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Original Research Article

The Effects of Ethanolic Extract of Premature *Carica papaya* (Pawpaw) Fruits on the Reproductive System of Female Wistar Rats

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Abstract

In recent times, different plant extracts have been reported to exert fertility enhancing effects. *Carica papaya* fruit is known for its use in preparation of herbal remedies. The aim of this study is to investigate the effects of *Carica papaya* on the reproductive system of female Wistar rats. Sixteen (16) female Wistar rats with regular oestrous cycle were used for this study. Animals were grouped into four (4) groups (control, low dose, middle dose, and high dose) of 4 animals each. *Carica papaya* fruit extract was administered for 14 days while monitoring the oestrous cycle. Twenty hours after the last administration, animals were sacrificed, and blood samples taken. Blood samples were collected for hormonal assay. Data obtained was expressed as Mean ± standard deviation, values were considered statistically significant when $P \leq 0.05$. Phytochemical analysis of premature *Carica papaya* fruit revealed the presence of alkaloids, terpenes, sterols and terpenoids were heavily present. Estrous cycle was not altered. Serum FSH and LH increased. The extract is likely to possess fertility enhancing properties.

Keywords: Infertility, phytochemical studies, oestrous cycle.

INTRODUCTION

Infertility is prevalent worldwide affecting about 5-8% of couples. Its prevalence in sub-Saharan Africa is high with 10-30% of couples affected in Nigeria (Chimbatata *et al.*, 2016). Hence, it has become serious health challenge with a lot of negative psychosocial and other consequences such as stigma, deprivation and neglect, violence, marital problems and mental health issues (Okonufua, 2003). The high cost of infertility treatments is associated with catastrophic health expenditure and many women discontinue treatment as a result. Premature Carica papaya has been used to treat menstrual pain, digestion and heart disease due to its antioxidant effect in oxidative stress. Hence, the need for further studies.

In recent times, different plant extracts have been reported to exert fertility enhancing effects. *Lophira Lanceolata, Cochlospermum planchonii, Kaempferia parviflora*, among others, have been reported to enhance fertility (Chaturapanich *et al.*, 2008; Al-Sa'aidi *et al.*, 2009; Etuk and Muhammad, 2009; Abu *et al.*, 2012). The active compounds such as phenols, alkaloids, saponins, and most especially flavonoids are known to have estrogenic (Das *et al.*, 2004) and androgenic activity (Yousef *et al.*, 2005).

Carica papaya fruit is a big oval in shape and is sometimes called pepo-like berries since they resemble melons by having a central seed cavity. In Nigeria, the fruit is known for its use in the manufacture of meat tenderizers and various herbal remedies used for the treatment of indigestion problems and the treatment of ulcers.

This study therefore aims to investigate the potential effect of *Carica papaya* on the female reproductive system.

MATERIALS AND METHOD

Fruit of premature *Carica papaya* was identified by Department of Plants Science and Biotechnology, Rivers State University. A voucher specimen number of RSUPB043 / *Carica papaya* fruit was assigned.

Preparation of Extracts

The premature fruit of *Carica papaya* was peeled and sliced into tiny pieces then air-dried in an air oven and grounded into powdered form. The powdered form was extracted with ethanol in a Soxhlet apparatus at 45ºC for 48 hours. The extract was preserved in an airtight glass jar and placed on a shelf in a cool dry place.

Procedure for Phytochemical Screening

To determine the presence of various phytoconstituents, a preliminary phytochemical study with the extracts was carried out to identify the following markers: alkaloids, saponins, tannis, flavonoid, terpenes, simple sugars, anthraquinones, sterols, terpenoids. Phytochemical screening was performed according to standard methods (Hikino *et al.*, 1984; Sofowara and Harborne, 1978).

Experimental Protocol

Sixteen (16) adult albino female Wistar rats, Rattus novegicus (100-122g body weight) were purchased from University of Port-Harcourt, Rivers State, Nigeria and, housed according to laboratory animal housing standards at the animal house, Faculty of Basic Medical Sciences, Rivers State University, Nigeria.

