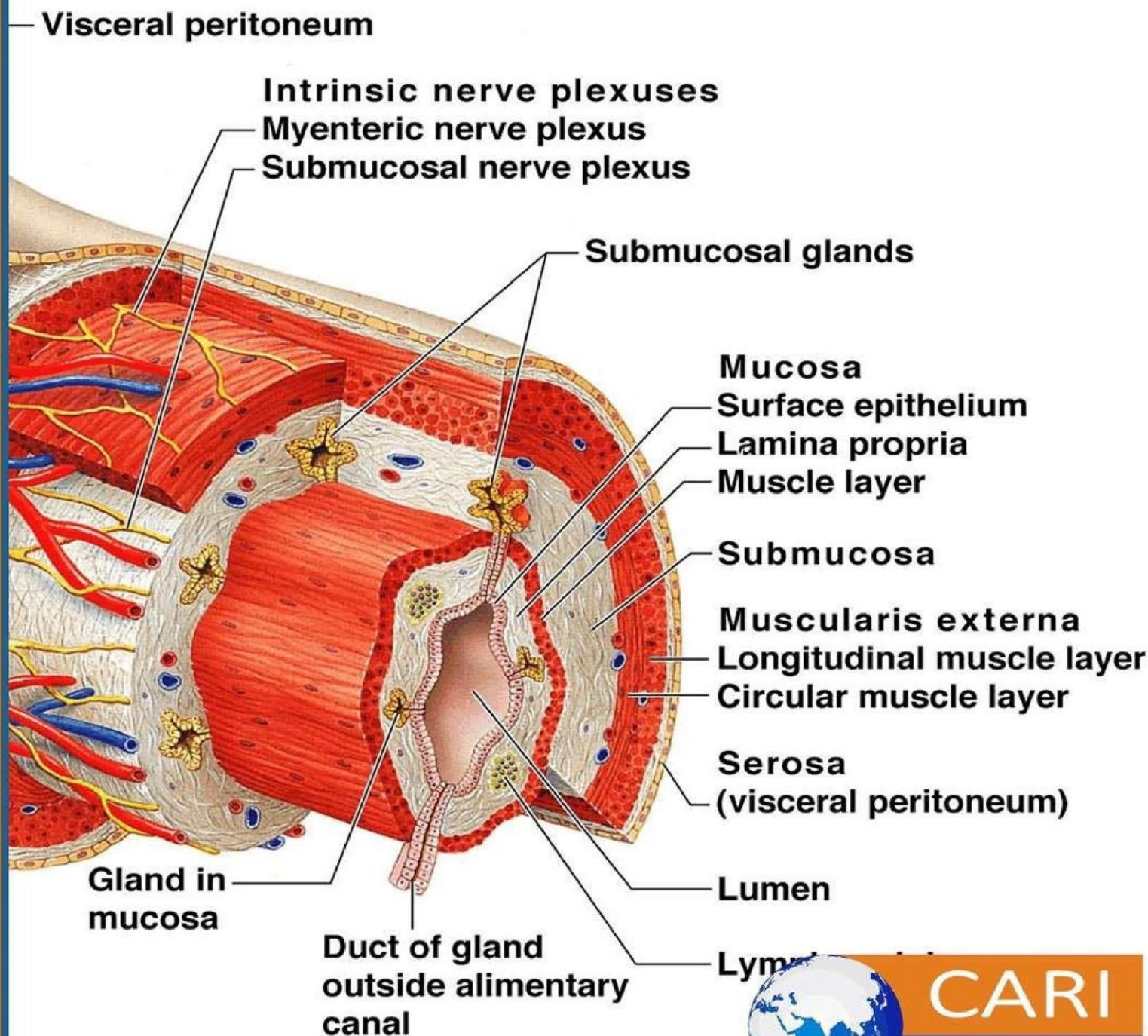


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THE EFFECT of ETHANOLIC EXTRACT of *Justicia secunda* (BLOOD ROOT) on the VAGINAL OPENING and OESTROUS CYCLE of FEMALE WISTAR RATS



## THE EFFECT of ETHANOLIC EXTRACT of *Justicia secunda* (BLOOD ROOT) on the VAGINAL OPENING and OESTROUS CYCLE of FEMALE WISTAR RATS

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### ABSTRACT

**Purpose:** This study investigates the effect of the ethanolic extract of *Justicia secunda* (Blood root) on the vaginal opening and oestrous cycle of female wistar rats.

