

Effects of Long-Term Consumption of *Xylopiya aethiopic* and *Tetrapleura tetraptera* Extracts on the Follicle Stimulating Hormone and Luteinizing Hormone of Female Swiss White Mice

Bright Ichechi Owhorji^{1*}, Gideon Emeghara¹, Tee Popnen Godwin¹, Simeon Chijioke Amadi², Inusa Amike³¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpulu-Oroworukwo, Port Harcourt²Department of Obstetrics and Gynecology, Rivers State University Teaching Hospital³Department of Obstetrics and Gynecology, University of Port Harcourt Teaching HospitalDOI: <https://doi.org/10.36347/sjams.2025.v13i08.006>

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*Corresponding author: Bright Ichechi Owhorji

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpulu-Oroworukwo, Port Harcourt

Abstract

Original Research Article

Increased growth in population, as seen most in developing economies of the world has resulted to a huge burden on the population. 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 effects of seed extracts of *Xylopiya aethiopic* (*Uda*) and *Tetrapleura tetraptera* (*Uhio*) on selected reproductive hormones using female Swiss White mice. Twenty (24) adult female Swiss White mice weighing 45g-55g were used. The female mice were randomly grouped into 4 with 5 animals in each group. Group 1 which was the control had only rat feed and distilled water. Group 2 had 100mg/kg of *Xylopiya aethiopic*. Group 3 had 100mg/kg of *Tetrapleura tetraptera*, and group 4 had 50mg/kg of *Xylopiya aethiopic* & 50mg/kg of *Tetrapleura tetraptera* daily. Administration of the extracts was 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 follicle stimulating hormone and luteinizing hormone. The results showed that group 2 had decreased serum levels of FSH and LH. Group 3 and group 4 had significantly increased serum level of LH and FSH. The increase seen in group 4 could likely be from the effect of *Tetrapleura tetraptera*. This result shows that *Xylopiya aethiopic* taken alone could reduce fertility, thereby helping birth control in breastfeeding mothers, who are the most consumers of this plant in tropical Africa. This also shows that taking a combination of both could enhance fertility in breastfeeding mothers.

Keywords: *Xylopiya Aethiopic*, *Tetrapleura tetraptera*, Follicle Stimulating Hormone, Luteinizing hormone, Swiss White Mice.

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INTRODUCTION

Infertility is a global reproductive problem marked by the difficulty to achieve conception after one year of unprotected and regular sexual activity (WHO, 2023). 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 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

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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. 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 year 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. In sub-Saharan Africa, most women take *Xylopiya aethiopicia* and *Tetrapleura tetraptera* as spices during postpartum period. Several studies have been carried out on consumption *Xylopiya aethiopicia* (Uda), and its use 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). *Tetrapleura tetraptera* seed extract increases sexual desire and motivation, as well as enhance copulatory efficacy by prolonging the duration of sexual intercourse but impairs male fertility (Adienbo and Krukru, 2021). The use of *Xylopiya aethiopicia* and *Tetrapleura tetraptera* has been associated with reported alterations of female fertility due to their vast uses (Stadtlander *et al.*, 2013; Ugiomoh *et al.*, 2023). This study was on the effect of combined consumption of *Xylopiya aethiopicia* and *Tetrapleura tetraptera* on selected reproductive hormones (FSH & LH) in mice. Hormones are chemical substances produced by glands from the endocrine system that aids 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 effects accounting for the elevated incidence in various hormonal imbalances, male infertility and sexual disorders.

MATERIALS AND METHODS

Materials for the study included 20 healthy female Swiss White mice weighing 45g-55g, weighing scale, *Xylopiya aethiopicia* and *Tetrapleura tetraptera* seed extracts, rat feed, Water, Ethanol, Ketamine,

Refrigerator/freezer (-20°C and -80°C), blood collection tubes (plain bottles), Microcentrifuge tubes, Gloves, Surgifield (SM-300A) Microplate Reader for hormonal assay, rat 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 (23.3–25.9°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 *Xylopiya aethiopicia* and *Tetrapleura tetraptera* were obtained from Mile 3 market in Port Harcourt, Rivers State. Both seeds were identified by a certified Botanist from the Rivers State University. Both seeds were separated, cleaned and healthy seeds selected. The seeds of *Xylopiya aethiopicia* and *Tetrapleura tetraptera* were then sun dried and milled into fine powder using a milling machine separately.

The powder form of seeds was individually subjected to cold extraction by soaking each 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.