Rats were fed with standard rat feeds; the rats had access to unlimited and unrestricted water before and during the experiment. They were also acclimatized for 14 days (12h light/dark cycle) before the commencement of the experiment. The rats were randomized into four groups, and each consisted of five rats, which based on their weights were administered the extract orally in low, medium, and high doses except the control which was given only distilled water. The rats were identified by defining marks placed on their head, tail, and back.

Oestrus Cycle

The oestrous cycle stages and duration was determined according to the methods reported by Goldman *et al.*, (2007). Proestrous was defined by smears possessing more of nucleated epithelial cells, Oestrus was defined as smears with many cornified epithelial cells, Metaoestrous equal proportion of epithelial cell, cornified and leucocytes while dioestrus phase was defined by smears with presence of leucocytes.

Animal Sacrifice

On the 15th day, the body weight of the rats was obtained using a digital weighing balance; the animals were anaesthetized using chloroform and humanely sacrificed. Blood was collected, the uteri dissected out and surrounding tissues removed and washed with normal saline. The uteri were blotted on filter paper and weighed quickly on a sensitive balance and fixed in Bouin's solution for 24 hours. The paraffin embedded tissues were cut at 5mm thickness and stained with haematoxylin-eosin solution. The sections were examined microscopically for histological observation.

LD50

LD 50 was determined using Lorke's method. Phase 1: 9 animals were divided into 3 groups of 3 mouse each, each group was administered with 500mg/kg, 1000mg/kg and 2000mg/kg, and observed for 24 hours. Phase 2: 9 animals were divided into 3 groups of 3 mouse each, each group was administered with 3000mg/kg, 4000mg/kg and 5000mg/kg and observed for 24 hours.

Statistical Analysis

Data was expressed as Mean ± SD. Mean difference between the treated groups and the control was tested using one-way Analysis of variance. Values will be considered statistically significant when $P \leq 0.05$. Significant difference was assessed between control group and treated group using Post Hoc Test (Bonferroni). Computer software package SPSS version 25 was used.

RESULTS

Table 1: Showing the Major Ingredients of *Carica Papaya*

Parameters	Results
Alkaloid	+++
Flavonoid	+
Saponin	+
Tannins	+
Terpenes	+++
Anthraquinone	-
Sterols	+++
Terpenoids	+++
Simple sugars	+

Key: Heavily present: +++; Slightly present: ++; present: +; Absent: -

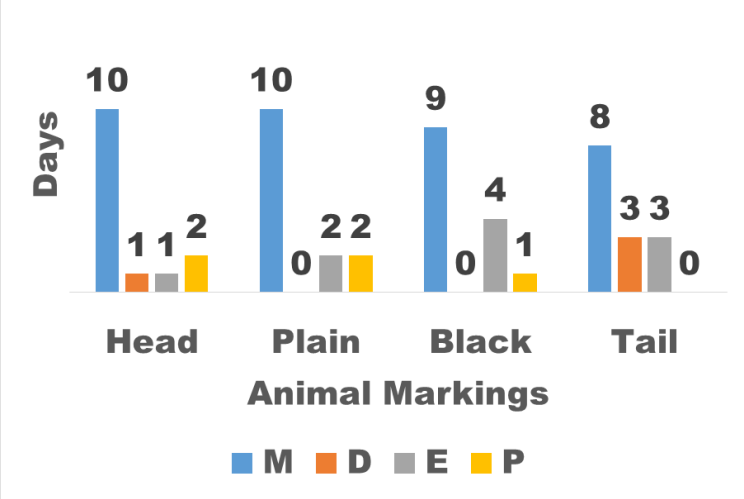

Figure I: Effect of Premature Plantain on estrous cycle (control)

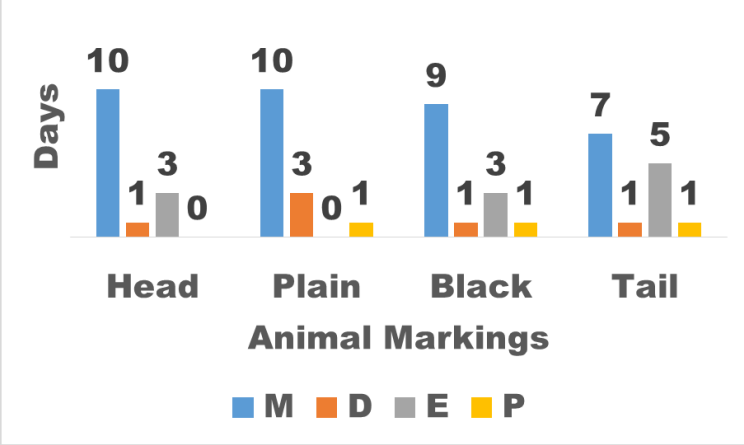

Figure II: Effect of Premature Plantain on estrous cycle (low dose)

