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Effects of Chronic Consumption of *Xylopia aethiopica* Seed Extracts on Oestrogen, Progesterone and Prolactin in Female Swiss White Mice

Gideon Ikenna Emeghara[1*], Bright Ichechi Owhorji[2], Simeon Chijioke Amadi[1], Alpheaus Gogo Mba[1], Inusa Amike[3]

[1]Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, College of Basic Medical Sciences, Rivers State University. Nkpolu Oroworukwo
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, Rivers State University
[3]Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital Port Harcourt

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***Corresponding author:** Gideon Ikenna Emeghara
Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, College of Basic Medical Sciences, Rivers State University. Nkpolu Oroworukwo

Abstract **Original Research Article**

Increased growth in population, as seen most in developing countries of the world has resulted to a huge burden on their economy. Several studies have reported significant roles played by medicinal plants to improve or control fertility and reproductive activities. The aim of this study was to assess the effect of seed extract of *Xylopia aethiopica* (Uda) on Oestrogen, Progesterone and Prolactin using female Swiss White mice. Twenty-two (22) adult female Swiss mice were used. The mice were randomly grouped into 2 groups with 11 animals in each group. Group 1 which was the control had only rat chow and water; group 2 had 100mg/kg Uda. They had these daily. The extracts were administered by oral gavage for 35 days. The mice were anaesthetised using ketamine on the 35th day and blood was collected in plane sample bottles via cardiac puncture and assayed for progesterone, oestrogen and prolactin. The results showed that extracts of *Xylopia aethiopica* significantly decreased the serum levels of oestrogen (41.8 ± 7.6), progesterone (4.3 ± 0.6), and prolactin (0.4 ± 0.1) compared to the control 62.2 ± 1.9, 6.2 ± 0.9 and 0.7 ± 0.1 respectively.

Keywords: *Xylopia aethiopica,* Oestrogen, Progesterone, Prolactin, Swiss White mice.

INTRODUCTION

Subfertility is a global reproductive problem marked by the difficulty to achieve conception after one year of unprotected and regular sexual activity (WHO, 2023). Population control is an issue in the sub-Saharan Africa. Birth control has become a programme of some governments. Infertility affects greater than 10% of our world's total population and Sub-Saharan Africa accounts for over 30%. (Menashe-Oren 2023; Macrotrends *et al.*, 2023). It is estimated by the WHO that 80% of the world's population depends on non-conventional extracts for medical treatment. It is commoner in the developing nations as well as in the developed countries where modern medicines are also used (Rickert *et al.*, 1999). The WHO estimated that about 80% of the world's population use herbal remedies, because they possess fewer side effects compared to orthodox drugs (Desai *et al.*, 2019). Several studies have reported significant roles played by medicinal plants to improve or regulate fertility and reproductive behaviour.

Scientific evaluation of any plant for medicinal purpose requires detailed information on its affordability, accessibility, safe dose and the toxicity level. Traditional use of any plant for medicinal purposes requires safety of such plants and hence needs to be screened for their toxicity level. Women have a wide range of contraceptive choices ranging from daily oral medications to intrauterine devices implanted for sterilization. Research and family planning organizations have, for a long time, focused upon female methods of contraception because women bear a disproportionate portion of the health and economic consequences of childbearing and rearing. In several countries and all through the ages, medicinal plants have been widely used to enhance or regulate fertility. Many herbal drugs are used to control fertilization with considerable success, besides the use of chemicals as antifertility agents in controlling human population (Anitha *et al*, 2013). In Nigeria, the folkloric uses of plant preparations for reproduction related purposes are well known and documented (Akaneme *et al.*, 2008). Elsewhere, Gupta *et al.,* (2004) reported that herbal contraceptives are used because of affordability, ease of availability from local