**Methodology:** Thirty (30) female wistar rats of 18-33g average weight was used. They were divided into six groups of five animals each. Group A received distilled water while animals in group B-F received 250, 300, 350, 400 and 450mg/body weight (kg) respectively of the ethanolic extract of the plant. Monitoring of the onset and completion of vaginal opening was done and the estrous cycle was also monitored throughout 3 weeks of extract administration. Early onset of vagina opening was seen in the treated group compared to the control. The oestrous cycle showed irregularities when compared to the control data were expressed as means  $\pm$  standard error of mean and analyzed by analysis of variance (ANOVA).

**Findings:** This result showed that the plant extract causes an irregular oestrous cycle and precocious pubertal onset in female wistar rats.

**Unique contribution to theory, practice and policy:** The plant extract of *Justicia secunda* likely to induce precocious puberty in young female wistar rats and irregular cyclicity in the oestrous cycle. This implies that *Justicia secunda* is likely to have contraceptive properties.

**Key words:** *Oestrous cycle, Puberty, Menstrual irregularities*

### INTRODUCTION

*Justicia* is the largest genus (Wasshausen *et al.*, 2004) of all 250 genus belonging to the *Acanthaceae* family. *Justicia* species are perennial herbs or subshrubs that are either erect or scandent. The leaves have cystoliths and are petiolate, with an entire leaf margin. Spikes or panicles cimas form the inflorescences, while solitary, terminal, or axillary flowers are uncommon.

Bracts and bracteoles are often prominent and imbricate. *Justicia* species are easily distinguished by their bilabial corolla, which has a two-lobed posterior lip, a three-lobed anterior lip, two stamens, a four-seed capsule, and a basal sterile part (Kitadi *et al.*, 2019). *Justicia secunda* Vahl is a creeping perennial herb (Iroha *et al.*, 2021) that belongs to the family *Acanthaceae*, order Scrophulariales, superorder Lamiflorae (sensu Dahlgren) (Kitadi *et al.*, 2019). In Barbados and Venezuela, it is also known as "Blood root" and "Sanguinaria" (Onoja *et al.*, 2017). It is a South American native tropical herbaceous plant that is now growing in other tropical or subtropical African countries including the Democratic Republic of the Congo (Kitadi *et al.*, 2019).

Many parts of this plant are used in traditional medicine to treat diabetes, cancer, respiratory and gastrointestinal ailments, inflammation, and the central nervous system as hallucinogens, somniferous agents, sedatives, and depressors, as well as epilepsy and other mental problems (Kitadi *et al.*, 2019). Other species of *Justicia* are used to treat headaches, fevers, sedatives, analgesics, bronchitis, arthritis, vaginal discharges, dyspepsia, and eye difficulties, as well as sedatives, analgesics, bronchitis, arthritis, bronchitis, arthritis, vaginal discharges, dyspepsia, and eye problems. Various extracts of *Justicia* have been shown to exhibit biological activity, and a vast number of phytochemicals have been extracted from various parts of those plants (Kitadi *et al.*, 2019).

The female reproductive system is extremely sensitive to several factors such as lifestyle, radiation, drugs and toxicants (Fucic *et al.*, 2012). Exposure to any of these factors could lead to functional alterations (Knapp *et al.*, 2012). Endocrine disrupting chemicals could impact on the synthesis and mechanism of action of sex hormones. The sexual maturation of a female rat is generally defined by vaginal opening (Lewis *et al.*, 2002). The oestrous cycle in rodents is similar to the menstrual cycle in humans. The oestrous cycle has four phases, namely proestrous, estrous, metaestrous and diestrous and lasts for 4 to 5 days (Auta *et al.*, 2016). In the proestrous, the vaginal opening appears full, swollen and moist, tissues are pink, with striations in both the dorsal and ventral lips of the vulva. In estrus, these appearances are similar but less. However, striations are more. In metaestrous, the vagina opening is pale. The features of diestrous include wet vagina opening which is closed with no swelling. Any disturbance that disrupts the regular functioning of the reproductive organs has an impact on the animal's ability to reproduce (Knapp *et al.*, 2012). As a result, scientists are studying the effects of various plant extracts on the reproductive system. There has been no research made on the effect of this plant on the vaginal opening and oestrous cycle of female wistar rats, hence, the need for this study.

