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Original Research Article

Effect of ethanolic extract of *Justicia secunda* (blood root) leaves on reproductive organs and hormones in female Wistar rats

Elile P. Okpara[1], Progress D. Victor[2]*, Edith Reuben[1], Pearl C. Ajie[2], Lolia T. Fiala[1]

[1]Department of Human Physiology, [2]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria

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***Correspondence:**
Dr. Progress D. Victor,
E-mail: Progress.Victor@ust.edu.ng

ABSTRACT

Background: Several environmental chemicals are suspected to be responsible for adverse health effects on the reproductive system. Poisonous plants grow in most communities found on range lands and pastures. One major effect on consumption of these plants is on reproduction which includes birth defect, abortion and interference with Oogenesis, spermatogenesis, libido and estrous cycle. *Justicia secunda* (blood root) as the name implies is known for its anti-anemic properties.
Methods: Animals were grouped into 6 groups with 6 rats in each group. They were housed in metal cages at room temperature and had access to commercial standard rodent pellets and clean water. Group 1 (control 0.00 mg/kg.) was given distilled water, groups 2-6 were given 99% ethanolic extract of *Justicia secunda* leaves orally at dose levels of 300, 400, 400, 450 and 450 mg/day, respectively for 42 days.
Results: The result of this study indicates that ethanolic extract of *Justicia secunda* (blood root) was able to increase the level of FSH significantly at medium dose group. Significant increase in oestrogen level observed in this study.
Conclusions: ethanolic extract of *Justicia secunda* (blood root) leaves is likely to cause an increase in the secreting ability of cells of the anterior pituitary gland producing FSH or cells of the hypothalamus producing gonadotrophin releasing hormones. The plant is likely to contain steroids.

Keywords: Estrogen, FSH, Reproductive hormones

INTRODUCTION

The female reproductive hormones include: follicle stimulating hormone, lutenizing hormone, oestrogen, progesterone, prolactin. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are gonadotropins (glycoprotein-based) released by the anterior pituitary in response to stimulation by gonadotropin-releasing hormone (GnRH) and released by the hypothalamus. FSH stimulates the growth of ovarian follicles in the ovary prior to the release of an egg at ovulation and promotes estradiol production in females. In women, LH stimulates estrogen and progesterone production from the theca internal cells in the ovaries.[1] A surge of LH in the mid menstrual cycle is responsible for ovulation, and continued LH secretion subsequently stimulates the corpus luteum to produce progesterone. The anterior pituitary gland is responsible for the release of prolactin. The rates of infertility in less industrialized nations are markedly higher and infectious diseases are responsible for a greater proportion of infertility.[2]

Several environmental chemicals are suspected to be responsible for adverse health effects on the reproductive system.[3] Poisonous plants grow in most communities found on range lands and pastures. One major effect on consumption of these plants is on reproduction which includes estrus behaviour, estrous cycle length,

embryonic and fetal viability, and conception.[4] *Justicia secunda* (blood root) as the name implies is known for its anti-anemic properties but no study have been done on the effect of this plant on reproduction.[5] Hence the need for this study.

METHODS

The materials used throughout the research include: absolute ethanol, distilled water, beakers, measuring cylinder, weighing scale, cotton wool, soxhlet extractor, hot air oven, syringes (1 ml), oral gavage tube, 6 cages.

Experimental location

The experiment was carried out in the animal house of the department of human physiology, faculty of basic medical sciences, Rivers State University, Rivers state, Nigeria. And lasted for 6 weeks (42 days).

Type and duration of the study

It was an experimental study. This study was carried out from August 2021 to February 2022.

Experimental protocol

Twenty-four (24) 3-weeks old female Wistar albino rat weighing 18-33 g used in this study were purchased from the animal house of the department of physiology, Rivers State University, Rivers state, Nigeria. Animals were grouped into six (6) groups, with each group containing four (4) animals.

Animals had access to commercial standard rodent pellets and clean water which was changed twice daily. Their cages were cleaned daily. The animals were acclimatized for fourteen days before commencement of the experiment. The experiments were conducted according to the approved Institutional animal care guidelines of the Rivers State University, Nigeria.

