Original Research Article

Alterations in Female Reproductive Hormones of Wistar Rats sequel to the Administration of *Xylopia aethiopica* Fruit

ABSTRACT

Aim: This present study was designed to examine the impact of *Xylopia aethiopica* fruit on the reproductive hormones of female Wistar rats.

Methodology: The fruits of *Xylopia aethiopica* were air-dried and extracted by Soxhlet extractor using ethanol as solvent. The lethal dose of the plant extract was assessed by standard method. Thirty adult female Wistar rats were grouped into 5 of 6 rats each. Animals in groups A, B, C, and D were exposed to 130, 259, 389 and 518 mg/kg b. wt. of *X. aethiopica* fruit extract respectively, while those in group E received normal animal feeds and water only. The administration was done once daily for 28 days via oral route. Reproductive hormones were assay using ELISA techniques.

Results: A non-significant increase was observed in the blood concentration of follicle stimulating hormone (FSH) when animals exposed to 130 mg/kg b. wt. of *Xylopia aethiopica* fruit extract were compared with those in the control group. Increase in the dose of *Xylopia aethiopica* fruit extract resulted in a decrease in serum FSH levels. Administration of the extract for 28 days led to a dose-dependent decline in the blood level of luteinizing hormone (LH). The result of this study indicates the extract decreased serum level of progesterone in female rats at low doses of 130 mg/kg and 259 mg/kg when compared with those in the control group. However, increasing the dosage of the extract increases the serum progesterone concentrations. Administration of *Xylopia aethiopica* extract to animals for 28 days led to a dose-dependent decline in the serum concentration of estrogen. This decrease was significant ($P<0.05$) when the estrogen levels of animals exposed to 389 mg/kg and 518 mg/kg of *Xylopia aethiopica* extract were respectively compared with those in the control group. Conversely, *Xylopia aethiopica* extract increases serum levels of prolactin with respect to dosage.

Conclusion: The impact of *Xylopia aethiopica* extract on female reproductive hormones observed in this study showed that the extract might be a potent contraceptive. Contraceptives of plant origin should be generally acceptable because they are less expensive with minimum adverse effects than synthetic agents.

Keywords: Contraceptive; female reproductive hormones; infertility; *Xylopia aethiopica* fruit

1. INTRODUCTION

Xylopia aethiopica has a great patronage in both nutrition and ethnomedicine. The plant which also known as African Negro pepper, is popular among traditional medicine practitioners and traditional birth attendants (TBA) who utilize the fruit preparations to cause the discharge of placental after a woman has giving birth [6]. A preparation of the stem bark or fruit is helpful in the management of bronchitis, stomach aches, asthma, and dysenteric conditions [1]. The seed extract is helpful as a vermifuge for roundworms [2]. Several postnatal women eat the aqueous preparation of the fruit for its perceived antiseptic properties. Some of the women have been reported to sometimes come to the hospitals with characteristics which suggest complications in organ [3]. Medicinal plant extracts with a therapeutic property has the tendency of wrong prescription and sometimes, overdosed. The fact that *Xylopia aethiopica* is a

natural product does not automatically confers on it safety and might be risky to its consumers. Chemical ingredients of the plant are perceived to be useful in preventing and managing cancerous tumors [4]. *Xylopia aethiopica* fruit is known to have alkaloids, terpenoids, flavonoids, and organic oils [5,6].

Fig. 1. *Xylopia aethiopica* Fruit [7]

Xylopia aethiopica is characterized with numerous chemical components with various medicinal potentials [8]. The chemical components of this plant have been investigated to include saponins, sterols, carbohydrates, glycosides, mucilage, acidic compounds, tannins, balsams, cardiac glycosides, volatile aromatic oils, phenols [9,10], alkaloids, rutin and fixed oils [11,12]. The plant has also be known to contain vitamins such as vitamin A, vitamin B, vitamin C, vitamin D, and vitamin E, and proteins as well as several minerals such as copper, manganese and zinc [10,12]. The impact of the fruit on b. wt. and glucose concentration [13] and lipid profile [14] of animals has been reported. The fruit has also been reported to induce hepatotoxicity [15], renal toxicity [16] as well as oxidative stress [17]. Recently, Ogbuagu et al. [18] reported that the fruit extract of *Xylopia aethiopica* adversely perturbed sperm qualities in male Wistar rats. This study was therefore aimed at investigating its effect on the reproductive hormones of female Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

The *Xylopia aethiopica* fruits were purchased from a market in Aba, Abia State. They were authenticated by Prof. (Mrs.) Margaret Bassey of Botany and Ecological Studies Department, University of Uyo. It was assigned a voucher number of UU/PH/4e and deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa-Ibom State, Nigeria.

