OPEN ACCESS
Scholars International Journal of Anatomy and Physiology
Abbreviated Key Title: Sch Int J Anat Physiol
ISSN 2616-8618 (Print) |ISSN 2617-345X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Tetrapleura tetraptera and *Xylopia aethiopica* Consumption Fluctuate Estrogen Levels

Jimmy Etukudo Okon[1*], Bassey Grace Edet[2], Nwachukwu Favour Obed[3], Amagwu, Patrick Iberi[4]

[1,2,3]Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Nigeria
[4]Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo, Nigeria

DOI: 10.36348/sijap.2023.v06i06.002 | **Received:** 17.04.2023 | **Accepted:** 25.05.2023 | **Published:** 24.06.2023

***Corresponding author:** Jimmy Etukudo Okon
Department of Medical Physiology, Faculty of Basic Medical Sciences, University of Uyo, Nigeria

Abstract

Extract of *Tetrapleura tetraptera* and *Xylopia aethiopica* have been used in the management and treatment of various ailments. This study investigated and compared the effects of ethanolic extract of both plants on estrogen in female wistar rats. Twenty-four rats were randomly divided into eight groups (n=3): one group for control and groups 2-8 served as experimental test groups. At the end of twenty-five days of oral administration of *Tetrapleura tetraptera and Xylopia aethiopica* extract, the test rats were sacrificed under chloroform anaesthesia and estrogen was measured using enzyme-linked immunosorbent assay (ELISA) method. Results showed significant reduction in serum estrogen after administration of low dose of *Tetrapleura tetraptera* in Group 2 rats at $P < 0.05$, but a non-significant increase and decrease in serum estrogen level in Groups 3 and 4 respectively at $P < 0.05$. *Xylopia aethiopica* extract caused a non-significant decrease in serum Estrogen level in Groups 5 and 6, but a non-significant increase in estrogen level of Group 7 at $P < 0.05$. In contrast, serum estrogen was significantly increased in Group 8 rats at $P < 0.05$ when *Tetrapleura tetraptera* and *Xylopia aethiopica* extracts were administered simultaneously. In conclusion, results obtained in this study suggest that individually, both *Tetrapleura tetraptera* and *Xylopia aethiopica* can affect the secretion and concentration of estrogen. The combination of extract of both plants could exert a positive effect on female fertility by increasing the levels of Estrogen.

Keywords: *Tetrapleura tetraptera, Xylopia aethiopica, Estrogen, infertility, Female infertility, Herbal medicine*.

INTRODUCTION

Infertility is a prevalent reproductive health issue characterized by the inability to achieve pregnancy after 12 months of regular unprotected intercourse (World Health Organization, 2023). It affects more than 10% of the global population and approximately 30% of the population in Sub-Saharan Africa (Menashe-Oren & Sánchez-Páez, 2023; Macrotrends, 2023). The causes of infertility vary, with ovulatory disorders (25%), fallopian tubal damage (20%), uterine or peritoneal abnormalities (10%), and endocrine system disorders (resulting in hormonal imbalances) being the most common. However, 30% of cases remain unknown (Samare-Najaf *et al.,* 2023; Ennab & Atiomo, 2023).

In Nigeria, traditional plant preparations have long been utilized for reproduction-related purposes and are well- documented (Al-Tawalbeh *et al.,* 2023; Salmany *et al.,* 2023; Magtalas *et al.,* 2023; Nsagha *et al.,* 2020). Tetrapleura tetraptera and Xylopia aethiopica are two such medicinal plants known for their ethno-medicinal uses and are reported to influence endocrine activities due to their hormonal balancing effects and various phytochemicals and minerals like: flavonoids, tannins, alkaloids, sugar, saponins, folic acid, crude proteins, copper, steroids, cardiac glycosides, oxalates, phytate, magnesium, phosphorus, potassium, calcium, iron, sodium, zinc, phenolic compounds and vitamins A, B, C D and E (Okata-Nwali *et al.,* 2023; Falana *et al.,* 2023; Jimmy & Ekpo, 2016; Tiwari & Sahu, 2017).). Saponins and flavonoids have been found to inhibit aromatase enzyme activity, affecting estrogen production (Omodamiro *et al.,* 2012; Ogbuagu *et al.,* 2020). These plants have been traditionally used as natural contraceptives for child spacing and in postpartum therapy to aid recovery, uterus shrinking, expulsion of retained placenta, and promotion of lactation (El Izzi *et al.,* 1990).

