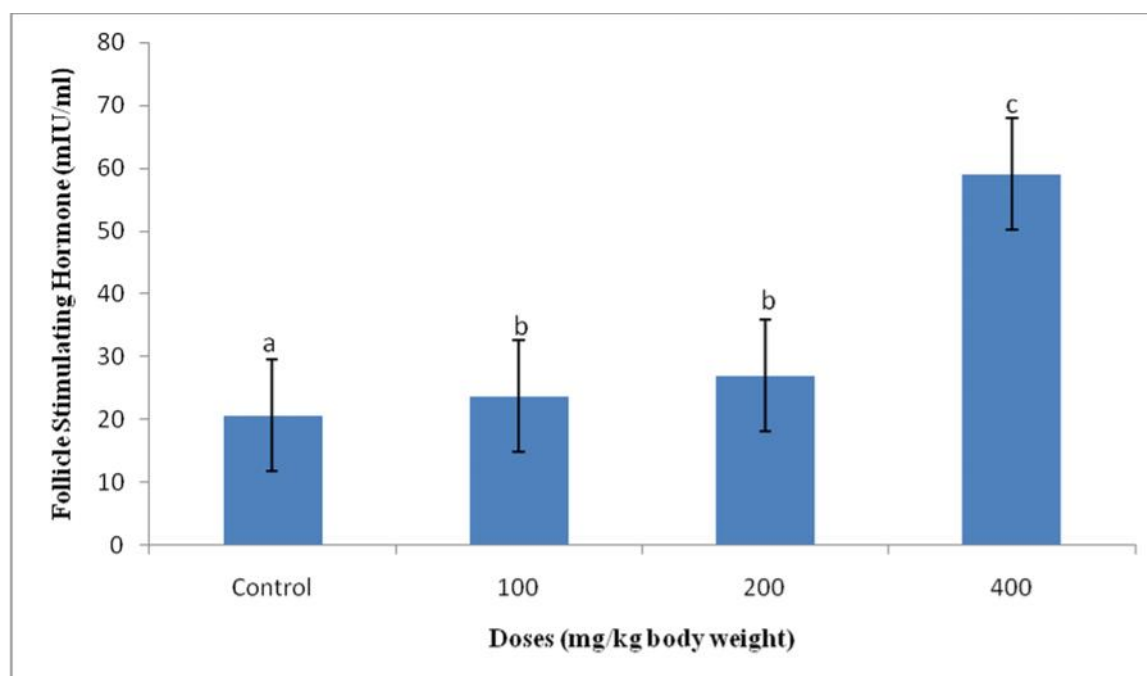


Fig. 4: Effect of ethanolic extract of *Dioscorea villosa* tuber on female rat serum follicle stimulating hormone concentration

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. This

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

208 Luteinizing hormone (LH) is produced in the anterior pituitary called gonadotrophs. LH is
209 responsible for final follicular growth and ovulation. LH activates secretion of progesterone by
210 converting granulosa and theca cells to progesterone-secreting cells [43]. In females, ovulation of
211 mature follicles in the ovary is induced by a large surge of LH secretion during the pre-ovulatory
212 periods. The reduction in the serum LH levels may be explained by an inhibitory effect of the
213 extract on the release of LH which may trigger disruption of ovulation. This may result in
214 impairment of oestrous cycle; hamper conception and normal reproduction in the females [44].
215 The ethanolic extract of *Dioscorea villosa* tuber decreased serum levels of LH most likely by
216 suppressing the release of gonadotropic-releasing hormones (GnRH) by the hypothalamus. This
217 shows that the extract has the ability to inhibit ovulation and this will prevent pregnancy.

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

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

233 Follicle stimulating hormone (FSH) is the central hormone of mammalian reproduction, essential
234 for gonadal development and maturation at puberty as well as gamete production during the

235 fertile phase of life [52]. FSH stimulates the ovaries to secrete estrogen. FSH stimulates the early
 236 growth of primary follicle up to the antral stage. During this antral stage, the antral fluid
 237 produced and secreted by the granulosa cells have high estrogen concentration [53].
 238 Administration of the ethanolic extract of *Dioscorea villosa* tuber at 400 mg/kg body weight
 239 which significantly increased the serum FSH in the rats compared to the control group might
 240 have caused the slight increase in serum estrogen level which was observed at 400 mg/kg body
 241 weight; ~~which compared favourably with the control~~. The decreased serum estradiol
 242 concentration at 100 and 200 mg/kg body weight might be a pointer to the fact that *Dioscorea*
 243 *villosa* extract will only have an effect on serum estradiol concentration when administered at a
 244 high dose (400 mg/kg body weight). The extract increased ~~the~~ serum FSH levels at 400 mg/kg
 245 body weight by stimulating the hypothalamus to release high quantities of GnRH. The ethanolic
 246 extract of *Dioscorea villosa* tuber has properties that qualify it to be used as an oral contraceptive
 247 because it has the ability to increase or decrease female reproductive hormones. This hormonal
 248 imbalance (alteration) might ~~have consequential effect on~~ ovulation ~~which would~~ hinder
 249 conception, ~~therefore suggesting that the extract could act as a contraceptive~~.

