



Tetrapleura tetraptera (Schum and Thonn) Taub Fruit Extract Impairs Reproductive Hormones and Fertility Parameters in Female Experimental Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author OMA designed the study, performed the statistical analyses, wrote the protocol and wrote the first draft of the manuscript. Author OSE managed the analysis of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The increasing rate of infertility in humans has necessitated the need to assess commonly used medicinal plants to ascertain their effects on reproductive functions. The aim of this study was to determine the effects of *tetrapleura tetraptera* fruit extract on reproductive hormones and fertility in females.

Methods: A total of seventy two (72) adult female *wistar* rats weighing 160-180 g were randomly assigned into four groups of 18 animals each. Group1 (control) was administered distilled water, groups II, III and IV were daily treated with the extract at doses of 75, 150 and 300 mg/kg body weight, respectively, orally for 28 days. Thereafter, 6 rats from each group were sacrificed during oestrous phase and serum used for analyses of reproductive hormones; while remaining 12 females from each group were each cohabited with males (2:1), sacrificed at gestational age 16 and fertility parameters were determined.

Results: The results show that in the test groups, there was significant ($P < 0.05$) reduction in the levels of FSH, LH and Estrogen, with a significant ($P < 0.05$) increase in progesterone, when

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compared with the control. Also, the number of pregnant female rats, number of foetuses, and fertility index significantly ($P < 0.05$) increased in the test animals, compared with the control.

Conclusion: We concluded that *Tetrapleura tetraptera* fruit extract impairs reproductive hormones and fertility parameters in female rats, and therefore should be consumed with caution.

Keywords: *Tetrapleura tetraptera*; reproductive hormones; fertility index; antifertility; infertility.

1. INTRODUCTION

Infertility, the inability of a sexually active, non-contracepting couple to attain pregnancy in one year, [1] is estimated to affect 1 in every 13 couples globally [2] while in developing countries 1 in every 4 couples is infertile [3]. Such infertile couples suffer from attendant high social, psychological and financial burden. Also, a large number of chemical compounds, including plant extracts are used to control fertilization [4] as well as alter the concentrations and functions of female reproductive hormones [5,6,7]. In Nigeria, the folkloric uses of plant preparations for reproduction-related purposes are well known and documented [8,9].

Since plant preparations play important role in fertility regulation, assessment of commonly used medicinal plants to ascertain their effects on reproductive functions, especially on fertility and reproductive hormones becomes very necessary, especially for the infertile couples desiring conception. One of such important plant is *Tetrapleura tetraptera*, commonly known as Aridan. It is a flowering plant belonging to pea or Mimosaceae family, and is found in tropical Africa lowland forests, especially from West Africa. *Tetrapleura tetraptera* has many medicinal uses, from its leaves, fruits, bark and pods. Different ethnic groups have different uses for *T. Tetraptera*. In the Niger Delta region of Nigeria, the fruits and seeds are used for treatment of various ailments including malaria [10,11]. In southern parts of Nigeria, the pods are commonly used spices in preparing local dishes and special delicacies [12,13]. In northern Nigeria, the fruits are also used in preparing soup for mothers after delivery in order to prevent post-partum contraction of the uterus [14], as well as for stimulation of lactation [15,16]; thus suggesting possible effects of the fruits on the female reproductive system. We therefore hypothesise that *T. Tetraptera* fruit extract has significant effects on reproductive functions in females. The objectives of this study therefore were, to evaluate the effects of *T. Tetraptera* fruits on serum reproductive hormones and

fertility parameters in females, using *wistar* rats as animal model.

2. MATERIALS AND METHODS

2.1 Plant Materials and Extraction

Dry fruits of *Tetrapleura tetraptera* purchased from oil mill market in Port Harcourt, Rivers State, Nigeria, were identified and authenticated by taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria, with herbarium number, UPH/C/104. The fruits were washed clean, dried, sliced into small pieces and then machine-grounded into fine, powdery particles.

Using the cold extraction method, 3000g of the finely crushed plant sample was stocked in 9L hydro-methanol solvent (20:80) in an aspirator jar. The mixture was shaken vigorously for 30 minutes and was left to stand for 72 hours at room temperature ($25 \pm 2^\circ\text{C}$). The resulting solution was then filtered using a wire gauze and a sieve with tiny pores (0.25mm). The filtrate was concentrated to dryness at 40°C by vaporizing in an oven, and then stored in a refrigerator. The percentage yield of the extract was 24.55%.