2.2 Extraction of Plant Materials

Extraction of the plant was carried out based on the outlined method in Ogbuagu et al. [7]. The fruits were rinsed under flowing tap water to eliminate contaminants and air-dried. The plant material was milled by laboratory blender. The pulverized plant material was macerated in 250 mL of 99.8% ethanol (Sigma Aldrich) contained in a flask attached to a Soxhlet extractor coupled with condenser and heating mantle (Isomantle). It was then poured into the sample holder (thimble) and inserted in the apparatus. The side arm is lagged with glass wool. The mixture was heated using the heating mantle (Isomantle) at 60 °C and as the temperature rises it starts to evaporate, going via the extractor to the condenser. The condensate dropped into the reservoir keeping the thimble. As soon as the solvent gets to the siphon it emptied itself into the flask and the process repeats itself. The procedure goes on until it is exhaustively extracted. The process runs for a total of 13 hours. As soon as it was set up, it was allowed to run without interruption as long as water and power supply were not interrupted. The apparatus was switched on and off and overnight running was not allowed, and the time for the complete process split over some days. The extract was poured into 1000 mL beaker and concentrated to dryness in water bath (A3672- Graffin Student Water Bath) at 35 °C. The total weight of the marc (residue) and the concentrated extract were noted. Several days was spent on the entire process. The evaporated extract was kept in the refrigerator until when the need for it arise.

2.3 Determination of Median Lethal Dose (LD_{50})

The lethal dose (LD_{50}) of the fruit extract was determined using albino mice according to the method described by Airaodion et al. [19]. This method involves two phases:

In Phase one, five groups containing five mice each weighing between 20 g and 27g were fasted for 18 hours. They were respectively treated with 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg body weight via intraperitoneal (i.p) route and were monitored for visible signs of toxicity and mortality for 24 hours. A dosage of 1000 mg/kg recorded 0% mortality while 2000 mg/kg, 3000 mg/kg 4000 mg/kg and 5000 mg/kg recorded 100% mortality within 24 hours. Based on the value of phase one, phase two was conducted.

In Phase two, twenty-five albino mice weighing between 20 and 27g were grouped into 5 of 5 mice per group and were fasted for 18 hours. Each group was administered 1200 mg/kg, 1400 mg/kg 1600 mg/kg, 1800 mg/kg and 2000 mg/kg b. wt. intraperitoneally (i.p) and was observed for physical signs of toxicity and mortality within 24 hours. 1200 mg/kg recorded 0% mortality while 1400 mg/kg, 1600 mg/kg, 1800 mg/kg and 2000 mg/kg recorded 100% mortality within 24 hours. The LD_{50} was computed as according to the formula below:

$$LD_{50} = \sqrt{ab}$$

Where a = highest dose giving 0% mortality

b = lowest dose giving 100% death

2.4 Experimental Design

Thirty female Wistar rats used in this study were purchased from the University of Uyo, Nigeria. They were acclimatized for seven days prior to the start of the treatment. The weights were determined and were separated into five groups of six rats each. Groups A, B, C, D served as the experimental groups, while group E served as the control. Animals in group A were exposed to 130 mg/kg b. wt. (10% of LD_{50}) of *X. aethiopica* fruit extract, those in group B were treated with 259 mg/kg b. wt. (20% of LD_{50}) of *X. aethiopica* fruit extract, those in group C were exposed to 389 mg/kg b. wt. (30% of LD_{50}) of *X. aethiopica* fruit extract, those in group D were treated with 518 mg/kg b. wt. (40% of LD_{50}) of *X. aethiopica* fruit extract, while those in group E (control) received normal animals feeds and water only. The treatment was done once daily for 28 days via oral route. After the treatment period, the animals were sacrificed under ether anaesthesia in a desiccator after an overnight fast. Blood was taken from the rats through cardiac puncture.

2.5 Estimation of Female Reproductive Hormones

Blood levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estrogen and prolactin were measured by enzyme-linked immunosorbent assay (ELISA) technique described by Tanv and Harkaran [20].

2.6 Statistical Analysis

Results were statistically analyzed using Graph Pad Prism software. Data were presented as Mean ± Standard Deviation (SD). The means were compared by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The results were considered to be significant at 95% confidence level ($p \leq 0.05$).

3. RESULTS

3.1 Median Lethal Dose (LD_{50}) Result

The visible signs of toxicity of *X. aethiopica* fruit extract observed in this study are excitation, decreased motor activity, paw licking, increased respiratory rate, gasping and coma which could be followed up by death. In the first phase of the median lethal dose determination, no death was observed in the group administered 1000 mg/kg b. wt. of *X. aethiopica* fruit extract. However, all the animals died in the groups exposed to 2000, 3000, 4000, and 5000 mg/kg b. wt. of *X. aethiopica* fruit extract respectively (Table 1). In the same vein, in the second phase of medial lethal dose determination, no death was recorded in the group treated with 1200 mg/kg b. wt. of *X. aethiopica* fruit extract while 100% mortality was recorded in the groups treated with 1400, 1600, and 1800 mg/kg b. wt. of *X. aethiopica* fruit extract respectively as presented in Table 1.