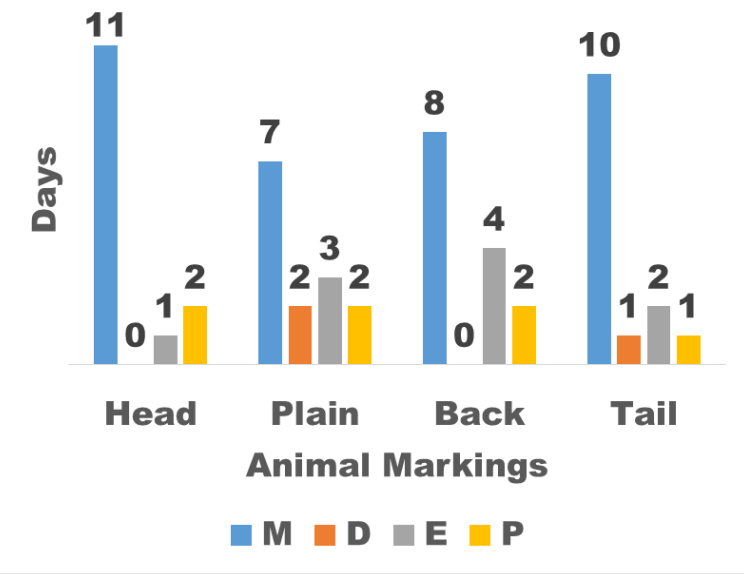

Figure III: Effect of Premature Plantain on estrous cycle (middle dose)

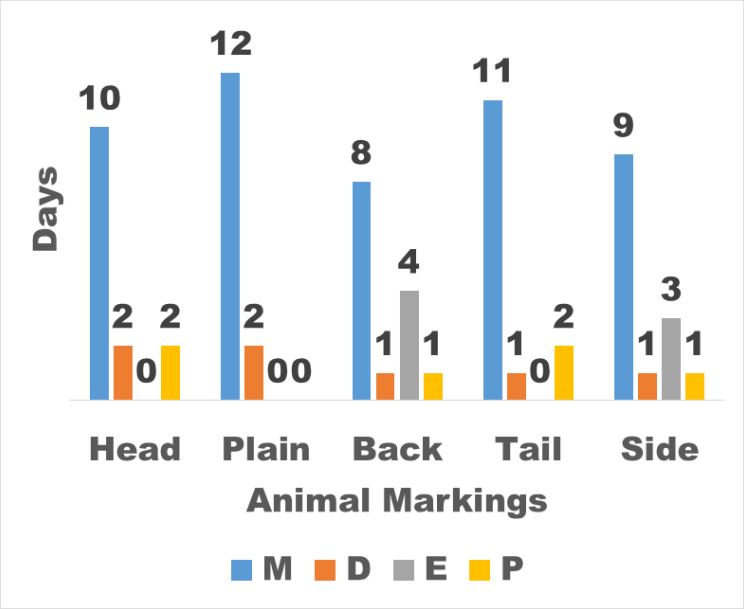

Figure IV: Effect of Premature Plantain on estrous cycle (high dose)

Table 2: Showing the Effect of *Carica Papaya* on Reproductive Hormones

Group	Control Mean ± S. D	Low Dose Mean ± S. D	Middle Dose Mean ± S. D	High Dose Mean ± S. D	F Value	P Value
F.S.H	0.350 ± 0.014	0.540 ± 0.014	0.595± 0.021	0.965± 0.035	252.349	0.00
L.H	0.590 ± 0.078	2.0450± 0.0778	1.275± 0.0778	1.815 ± 0.0212	190.856	0.00
OESTROGEN	78.50 ± 2.121	131.0 ± 2.828	82.0 ± 1.414	58.0± 1.414	464.434	0.00
PROGESTERONE	25.0 ± 1.414	24.0 ± 1.414	22.50 ± 2.121	21.50.707	2.148	0.234