Plant material

Fresh leaves of *Justicia secunda* of the family *Acanthaceae* was obtained from a small garden opposite the department of forestry in Rivers State University, Port Harcourt in October 2021. It was identified and authenticated by the department of plant science and biotechnology of Rivers State University. An authentication number of RSU PB 041 was assigned.

Preparation of plant extract

The leaves of *Justicia secunda* (blood root) were washed and air-dried at room temperature. It was completely dried using the hot air oven at 45-50 degrees and blended into fine powder with Binatone blender model FP-850. 50 gm of *Justicia secunda* was extracted in 500 ml of 99% ethanol using a Soxhlet extractor.

Experimental design/setup

Thirty female Wistar rats were used for this study. Experimental animals were divided into six groups of four rats each. This experiment lasted for 42 days.

Table 1: Table showing experimental design animal sacrifice.

Groups	Dose and extract administration
Group 1 (control)	Distilled water
Group 2	250 mg/body weight (kg) of ethanolic extract of *Justicia secunda*
Group 3	300 mg/body weight (kg) of ethanolic extract of *Justicia secunda*
Group 4	350 mg/body weight (kg) of ethanolic extract of *Justicia secunda*
Group 5	400 mg/body weight (kg) of ethanolic extract of *Justicia secunda*
Group 6	450 mg/body weight (kg) of ethanolic extract of *Justicia secunda*

Animal sacrifice

The animals were anaesthetized one after the other with chloroform. Cotton wool was soaked with chloroform and put in the desiccator. Animal was then put in the desiccator, and allowed to get into the stage of deep anesthesia, where the animal was unconscious. The animal was pinned on dissecting board in anatomical position. The animal was then dissected and the following organs were harvested: blood (collected through the vein of the forelimb) ovary and uterus.

Experimental procedures

Analysis of the female reproductive hormones was also carried out. The level of progesterone and prolactin in ng/ml, oestrogen tested in pg/ml was tested using Tiets NW Method. Follicle stimulating hormone and luteinizing hormone in m/u/ml using Layman LC method.

Procedure for tissue processing

Histological and morphometric evaluation were carried out on the ovaries and uteri.

Fixation

The ovaries and uteri were fixed with Bouin's fluid for twenty- four (24) hours and transferred into 10% formalin.

Dehydration

Graded percentages of alcohol (ethanol) were used to dehydrate the tissues. These include 50%, 70%, 95% and absolute ethanol 1, 2 and 3. The tissues lasted 2 hours

each in 50%, 70% and 95%, and lasted 6hours each in absolute ethanol 1, 2 and 3.

Clearing

The tissues were cleared in xylene 1, 2 and 3, each lasted for 1 hour.

Infiltration/impregnation

The tissues were infiltrated in 60°C molten paraffin wax 1 and 2 in laboratory oven- New life (NL-9052-1). Each lasted for 1 hour.

Embedding

Tissues were embedded in 70°C molten paraffin using tissue cassettes and metal embedding moulds, and allowed to block (solidify) immediately in freezer.

Sectioning

Tissues were sectioned with microtome- Leica RM 2135 using 2 microns. The ribbons were spread on glass slides with 20% ethanol. The ribbons were then floated in a warm water (about 30 to 40°C) in water bath- Raymond Lamb water bath (E65). Sectioned ribbons were later picked with properly labelled glass slides and placed on hot plate- Raymond Lamb under temperature of 60°C.

Dewaxing

Sectioned tissue slides were dewaxed under 80°C temperature in the laboratory oven.

Staining

Dewaxed tissue slides were sent to water. That is passing the dewaxed tissues from xylene to absolute ethanol to 95% to 70% to 50% and finally to water, and allowed to bluing. Tissue slides were stained in Cole hematoxylin for 10 min, rinsed in water and differentiated with 1% acid alcohol. Then stained in 1% eosin for 5 min, and rinsed in water. Stained slides were rinsed in absolute ethanol.

Mounting

Slides were rinsed in xylene, mount with DPX mountant and then cover slipped.