The LD_{50} was computed as according to the formula below:

$$LD_{50} = \sqrt{ab}$$

Where a = highest dose giving 0% mortality

b = lowest dose giving 100% death

Thus, a = 1200 mg/kg

b = 1400 mg/kg

LD_{50}= 1296.15 mg/kg

3.2 Impact of ethanolic extract of *Xylopia aethiopica* fruit on Female Reproductive Hormones of Animals after 28 days of Treatment

A unnoticeable increase was seen in the blood concentration of FSH when animals exposed to 130 mg/kg b. wt. (low dose) of *Xylopia aethiopica* fruit extract were compared with those in the control group (P = 0.05), as presented in Table 2. Increase in the dose of *Xylopia aethiopica* extract resulted in a decrease in serum FSH levels. This decrease was significant when the FSH levels of animals treated with 389 mg/kg and 518 mg/kg b. wt. of ethanol extract of *Xylopia aethiopica* fruit were respectively compared with those in the control group (P<0.05). Administration of ethanol extract of *Xylopia aethiopica* fruit to animals for 28 days led to a dose-dependent decrease in the serum level of luteinizing hormone (LH). This decrease was only significant (P = 0.01) when the LH levels of animals treated with 259, 389 and 518 mg/kg of *Xylopia aethiopica* extract were respectively compared with those in the control group. The result of this study indicates that extract of *Xylopia aethiopica* decreased serum level of progesterone in female rats at low doses of 130 mg/kg and 259 mg/kg. The decrease was only significant (P<0.05) at the lowest dose of 130 mg/kg when compared with those in the control group. However, increase in the dose of the extract increases the serum progesterone concentrations. The increase became significant (P = 0.02) when the concentration of serum progesterone in animals treated with 518 mg/kg of *Xylopia aethiopica* extract were compared with those in the control group. Administration of *Xylopia aethiopica* extract to animals for 28 days led to a dose-dependent decrease in the serum level of estrogen. This decrease was only significant (P<0.05) when the estrogen levels of animals treated with 389 mg/kg and 518 mg/kg of *Xylopia aethiopica* extract were respectively compared with those in the control group. Conversely, *Xylopia aethiopica* extract increases serum levels of prolactin in a dose-dependent manner. The increase was however nonsignificant (P<0.05) when the prolactin levels of animals treated with 130 mg/kg (low dose) of *Xylopia aethiopica* extract were compared with those in the control group.

Table 1. The Median lethal dose (LD_{50}) of *Xylopia aethiopica* fruit extract

Study Phase/ (Animal)	Dosage of Extract (mg/kg) b.w	No of Mice per Group	No. of Death Recorded	% Mortality
PHASE ONE				
I	1000	5	0	0
II	2000	5	5	100
III	3000	5	5	100
IV	4000	5	5	100
V	5000	5	5	100
PHASE TWO				
I	1200	5	0	0
II	1400	5	5	100
III	1600	5	5	100
IV	1800	5	5	100
V	2000	5	5	100

LD_{50}= 1296.15 mg/kg

Table 2. Impact of ethanol extract of *Xylopia aethiopica* fruit on Female Reproductive Hormones of Animals after 28 days of Treatment

Group	A	B	C	D	E	
Dose of extract (mg/kg)	**130**	**259**	**389**	**518**	**Control**	**P Value**
FSH (mg/dL)	20.52±2.15	16.84±3.08	11.47±2.26*	9.33±1.42*	18.77±2.26	0.05
LH (mg/dL)	31.89±7.08	29.03±3.82*	24.41±3.33*	20.72±3.06*	34.98±5.07	0.01
Pg (ng/dL)	11.03±2.62*	16.67±3.27	23.55±2.99	28.22±4.01*	19.71±2.13	0.02
Estrogen (mg/dL)	29.11±3.38	26.45±4.10	24.06±2.44*	20.98±3.04*	33.02±7.25	0.04
Prolactin (ng/dL)	25.09±2.35	30.27±4.05*	32.33±3.62*	38.02±3.84*	20.14±4.04	0.05

Results are presented as Mean±S.D, where n = 6. Values with * are statistically significant at p value ≤ 0.05 when compared with the control group.

Legend: FSH = Follicle Stimulating Hormone, LH = Luteinizing Hormone, Pg = Progesterone

4. DISCUSSION

The acute toxicity study of the plant extracts recorded 100% mortality at a dose of 1400 mg/kg bodyweight and above (Table 1). This shows that the fruit of *Xylopia aethiopica* might be highly toxic. The visible signs of toxicity seen in the animals are excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