Histological Plates Showing the Effect of Premature Carica Papaya on the Ovary

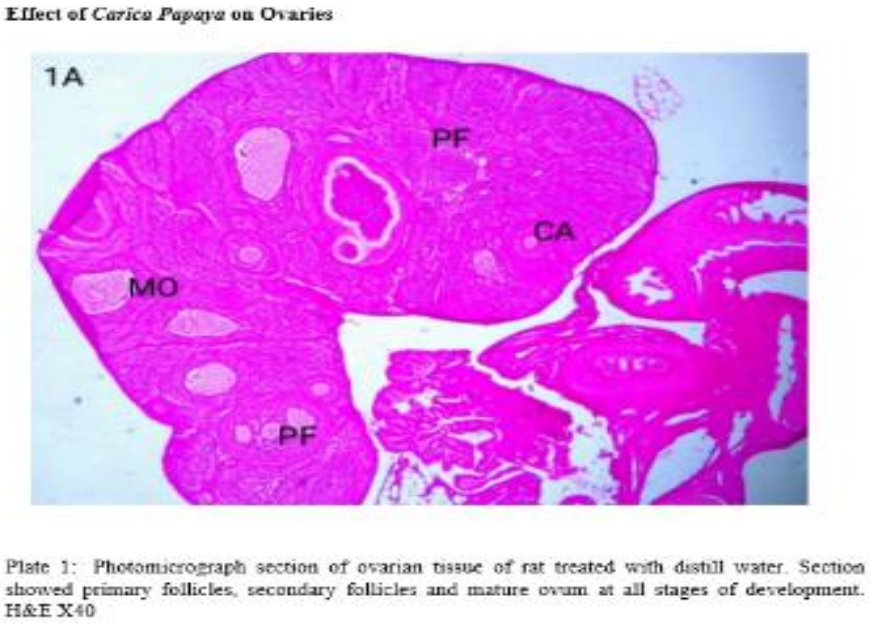

Plate 1: Photomicrograph section of ovarian tissue of rat treated with distill water. Section showed primary follicles, secondary follicles and mature ovum at all stages of development. H&E X40

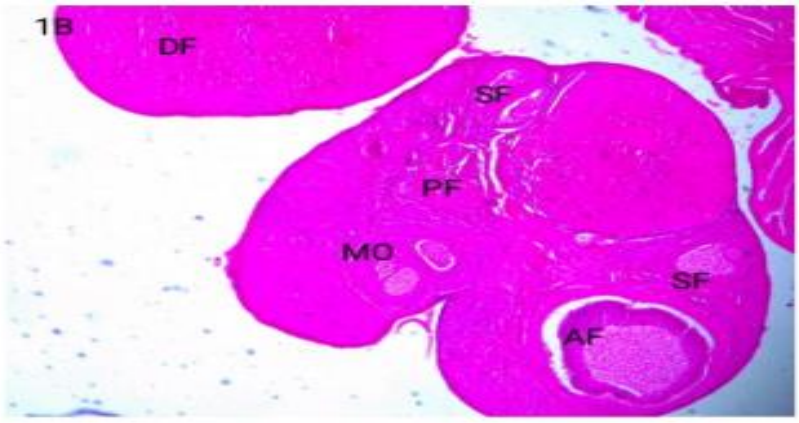

Plate 2: Photomicrograph section of Ovary tissue administered low dose, showing some mature ova (MO), Primary follicles, secondary follicles at different stages of development (GF), atretic follicles (AF) and corpus albicans. Degenerating follicles are also seen (DF). (H&E X100).