## **MATERIALS AND METHODS**

Thirty (30) female wistar rats weighing 18 to 33g were used for the study.

### **Preparation of Plant Extraction**

Fresh leaves of *Justicia secunda* was air-dried and grinded into fine powder using Binatone blender and 50g of pulverized leaves were extracted in 99.5% ethanol using soxhlet apparatus.

### **Experimental Design**

A total of 30 immature female rats were randomized into six groups and extract was given based on their mean body weight

- I. Group A (control): Distilled water
- II. Group B: 250mg/body weight (kg)
- III. Group C: 300mg/body weight (kg)
- IV. Group D: 350mg/body weight (kg)
- V. Group E: 400 mg/body weight (kg)
- VI. Group F: 450mg/body weight (kg)

### **Confirmation of Vagina Opening**

Each rat was physically evaluated to assess for commencement of vagina opening. As soon as vagina opening was confirmed, determination of oestrous cycle began.

### **Determination of Estrous Cycle**

Vaginal smear of the female rats were collected with the aid of a micro dropping pipette, normal saline and distilled water. The vaginal smear was dropped on a microscope slide and examined daily during the early hour of the day (7am – 9am). The wet smear was viewed under a light microscope. The proportion among the cells observed, was used to determine the different stages of the estrous cycle of the experimental animal. The oestrous cycle phases and duration were determined according to the methods reported by Goldman *et al.* (2007). Proestrous phase was determined by smears possessing more of nucleated epithelial cells. Estrous phase was determined as smears with a large number of cornified epithelial cells. Metaestrous phase showed equal proportion of epithelial cell, cornified cells and leucocytes while diestrous phase was determined by smears with leucocytes. The staining of the smear for microscopic analysis was done according to the method used by Shorr (1941).

The wet vaginal smear for each rat was allowed to dry. Then fixed using 95% ethanol, and after that the slide was stained using Leishman stain. Ten minute later, each slide was washed using buffer. potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) and solution disodium hydrogen phosphate

( $\text{Na}_2\text{HPO}_4$ ). The washed slides were allowed to dry, then covered slip using Dibutylphthalate Polystyrene Xylene Mountant (DPX) and Xylene. This procedure was done daily for twenty-one days. The covered slipped slides were viewed using electron microscope and photographs were taken by a photomicrograph for each wistar rat in each group.