The results of the impact of ethanolic fruit extract of *Xylopia aethiopica* on female reproductive hormones of Wistar rats after twenty-eight days of exposure are shown in table 2**.** The ability of pre-ovulatory follicles to mature as well as for ovulation to occur is controlled by the effects of ovary as well as ovarian hormones. Perturbations in the levels of these hormones result in abnormality in ovarian functions as well as the period of estrous cycle [21]. These perturbations in hormones could be a reflection of several components of plants [22]. Maintaining homeostasis in human reproductive hormones is vital [23]. To achieve a success in fertility during the first 14 days of the menstrual cycle, concentrations of estrogen increase and influence the growth and elevation of uterine cells. Sequel to the FSH, an ovum starts developing within one of the ovaries. On the 14th day of a 28-day cycle, an ovary is released in following the action of LH, and a rise in the concentration of progesterone happened during the 14 days (day 15 to 28) of the menstrual cycle, which leads to an elevation in the thickness of the lower limb [24]. In this present study, a nonsignificant elevation was seen in the blood concentration of follicle stimulating hormone (FSH) when animals exposed to 130 mg/kg b. wt. (low dose) of ethanol extract of *Xylopia aethiopica* fruit were compared with those in the control group. Increase in the dose of *Xylopia aethiopica* extract resulted in decrease in serum FSH levels. This decline was noticeable ($P<0.05$) when the FSH levels of animals exposed to 389 mg/kg and 518 mg/kg b. wt. of *Xylopia aethiopica* extracts were respectively compared with those in the control group. FSH is the main reproductive hormone in mammals, useful in the development of the gonad and maturation at puberty and the production of gamete in the fertile phase of life [25]. It enhances the growth and maturation of ovarian follicles by exerting it action directly on granulose cell receptors [22]. The decline in the FSH concentration by fruit of *Xylopia aethiopica* at high doses might hinder production of follicles as well as its maturation in the pre-ovulatory phase [26]. The decline in the blood levels of FSH at these doses is similar to the study of Nnodim *et al.* [27] who observed a decrease in serum FSH when animals were treated with high doses of *Xylopia aethiopica* fruit extract. It could also mean that the extract at the administered doses could have exhibited its potential on the hypothalamus because the release of these hormones is controlled by the gonadotropic releasing hormone released by the hypothalamus [28]. The decline seen in the concentration of FSH could have a negative effect on conception in female animals and possibly humans. The major role of this hormone is to induce gametogenesis as well as the development of follicles in females [29]. In

females, FSH facilitates the maturation of follicular cells of the ovary. Steroidogenic functions in females are dependent on other hormonal factors such as LH while FSH induces the modification of androgens into estrogens [30]. The decrease in circulating FSH seen in this study could also be traced to the overall effect of metabolism. Since steroids reduce FSH synthesis and extracts of *Xylopia aethiopica* fruit contains steroids [7], it might be suggestive that steroids could also possibly be responsible for decrease in circulating FSH level seen in this present study.

Luteinizing hormone (LH) is known to induce the release of reproductive steroids from the gonads [31]. In females, ovulation of follicles that are matured is stimulated by a surge in the release of LH prior to the ovulation periods. Different scholars have proved that the release of LH surges at the pre-estrous stage were responsible for ovulation [22, 26]. Anything capable of stopping this secretion could unhinged ovulation by reducing the volume of matured follicles or cause a disruption in the estrous cycle [32,33]. The significant decline seen in the concentration of blood LH in this study (especially at high doses) might indicate the extract inhibits the secretion of LH which could have resulted in the perturbation of ovulation. This could lead to impairment of estrous cycle, impedes conception as well as distort normal reproduction in females. Thus, it is likely that *X. aethiopica* fruit contains anti-gonadotropic agents which might adversely impact the estrous cycle and distort reproduction in females [34].The decline in the blood level of LH seen in this present study was dose-dependent.

Progesterone aids the regulation of the menstrual cycle which occurs monthly. It is involved in the preparation of the body in order to achieve conception and increase sexual desire [35]. Progesterone helps in the maturation of lactating glands during pregnancy. A surge in the concentration of progesterone was perceived to be behind the symptoms seen in premenstrual syndrome (PMS). The feedback inhibition of the release of Gonadotropin-releasing hormone (GnRH) by estrogens and progesterone forms the foundation for the development of most contraceptives. This inhibition of Gonadotropin-releasing hormone disallowed a rise in LH and ovulation during the cycle [36]. Observations from this present investigation revealed that the extract of *X. aethiopica* decreased serum level of progesterone in female rats at low doses of 130 mg/kg and 259 mg/kg. The decrease was more noticeable ($P<0.05$) at the lowest dose of 130 mg/kg when compared with those in the control group. The decline observed in blood progesterone concentration by *Xylopia aethiopica* extract at these doses might have negative impact on ability to achieve conception in females; hamper ovulation which might lead to annovulation and sequelae [22]. Alkaloids had shown to hamper the production of progesterone in the cell [37]. Thus, the decreased progesterone concentration by *Xylopia aethiopica* could be due to the present of alkaloidal in the fruit. This result is in line with the findings of Onuka *et al.,* [38] who observed an increase in the level of progesterone when animals were treated with low doses of *Xylopia aethiopica* fruit extracts. However, increase in the dose (388.5 and 518 mg/kg) of the extract in this study increased the progesterone concentrations. The increase became significant ($P<0.05$) when the concentration of serum progesterone in animals exposed to 518 mg/kg of *Xylopia aethiopica* extract were compared with those in the control group. The phytochemical evaluation of *Xylopia aethiopica* fruit revealed that it contains alkaloids, saponin, tanins, Coumarin, phlobatannins, anthraquinones, steroids, flavonoids, cardiac glycosides and so on [7]. Yun *et al.,* [39] cited in Egba *et al.,* [26] had reported that high dose of extract of *Xylopia aethiopica*, containing saponins lowered blood androgens as well as 17β-estradiol, but elevated the concentrations of progesterone, indicating that presence of saponins might affect the synthesis of steroid in the ovary. High level of progesterone has shown antiestrogenic impact on the cells of myometrium, thereby reducing their excitability, their sensitivity to oxytocin, and their spontaneous electrical activity while elevating their membrane potential [40]. The rise observed in the blood level of progesterone in this research sequel to administration of high dose of *Xylopia aethiopica* fruit extract was consistent with the findings of Nnodim *et al.,* [27] who investigated the effects of *Xylopia aethiopica* fruits on reproductive hormonal level in rats. Decrease in blood concentrations of FSH and LH had been reported to increase progesterone concentration in Wistar rats [41]. Therefore, the increase observed in the progesterone level of experimental rats used in this study following the