2.2 Phytochemical Screening

In order to determine the presence of various plant constituents in the plant extract, a preliminary phytochemical screening was done as previously described [17].

2.3 Experimental Animals

Healthy, non-pregnant adult female *wistar* rats weighing 160-180g were obtained from the animal house facility of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt. The rats were housed in wire-meshed wooden cages at natural environmental conditions of temperature ($25 \pm 2^\circ\text{C}$) and natural light: dark cycle, allowed to acclimatise for 10 days, and had free access to standard rat chow and water *ad libitum*. They were cared for and managed in accordance with

the guidelines of the U.S. National Institute of Health (NIH) on the care and use of laboratory animals (NIH Publication, No. 85-23, revised 1985).

2.4 Experimental Design

A total of seventy two (72) female *wistar* rats showing regular 4-5 days oestrus cycles were randomly assigned into four groups of 18 animals each. Group 1 (control) was administered 1 ml of distilled water; groups II, III and IV were daily treated with the *Tetrapleura tetraptera* extract at doses of 75, 150 and 300 mg/kg body weight, respectively. A stock solution of the extract was constituted in distilled water to give the doses required for the study. All administrations were done by oral gavage once a day (8.00 am - 10.00 am), for 28 days, starting when animals were in the proestrus phase of the oestrous cycle.

2.5 Collection and Analyses of Samples

On day 28 of the study, after the last administration, all animals in proestrous phase were fasted overnight, sacrificed the following day using chloroform anaesthesia while in oestrous phase. A total of 6 rats from each group were sacrificed. Blood samples (4 ml) were collected by cardiac puncture into plain sample bottles. The blood was centrifuged for 15 minutes at 2000 rpm, the serum portions separated and stored at -20°C, then later used for hormone analyses.

2.6 Effect of *Tetrapleura tetraptera* Fruit Extract on Reproductive Hormones

The quantitative determination of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone was done using enzyme-linked immunoassay technique (ELISA), as previously reported [18,19].

2.7 Effect of *Tetrapleura tetraptera* Fruit Extract on Fertility

To evaluate the effect of the extract on female fertility, twelve (12) female rats from each group were cohabited with males of proven fertility, in a female:male (2:1) ratio, in separate cages for 10 days. Administration of extract continued throughout the mating period of the study. The female rats were observed every morning to confirm mating by checking for the presence of vaginal mucous plug, and for spermatozoa in

vaginal smear using a light microscope. Those found positive were counted as first day of pregnancy, and such female rats were isolated in separate cages, extract treatment discontinued, sacrificed on day 16 of pregnancy and hysterectomy done. The number of pregnant rats in each group, and the number of foetuses in each female rat were counted, and the fertility index and relative difference in fertility for each group calculated.

2.8 Statistical Analysis

Statistical analysis of the data was done using statistical package for social sciences (SPSS) software (version 25.0). Differences in mean between groups was evaluated using Analysis of variance (one-way ANOVA) followed by Dunnett multiple comparison test. Results were considered statistically significant at $P < 0.05$ and 95% confidence level. The results are presented as mean and standard error of mean ($M \pm S.E.M$).

3. RESULTS

3.1 Phytochemical Constituents of *T. Tetrapleura* Fruit Extract

The results of the phytochemical screening showed that the extract of *Tetrapleura tetraptera* contains alkaloids, tannins, saponin, flavonoids, cardiac glycosides, phlobatanins, phytates and polyphenols.

3.2 Effect of *Tetrapleura tetraptera* Fruit Extract on Serum Reproductive Hormones

A dose-dependent reduction in the serum levels of gonadotropic hormones (Fig.1) was observed: Luteinizing hormone significantly ($P < 0.05$) decreased in all the extract-treated groups, while Follicle stimulating hormone decreased at 75 mg/kg b.w ($p > 0.05$); 150 mg/kg b.w ($p < 0.05$) and 300 mg/kg b.w ($p < 0.05$) extract-treated groups respectively, compared to the control group animals. For the female sex hormones (Fig. 2), Estrogen decreased in the test groups treated with 75 mg/kg b.w ($p > 0.05$), 150 mg/kg b.w ($p < 0.05$) and 300 mg/kg b.w ($p < 0.05$) respectively, compared to the control group animals. Progesterone, on the other hand, increased significantly ($P < 0.05$) in all the extract-treated groups, compared to the control group animals.