250 5. CONCLUSION

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

255 RECOMMENDATION

256 It is therefore recommended that further studies should be conducted to isolate and characterize
 257 the bioactive fractions of the ethanolic extract of *Dioscorea villosa* tuber. The possible molecular
 258 mechanism of action of its bioactive fractions should be elucidated. Its contraceptive effect
 259 should also be compared with that of a reference contraceptive drug that would serve as positive
 260 control.

261 REFERENCES

- 262 1. Hanson SJ, Burke H, and Anne E. "Fertility control: contraception, sterilization, and
 263 abortion". In Hurt, K. Joseph., Guile, Mathew W., Beinstock, Jessica L., Harold, E.,

- 264 Wallach, Edward, E. The Johns Hopkins manual of gynaecology and obstetrics (4th
265 edition). Philadelphia: Wolters Kluwer Health/Lippincott Williams Kluwer. 2010. Pp.
266 382-395.
- 267 2. Jones RK. Hormonal contraceptives offer benefits beyond pregnancy prevention-Beyond
268 birth control, the overlooked benefits of oral contraceptive pills. American Society of
269 obstetricians and gynaecologists. Hum. Reprod. 2009; 13 (12):3371-8.
- 270 3. Speroff L, Darney R, and Philip D. "Oral contraception". A clinical guide for
271 contraception (5th edition). Philadelphia: Lippincott Williams & Wilkins. 2011. Pp. 19-
272 152. ISBN 978-1-60831-610-6.
- 273 4. Susan SR, and Terri K. "Common Reproductive Issues". Contraception. Lippincott
274 Williams & Wilkins. 2009. Pp. 119.
- 275 5. Chin HB, Sipe TA, Elder R, Mercer SL, Chattopadhyay SK, Jacob V, Wethington HR,
276 Kirby D, Elliston DB. "The Effectiveness of Group-Based Comprehensive Risk-
277 Reduction and Abstinence Education Interventions to Prevent or Reduce the Risk of
278 Adolescent Pregnancy, Human Immunodeficiency Virus, and Sexually Transmitted
279 Infections". American Journal of Preventive Medicine. 2012; 42(3): 272-294.
- 280 6. Berman JR, Berman L, Goldstein I. Female sexual dysfunction: incidence,
281 pathophysiology, evaluation, and treatment options. Urology. 1999; 54: 385–391.
- 282 7. American Psychiatric Association (APA). Diagnostic and Statistical Manual of Mental
283 Disorders: DSM-IV, 1994. American Psychiatric Association, Washington, DC.
- 284 8. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence
285 and predictors. JAMA. 1999; 281: 537–544.
- 286 9. Levin RJ VIP. Vagina, clitoral and periurethral glansFan update on human female genital
287 arousal. Exp Clin Endocrinol. 1991; 98: 61–69.
- 288 10. Giuliano F, and Rampin O. Central neural regulation of penile erection. Neurosci
289 Biobehav Rev. 2000; 24: 517–533.
- 290 11. Ottesen B. et al. Vasoactive intestinal polypeptide (VIP) provokes vaginal lubrication in
291 normal women. Peptides. 1987; 8: 797–800.
- 292 12. Goldstein I, and Berman JR. Vasculogenic female sexual dysfunction: vaginal
293 engorgement and clitoral erectile insufficiency syndromes. Int J Impot Res. 1998;
294 10(Suppl 2): S84–S90.
- 295 13. Zemel P. Sexual dysfunction in the diabetic patient with hypertension. Am J Cardiol.
296 1988; 61: 27H–33H.
- 297 14. Park K et al. Vasculogenic female sexual dysfunction: the hemodynamic basis for vaginal
298 engorgement insufficiency and clitoral erectile insufficiency. Int J Impot Res. 1997; 9:
299 27–37.

