

## Effect of ethanolic stem bark extract of *Annona muricata* (soursop) on reproductive hormones and ovary in female Wistar rats

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### Abstract

The effect of ethanol extract of stem bark of *Annona muricata* (soursop) on reproductive hormones and ovaries of Wistar rats was studied. The phytochemical analysis revealed the presence of alkaloids, flavonoids, flavones, flavonols, xanthenes, flavanonols, flavonones, and condensed tannins. Twenty (20) experimental animals were randomly grouped into five groups of 4 rats per group and were allowed to acclimatize for 2 weeks. Rats in group A served as the control and were fed with rat feed and water, rats in group B served as positive control and received low dose clomiphene citrate (20 mg/kg) daily for 21 days, rat feed and water, rats in groups C to E were fed with normal rat feed, water and received different doses of fresh stem bark of *Annona muricata* extract at 250, 500 and 1000 mg/kg respectively for 21 days. The animals were sacrificed under anesthesia after the experiment and the ovaries were surgically extracted, weighed and sent for histology. Blood samples were collected for analysis (hormonal assay of luteinizing hormone (LH), estrogen, follicle stimulating hormone (FSH), progesterone and prolactin. This was carried out using enzyme linked immunosorbent assay (ELISA) technique. The results showed that there were significant changes in the hormone level of the test groups when compared with the control rats. Significant increase was recorded in progesterone, estrogen, prolactin, follicle stimulating hormone and luteinizing hormone  $p < 0.05$ . Hence *Annona muricata* improves fertility in female Wistar rats. There were no histological changes in the ovaries and hence, it was concluded that *Annona Muricata* does not cause organ damage with increasing dose.

**Keywords:** *Annona muricata*; FSH; LH; Progesterone; Prolactin; Estrogen

### 1. Introduction

In Nigeria most herbal products are not registered as drugs and legislation for distribution and purchase of herbal medicines is not as stringent as it is for conventional medicines. There is increase worldwide in the use of herbal medication especially amongst couple with infertility issues <sup>1,2</sup>. The use of herbal medication from plants or other sources contain active ingredients perceived to have therapeutic uses. The increase in the use of herbal medication by pregnant women in most African countries especially Nigeria is believed to be due to the relatively low cost, cultural belief, increase in media publicity<sup>3,4</sup>. Because of paucity of information on the risks and benefits associated with the use of herbal medication in pregnancy and also due to absence of pre- registration clinical trials and post approval

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surveillance, the use of herbal medication especially in productive period is of special concern and may pose dangers to the fetus<sup>4</sup>. Infertility has posed serious problems all over the world especially in countries with declining population. There has been increase in the successful management of infertility, these methods are however expensive (In vitro-fertilization and intrauterine insemination) and cannot be afforded by most people in the 3<sup>rd</sup> world countries<sup>5</sup>. This study, therefore, was aimed to investigate the effect of *Annona muricata* stem bark on female reproductive hormones.

*Annona muricata* is widely known as soursop due to the sour and sweet taste of its fruit. It is also known as prickly custard apple due to its taste. This plant has the taxonomic classification of the kingdom of plantae, the division of angiosperms (magnoliophyta), the class of magnolids, the order of magnoliales, the family of annonaceae, the genus of annona and the species of *A. muricata* L.<sup>6,7</sup>. The Annonaceae family consists of approximately 130 genera and 2300 species, while the genus *Annona* comprises over 70 species among which *A. muricata* is the most widely grown<sup>8,9</sup>.

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## 2. Materials and method

### 2.1. Ethical approval

Ethical approval for the research was sought and obtained from the Ethical committee of Abia State University Uturu.

### 2.2. Animals for the study

Twenty (20) male adult Wistar rats (150 g – 200 g) were obtained from animal farm, the Animal House of the Physiology located at the Department of Human Physiology, Abia State University Uturu. They were acclimatized for two weeks, having free access to water and food.

### 2.3. Phytochemical analysis

Phytochemical analysis as was reported by<sup>10</sup> showed the presence of alkaloids, flavonoids, flavones, flavonols, xanthenes, flavanonols, flavonones, and condensed tannins.