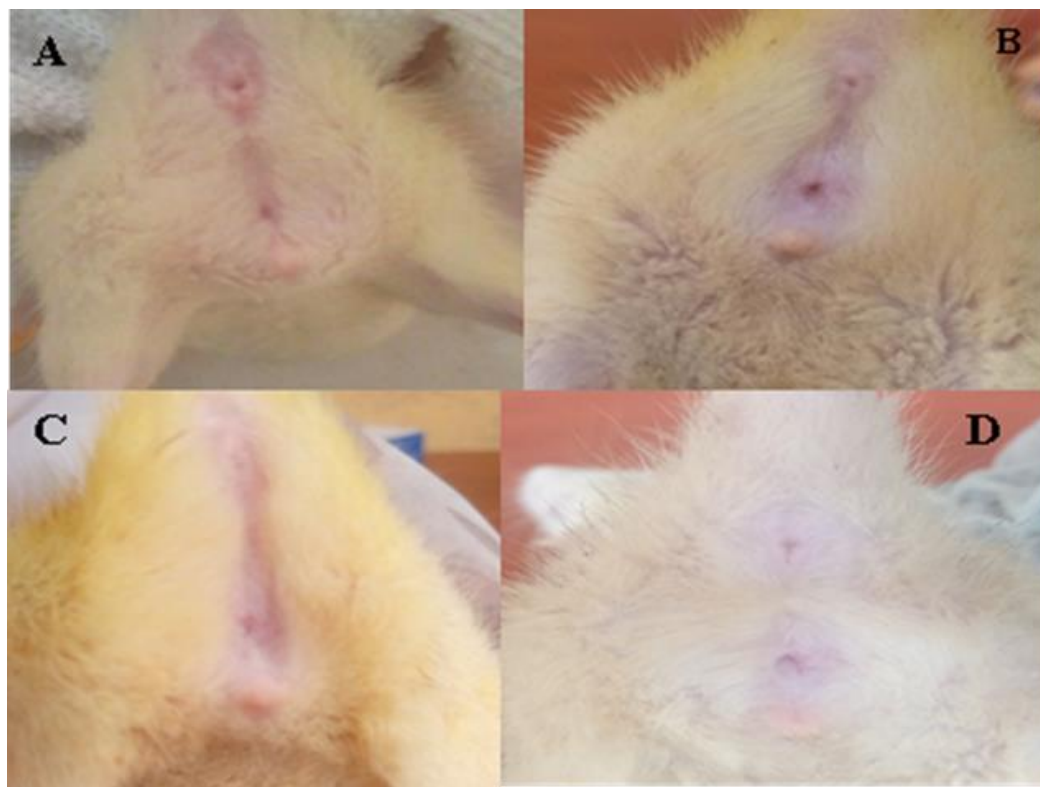
### **Statistical Analysis**

All the obtained data were expressed as means  $\pm$  standard error of mean and analyzed by analysis of variance (ANOVA) and Least Significance Difference (LSD) Post Hoc test, using Statistical Package for the Social sciences (SPSS) version 22.0. Differences were considered significant if P-value  $\leq 0.05$ .