sources and less side effects. In some cases, users may not know the actual effects of these herbal products. There has been an increase in demand for the phytopharmaceuticals all over the world because the allopathic drugs have more side effects (Nath and Deb, 2015). Plant-sourced substances have been documented to affect reproductive functions and alter the physiology of the endocrine system (Al-Tawalbeh *et al.*, 2023). Contraception methods have suffered some draw back over the years due to some undesirable side effects like menstrual abnormalities. A search for new, safe, effective and affordable form of contraception has remained top priority necessitating the exploration and screening of medicinal plants. This study was on the effect of *Xylopia aethiopica* on Oestrogen, Progesterone and Prolactin in mice. Several studies have been carried out on consumption *Xylopia aethiopica* (*Uda*), and it's used as spice for pepper soup (Tairu *et al.*, 2000). It is believed that it helps in contraction of the uterus (Durugbo *et al.*, 2013). Traditionally, it has also been used for inducing lochia postpartum, management of rheumatism, asthma, headache, bronchitis, neuralgia and colic pain (Woode *et al.*, 2011). The use of *Xylopia aethiopica* has been associated with reported alterations of female fertility due to their vast uses (Stadtlander *et al.*, 2013; Ugiomoh *et al.*, 2023). Hormones are chemical substances produced by glands from the endocrine system that aid communication between cells. Hormones produced from the bedrock of the endocrine system, regulates fertility, growth, change in mood and others. Plant extracts have been shown to possess the ability to interfere with the functions of the endocrine system, thereby exhibiting an androgenic antagonistic effect responsible for the elevated incidence in various hormonal imbalance, male infertility and sexual disorders.

MATERIALS AND METHODS

Materials for the study included 22 healthy female Swiss White mice, *Xylopia aethiopica* seed extracts, mice feed, Water, Ethanol, (solvent for extraction), Ketamine, Refrigerator/freezer (-20°C and -80°C), Blood collection tubes (plain tubes for serum collection), Microcentrifuge tubes, Gloves, Surgifield (SM-300A) Microplate Reader for hormonal assay, mice cage and sawdust. The mice were obtained from a livestock farm in the Department of Pharmacology, Rivers State University, Rivers State. They were kept 12 hours in darkness and 12 hours in light condition. The temperature was (24.3–28.9°C) and the humidity in the room was (55–60%). All rats were fed a standard diet and water *ad libitum* and were acclimatised for 2 weeks prior to the commencement of the experiment.

Study Area:

The study took place in the animal house of the Faculty of Basic Medical Science, Rivers State University, Nigeria. Under the human-animal care requirements outlined in the 'Guide to the care and use of animals in research and teaching' as approved by the National Institute of Health (NIH), for the care and use of laboratory research animals in experimental research, all animal experiments were carried out (NRC, 2016).

Preparation and Storage: The seeds of *Xylopia aethiopica* was obtained from Mile 3 market, Port Harcourt. The seeds were separated, cleaned and healthy ones selected. The seeds were then sun dried and milled into fine powder using a milling machine.

The powder form of the seeds was subjected to cold extraction by soaking 1kg of it into an aspirator jar containing 3L of ethanol and shaken vigorously for 30 minutes. Then left to stand for 24 hours. The dried extract was stored in airtight plain sample bottles (Odesanmi *et al*; 2011) and then stored in a refrigerator.

METHOD

The median lethal dose LD50 of *Xylopia aethiopica* was found to be 3,464 mg/kg (Ayodele *et al*, 2019). Experimental design: The study was a longitudinal experimental design using standard methods for analysis. The animals were weighed weekly, while they were permitted to feed and consume water liberally. The study used twenty (22) healthy female Swiss white mice weighing between 45- 55grams. The female Swiss mice were grouped into 2 groups with 11 mice in each group.

Group 1- Rat feed and distilled water.
Group 2- *Xylopia aethiopica* 100mg/kg body weight.

Determination of Oestrous Cycle: The oestrous cycle stages and duration was determined according to the method proscribed by Goldman *et al*. (2007). Proestrus was defined by smears possessing more nucleated epithelial cells. Oestrous characterised as smears with a more significant number of cornified epithelial cells, metestrus equal proportion of epithelial cell, cornified and leucocytes. In contrast, smears defined the dioestrus stage with the presence of leucocytes. The staining of the smears for the microscopic analysis was done according to Shorr's method (1941). Micro drop pipette, normal saline and distilled water, was used to collect vaginal smear from the female rats. The smear was dropped on a microscope slide and examined daily in the morning (7 am-9 am) under a light microscope. The cells' proportion was observed and used to determine the various phases of the experimental animals' oestrous cycle. Next, the wet smear from each rat was dried and fixed with 95% ethanol, and the slide was stained with Leishman stain, wash with buffer solution (K2OH and Na2OH) after 10minutes. The slide was dried then covered with coverslip using Manta (D PX) and Xylene. The coverslip was viewed and snaped using an electron microscope for each Wistar rat in each group. The animals were anaesthetised using ketamine at day 35. Blood was collected in-plane sample bottles via cardiac puncture for Oestrogen, Progesterone and Prolactin.

Ethical Approval: Ethical application form was duly completed, submitted, and approved by the College of Medical Sciences, Rivers State University, with Ref number; RSU/FBMS/REC/23/010.