### 2.4. Preparation of the extract

Fresh stem bark extracts of *Annona muricata* were air dried at room temperature for two weeks. They were pulverized with a mechanical grinding machine (GX 160 Delmar 5.5 HP). They were then ground to powdered form using electric blender. 200 g of the dried powdered form was then soaked in 70% ethanol for 48 hours. The mixture was then filtered and evaporated to dryness using water bath at 40°C. The extract (0.02 g, 0.04 g and 0.08 g respectively) was then diluted with appropriate milliliter of distilled water to get the appropriate stock solution (mg/ml). 100 mg/kg, 200 mg/kg and 400 mg/kg were calculated using the formula below.

Dose (ml) = (required dose (mg/kg) × weight of animal (kg)) / stock (mg/ml). The prepared extract was stored in a refrigerator at 4°C until time for use.

### 2.5. Experimental design

The rats were divided into five groups of five rats per group (control group and groups 2 to 5). Twenty rats weighed between 150 g and 180 g. They were allowed to acclimatize for 2 week. The first group served as the control and were given food and water *ad libitum* throughout the period of the research, group 2 were given 20 mg/kg of clomiphene citrate (standard drug), groups 3 to 5 received different doses of the extract; 250 mg/kg was given to group 3 and they served as low dose group, 500 mg/kg was given to group 4 and they served as medium dose, while 1000 mg/kg was given to group 5 and they served as high dose group. The extract was administered for 21 days using orogastric cannula. The dosage were however dependent on the outcome of the toxicity test (LD50) of the extract according to<sup>11</sup>. After 21 days of administration of extract, they were anesthetised and blood samples were taken by ocular puncture and sent to the lab God heal-lab, medical and diagnostic services ltd. no 40 brass Aba, Abia state, Nigeria for hormonal assay (FSH, LH, oestrogen, progesterone and prolactin).

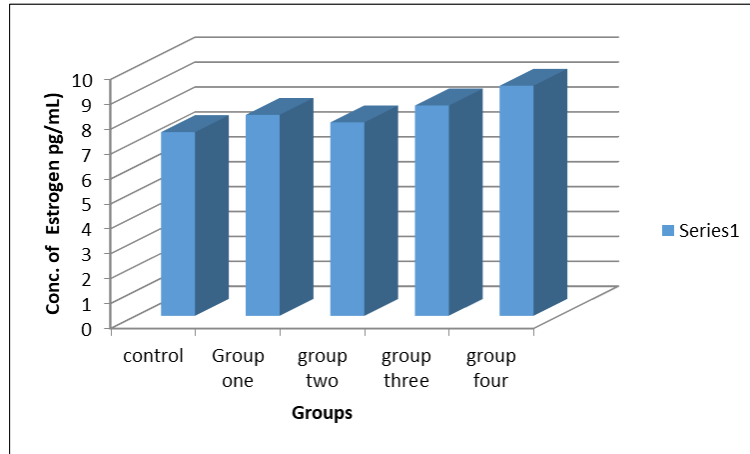
### 2.6. Statistical analysis

The data obtained were analyzed using statistical package for social sciences (SPSS-24). Results were presented as mean ± standard error of mean (SEM) of sample replicates.  $p < 0.05$  was considered to be statistically significant.

### 3. Results

#### 3.1. Estrogen

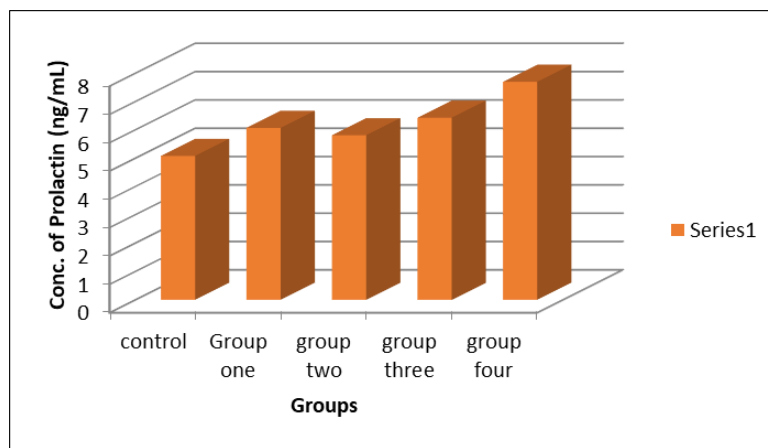
There was significant increase in the estrogen level in groups 1, 2, 3, and 4 when compared with the control group,  $p < 0.05$ . The mean value of control group was  $7.3818 \pm 0.0014$ , that of group one was  $8.0738 \pm 0.0018$ , group two was  $7.77 \pm 0.0008$ , group three was  $8.45 \pm 0.001$  and group four was  $9.24 \pm 0.34$ .