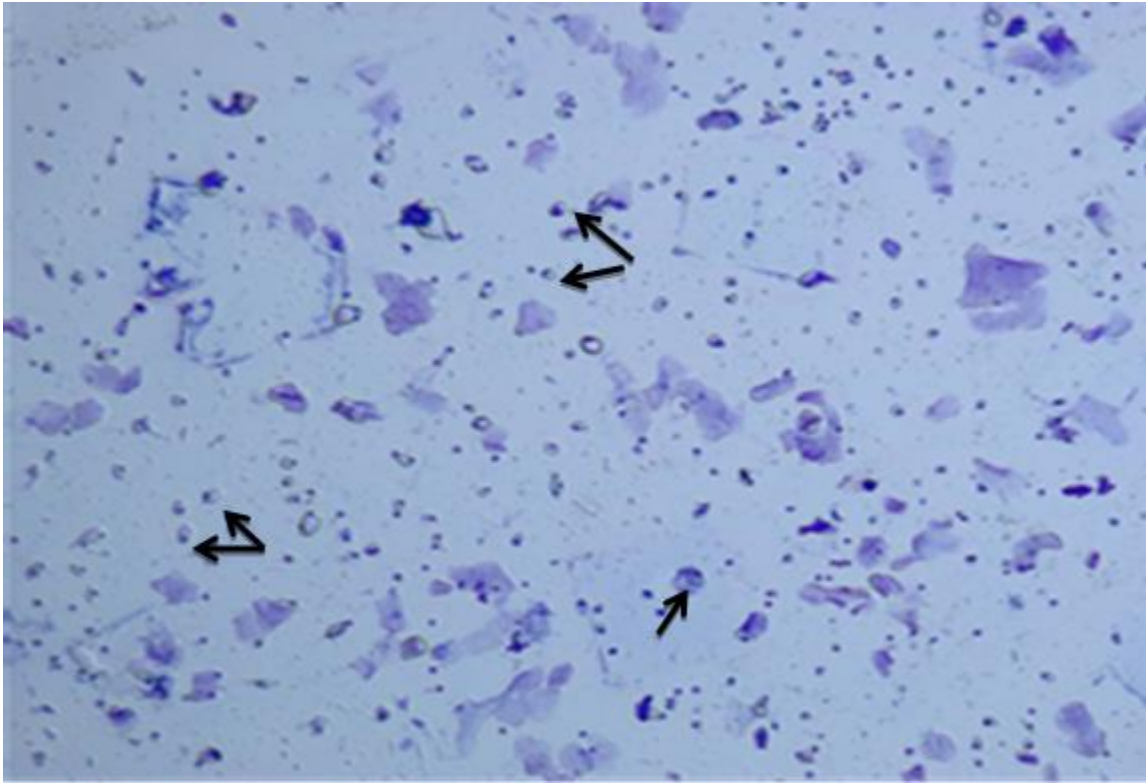


**Figure I: Plastic Micro Pasteur Pipette with Tapered End**

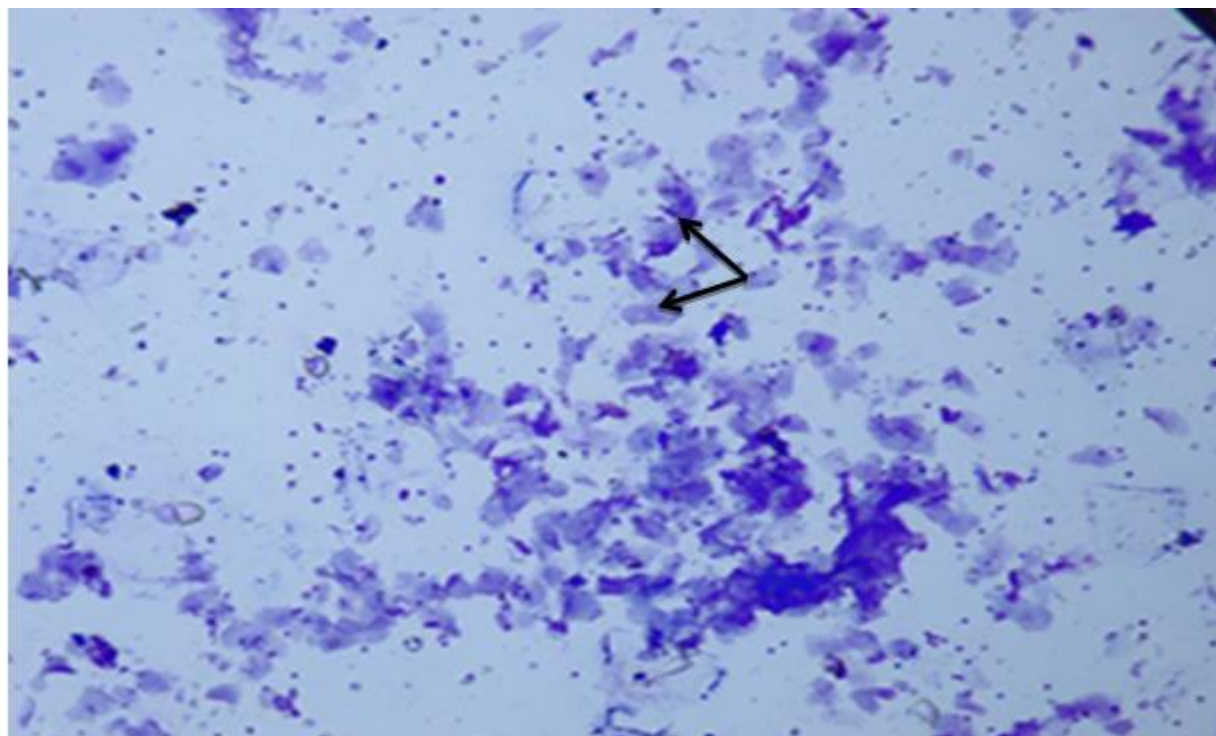
## RESULTS



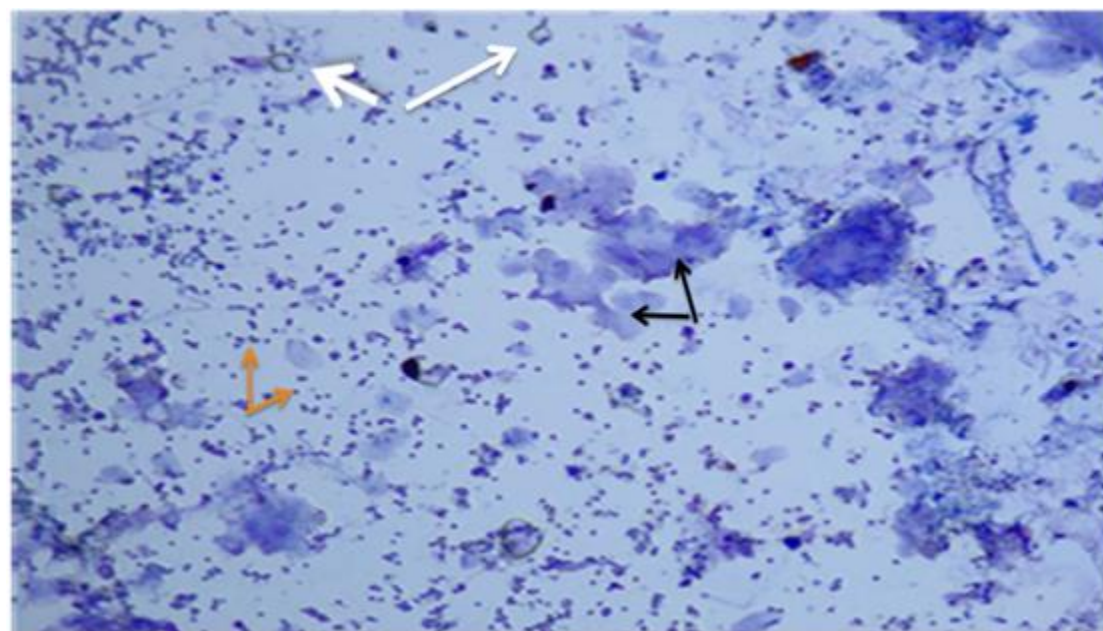
**FIGURE II: Appearance of the Vagina in Different Phases of Oestrous Cycle of a Female Wistar Rat. (a)-Proestrous, (b)-Estrous, (c)- Metestrous, (d)- Diestrous**