administration of high dose of *Xylopia aethiopica* might be due to the reduction in the concentrations of FSH and LH observed in this study. Increase in serum progesterone concentrations had been speculated to indirectly predispose animals to teratogenicity and carcinogenesis [42]. Therefore, the elevation observed in the blood level of progesterone in this study at high doses suggested that extracts of *Xylopia aethiopica* might indirectly predispose its consumers to teratogenicity and carcinogenesis when consumed in high dosage.

Estrogen is known to facilitate the development of the linings of the uterus, leading to its thickness prior to ovulation. Estrogen is also involved in making reproductive organs mature. Estrogen and FSH increase the number of cells of the granules when follicles are developing. Herbal remedies possessing estrogenic agents are known to affect the action pituitary by lowering the release of LH and FSH thereby hindering ovulation. Hence, the decline in the blood levels of estrogen seen in this study might be linked to a reduction in the activity of aromatase or substrate supplementation during the production of estrogen [43]. Such a reduction in the concentration of estrogen could impair ovulation as well as the sustenance of pregnancy [43]. Kadohama *et al.* [44] had observed that various alkaloids from plant origin impair the activity of the enzyme, aromatase. It is therefore likely that the phytochemical content of extract of *Xylopia aethiopica* fruit reported by Ogbuagu et al. [7] might inhibit the activity of the endocrine system and subsequently cause an imbalance in reproductive hormones. The results of this present study could be useful in the development of female contraceptive from herbal origin. The reduction in the serum concentration of estrogen seen in this study is consistent with the report of Adienbo *et al.,* [45] who observed a dose-dependent decline in the number of pregnant females in all their experimental groups when they reported the contraceptive efficacy of hydro-methanolic fruit extract of *Xylopia aethiopica* in male albino rats.

Ovaries play crucial roles in estrogen synthesis and several things could perturb its functions. In the process of synthesizing follicles, thecal and granulosa cells take part in the estrogen synthesis. Theca cells can not directly synthesize estrogen, thus in developing follicles, androgens are secreted from the thecal cells and moved to the granulosa cells in which P_{450} aromatase modifies androgens to estrone and 17-beta estradiol [46]. Apart from saponin, flavonoids have also been reported to be one of the phytochemicals in *Xylopia aethiopica* fruit extract [7]. Reports have revealed that both saponins and flavonoids impair the activity of aromatase in human preadipocyte [47] and the impairment of aromatase activity is known decrease the production of estrogen to zero level [48]. Furthermore, estrogen derived from the modification of androgen forms a major portion of estrogen pool. In addition, the production of androgen from the thecal cells is predominantly regulated by LH from the pituitary [49]. Thus, the reduction in the levels of LH seen in this study might inhibit the production of androgen and impair the derivation of estrogens from androgens in the cell of the granule leading to a decline in the concentration of estrogen. This result is consistent with the reports of Onuka *et al.,* [38] who observed a decrease in estrogen concentration sequel to *Xylopia aethiopica* administration. FSH has also been reported to stimulate the conversion of androgens to estrogens [30]. The decrease in the level of estrogen might also be attributed to the observed decrease in serum FSH level in this study. The synthesis of estrogen by corpus luteum takes place due to a relationship between various endocrine glands and enzymes [50]. It is most probable that the extract of *Xylopia aethiopica* fruit used in this study had the propensity to decrease the level of estrogen by obstructing its synthesis.