**Figure 1** Mean concentration of estrogen

#### 3.2. Prolactin

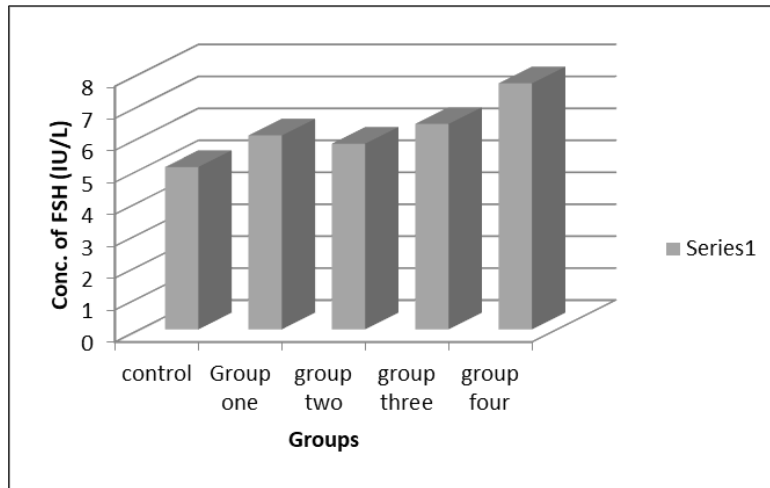
There was significant increase in the prolactin level of the groups 1, 2, 3, and 4 when compared with the control group,  $p < 0.05$ . The mean value of control group was  $8.26 \pm 0.004$ , that of group one was  $9.15 \pm 0.00$ , group two was  $8.76 \pm 0.005$ , group three was  $9.02 \pm 0.007$  and group four was  $10.0 \pm 0.00$ .



**Figure 2** Mean concentration of prolactin

#### 3.3. FSH

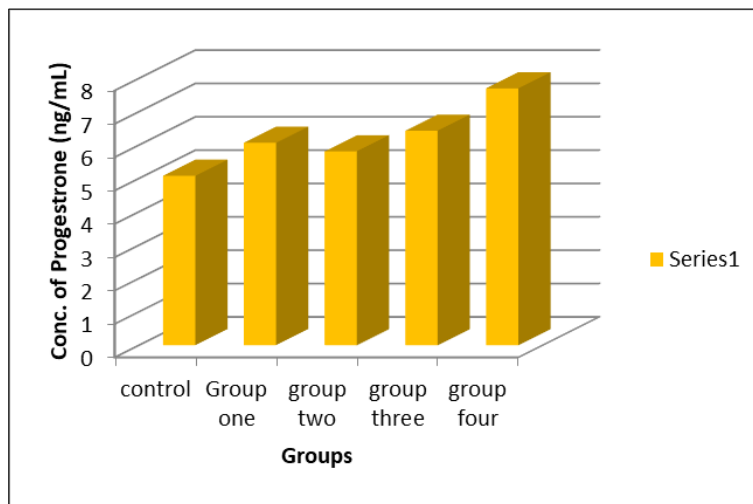
There was significant increase in the FSH level of the groups 1, 2, 3, and 4 when compared with the control group,  $p < 0.05$ . The mean value of control group was  $7.94 \pm 0.004$ , that of group one was  $9.50 \pm 0.002$ , group two was  $8.29 \pm 0.12$ , group three was  $9.37 \pm 0.000$  and group four was  $10.14 \pm 0.03$ .