Studies have reported the effects of Tetrapleura tetraptera and Xylopia aethiopica on reproductive hormones. Tetrapleura tetraptera has been shown to decrease estrogen levels, exhibit aphrodisiac potential and impact spermatogenesis (Agbai *et al.*, 2019; Adelakun *et al.*, 2021). Xylopia aethiopica has been found to decrease estrogen (Chibuogwu *et al.*, 2022) progesterone, and luteinizing hormone (LH) levels (Onuka *et al.*, 2017), increase prolactin levels (Ehigiator & Adikwu, 2020; Ogbuagu *et al.*, 2022), and reduce LH, follicle-stimulating hormone (FSH), and testosterone levels (Adienbo *et al.*, 2021). However, there are contrasting findings regarding the effect of Xylopia aethiopica on estrogen levels (Ehigiator & Adikwu, 2020; Ogbuagu *et al.*, 2022).

Estrogen plays a crucial role in fertility by facilitating the development of the uterine lining and promoting the maturation of reproductive organs (Balló *et al.*, 2023). It is commonly used in fertility treatments. Tetrapleura tetraptera and Xylopia aethiopica have been documented for their role in fertility regulation and reproduction due to their diverse ethno-medicinal uses (El Izzi *et al.*, 1990; Stadtlander *et al.*, 2013; Ugiomoh *et al.*, 2023). However, there is a lack of investigation on their combined physiological effect on estrogen levels. Therefore, this study aims to examine and compare the impact of ethanolic extracts of Tetrapleura tetraptera and Xylopia aethiopica on estrogen levels in female Wistar rats.

Experimental Section

Collection and Identification of Plant Materials

The dried fruits of *Tetrapleura tetraptera* and *Xylopia aethiopica* were purchased from the market and were identified and authenticated at the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo Town Campus, Uyo.

Preparation of Ethanolic Extract of *Tetrapleura tetraptera* and *Xylopia aethiopica*

The dried fruits of *Tetrapleura tetraptera* and *Xylopia aethiopica* were chopped into small pieces and pounded into smaller, coarse pieces using a wooden mortar and pestle. The coarse forms of *Tetrapleura tetraptera* and *Xylopia aethiopica* were weighed to be 476.7g and 446.6g respectively. Each was macerated in 60% ethanol and 40% distilled water and allowed to stand for 72 hours in an air tight container, after which each plant materials were filtered and the filtrate was concentrated at 45°C in a water bath and left to dry. Concentrated forms of *Tetrapleura tetraptera* were again weighed to be 117.6g and *Xylopia aethiopica* to be 133.2g. After concentration, the extracts were preserved in a refrigerator until used.

Acute Toxicity Studies (Ld_{50})

Acute Toxicity (LD_{50}) was determined using thirty-three (33) mice which were weighed between 18g-32g and divided into 11 groups of three mice (n=3). *Tetrapleura tetraptera* and *Xylopia aethiopica* extract were administered intraperitoneally using the Method of Lorke's (1983). From the results obtained, the calculated median lethal dose of *Tetrapleura tetraptera* extract was 1369.3mg/kg and *Xylopia aethiopica* was 2121.3mg/kg. This formed the dosage for administration on the rats.

Preparation of Stock Solution

1g of ethanolic extract of *Tetrapleura Tetraptera* and *Xylopia Aethiopica* were each dissolbed in 10ml of distilled water as follows:
(1g ÷ 10ml) = 1000mg, where 1g = 1000mg.
Stock solution = 100mg/ml
Therefore, actual dosage = [weight of animal (kg) × dose of drug (mg/kg)] ÷ Stock concentration (mg/ml).