**Plate 1: Proestrous Phase showing Domination of the Nucleated Epithelial Cells**

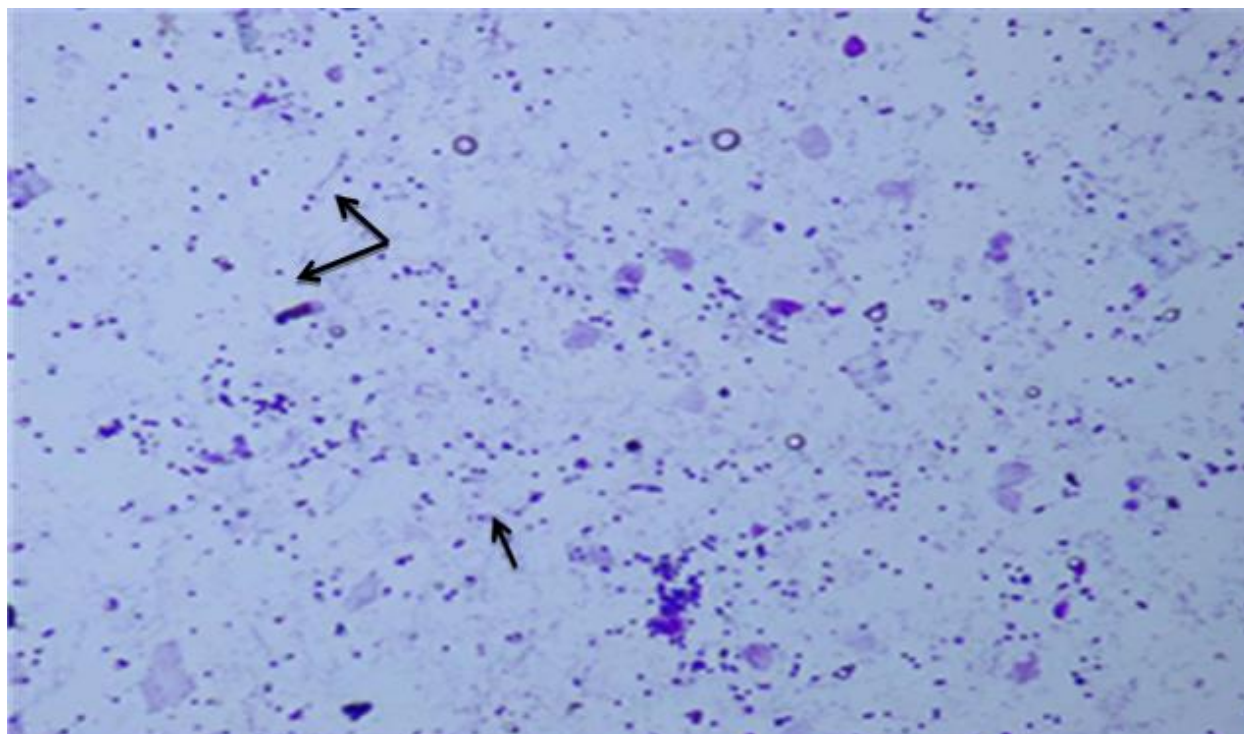


**Plate II: Estrous Phase Showing Dominance of Cornified Epithelial Cells**



**Plate III: Metestrous Phase of Treated Rats showing Nucleated Epithelial Cell (White Arrow), Cornified Cells (Black Arrow) and Leucocytes (Brown Arrow)**