**Figure 3** Mean concentration of FSH

### 3.4. Progesterone

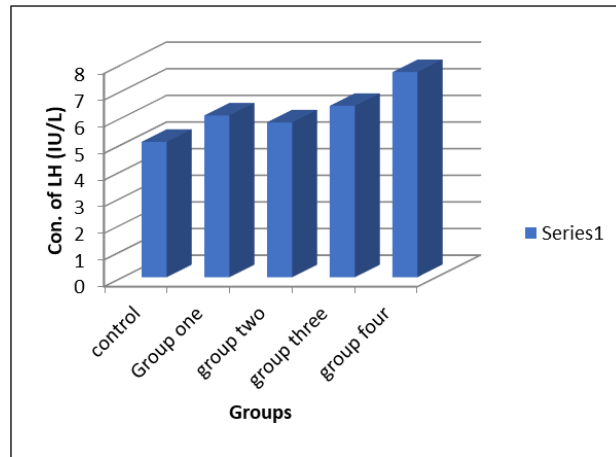
There was significant increase in the progesterone level of the groups 1, 2, 3, and 4 when compared with the control group,  $p < 0.05$ . The mean value of control group was  $6.10 \pm 0.05$ , that of group one was  $7.28 \pm 0.12$ , group two was  $6.35 \pm 0.002$ , group three was  $7.24 \pm 0.005$  and group four was  $8.15 \pm 0.005$ .



**Figure 4** Mean concentration of progesterone

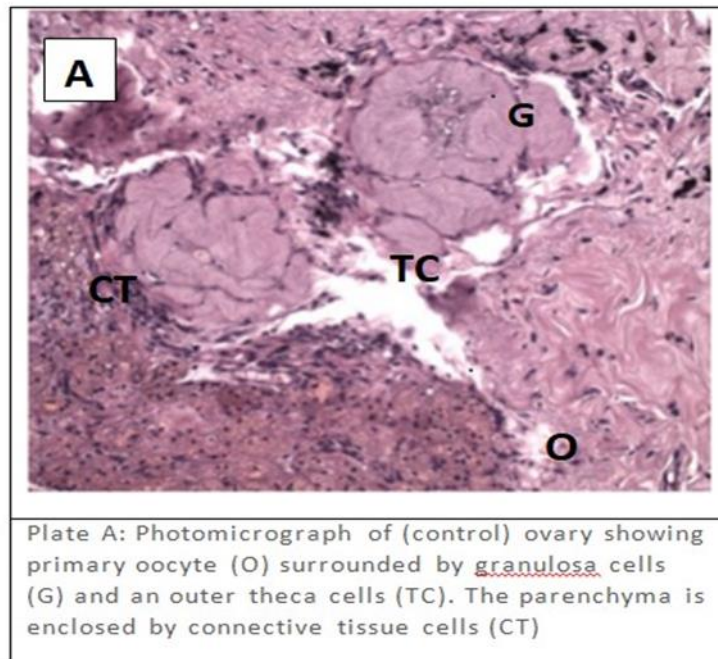
### 3.5. LH

There was significant increase in the LH level of groups 1, 2, 3, and 4 when compared with the control group,  $p < 0.05$ . The mean value of control group was  $5.08 \pm 0.004$ , that of group one was  $6.07 \pm 0.015$ , group two was  $5.81 \pm 0.005$ , group three was  $6.43 \pm 0.003$  and group four was  $7.70 \pm 0.004$ .



**Figure 5** Mean concentration of LH

### 3.6. Histology



**Figure 6** Photomicrograph of ovary of wistar rat feed with extract of *Annona muricata* (H&E X400), group A.

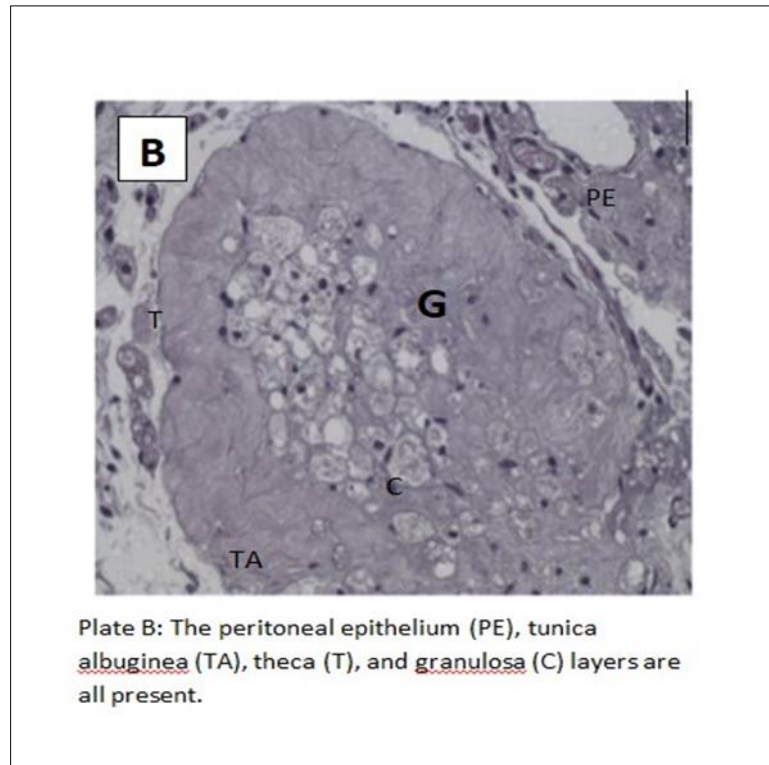


Figure 7 Photomicrograph of ovary of wistar rat feed with extract of *Annona muricata* (H&E X400), group B.