3.3 Effect of *Tetrapleura tetraptera* Fruit Extract on Fertility Parameters in Female Wistar Rats

The result for the effect of the extract on fertility (Table 1), shows significant ($P < 0.05$) reductions in the number of pregnant female rats, number of

foetuses and, the fertility index respectively, in the test groups, compared to the control group. The relative reduction in fertility was by 18.18% for the 75 mg/kg extract treated group ($p > 0.05$), and 45.46% for both the 150 mg/kg ($p < 0.05$) and 300 mg/kg ($p < 0.05$) groups respectively, compared to the control group rats.

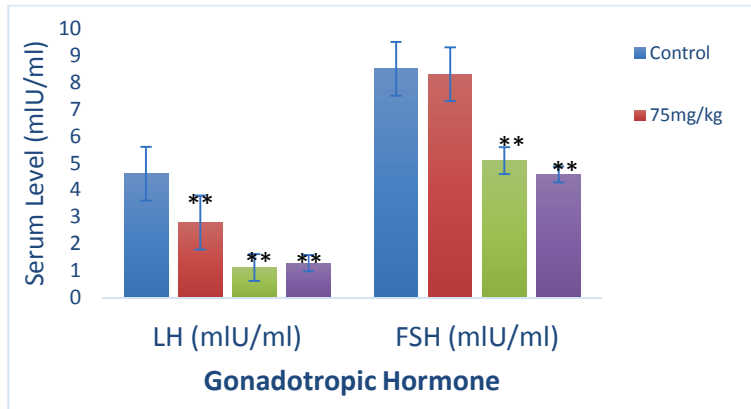


Fig. 1. Effect of *Tetrapleura tetraptera* fruit extract on serum gonadotropin hormones in female wistar rats. Bars represent mean value. (N = 6); where $P \leq 0.01^{**}$

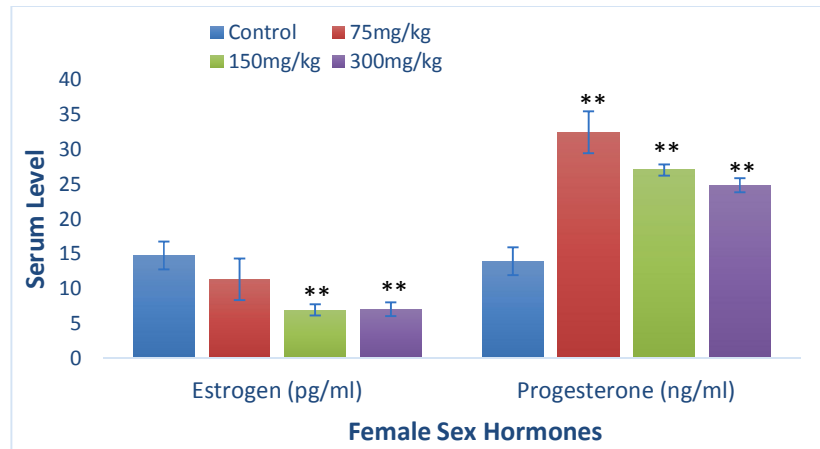


Fig. 2. Effect of *Tetrapleura tetraptera* fruit extract on serum female sex hormones in wistar rats. Bars represent mean value; (N = 6); where $P \leq 0.01^{**}$

Table 1. The Effects of *tetrapleura tetratera* fruit extract on fertility parameters of female wistar rats

Treatment group	Number mated	Pregnant females	Number of foetuses (mean±SEM)	Fertility index (%)	Relative reduction in fertility (%)
Control	12	11	6.00 ± 0.63	91.67	
75 mg/kg	12	9	5.00 ± 0.03	75	-18.18
150 mg/kg	12	6	3.17 ± 0.72*	50*	- 45.46*
300 mg/kg	12	6	4.17 ± 0.93*	50*	- 45.46*