The median lethal dose LD50 of *Xylopiya aethiopicia* was found to be 3,464 mg/kg and the median lethal dose, LD50 of *Tetrapleura tetraptera* was found to be 244.94 mg/kg (Jimmy *et al.*, 2016).

The study was a longitudinal experimental design using standard methods for analysis. The animals were weighed weekly. They were permitted to feed and consume water liberally. The study used twenty (20) healthy female Swiss white mice weighing between 45-55grams. The female Swiss mice were grouped into four groups with five mice in each group.

Group 1- Rat feed and distilled water.
Group 2- *Xylopiya aethiopicia* 100mg/kg body weight.
Group 3- *Tetrapleura tetraptera* 100mg/kg body weight.
Group 4- *Xylopiya aethiopicia* 50mg/kg body weight plus *Tetrapleura tetraptera* 50mg/kg body weight.

The four different groups of experimental animals were fed with the extracts as documented above for 35 days.

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, washed with buffer solution (K₂OH and Na₂OH) after

10minutes. The slide was dried then covered with coverslip using Manta (DPX) and Xylene. The coverslip was viewed 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 FSH and LH.

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 were expressed as mean \pm Standard Error of Mean (\pm SEM). The statistical significance was determined using analysis of variance (ONE WAY ANOVA) and t-test with the IBM SPSS Statistics version 23.0. A Probability-value of less than 0.05 was assumed to denote a significant difference.

RESULTS

Table 1: Result of FSH and LH for *Xylopiya aethiopic* 100mg/kg body weight and the Control group.

	Hormone levels	Hormone levels	Mean Difference	t-test (p Value)
	Control	<i>X. aethiopic</i>	(95% CI)	
	Mean \pm SD	Mean \pm SD		
FSH	0.4 \pm 0.0	0.3 \pm 0.0	0.1 (0.1 – 0.2)	10.12 (0.001*)
LH	0.3 \pm 0.0	0.2 \pm 0.0	0.1 (0.1 – 0.1)	6.57 (0.001*)

FSH – Follicle stimulating hormone; LH – Luteinizing hormone; *statistically significant.

Table 1 shows the mean values of reproductive hormones of study animals after 5 weeks of administration of *X. aethiopic* seed extract. There was

significant reduction ($P < 0.05$) in the values for Luteinizing hormone and Follicle Stimulating Hormone, when compared with control.

Table 2: Result of FSH and LH for *Tetrapleura tetraptera* 100mg/kg body weight and the Control group.

	Hormone levels	Hormone levels	Mean Difference	t-test (p Value)
	Control	<i>T. tetraptera</i>	(95% CI)	
	Mean \pm SD	Mean \pm SD		
FSH	0.4 \pm 0.0	0.9 \pm 0.1	-0.5 (-0.6 – -0.4)	13.15 (0.001*)
LH	0.3 \pm 0.0	0.6 \pm 0.1	-0.3 (-0.4 – -0.3)	11.48 (0.001*)

FSH – Follicle stimulating hormone; LH – Luteinizing hormone; *statistically significant.

Table 2 shows the effect of *Tetrapleura tetraptera* extract on FSH and LH. There was significant

p-value ($P < 0.05$) increase in the values for FSH and LH when compared with the control.

Table 3: Result of FSH and LH for combined consumption of *Xylopiya Aethiopic* 50mg/kg body weight & *Tetrapleura tetraptera* 50mg/kg body weight and the Control group.

	Hormone levels	Hormone levels	Mean Difference	t-test (p Value)
	Control	<i>X. aethiopic</i> + <i>T. tetraptera</i>	(95% CI)	
	Mean \pm SD	Mean \pm SD		
FSH	0.4 \pm 0.0	0.6 \pm 0.1	-0.2 (-0.3 – -0.1)	7.16 (0.001*)
LH	0.3 \pm 0.0	0.5 \pm 0.1	-0.2 (-0.3 – -0.2)	7.15 (0.001*)

FSH – Follicle stimulating hormone; LH – Luteinizing hormone; *statistically significance.

Table 3 shows the effect of combined intake of *Xylopiya aethiopic* and *Tetrapleura tetraptera* on FSH

and LH. There was significant p value ($P < 0.05$) increase in the FSH and LH when compared with the control.