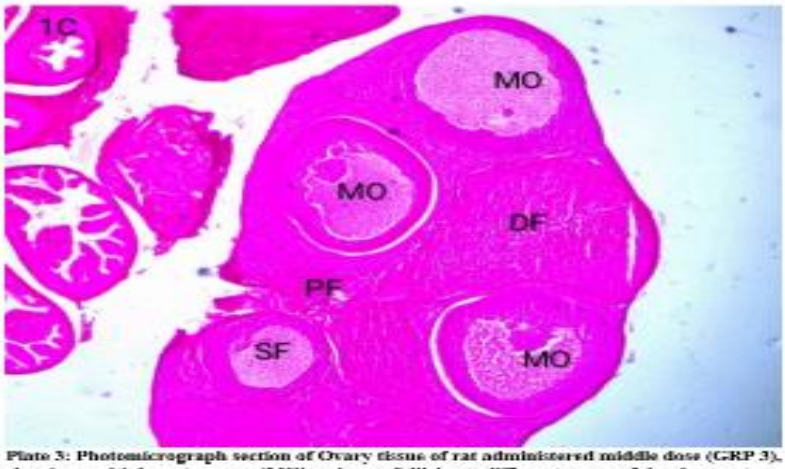

Plate 3: Photomicrograph section of Ovary tissue of rat administered middle dose (GRP 3), showing multiple mature ova (MO), primary follicles at different stages of development (GF), secondary follicle (SF) and degenerating. (H&E X100).

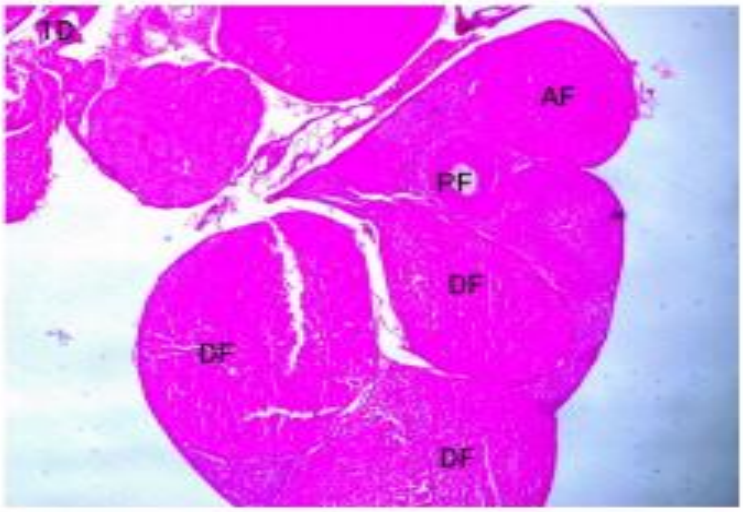

Plate 4: Photomicrograph section of Ovary tissue from rat administered high dose (Grp 4). Section showed few viable follicles (PF). There are high non viable follicles (degenerating DF) and atretic follicles (AF). (H&E - X100).
Effects of the extract on histology of the Uterus.

Histological Plates Showing the Effect of Premature Carica Papaya on the Uterus

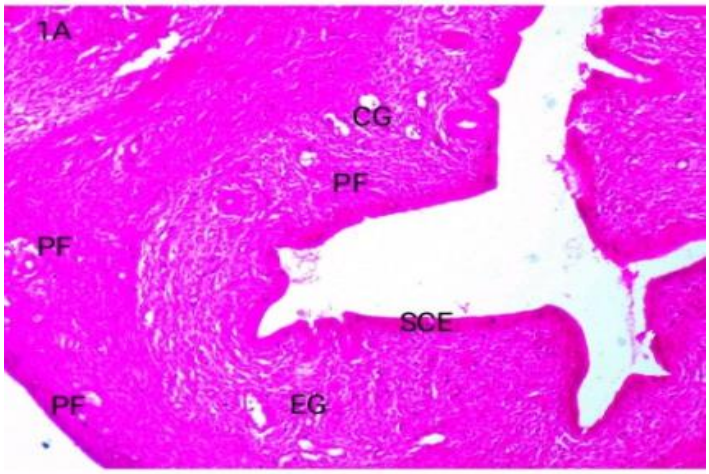

Plate 5: Photomicrograph of rat uterine tissue section of normal control rat given distill water Grp 1. Section showed Normal perimetrium, myometrium (MM), endometrium, (EM) endometrial glands (EG and CG)), lamina propria and surface simple columnar epithelium (SCE) can be seen. H&E X100