Experimental Animals

Twenty-four female albino wistar rats weighing between 143g- 248g were used in this study. These animals were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Akwa Ibom State, Nigeria, following the institutional ethical approval. They were kept under standard laboratory conditions, fed with commercial grower mash. Water and feeds were provided ad libitum. The animals were left for two weeks to acclimatize before experimentation.

Experimental Design

The animals were divided into 8 groups of 3 animals each (n=3) according to their body weight. The extracts were administered orally and administration lasted for 25 days.

Group 1: Control group, administered distilled water.
Group 2: Administered 136.9mg/kg/day of ethanolic extract of *Tetrapleura tetraptera.*
Group 3: Administered 273.8mg/kg/day of ethanolic extract of *Tetrapleura tetraptera*.
Group 4: Administered 410.8mg/kg/day of ethanolic extract of *Tetrapleura tetraptera*.
Group 5: Administered 212.1mg/kg/day of ethanolic extract of *Xylopia aethiopica* extract.
Group 6: Administered 424.3mg/kg/day of ethanolic extract of *Xylopia aethiopica* extract
Group 7: Administered 636.4mg/kg/day of ethanolic extract of *Xylopia aethiopica* extract.
Group 8: Administered 273.8mg/kg/day of ethanolic extract of *Tetrapleura tetraptera* and 424.3mg/kg of ethanolic extract of *Xylopia aethiopica* simultaneously.

For each animal, the dosage of the drug to be given was calculated per weight of the animal:

Actual dosage = [weight of animal (kg) × dose of drug (mg/kg)] ÷ Stock concentration (mg/ml)

Collection of Blood Samples

At the end of twenty-five (25) days, 24 hours after the last dose of the extract and distilled water was administered, the animals were weighed again and sacrificed under chloroform anaesthesia. About 5ml of blood was collected from the rats via cardiac puncture into labeled plain vacutainer tubes and centrifuged. The serum samples obtained were then stored at appropriate temperature until they were profiled for Estrogen.

Assay Method for Estrogen

The quantitative determination of estrogen levels in serum were measured using Enzyme-Linked Immunosorbent Assay (ELISA) method.

Statistical Analysis

Results were expressed as mean ± Standard Error of Mean (SEM). Statistical significance of difference observed between control and experimental groups was analyzed using one-way Analysis of Variance (ANOVA). Any significant ANOVA was further analyzed by Tukeys post hoc test. P values < 0.05 were considered statistically significant.

RESULTS

The findings of this present study on the comparative effect of *Tetrapleura tetraptera* and *Xylopia aethiopica* on estrogen level of female Wistar rats are presented below:

Table 1: Effect of *Tetrapleura tetraptera* on estrogen

Group	***Dose***	***Dosage of extract administered (mg/kg)***	***Mean Estrogen (miU/ml)***	***P-value***
Group 1	Control		14.18 ± 0.50	0.05
Group 2	Low dose	136.9	11.38 ± 0.04*	0.05
Group 3	Middle dose	273.8	15.62 ± 0.11	0.05
Group 4	High dose	410.8	12.44 ± 0.07	0.05

*Significant at P<0.05

Table 1 table showing the effect of low, middle and high doses of *Tetrapleura tetraptera* on estrogen level. Only the low dose has a statistically significant effect on estrogen level when compared with the control group at P<0.05

Table 2: Effect of *Xylopia aethiopica* on estrogen

Group	***Dose***	***Dosage of extract administered (mg/kg)***	***Mean Estrogen level (miU/ml)***	***P-value***
Group 1	Control		14.18 ± 0.50	0.05
Group 5	Low dose	212.1	9.27 ± 0.18	0.05
Group 6	Middle dose	424.3	8.85 ± 0.15	0.05
Group 7	High dose	636.4	15.36 ± 0.44	0.05

Table 2 table showing the effect of low, middle and high doses of *Xylopia aethiopica* on estrogen level. Each of the dose has no statistically significant effect on estrogen level when compared with the control group at P<0.05.