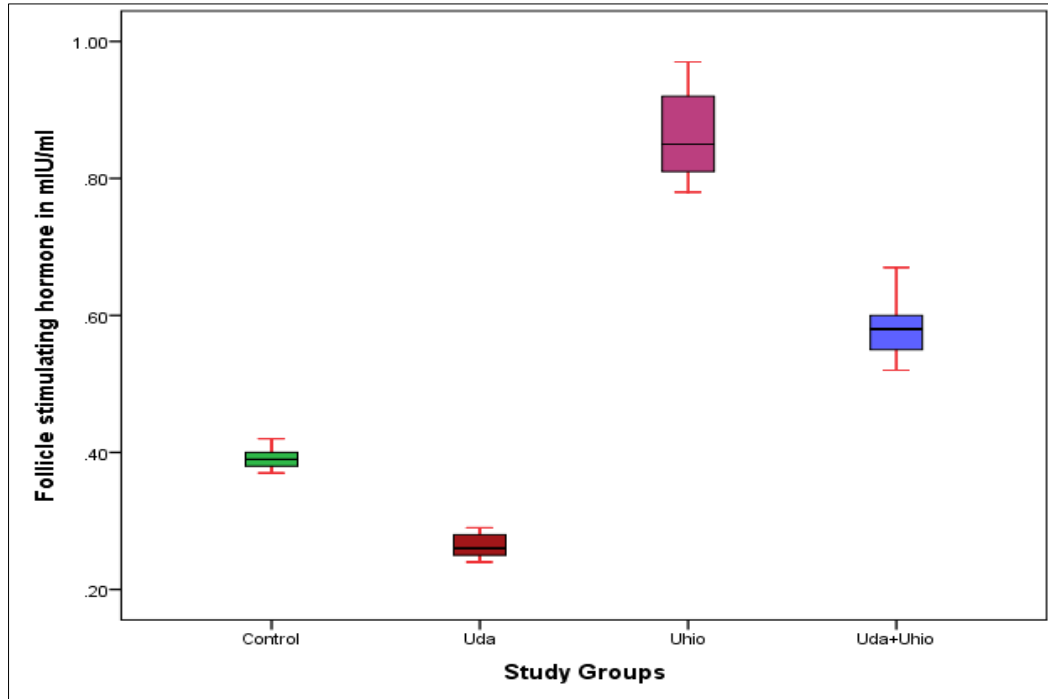


Figure 1: Follicle Stimulating Hormone of Female Swiss Mice on the *Xylopi aethiopia* (Uda), *Tetrapleura tetraptera* (Uhio), *Tetrapleura tetraptera* + *Xylopi aethiopia* (combined) and Control groups

Figure 1 Compares the levels of Follicle stimulating hormone of female Swiss mice on the *Xylopi aethiopia*, *Tetrapleura tetraptera*, *Tetrapleura tetraptera* + *Xylopi aethiopia* and Control groups. FSH was highest among the group fed with only

Tetrapleura tetraptera (Uhio) extracts, followed by the group fed with combined extract. Those fed with *Xylopi aethiopia* had the lowest FSH level, even lower than those of the control group.