Statistical analysis: Data obtained from this study was expressed as mean ± Standard Error of Mean (±*SEM).* The statistical significance was determined using analysis of variance (ONE WAY ANOVA) and t-test with the statistical package for social sciences (SPSS) version 23.0. A Probability-value of less than 0.05 was assumed to denote a significant difference.

RESULTS

Table 1: Comparison of Oestrogen between the Test dose (*Xylopia aethiopica*) and the Control groups

Hormone tested	Hormone level	Hormone level	Mean Difference	t-test (p Value)
	Control	*X. aethiopica*	(95% CI)	
	Mean ± SD	Mean ± SD		
Oestrogen(ng/ml)	62.2 ± 1.9	41.8 ± 7.6	20.4 (12.4–28.4)	5.85 (0.001*)

*Statistically significant

Reproductive Hormone Values in Study Animals

Table 1 shows the mean values of reproductive hormone oestrogen of study animals after 5 weeks of administration of *Xylopia aethiopica* seed extract. There was significant reduction (P<0.05) in the values for oestrogen when compared with the control.

Table 2: Comparison of Progesterone between the Test dose (*Xylopia aethiopica*) and the Control groups

Hormone tested	Hormone level	Hormone level	Mean Difference	t-test (p Value)
	Control	*X. aethiopica*	(95% CI)	
	Mean ± SD	Mean ± SD		
Progesterone(ng/ml)	6.2 ± 0.9	4.3 ± 0.6	2.0 (0.8 – 3.1)	4.06 (0.004*)

*Statistically significant

Reproductive Hormone Values in Study Animals

Table 2 shows the mean values of reproductive hormones progesterone of study animals after 5 weeks of administration of Xylopia aethiopica seed extract. There was significant reduction (P<0.05) in the values for progesterone when compared with the control.

Table 3: Comparison of serum Prolactin between the Test group (*Xylopia aethiopica*) and the Control group

Hormone tested	Hormone level	Hormone level	Mean Difference	t-test (p Value)
	Control	*X. aethiopica*	(95% CI)	
	Mean ± SD	Mean ± SD		
Prolactin(ng/ml)	0.7 ± 0.1	0.4 ± 0.1	0.3 (0.2 – 0.4)	7.57 (0.001*)

*Statistically significant

Reproductive Hormone Values in Study Animals

Table 3 shows the mean values of reproductive hormones of study animals after 5 weeks of administration of Xylopia aethiopica seed extract. There was significant reduction (P<0.05) in the value for prolactin when compared with the control.

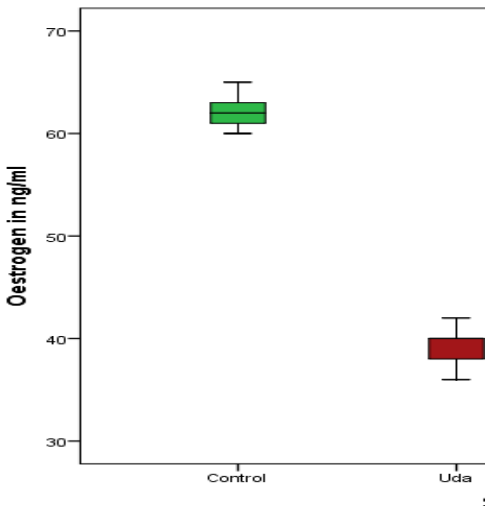

Figure 1: Comparing oestrogen level of Female Swiss mice on *Xylopia aethiopica* [Uda] and Control groups

Figure 1 Compares the levels of Oestrogen level of female Swiss mice on the Uda and Control groups. Oestrogen was significantly reduced when compared with those on distilled water and normal feed (control group).