Statistical analysis

Data was expressed as mean±SEM and the significance level was set at $p \leq 0.05$. Data were analyzed using analysis of variance (ANOVA) and of post hoc Fisher's least significant difference (LSD) test using software, Statistical Package for Social Sciences (SPSS) version 28.

RESULTS

Gradual reduction in weight of the uteri in the treatment groups, but no statistical significance (Table 1). Estrogen and FSH levels increased in the treatment groups when compared to the control (Table 2).

Table 2: Effect of ethanolic extract of *Justicia secunda* on the weight of the uterus.

Groups	Weight (kg) mean±SEM
Group 1	0.06±0.009
Group 2	0.09±0.015
Group 3	0.08±0.020
Group 4	0.08±0.026
Group 5	0.08±0.004
Group 6	0.06±0.004

n=4, *p≤0.05 statistically significant compared to control.
Key: Group 1= control group, Group 2= treated with 300 mg, Group 3= treatment with 400 mg, Group 4= treated with 400 mg, Group 5= treated with 450 mg, Group 6= treated with 450 mg

Effect of Justicia secunda on the ovaries

This section showed ovarian follicles at different stages of development [primordial follicles, primary follicles (GF), secondary follicle (SF) and mature ovum and atretic follicles (ATF). H and Eat 40X] (Figure 1). This section showed many degenerating (DF) and atretic follicles at all stages (ATF). There are few viable primordial, primary and secondary follicles (PF). H and E at 40X (Figure 2). This section showed ovarian follicles at different stages of development. No corpus observed but there is observable medulla lesion. Secondary follicle (SF), mature ovum (MO) and atretic follicles (ATF). H and E at 40X (Figure 3).

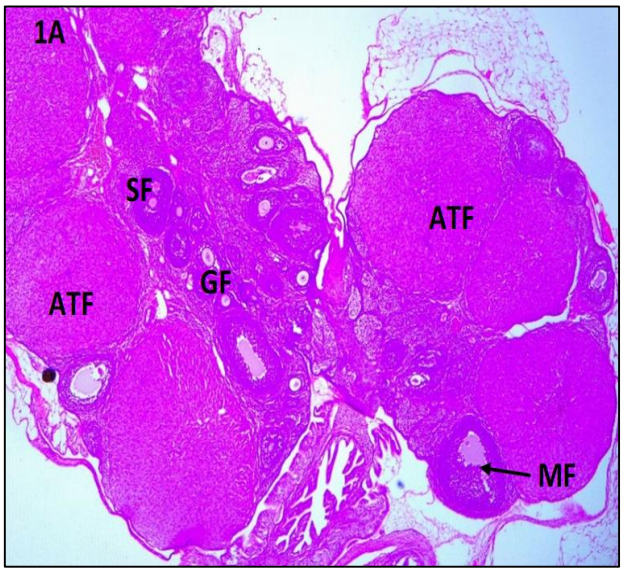

Figure 1: Photomicrograph section of ovarian tissue from rats from G1.

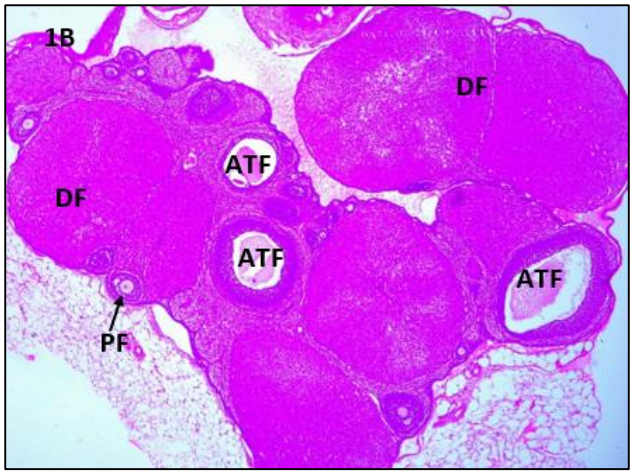

Figure 2: Photomicrograph section of ovarian tissue from rats from G2 group.