Plate 6: Photomicrograph of rat uterine tissue section of Group 2 treated with low dose. Section showed myometrium (MM) and endometrium tissues (EM) with some simple coiled glands (CG) and enlarged tubular glands (EG) with simple columnar epithelial cells (SCE). H&E X100

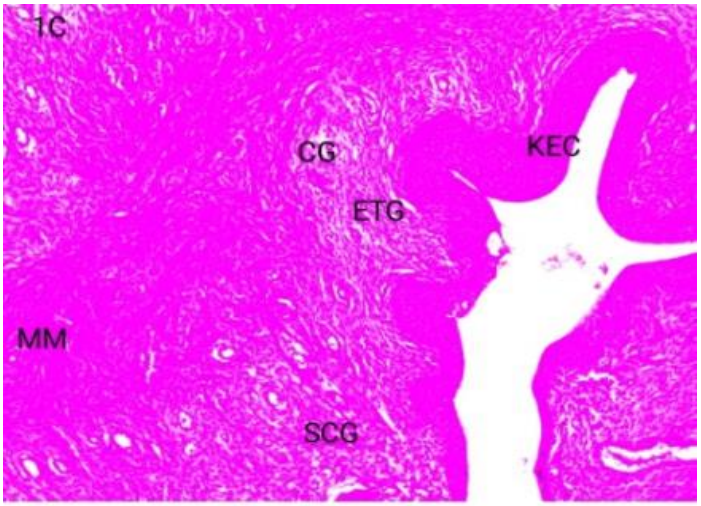

Plate 7: Photomicrograph section of ovarian tissues from rats treated with middle dose of. The uterine tissues showed normal perimetrium (PM) and myometrium (MM) but the endometrial epithelial tissues shows stratified squamous cells with keratinization. Some coiled (CG) and enlarged tubular glands (ETG) are present. H&E X100

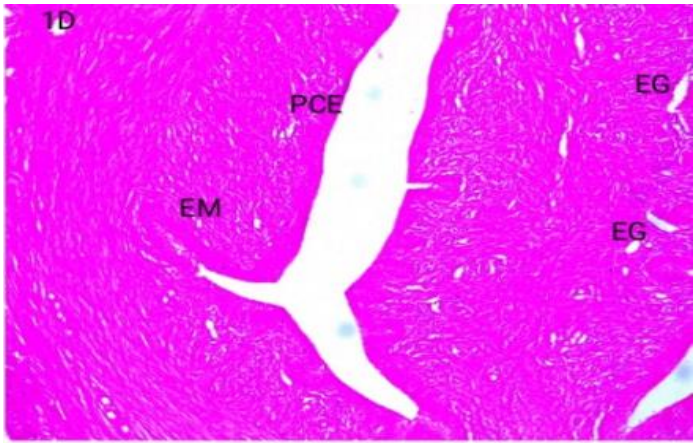

Plate 8: U X100 G4 Photomicrograph Section of rats' endometrial tissues treated with group 4. Section showed endometrium (EM). The endometrial glands are tortuous with pseudostratified columnar epithelium (PCE) with less blood vessels. Proliferative phase with more enlarged gland (EG) H&EX100

Histological Plates Showing the Effect of Premature Carica Papaya on the Anterior Pituitary Gland

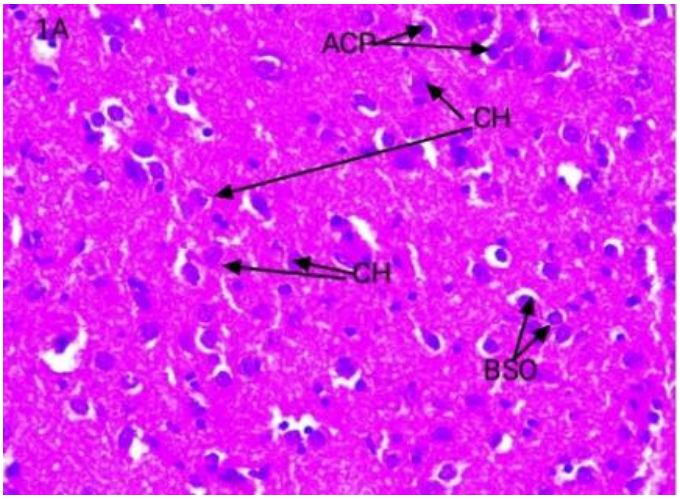

Plate 9: P X400 Photomicrograph section of Pituitary gland from rat tissue given distill water (Control). Section showed normal chromaphobes (CH), Acidophils (ACD) and Basophils (BSO) cells of the adnohypophysis. H&E X400