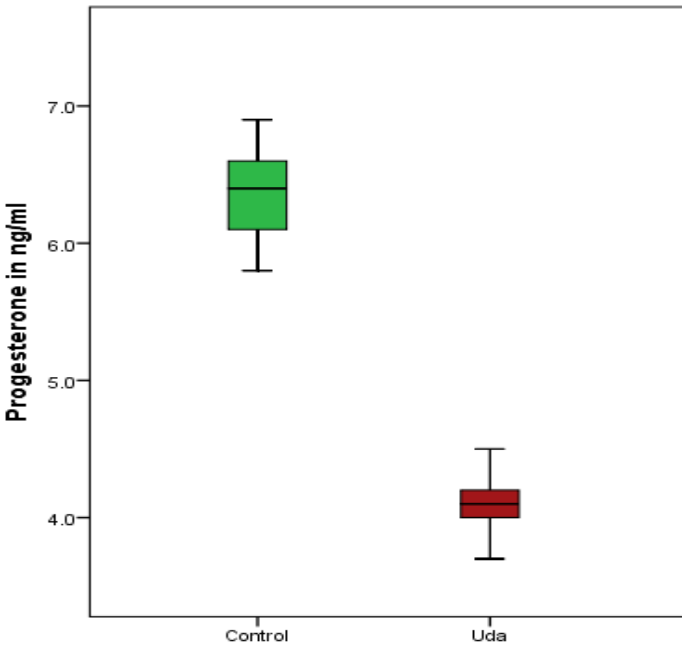

Figure 2: Comparing Progesterone level in Female Swiss mice on *Xylopia aethiopica* and Control groups

Figure 2 Compares the levels of Progesterone level of female Swiss mice on the Uda and Control groups. Progesterone was markedly reduced in those fed with Uda when compareded with those on distilled water and normal feed (control group).

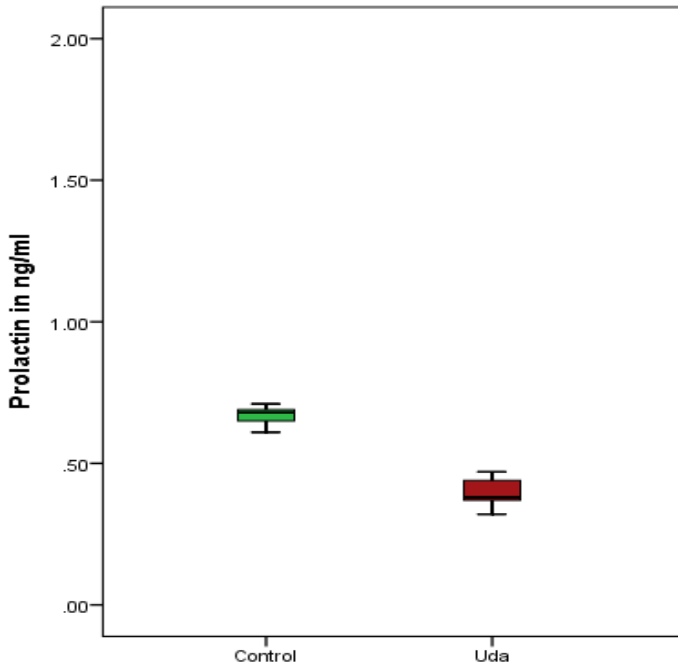

Figure 3: Comparing Prolactin level of female Swiss Mice on *Xylopia aethiopica* and Control groups

Figure 3 Compares the levels of Prolactin level of female Swiss mice on the Uda and Control groups. Those fed with Uda had reduced Prolactin level lower than those on distilled water and normal feed (control group).

DISCUSSION

This experiment was done to analyse the effect of *Xylopia aethiopica* on Oestrogen, Progesterone and Prolactin by using female Swiss white mice as the experimental animals. Plant-sourced substances have been documented to affect reproductive functions and alter the physiology of the endocrine system (Al-Tawalbeh *et al.,* 2023). Extracts of *Xylopia aethiopica* have also been studied and reported to affect the secretion and body serum levels of reproductive hormones (Adienbo *et al.,* 2021; Ogbuagu *et al.,* 2022). The current study result showed that the level of serum Oestrogen, Progesterone, Prolactin were significantly decreased (41.8 ± 7.6, 4.3 ± 0.6, 0.4 ± 0.1) in the group 2 mice administered with *Xylopia aethiopica* (Uda) seed extract. This was a significant decrease ($P < 0.05$) when compared with the control (62.2 ± 1.9, 6.2 ± 0.9, 0.7 ± 0.1). This result agrees with the work done by Nnodim, *et al.,* 2013; Onuka *et al.,* 2017; Godam *et al.,* 2021. However, there are also contradicting results regarding the effect of *Xylopia aethiopica* on oestrogen levels (Ehigiator & Adikwu, 2020). The reduced levels of serum oestrogen in this study can be connected to the decrease in the ovarian aromatase activity, which is necessary for the production of oestrogen.

CONCLUSION

The findings in this study shows that administration of *Xylopia aethiopica* (Uda) on female mice caused significant changes in the level of the hormones. Oestrogen, progesterone and prolactin were significantly decreased by *Xylopia aethiopica* extract administration.

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