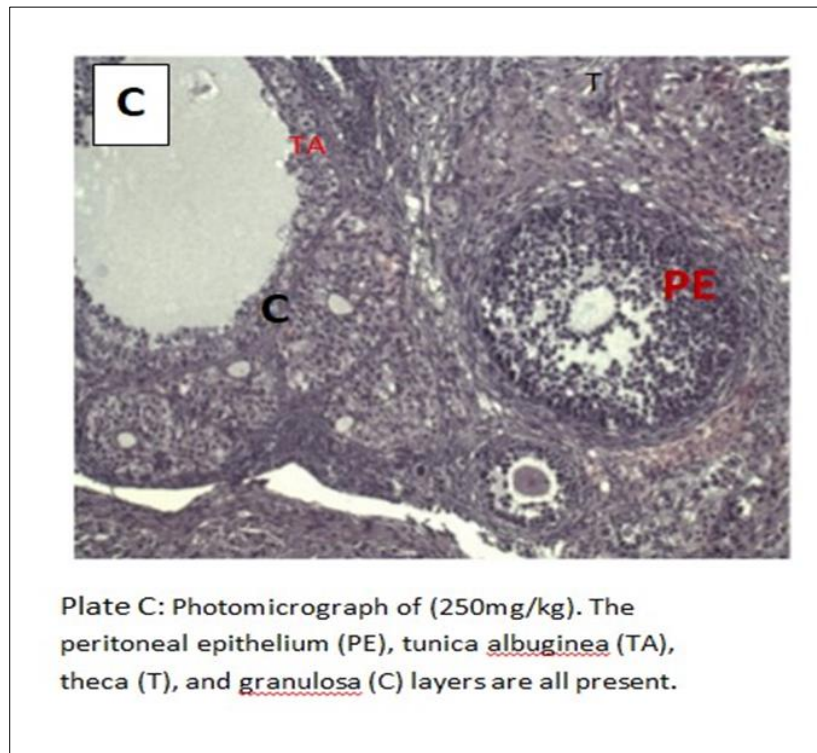
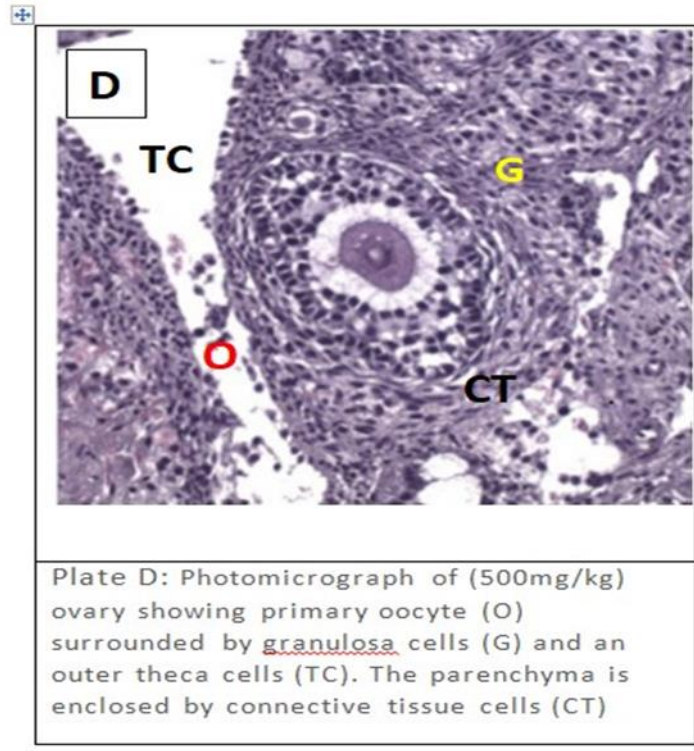
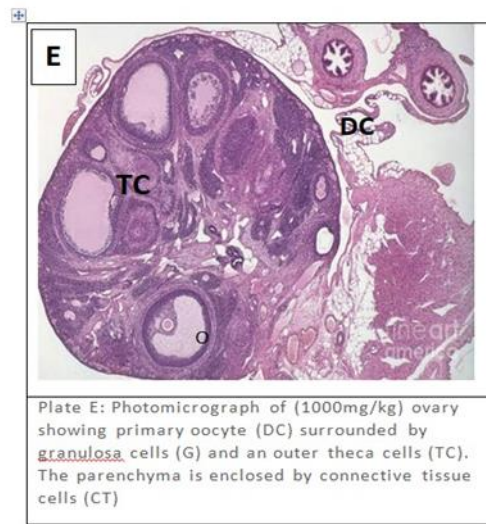