A rise in prolactin level seen in this study might be due to the impact of the extract which is likely functioning as an antagonist of dopamine. The elevated level of prolactin observed in this study confirms the folklore utilization of the fruit to induce lactation. The result of this study is consistent with the reports of Onyebuagu *et al.,* [34], Anacletus *et al.,* [51] and Ehigiator and Adikwu [52] who respectively reported an increase in blood prolactin level when they administered different doses of *Xylopia aethiopica* extracts on female Wistar rats. The increased blood prolactin was due to a rise in the production and release of prolactin in the anterior pituitary sequel to hormonal factors [53]. The blood level of prolactin per time is affected by several factors regulating synthesis [54]. These hormonal imbalances observed in this study might be influenced by chemical component of the extract. Yakubu *et al.* [22] and Benie *et al.* [32] independently reported that

phytochemical analysis has shown that plant extracts contained chemical components that may influence the control of estrous cycle, conception and reproduction. Alkaloids and flavonoids have been reported to decrease plasma levels of estrogen [54]. Therefore, the presence of these phytochemicals in *Xylopia aethiopica* fruit may be responsible for the perturbations in the concentrations of the circulating hormones seen in this study. Our previous study [18] showed that extracts of *Xylopia aethiopica* fruit reduced sperm qualities in Wistar rats and might result in infertility in male rats. This is suggestive that the fruit has the propensity to induce infertility in both male and female rats.

5. Conclusion

The effect of *Xylopia aethiopica* extract on female reproductive hormones observed in this study showed that the extract might be a potent contraceptive. Plant products as contraceptive will be more acceptable for economic reasons and for the fact that they are associated with minimum side effects than synthetic agents.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

REFERENCES

1. Burkill HM. Brief description and details of the uses of over 4,000 plants. Royal botanical garden, kew; 2004.

2. Dalziel JM. The useful plants of Tropical West Africa.Crown overseas Agents Colonies, London. 1975;461.

3. Ezekwesili C, Nwodo O, Eneh F. Ogbunugafor H. Investigation of the chemical composition and biological activity of *Xylopia aethiopica* Dunal (Annonacae).African Journal of Biotechnology. 2010;9(43):7352-7356.

4. Del-Rio A, Obdulio BG, Castillo J, Main FR, Ortuno A. Uses and properties of Citrus flavonoids. Journal of Agricultural Food Chemistry. 1997;45:4505-4515.

5. Iwu MM. Handbook of Africa Medical Plant.CRCPress, London. 1993;435.

6. Shanmugam P, Anura W, Nedra KD, Gavin C, Wijesundara DSA, Raymond A, Veranja K. Antioxidant constituents from Xylopia championii. Pharmaceutical Biology. 2008;46(5):352-355.

7. Ogbuagu EO, Ogbuagu U, Unekwe PC, Nweke IN, Airaodion AI. Qualitative determination of the phytochemical composition of ethanolic extract of *Xylopia aethiopica* fruit. Asian Journal of Medical Principles and Clinical Practice. 2020; 4(3):1-5

8. Nwafor A, Adienbo MO, Egwurugwu JN. *In vivo* effects of *Xylopia aethiopica* on hemorheological parameters in Guinea pigs. African. Journal of Applied Zoology/ Environmental Biology. 2009;11:79-81.

9. Esekhiagbe M, Agatemor MMU, Agatemor C. Phenolic content and antimicrobial potentials of *Xylopia aethiopica* and Myristica argentea. Macedonian Journal of Chemistry and Chemical Engineering. 2009;28(2):159-162.

10. John-Dewole O, Agunbiade S, Alao O, Arojojoye O. Phytochemical and antimicrobial studies of extract of the fruit of *Xylopia aethiopica* for medicinal importance. Journal of Biotechnology and Pharmaceutical Research. 2012; 29(6):118-122.

11. Asekun O, Kunle O. The chemical constituents of the fruit essential oil of *Xylopia aethiopica* (Dunal) A. Rich from Nigeria.Journal of Essential Oil Bearing Plants. 2004;7(2):186-189.

12. Nwaichi E, Igbinobaro O. Effects of some selected spices on some biochemical profile of Wister albino rats. American Journal of Environmental Engineering. 2012;2(1):8-11.

13. Ogbuagu EO, Nweke IN, Unekwe PC, Airaodion AI, Ogbuagu U. Weight gain reduction and hypoglycemic effects of *Xylopia aethiopica* fruit extract on Wistar rats. International Journal of Research and Reports in Hematology. 2020;5(3):1-8.

14. Ogbuagu EO, Unekwe PC, Airaodion AI, Nweke IN, Ogbuagu U. Hypolipidemic propensity of ethanolic extract of *Xylopia aethiopica* fruit in Wistar rats. Asian Journal of Research in Cardiovascular Diseases. 2020;3(5):1-11.

15. Ogbuagu EO, Unekwe PC, Airaodion AI, Nweke IN, Ogbuagu U. Hepatotoxic effect of *Xylopia aethiopica* fruit in Wistar rats. International Research Journal of Gastroenterology and Hepatology. 2021; 4(1):1-16

16. Ogbuagu EO, Airaodion AI, Ogbuagu U, Nweke IN, Unekwe PC. Nephrotoxicity of ethanol extract of *Xylopia aethiopica* fruit in wistar rats. International Journal of Advances in Nephrology Research. 2021;4(1):1-16.