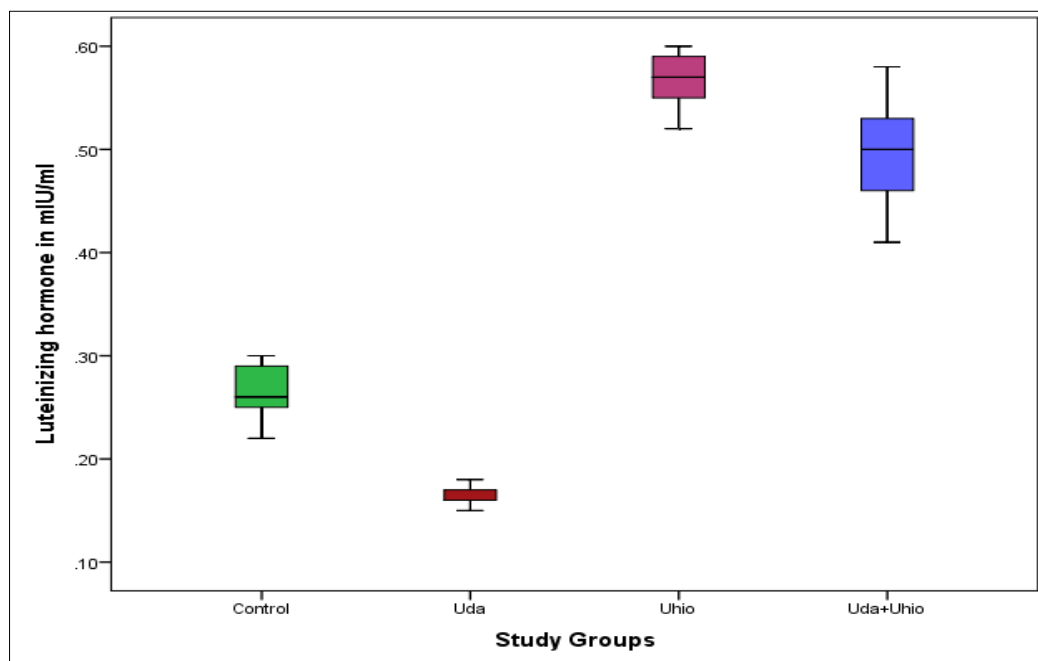


Figure 2: Luteinizing Hormone level of female Swiss mice on *Xylopi aethiopia* (Uda), *Tetrapleura tetraptera* (Uhio), *Tetrapleura tetraptera* + *Xylopi aethiopia* (combined) and Control groups

Figure 2 Compares the levels of Luteinizing Hormone of female Swiss mice on the *Xylopi aethiopia* (Uda), *Tetrapleura tetraptera* (Uhio), *Xylopi*

aethiopia + *Tetrapleura tetraptera* and Control groups. LH was highest among experimental animals fed with only *Tetrapleura tetraptera* (Uhio) extracts, followed by

the one fed with combined extracts. Those fed with *Xylopiya aethiopicia* (Uda) only had LH level lower than all including the control group.

DISCUSSION

This experiment was done to analyse the effect of *X. aethiopicia* and *T. tetraptera* administered individually, and in combined form on selected reproductive hormones using female Swiss white mice as the experimental animals. The study of *Xylopiya aethiopicia* on selected reproductive hormones showed that the level of serum Follicle Stimulating Hormone and Luteinizing Hormone were significantly decreased in group administered with *Xylopiya aethiopicia* (Uda) seed extract. There was significant decrease ($P < 0.05$) in the levels of all hormones when compared with the control and other groups. This decrease in this study is like other works done by Nnodim, *et al.*, 2013; Onuka *et al.*, 2017; Godam *et al.*, 2021. *Xylopiya aethiopicia* was noticed to reduce luteinizing hormone and luteinizing hormone levels (Adienbo *et al.*, 2021). Saponins, as found in *Xylopiya aethiopicia*, when administered can alter LH levels by LH gonadotropins inhibition (Agbai *et al.*, 2017; Adienbo *et al.*, 2021) which can lead to, reduction in the level of Oestrogen. LH from the pituitary gland regulates production of androgens from the theca cells. A decrease may lead to low concentration of progesterone and estrogen (Jimmy *et al*; 2023). In this study, consumption of seed extract of *T. tetraptera* (Uho) caused a significant increase in the serum Follicle Stimulating Hormone and Luteinizing Hormone. The result gotten showed a significant rise ($p < 0.05$) in levels of LH & FSH in the group fed with *Tetrapleura tetraptera* when compared with the control group. This may be because of negative feedback mechanism of decreased progesterone and oestrogen levels from the tissues of the ovaries due to the adverse effect of *Tetrapleura tetraptera* toxicity. In this study, the oral administration of combined extracts of *Xylopiya aethiopicia* and *Tetrapleura tetraptera* caused a significant rise in the levels of Luteinizing Hormone and Follicle Stimulating Hormone. A study done by Obulor *et al.*, (2022) on oral administration of lambda cyhalothrin and the three local spices (20 mg/kg/ body weight/day of *Tetrapleura tetraptera*, *Piper guineense*, and *Xylopiya aethiopicia*) on mice showed decreased levels of FSH and LH. This study suggests that the significant increase of FSH and LH in experimental mice on *Tetrapleura tetraptera* and *Xylopiya aethiopicia* combined may be due to ovarian failure, leading to a possible loss of negative feed-back mechanism of oestradiol on the hypothalamic-hypophysial axis. These results show that breastfeeding mothers in sub-Saharan Africa who take *Xylopiya aethiopicia* as a spice during postpartum period may benefit from it as a contraceptive while those who take *Tetrapleura tetraptera* may not. It also shows that *Tetrapleura tetraptera* may be of help in enhancing fertility in sub fertile women.

CONCLUSION

Whereas the mice on *Xylopiya aethiopicia* only had significant decrease in both hormones, those fed with *Tetrapleura tetraptera* only and those fed with combined extracts of *Xylopiya aethiopicia* and *Tetrapleura tetraptera* had similar results on the hormones, which was an increase in the hormone levels.

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