Data is represented as mean±SEM. (N = 12); where $P < 0.05$ is statistically significant when compared to the control group. Fertility index (%) = (No of pregnant rats/No of rats that mated) x 100

4. DISCUSSION

From this study, *Tetrapleura tetraptera* fruit extract caused a reduction in the serum levels of FSH, LH and Estrogen while, progesterone increased. Also, there was a reduction in all the fertility parameters. The gonadotropins (FSH and LH) are secreted by the gonadotropic cells in the anterior pituitary gland, and regulated by gonadotropin releasing hormone (GnRH) secreted by the hypothalamus. Also, the female sex hormones are primarily produced in the ovaries under the influences of the gonadotropic hormones, through feedback mechanisms. Therefore, disruptions in these regulatory mechanisms could alter the serum levels of FSH, LH, oestrogen and progesterone. The reductions in FSH and LH levels in the test animals suggest that the extracts possibly acted at the hypothalamo-pituitary axis by either inhibiting or impairing the GnRH-secreting cells in the hypothalamus and, or by affecting the gonadotropic cells in the anterior pituitary gland, thereby impairing their secretions.

In females, Follicle stimulating hormone (FSH) controls growth and maturation of follicles produced in ovaries by directly acting on granulosa cells, while Luteinizing hormone (LH) stimulates secretion of estrogen in the ovaries as well as helping in ovulation of mature follicles. Maturation of pre-ovulatory follicles and ovulation are therefore under combined and balanced influences from gonadotropic hormones [20]. Therefore, the reduction in FSH may impede or hinder folliculogenesis thereby delaying maturation of ovarian follicle, especially in the pre-ovulatory phase [21], and therefore affecting conception; while the reduction in LH secretion could result to disruption of ovulation, and hence, impairment of estrous cycle and conception. These effects are evidenced in the observed reductions in number of pregnant females and fertility index. Similar studies have reported that some plant extracts caused a decrease in serum levels of FSH and LH [7,22]. Our results also agree with findings of Jarry et al, [23] where triterpenoid glycoside in methanolic and lipophilic extract of *Cimicifua racemosa* caused reduction of LH concentration. This suggests that the glycosides contained in *T. tetraptera* could also be implicated in the antigonadotropic effects observed in this study.

This study also shows that oestrogen level significantly decreased in the test animals, similar to the report of Osunuga et al [24] who

observed that leaf extracts of *Momordica Charantia* reduces estrogen levels of adult female *wistar* rats. Leuteinising hormone is known to stimulate ovarian follicles to synthesise testosterone which is then aromatised to estrogen by aromatase enzyme [25,26]. Therefore, the reduction in estrogen level observed in this study could be due to the decrease in LH level and, or by direct effect of the extract on the granulosa cells in the ovary, possibly by the alkaloids and flavonoids content of the extract which have been reported to reduce estradiol concentrations [27,28]. Progesterone level, on the other hand, was observed to significantly increase in the test groups, suggesting that the fruit extract of *Tetrapleura tetraptera* possibly stimulated an increased secretion of progesterone by the ovary or altered its regulatory mechanisms resulting to prolongation of the secretory functions of the corpus luteum. This agrees with the findings of Oluyemi et al. [29].

These effects of *Tetrapleura tetraptera* fruit extract in altering the serum levels of FSH, LH, Estrogen and Progesterone may explain the observed reductions in the fertility parameters: number of pregnant rats, number of foetuses, and fertility index in the extract-treated animals. *Tetrapleura tetraptera* contains flavonoids, alkaloids and saponins [30], which are known to exhibit antifertility activity [31,32] by directly influencing decrease in pituitary secretion of LH and FSH, and therefore impairing folliculogenesis, ovulation [32] and eventually fertility.

5. CONCLUSION

This present study has clearly shown that *Tetrapleura tetraptera* fruit extract impairs reproductive capacity in experimental animals by reductions in FSH, LH, oestrogen, pregnancy, foetuses and fertility index, with a sustained increase in progesterone level. Therefore, the fruits should be consumed with caution, especially by couples desiring conception.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Ethical approval was sort for and given by the research ethical committee of the university of Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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