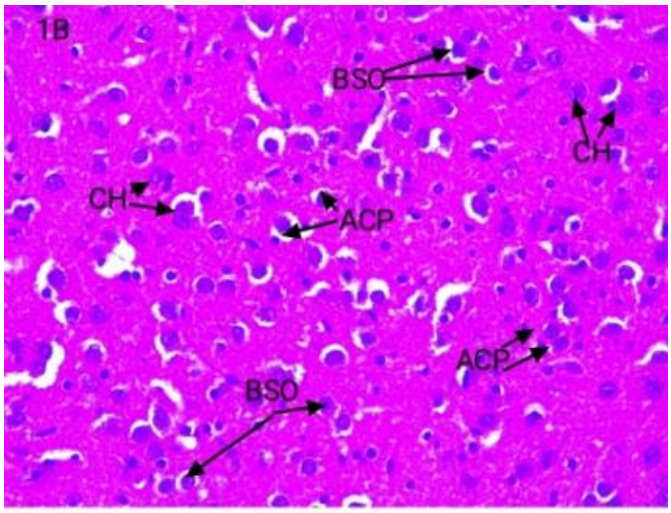

Plate 10: P X400 Photomicrograph section of Pituitary gland from rat tissue given low dose of extract. Section showed more chromaphobes (CH), Acidophils (ACP) and Basophils (BSO) cells of the adenohypophysis. H&E X400

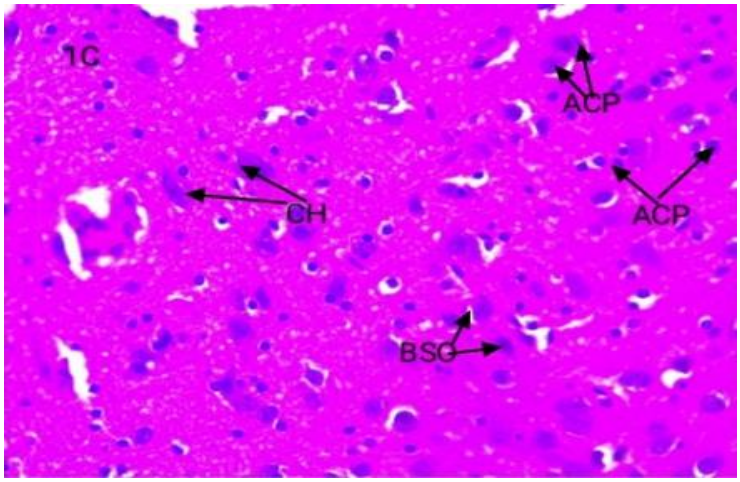

Plate 11: P X400: Photomicrograph section of Wistar rat treated with medium dose show representative adenohypophysis with a moderately increased number of chromophil and chromophobe (CH) and less acidophils (ACP) and basophils (BSO) cells x400