Table 3: Effect of *Tetrapleura tetraptera* and *Xylopia aethiopica* on estrogen

Group	***Dose***	***Dosage of extract administered (mg/kg)***	***Mean Estrogen level (miU/ml)***	***P-value***
Group 1	Control		14.18 ± 0.50	0.05
Group 8	Middle dose of *T. tetraptera* + middle dose of *X. aethiopica*	273.8 + 424.3	21.69 ± 0.002*	0.05

*Significant at P<0.05

Table 3 table showing the effect of combined middle doses of *Tetrapleura tetraptera* and *Xylopia aethiopica* on estrogen level. The combined middle dose significantly increased estrogen level at P<0.05.

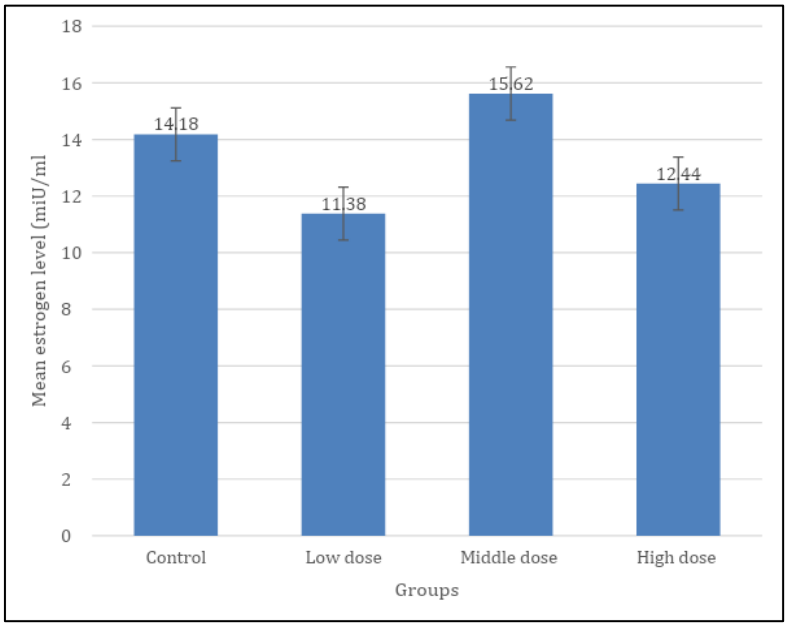

Figure 1: Comparative effect of *Xylopia aethiopica* on estrogen level

Figure 1 bar chart showing the effect of *Tetrapleura tetraptera* on estrogen compared with control group. In the figure 1 above, low dose of *Tetrapleura tetraptera* (11.38 ± 0.04 miU/ml) caused significant reduction (at P < 0.05) in estrogen levels when compared with the control group (14.18 ± 0.50 miU/ml). Middle dose (15.62 ± 0.11 miU/ml) and high dose (12.44 ± 0.07 miU/ml) of *Tetrapleura tetraptera* did not cause any significant effect on estrogen level of when compared with the control group (14.18 ± 0.50 miU/ml) at P < 0.05.