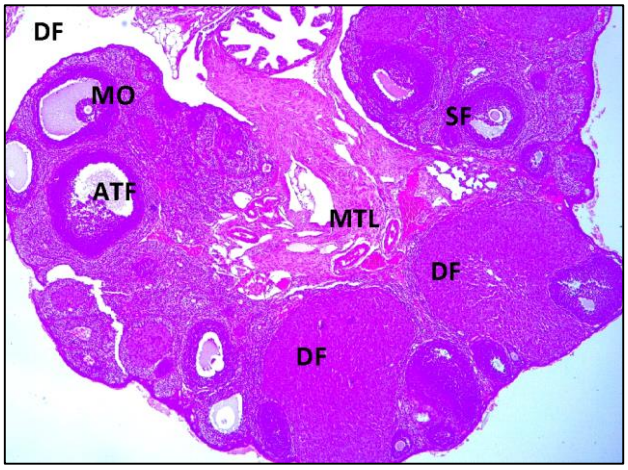

Figure 3: Photomicrograph section of ovarian tissue from rats from G6 group.

Table 3: Effect of ethanolic extract of *Justicia secunda* on the female reproductive hormones.

GROUP	FSH m/u/ml mean±SEM	LH m/u/ml mean±SEM	PRL ng/ml mean±SEM	E_2 pg/ml mean±SEM	PROG ng/ml mean±SEM
Group 1	0.48±0.07	0.77±0.28	0.94±0.27	37.35±10.68	15.03±019
Group 2	0.40±0.10	0.94±0.28	1.08±0.11	57.75±2.32*	20.13±4.12
Group 3	1.65±0.25*	0.92±0.37	1.56±0.26	47.00±1.29	16.63±1.56
Group 4	1.31±0.36*	0.81±0.32	1.10±0.12	47.00±4.30	22.50±3.89
Group 5	0.51±0.10	1.05±0.27	1.02±0.13	58.50±3.97*	20.23±3.69
Group 6	0.56±0.12	1.14±0.27	1.55±0.55	46.00±4.81	23.38±1.88

n=4, *p≤0.05 statistically significant compared to control. Key: Group 1= control group, Group 2= treated with 300 mg, Group 3= treatment with 400 mg, Group 4= treated with 400 mg, Group 5= treated with 450 mg, Group 6= treated with 450 mg, FSH- Follicle stimulating hormone, LH- Luteinizing hormone, PRL- Prolactin, E_2- oestrogen, PROG- progesterone.

DISCUSSION

Gradual reduction in weight of the uteruses were observed in the present study, but no statistical significance as seen in Table 2. This reduction could be attributed to the effect of the plant extract on the uteri.

In the present study, histomorphological changes (lesion in the medulla) was observed in tissue section of the ovaries as seen in Plate 3. This implies that the plant extract had direct effect on the ovaries. The result from the present study agrees with Asuquo et al.[6] They reported that the histomorphological changes observed in treated rats may be due to the direct effect of the extract on the organ.

Result from this research showed a significant increase of FSH in middle dose group as seen in Table 3, this implies that the plant extract may have exerted some effect on the cells of the anterior pituitary gland secreting FSH or cells of the hypothalamus secreting GnRH. This study agrees with Adebayo and Okonkwo et al.[7,8] They reported that extracts that influence the level of FSH may have exerted its effect on the anterior pituitary or hypothalamus, because FSH is regulated by gonadotrophic releasing hormone. This result however contrasted with the reports of Onyebuagu et al and Agbai et al.[9,10] They reported that plant extract did not cause any significant change in FSH.

Significant increase in oestrogen level observed in this study as seen in Table 2. This can be attributed to the presence of phytoestrogens and antioxidants in the plant extract. This result agrees with Shabanian et al.[11] Burton and Wells reported that increase in the level of estrogen may be due to the presences of phytoestrogens and antioxidant in the plant extract.[12]

In the control and lose dose groups, histomorphological changes was absent as seen in seen in Figure 1 and Figure 2 respectively.

CONCLUSION

In conclusion, ethanolic extract of *Justicia secunda* (blood root) leaves is likely to cause an increase in FSH. The plant may have steroidal effect.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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