- 300 15. Avis NE. et al. Is there an association between menopause status and sexual functioning?
301 Menopause. 2000; 7: 297–309.
- 302 16. Elvis-Lewis M. Should we be concerned about herbal remedies? J Ethnopharmacol.
303 2001. 75: 141-164.
- 304 17. Govaerts R, Wilkin P. and Saunders RMK. World Checklists of Dioscoreales. Yams and
305 their allies: 1-65. The Board of Trustees of the Royal Botanic Gardens, Kew, 2007.
- 306 18. Bhandari MR, and Kawabata J. Assessment of antinutritional factors and bioavailability
307 of calcium and zinc in wild yam (*Dioscorea* spp.) tubers of Nepal. Food Chem. 2004, 85:
308 281-287.
- 309 19. Roy A, and Geetha RV. *In vitro* α -amylase and α -glucosidase inhibitory activities of the
310 ethanolic extract of *Dioscorea villosa* tubers. Int J Pharm Bio Sci. 2013 Oct; 4(4): (P) 49-
311 54.
- 312 20. Hooker E. Final report on the amended safety assessment of *Dioscorea villosa* (wild
313 yam) root extract. Int J Toxicol. 2004, 23: 49-54.
- 314 21. Wojcikowski K, Wholmuth H, Johnson DW, Gobe G. *Dioscorea villosa* (wild yam)
315 induces chronic kidney injury via pro-fibrotic pathways. Food Chem Toxicol. 2008, 46:
316 3122-3131.
- 317 22. Bharanthi A, Yan-Hong W, Mei W, Zulfiqar A, Troy JS, Jerry Z, Ikhas AK.
318 Characterization of steroidal saponins from *Dioscorea villosa* and *D. cayenensis* using
319 Ultrahigh Performance Liquid Chromatography/Electrospray Ionization Quadrupole
320 Time-of-Flight Mass Spectrometry. Planta Med. 2014; 80(04): 321-329.
- 321 23. Sautour M, Miyamoto T, Lacaille-Doubois M. Steroidal saponins and flavan-3-ol
322 glycosides from *Dioscorea villosa*. Biochem System Ecol. 2006, 74: 60-63.
- 323 24. Komesaroff P, Black CV, Cable V, Sudhir K. Effects of wild yam extract on menopausal
324 symptoms, lipids and sex hormones in healthy menopausal women. Climacteric. 2001,
325 4(Suppl 2): 144-150.
- 326
- 327 25. Claudio ML, Adriani KL, Marcelia GD, Mairim RS, Denisson LO, Enrik BDA, Lucindo
328 JQ, Adriano ASA. Bioassay-guided evaluation of *Dioscorea villosa* – an acute and
329 subchronic toxicity, antinoceptive and anti-inflammatory approach. BMC
330 Complementary and Alternative Medicine. 2013, 13:195. doi: 10.1186/472-6882-13-195.
- 331 26. Harborne JD. Phytochemical methods: A guide to modern techniques of plant analysis.
332 Chapman & Hall, London. 1973. p. 279.
- 333 27. Walls ME, Wani MC, Brown DM, and Fullas F. Effect of tannins on screening of plant
334 extracts for enzyme inhibitory activity and techniques for their removal. Phytomedicine.
335 1996; 3,281–285.