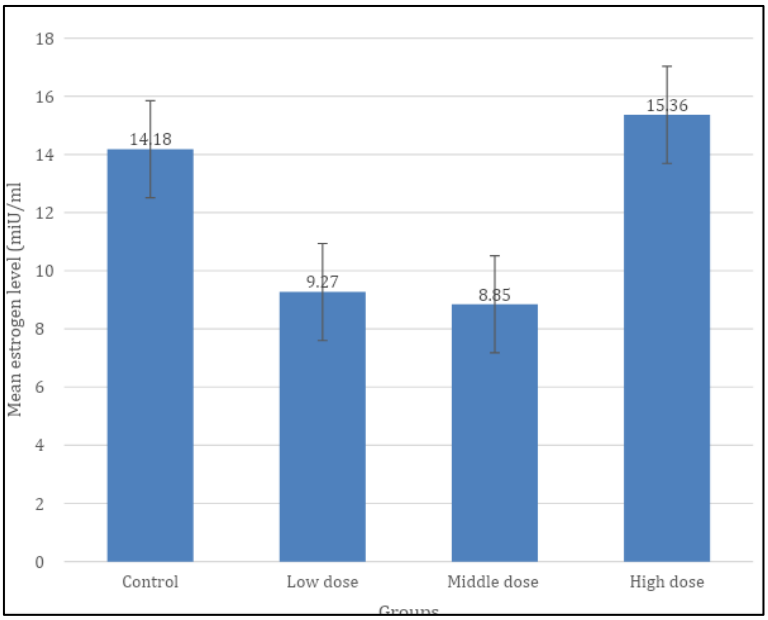

Figure 2: Comparative effect of Xylopia aethiopica on estrogen

Figure 2 bar chart showing the comparative e*ffect of Xylopia aethiopica on* estrogen.

In the figure 2 above on the comparative effect *of Xylopia aethiopica* on estrogen, there was no statistically significant difference in estrogen levels in the low dose (9.27 ± 0.18 miU/ml) and medium dose (8.85 ± 0.15 miU/ml) when compared with the control (14.18 ± 0.50 miU/ml) at P < 0.05. However, high dose of *Xylopia aethiopica* caused an increase in estrogen level in the high dose group (15.36 ± 0.44 miU/ml), which was not significant when compared with control group (14.18 ± 0.50 miU/ml) at P < 0.05.

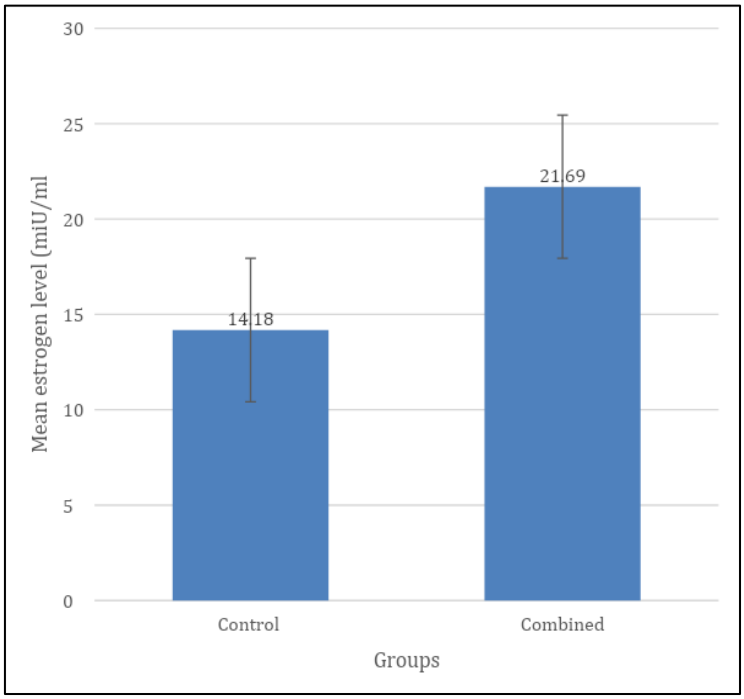

Figure 3: Comparative effect of combined middle doses of *Tetrapleura tetraptera and Xylopia aethiopica on estrogen*

Figure 3 bar chart showing the effect of combined doses of *Tetrapleura tetraptera and Xylopia aethiopica* on estrogen level compared with the control group. In the figure 3 above, combined middle doses of *Tetrapleura tetraptera* and *Xylopia aethiopica* extracts caused a statistically significant increase in estrogen level in the female Wistar rats (21.69 ± 0.002 miU/ml) compared to the control group (14.18 ± 0.50 miU/ml) at $P < 0.05$. This signifies a novel discovery of their combined potential in the treatment of female infertility.

DISCUSSION

Plant-derived chemicals have been reported to have the ability to influence endocrine activities and interfere with the normal functioning of the endocrine system (Al-Tawalbeh *et al.*, 2023). *Tetrapleura tetraptera* and *Xylopia aethiopica* have been reported to alter reproductive hormones secretions and concentration (Agbai *et al.*, 2019; Onuka *et al.*, 2017; Ehigiator & Adikwu, 2020; Ogbuagu *et al.*, 2022; Adienbo *et al.*, 2021).