**Plate IV: Diestrous Phase of Treated Rats showing Domination of the Leucocyte**

**Table 1: Effect of Oral Administration of Ethanolic Extract of *Justicia secunda* (Blood Root) on the Age of Vaginal Opening**

<b>Group</b>	<b>Age of Vaginal Opening (days) (mean±SEM)</b>
A (Control)	44.60±2.68
B (250mg/body weight)	38.20±0.58*
C (300mg/body weight)	38.0±0.45*
D (350mg/body weight)	37.80±0.37*
E(400mg/body weight)	37.60±0.24*
F(450mg/body weight)	37.60±0.24*

n=5, \*P≤ 0.05 is statistically significant compared to control

Rats at treated group showed a significant early vaginal opening,  $p < 0.05$  than the control. The high dose groups (400 and 450 mg/body weight) showed the earliest onset of vaginal opening ( $37.60 \pm 0.24$ ) which was significant compared to the control.

**Table II: Effect of the Ethanolic Extract of *Justicia secunda* (Blood Root) leaves on Oestrous Cycle Phases and Number of Cycles**

GROUP	PROESTROUS/(days) ) mean ±SEM	ESTROUS/(days) mean ±SEM	METESTROUS/(days) ) mean ±SEM	DIESTROUS/(days) mean ±SEM	NUMBER OF CYCLE/(days) mean ±SEM,
<b>Group A (Control)</b>	3.40± 0.51	9.00±1.00	9.20±0.20	3.40±0.51	3.80±0.20
<b>Group B (290mg)</b>	5.20±1.02	5.80±0.86*	12.40±0.68*	1.20±0.49*	1.60±0.25*
<b>Group C (380mg)</b>	4.60±0.87	5.40±0.75*	11.00±0.45*	4.60±01.03	2.20±0.20*
<b>Group D (420mg)</b>	4.00±0.55	6.40±0.81*	13.00±0.32*	1.60±0.60	1.60±0.25*
<b>Group E (450mg)</b>	3.40±0.93	5.20±0.58*	12.20±0.86*	4.20±0.49	2.20±0.37*
<b>Group F (470mg)</b>	3.20±0.20	7.80±0.97	10.60±0.68	3.20±0.67	1.40±0.24*

A prolongation of the proestrous phase is seen in group B to F while a reduction in this phase is seen in Group F. However, this change is insignificant ( $p < 0.05$ ) when compared to the control. The extract has a significant ( $p < 0.05$ ) effect on the estrous phase of Group B-E. A reduction of this phase is observed compared to the Control. The estrous phase is also reduced in group F but this reduction is insignificant. An opposite effect is seen in the metaestrous phase which shows a significant prolongation in Group B-F and an insignificant Prolongation. The diestrous phase appears to be prolonged in Group D and E than the control but insignificant. However, Group B, C and F shows a declined diestrous phase which is significant in Group B. Generally, there was a significant reduction in the number of oestrous cycles of the rats in all treated group

## DISCUSSION

In the present study, early vaginal opening was observed in treatment groups when compared with the control. Vagina opening was observed on day  $38.20 \pm 0.58$ ,  $37.80 \pm 0.37$  and  $37.6 \pm 0.24$  for low dose, middle dose and high dose groups respectively. The possible reason for the reduction in the age at vagina opening could be that the plant extract had a positive effect on hypothalamic-pituitary-ovarian axis thereby elevating gonadotropins and ovarian hormones. This agrees with Bend et al. (2018), they stated that precocious onset of puberty is caused by the simultaneous elevation of gonadotropins and consequently ovarian hormones on treated animals indicates the activation of the hypothalamic-pituitary-ovarian axis. Results from the present study also agrees with Lienou *et al.*, (2010). They reported that plants that contains estrogenic properties would induce precocious vaginal opening.