17. Ogbuagu EO, Ogbuagu U, Airaodion AI, Uche CL, Ezirim EO, Nweke IN, Unekwe PC. Effect of Ethanol Extract of *Xylopia aethiopica* Fruit on Oxidative Stress Indices of Wistar Rats. Asian Journal of Immunology. 2022;6(1):40-51.

18. Ogbuagu EO, Airaodion AI, Ogbuagu U, Ezirim EO, Nweke IN, Unekwe PC. Ethanol Extract of *Xylopia aethiopica* (African Negro Pepper) Fruit adversely perturbed Semen Qualities in Male Wistar Rats. International Journal of Research and Report in Gynaecology. 2022;5(2):44-55

19. Airaodion AI, Ekenjoku JA, Ogbuagu EO, Okoroukwu VN, Ogbuagu U. *Carica papaya* leaves might cause miscarriage. Asian Research Journal of Gynaecology and Obstetrics. 2019;2(2):1-9.

20. Tanv S, Harkaran S. Estimation of serum prolactin levels and determination of prevalence of hyperprolactinemia in newly diagnosed cases of subclinical hypothyroidism. Journal of family medicine and primary care. 2018;7(6): 1279.

21. Shivalingappa H, Satyanaranyan ND, Purohit MG, Sahranabasappa, A. &Patil, S. B. (2002).Effect of ethanol extract of *Rivea hypocraterifomis* on the estrous cycle of the rat. *Journal of Ethnopharmacology.*82: 11-17.

22. Yakubu, M. T., Akanji, M. A., Oladiji, A. T., Olatinwo, A. O., Adesokan, A. A., Yakubu, M. O., Owoyele, B. V., Sunmonu, T. O. & Ajao, M. S.(2008). Effect of *Cnidoscolousaconitifolius*(Miller) I.M. Johnston leaf extract on reproductive hormones of female rats. *Iranian Journal of Reproductive Medicine.*6(3): 149-155

23. Farahbod, F. &Soureshjani SH. Medicinal herbs affecting gonadotropin hormones in women: An updated systematic review. International Journal of Life Science and Pharma Research. 2018;8(1):20-28.

24. Vlckova R, Sopkova D, Andrejcakova Z, Valocky I, Kadasi A, Harrath AH. Dietary supplementation of yucca (*Yucca schidigera*) affects ovine ovarian functions. Theriogenology. 2017;88:158-165.

25. Ogbuagu EO, Airaodion AI. Tiger Nut (*Cyperus esculentus* L.) Boosts Fertility in Male Wistar Rats. *Asian Research Journal of Gynaecology and Obstetrics.* 2020;3(3): 8-18

26. Egba SI, Omodamiro OD, Obike JC, Ali ES. Influence on some female fertility hormonal response in Wistar albino rats: Possible contraceptive role for methanol leaf extract of *Ocimum gratissimum. Journal of Chemical and Pharmaceutical Research*. 2015;7(5):889-898.

27. Nnodim JK, Nwanjo HU, Okolie NJ, Opara AU, Nwosu DC, Okoroiwu I, Dike J, Okorie, H, Nwadike CN, Uduji HI. Effects of *Xylopia aethiopica* Fruits on reproductive hormonal level in rats.*Global Journal of Medicinal Plant Research,* 2013;1(1): 29-31.

28. Airaodion AI, Ngwogu AC, Megwas, AU, Ekenjoku JA, Ngwogu KO. Effect of common household insecticides used in Nigeria on rat male reproductive hormones. *Int. J. Res. Rep. Gynaecol.*, 2019;2(1):1-8.

29. Litwack G, Schmidt RJ. Biochemistry of hormones II: polypeptide hormones. In Textbook of Biochemistry with Clinical Correlations, 5th edn (Devlin, T.M., ed.), pp. 905-958. John Wiley and Sons, Inc., New York. 2001.

30. Omotuyi IO, Ologundudu A, Onwubiko VO, Wogu MD, Obi FO. *Hibiscus sabdariffa*Linn anthocyanins alter circulating reproductive hormones in rabbits (*Oryctolaguscuniculus*). *Journal of Diabetes and Endocrinology*. 2010;1(3):36-45