336

- 337 28. Odebiyi A, and Sofowora AE. Phytochemical screening of Nigerian medicinal plant. *Part*
338 *III, Lloydia*, 1978; 41, 234-246.
- 339 29. Awe IS, and Sodipo OA. Purification of saponins of root of *Bhlighia sapida* KOENIG-
340 HOLL. Nig J Biochem. and Mol. Biol. (Proceedings Supplement). 2001; 16: 201s-204s.
- 341
- 342 30. El-Olemy MM, Al-Muhtadi FJ, Afifi AA. Experimental Phytochemistry. A Laboratory
343 Manual. Riyadh, Saudi Arabia: King Saud University Press, 1994.
- 344 31. Mainasara MM, Aliero BL, Aliero AA, Yakubu M. Phytochemical and antibacterial
345 properties of root and leaf extract of *Calotropis procera*. Nig. J. Basic Appl. Sci. 2012;
346 20(1):1-6.
- 347 32. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, 2nd Edition, Spectrum
348 Books, Ibadan, Nigeria; 1993; 26-100.
- 349
- 350 33. The National Academies. Guide for the care and use of laboratory animals. Washington
351 DC: National Academy Press; 1996. p. 1-140.
- 352 34. Guyton AC, and Hall JE. Textbook of Medical Physiology (Tenth Edition). Harcourt
353 International Edition, W.B. Saunder Company, Philadelphia. 2000. Pp. 279-281.
- 354 35. Sharma P, Malhotra C, Taneja DK, Saha R. "Problems related to menstruation amongst
355 adolescent girls". Indian Journal of Pediatrics. 2008; 75 (2): 125–9.
- 356 36. Klump KL, Keel PK, Racine SE. "The interactive effects of estrogen and progesterone
357 on changes in emotional eating across the menstrual cycle". Journal of Abnormal
358 Psychology. 2013; 122 (1): 131–137.
- 359 37. Farnsworth NR, Bingel AS, Cordell GA, Craw FA, Fong HHS. Potential value of plants
360 as source of new antifertility agents. J Pharm Sci. 1971; 64: 717-54.
- 361 38. Prankash AO, Saxena V, Shukla S, Mathur R. Contraceptive potency of *Pueraria*
362 *tuberosa* D.C. and its hormonal status. Act Euro Fertil. 1985; 16(1):59-65.
- 363 39. Makonnen E, Zerihun L Assefa G, Rostom AA. Antifertility activity of *Ricinus*
364 *communis* seed in female guinea pigs. East Afr Med J. 1999; 76(6):335-7.
- 365 40. Gebrie E, Makonnen E, Zerihun L, Debella A. The possible mechanisms for the
366 antifertility actions of the methanolic root extract of *Rumex steudelli*. Afr Health Sci.
367 2005; 5: 119-25.
- 368 41. Kumar D, Kumar A, Prakash O. Potential antifertility agents from plants: a
369 comprehensive review. J Ethnopharmacol. 2012; 6: 40(1):1-32.
- 370 42. Boon H, and Smith M. "The complete natural medicine guide to the 50 most common
371 medicinal herbs". Robert Rose Inc., Toronto, Ontario, Canada. 2004.

- 372 43. Alleyne BJ and Richard PD. "Girls now reaching puberty before 10 - a year sooner than
373 20 years ago". The Daily Telegraph (London). 2010.
- 374 44. Al-Qarawi AA, Abdel-Rahman HA, El-Badry AA, Harraz F, Razig NA, Abdel-Magied
375 EM. The effect of *Cynomorium coccineum* and *Withania somnifera* on gonadotrophins
376 and ovarian follicles of immature Wistar rats. Phytother Res. 2000; 14: 288-290.
- 377 45. Montaserti A, Pourheydar M, Khazaei M, Ghorbani R. Anti-fertility effects of *Physalis*
378 *alkekengi* alcoholic extract in female rat. Iranian J Reprod Med. 2007; 5: 13-16.
- 379 46. Takasaki A, Tamura H, Miwa I, Taketani T, Shimamura K, Sugino N. "Endometrial
380 growth and uterine blood flow: a pilot study for improving endometrial thickness in the
381 patients with a thin endometrium". The Journal of Fertility and Sterility. 2010;
382 93 (6):1851-1858.
- 383 47. Stricker R, Eberhart R, Chevailler MC, Quinn FA, Bischof P. "Establishment of detailed
384 reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and
385 progesterone during different phases of the menstrual cycle". Journal of Clinical and
386 Chemical Laboratory Medicine. 2006; 44 (7): 883–337.
- 387 48. Telefo PB, Moundipa PF, Tchana AN, Tchouanguép-Dzickotze C, Mbiapo FT. Effects of
388 an aqueous extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera*
389 *verticillata* on some physiological and biochemical parameters of reproduction in
390 immature female rats. J Ethnopharmacol. 1998; 63: 193-200.
- 391 49. Levin Ellis R, and Hammes Stephen R. "Estrogens and Progestins". 12th ed. New York:
392 McGraw-Hill. 2011. Pp. 1163–1194.
- 393 50. Hsia SM, Yeh CL, Kuo YH, Wang PS, Chiang W. Effects of Adlay (*Coix lachryma-jobi*
394 L. var. ma-yuen Stapf.) Hull extracts on the secretion of progesterone and estradiol in
395 vivo and in vitro. Exptal Biol & Med. 2007; 232:1181-1194.
- 396 51. Glasier Anna. "Contraception". Endocrinology. 6th edition. Philadelphia: Saunders
397 Elsevier. 2010. Pp. 2417–2427.
- 398 52. Simoni M, and Nieschlag E. FSH in therapy: physiological basis, new preparations and
399 clinical use. Reprod Med Rev. 1995; 4:163–177.
- 400 53. Laura B. "Oral Contraceptives: An Update on its Health Benefits and Risks". Journal of
401 Pharmacology Association. 2001; 41(6):56-59.