In this present study, proestrous and metaestrous phases of the estrous cycle were prolonged while estrus phase was reduced. This disruption in oestrous cycle could be as a result of phytochemical constituent of plants used. This study is in agreement with Jubril et al. (2016). They reported that the disruption in oestrous cycle of rat may be due to the presence of alkaloids and saponin.

In conclusion, ethanolic extract of *Justicia secunda* leaves is likely to cause precocious puberty in young female rats and irregular cyclicity in the estrous cycle.

## REFERENCE

- Auta, T. & Hassan, A. T. (2016). Alteration in estrus cycle and implantation in *Mus musculus* administered aqueous wood ash extract of *Azadirachta indica* (neem). *Asian Pacific Journals of Reproduction*, 5(3), 188–92.
- Emma F. Bend, Pierre C. Oundoum Oundoum, Marie I. Ngaha Njila, Brice L. Koloko, Camille D. Nyonseu, Samuel H. Mandengue, Paul Moundipa, Theophile Dimo, Dieudonne Massoma Lembè. Effect of the Aqueous Extract of *Schumannia phyton magnificum* Harms on Sexual Maturation and Fertility of Immature (*K. schum*) Female Rat. *Pharmacology & Pharmacy* > Vol.9 No.10, October 2018

- Fucic, A., Gamulin, M., Ferencic, Z., Katic, J., Krayner von Krauss, M., Bartonova, A. & Merlo, D. F. (2012). Environmental exposure to xenoestrogens and oestrogen related cancers: reproductive system, breast, lung, kidney, pancreas, and brain. *Environmental Health: A Global Access Science Source*, 11 Suppl 1, S8. <http://doi.org/10.1186/1476-069X-11-S1-S8>
- Goldman, J. M., Murr, A. S. & Cooper, R. L. (2007). The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Research Part B Developmental and Reproductive Toxicology*, 1:84-97
- Iroha, B. Nkem. & Maduelosi, J. Ngozi. (2021). Corrosion Inhibitive Action and Adsorption Behaviour of *Justicia Secunda* Leaves Extract as an Eco-Friendly Inhibitor for Aluminium in Acidic Media. *Biointerface Research in Applied Chemistry*, 11(5), 13019-13030.
- Jubril, M.M., Adeiyi, S.O., Rayyanu, Z.S. (2016). Effects of Aqueous Stem Bark Extract of *Anogeissus leiocarpus* on the serum levels of Progesterone and Estradiol and the Estrous cycle of Wistar Rats. *Academia Anatomica International*
- Kitadi M. Julies, Lengbiye, M. Emmanuel, Gbolo Z. Benjamin, Inkoto L. clement, Muanyishay L. Christophe, Lufululuabo L. Georges, Tshibangu S. T. Damien, Tshilanda D. Dorothee, Mbala M. Blaise, Ngbolua Koto-te-Nyiwa, Mpiana T. Pius (2019). *Justicia secunda* Vahl species: Phytochemistry, Pharmacology and Future Directions: A Mini-Review. *Discovery Phytomedicine*. 6(4): 157-171.
- Knapp, K. M., Brogly, S. B., Muenz, D. G., Spiegel, H. M. L., Conway, D. H., Scott, G. B. & Read, J. S. (2012). Prevalence of congenital anomalies in infants with in utero exposure to antiretrovirals. *The Pediatric Infectious Disease Journal*, 31(2), 164–70. <http://doi.org/10.1097/INF.0b013e318235c7aa>
- Lewis, E. M., Barnett, J. F. Jr., Freshwater, L., Hoberman, A. M., and Christian, M. S. (2002). Sexual maturation data for Crl Sprague-Dawley rats: Criteria and confounding factors. *Drug Chem Toxicol* 25, 437–58
- Lienou, L.L., Telefo, B.P., Bayala, B., Yemele, M.D., Lemfack, C.M., Goka, C.S., Tagne, R.S., Moundipa, P.F. (2010). Effect of ethanolic extract of *Senecio bialfrae* on puberty onset and fertility in immature female rat. *Cameroon Journal of Experimental Biology*. 6:101-109.
- Shorr, E. A. (1941). New technique for staining vaginal smears: III, a single differential stain. *Science*, 94:545-6.
- Wasshausen, D. C. & Wood, J. R. I. (2004) Acanthaceae of Bolivia. *Control U S National Herb* .49, 1-152.