31. Airaodion AI, Ogbuagu EO, Ekenjoku JA, Okoroukwu VN, Ogbuagu U, Airaodion, EO. Antifertility effect of ethanolic leaf extract of *Carica papaya* in male Wistar

rats. *Merit Research Journal of Medicine and Medical Science.* 2019;7(10):374-381.
32. Benie T, Duval J, Thieulant ML. Effects of some traditional plant extract on rat oestrous cycle compared with clomid. *Phytotherapy Research*. 2003;17(7): 748-755.
33. Zahradnik HP, Hanjalic-Beck A, Groth K. Non-steroidal anti-inflammatory drugs and hormonal contraceptives for pain relief from dysmenorrhea: a review. *Contraception*. 2010;81(3): 185-196.
34. Onyebuagu P., Aloamaka CP, Igweh JC. *Xylopia aethiopica* Lowers Plasma Lipid Precursors of Reproductive Hormones in Wistar Rats. Int. J. Herbs and Pharm. Res. 2013; 2(4): 48-53.
35. Montaserti A, Pourheydar M, Khazaei M, Ghorbani R. Anti-fertility effects of *Physalisalkekengi*alcoholic extract in female rat. *Iranian J Reprod Med* 2007; 5: 13-16.
36. Norris DO, Carr JA. Vertebrate Endocrinology. Academic press. 2013; P. 126.
37. Gocze PM, Porpaczy Z, Freeman, D. A. Effects of alkaloids in cigarette smoke on human granulose cell progesterone synthesis and cell viability. *Gynecol Endocrinol.* 2006;10:223-228
38. Onuka AE, Okechukwu NC, Maxine KM. A Comparative Study between *Xylopia aethiopica* Dried Fruit Extract and Ibuprofen Inhibiting Effects on Some Reproductive Hormones Irrespective of the Estrous Cycle. *International Journal of Complementary & Alternative Medicine.* 2017;8(5):1-6.
39. Yun YW, Yuen BH, Moon YS. Effects of superovulatory doses of pregnant mare serum gonadotropin on oocyte quality and ovulatory and steroid hormone responses in rats. *Biol. Pharm. Bull.* 1987;26(11):1574-1580.
40. Al-Shemary NNA, Mousa SA, Muslim ZZ. Histological and hormonal study about the effect of aqueous extract of *Ocimum gratissmum* on female reproductive system in albino mice. *Journal of Pharmaceutical Science and Research*. 2018;10(4): 765-767.
41. Chinnappan SM, George A, Ashok G, Choudhary YK. Effect of herbal extract *Eurycomalongifolia* (Physta®) on female reproductive hormones and bone biochemical markers: an ovariectomised rat model study. *BMC Complementary Medicine and Therapies*. 2020;20(31):1-9.
42. Oze G, Onyeze GO, Ojiako O, Abanobi S, Ozims S, Ohiri A. Raised serum estrogen and progesterone concentrations in female rats treated with extract of Alstoniaboonei (De wild). *International Research Journal of Biochemistry and Bioinformatics*. 2012;2(6):135-141.
43. Hsia SM, Yeh CL, Kuo YH, Wang PS, Chiang W. Effects of Adlay (*Coixlachryma*-jobi L. var. ma-yuenStapf.) Hull extracts on the secretion of progesterone and estradiol *in vivo* and *in vitro*. *Experimental Biology and Med*ine. 2007232:1181-1194.
44. Kadohama N, Shintani K, Osawa Y. Tobacco alkaloid derivatives as inhibitors of breast cancer aromatase.*Cancer Lett*; 2013;75: 175-182.
45. Adienbo OM, Nwafor A, Ogbomade RS. Contraceptive efficacy of hydro-methanolic fruit extract of *Xylopia aethiopica* in male albino rats. *International journal of Advanced Biological and Biomedical Research***.** 20131(7): 718-727
46. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during ageing: from periphery to brain. Trends Mol Med. 2013;19(3): 197-209.
47. Golan M, Avitan A, Qutob M, Dweik H. Abu-Lafi S. Quillaja saponins inhibit tilapia aromatase activity *in vitro*. Cybium. 2008;32: 80-82.
48. Stadtlander T, Levavi-Sivan B, Kerem Z, Dweik H, Qutob M. Effects of a saponin fraction extracted from *Trigonellafoenum-graecum*L. 2012.
49. Balunas MJ, Su B, Brueggemeier RW, Kinghorn AD. Natural products as aromatase inhibitors. *Anticancer Agents Med Chem*. 2008;8(6): 646-682.
50. Daramola OI, Akindele OO, Bolarinwa AF, Yinusa-Raji, Y. Effects of *Telfairia Occidentalis* Seed Oil on Female Reproductive Functions in Wistar Rats.*Nigerian Journal of Physiological Sciences.* 2015;30:95-101.
51. Anacletus FC, Nwauche KT, Wopara I. Hyperprolactin activity of aqueous extract of *Xylopia aethiopica* on female wister albino rats. *Research Journal of Pharmaceutical Dosage Forms and Technology.* 2015;7(3):103-105.
52. Ehigiator BE, dikwu E. Toxicity study of ethanolic stem bark extract of *Xylopia*

aethiopica on fertility indices of male rats: An experimental study. International Journal of Reproductive BioMedicine. 2019;18(1):265–274.
53. Wass JA, Shalet SM. Oxford Textbook of Endocrinology and Diabetes. Oxford: Oxford University Press. 2002.
54. Bianco F, Basini G, Grasselli F. The plant alkaloid Sanguinarine affects swine granulose cell activity. *Reproductive Toxicology*. 2006;21(3): 335-340.