Figure 8 Photomicrograph of ovary of wistar rat feed with extract of *Annona muricata* (H&E X400), group C.





**Figure 9** Photomicrograph of ovary of wistar rat feed with extract of *Annona muricata* (H&E X400), group D.



**Figure 10** Photomicrograph of ovary of wistar rat feed with extract of *Annona muricata* (H&E X400), group E.

#### 4. Discussion

Because of paucity of information on the risks and benefits associated with the use of herbal medication in pregnancy and also due to absence of pre- registration clinical trials and post approval surveillance, the use of herbal medication especially in productive period is of special concern <sup>4</sup>. However, the histological studies done showed that *Annona muricata* had no negative or harmful effect on the ovaries of wistar rat.

This study revealed that consumption of *Annona muricata* significantly increased the gonadotropin hormones which could improve the chances of fertilization. The gonadotropin hormones (FSH and LH) function to stimulate the growth of primary follicle, maturation and release of the matured oocyte. Estrogen functions to promote proliferation and growth of specific cells responsible for secondary sexual characteristics. In addition, estrogen in combination with FSH function to promote LH receptors on the original granulosa cells, thus allowing LH stimulation to occur in addition to FSH stimulation. This would create more rapid increase in follicular secretion<sup>12</sup>. There was significant increase in the estrogen levels among the experimental groups (groups 1 to 4) as recorded in this study. This also promotes fertilization. Progesterone functions to promote secretory changes in the uterus. It also decreases the frequency and intensity of uterine contraction, thus plays a role in preventing expulsion of the implanted uterus. There was significant increase in the level of progesterone in groups one to four when compared with the control group. This also promotes fertilization and fertility. However, the level of prolactin was also increased among the experimental groups (groups 1 to 4). Hyperprolactinemia is one of the major causes of infertility<sup>13</sup>. Although the mechanism by which hyperprolactinemia causes infertility is not known. Elevated prolactin usually impact reproduction via action on gonadotropin hormones neurons of the hypothalamus and or on pituitary gland<sup>14, 15</sup>. It reduces the frequency and amplitude of LH release. This might suggest that prolactin's target may be the hypothalamus and pituitary gland<sup>16</sup>. In this study there was increase in both the gonadotropin and prolactin, and this may be because the increase in prolactin level was not so much to cause inhibitory effect on gonadotropin.

More so, the histological results showed no changes in the cellular makeup of the ovaries following administration of *Annona muricata* L. this goes further to show that the consumption of *Annona Muricata* is safe and does not pose any harmful effect on the reproductive organs. *Annona muricata* is safe for consumption, however, its effect on increased level of prolactin should be subjected to further studies.

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## 5. Conclusion

The findings from this research showed that *Annona muricata* L. had significant effect on the reproductive hormones of female wistar rats. The histological results showed no changes in the cellular makeup of the ovaries following administration of *Annona muricata* L. This goes further to show that the consumption of *Annona muricata* is safe and does not pose any harmful effect on the reproductive organs. This study also follows in line that *Annona muricata* significantly affected the progesterone, FSH, prolactin, and estrogen level of female wistar rats.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of ethical approval*

Ethical approval for the research was sought and obtained from the Ethical committee of Abia State University Uturu, Abia State, Nigeria.

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### Author's short biography



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Dr Cornelius. M. Nwozor was the former head of department ( HOD) of Physiology. He was recently promoted to the rank of Associate Professor of Human Physiology. Currently he is the Associate Dean, Faculty of Basic Medical Sciences.