The results of this present study of *Tetrapleura tetraptera* and *Xylopia aethiopica hormonal function* on estrogen show that the level of serum Estrogen was significantly decreased in test rats administered low dose of *Tetrapleura tetraptera* fruit extract. There was also a non-significant slight decrease in the Estrogen level in test rats administered with high dose of *Tetrapleura tetraptera* fruit extract when compared with the control. This decreasing effect of *Tetrapleura tetraptera* based on this study is similar to findings of Agbai *et al.*, (2019). They reported that *Tetrapleura tetraptera* caused a reduction in luteinizing hormone and estrogen during proestrous phase of the estrous cycle. It was also observed that in test rats administered middle dose of *Tetraplera tetraptera*, there was an increase in the estrogen level when compared to the control. This increase, however, was not significant.

Observations from this present study also revealed that low dose and high dose of *Xylopia aethiopica* extract decreased serum estrogen level. The decrease was not significant when compared with the control group. Several studies have reported this inhibiting effect of *Xylopia aethiopica* on estrogen levels (Agbai *et al.*, 2019; Onuka *et al.*, 2017; Ogbuagu, 2022). A study conducted by Chibuigwu *et al.*, (2022) reported that *Xylopia aethiopica* showed a significant increase in oestradiol and no effect on serum progesterone levels.

The low serum levels of estrogen seen in this study might be linked to a reduction in the activity of ovarian aromatase, which is critical for estrogen production, and could be from the phytochemical components of the extracts under study. This aligns with several reports that saponins and flavonoids inhibits aromatase enzyme in human preadipocytes (Omodamiro *et al.*, 2012; Ogbuagu *et al.*, 2020; El Izzi *et al.*, 1990; Stadtlander *et al.*, 2013). Saponins from *Xylopia aethiopica* administration have been found to impair LH levels by inhibiting its LH gonadotropes (Agbai *et al.*, 2017; Adienbo *et al.*, 2021; Izzi *et al.*, 1990) and consequently, a decrease in estrogen levels. Androgen synthesis from the thecal cells is predominantly regulated by LH from the pituitary.

Reduced LH levels flaws androgen synthesis and impairs estrogen derivation from androgens, leading to a decline in the concentration of estrogen (Ogbuagu *et al.,* 2022). The significant quantity of saponin recorded from the qualitative phytochemical analysis of *T. Tetraptera* and *X. aethiopica* extract in this study justifies this.

Combined middle doses of *Tetrapleura tetraptera* and *Xylopia aethiopica* pods extract showed significant increase in Estrogen level. It could be hypothesized that this increased serum Estrogen level might be a synergistic inhibitory effect or a summation of the inhibitory effect of both plants extract on Estrogen level when they are consumed simultaneously. The presence of aromatase enzyme activity in granulosa cells of the follicle, resulting in conversion of androgen to Estrogen might be implicated in the high estrogen so observed in this current study (Omodamiro *et al.,* 2012; Ogbuagu *et al.,* 2020). High levels of Estrogen exert a positive feedback on hypothalamic release of GnRH leading to a surge of FSH and LH thereby facilitating the growth of Graafian follicle in preparation of ovulation. Estrogen levels in the bloodstream have been known to be highest during ovulation in preparation for pregnancy. During ovulation, the Graafian follicle rupture with consequent discharge of ovum which enters the fallopian tube in preparation for fertilization (Ogbuagu *et al.,* 2020). Because an optimal balance between estrogen and preogesterone levels are required for pregnancy, further studies should be done to determine the effect of the combined extract of Tetrapleura tetraptera and Xylopia aethiopica on the Estrogen-Progesterone ratio as in the case of Estrogen dominance. It should also determine the effect of the combined extract of Tetrapleura Tetraptera and Xylopia aethiopica on fertilization, the mating tendency and the product of mating.

CONCLUSION

Based on the results in this study, it is likely that simultaneous consumption of *Tetrapleura tetraptera* and *Xylopia aethiopica* could improve a woman's chances of getting pregnant by increasing the levels of Estrogen.

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