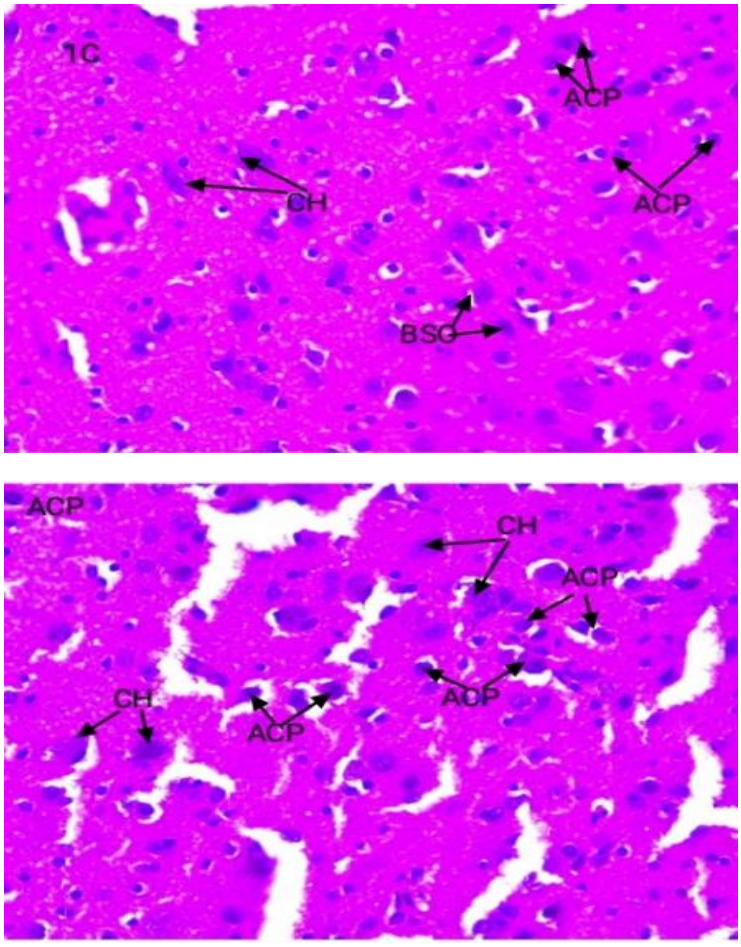

Plate 12: Photomicrograph section of pituitary gland tissue of Wistar rats from group 4 showed the pars distalis with decreased acidophil (ACP) and basophils (BSO) and a moderately increased number of chromophil and chromophobe (CH) cells, H&E x400

DISCUSSION

Phytochemical analysis of ethanolic extract of premature unripe *carica papaya* revealed that it contains flavonoids, alkaloids, terpenoids, terpenes, simple sugars, sterols, and tannins. This implies that the plant extract contains antioxidant and fertility enhancing effects. This agrees with Gupta *et al.,* (2013) and Tahvilzadeh *et al.,* (2006). They stated that Flavonoids and alkaloids possess potent antioxidant activity which may counteract with the Formation of Reactive oxidative Stress. This antioxidant activity may

be responsible for the fertility enhancing effects found in the extract.

In the present study, 14 days administration of *carica papaya* did not alter the oestrous cycle length/pattern. This implies that ethanolic extract of *carica papaya* had positive effect on the fertility. This result disagrees with Monima *et al.,* (2019), they reported that any interference which alter the normal functioning of agents of reproduction affects the ability to reproduce.

Hormonal assay of the groups treated with ethanolic extract of premature unripe Carica papaya fruit revealed a dose dependent increase in FSH following administration of the extract. This implies that the function of the uterus and ovaries were maintained. This was in line with Okonkwo *et al.,* (2021). They stated that FSH promotes follicle production and maintains the functions of the uterus.

A significant increase in serum LH levels was also observed in the present study. This implies that ovulation was maintained, the number of follicles was not altered and oestrous cycle was not altered. This was in contrast with (Benie *et al.,* 2003). According to them, reduction in serum LH levels disrupts ovulation, reduces the number of follicles, and alters oestrous cycle pattern.

An increase was also observed in the Serum oestrogen levels in the present study. This implies that the extract exerted an effect in the pituitary gonadal axis. This result was in contrast with Amah *et al.,* (2012), Koneri *et al.,* (2006). They reported a significant decrease in serum oestrogen levels and attributed to a distortion in the pituitary gonadal axis.

Histological analysis of the uteri conducted in the control and treated groups revealed that the administration of the extracts at low, medium, and high doses preserved cellular structures, but distorted the endometrial gland structures when compared to the control group. Enlarged tubular glands (Uterine glands) seen in this study imply that the extract preserved tubular glands, hence will support pregnancy. This result agrees with Gray *et al.,* (2001). They reported that uterine glands are required to support pregnancy.

CONCLUSION

In conclusion, this study reveals that ethanolic extracts of unripe premature carica papaya fruit is likely to possess fertility-boosting properties because it does not alter oestrous cycle and maintain ovulation. It also increased LH and FSH levels.

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