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The Effects of Ethanolic Extract of Premature *Musa Paradisiaca* (Plantain) Pulp on the Reproductive System of Female Wistar Rats

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ABSTRACT

Musa paradisiaca is a medicinal plant that is used widely in Nigeria for the preparation of various herbal remedies. This study investigated the effects of premature *Musa paradisiaca* (plantain) fruit on the reproductive hormones and characteristics of female wistar rats. Twenty female Wistar rats with regular oestrous cycle with an average weight of 145g were used. The animals were randomly grouped and divided into four groups of five animals each. *Musa paradisiaca* extract was administered for 14 days while monitoring the estrous cycle. Twenty hours after the last administration, animals in their proestrus were mated while animals on the oestrous phase were sacrificed, and blood samples taken. Phytochemical analysis of premature *Musa paradisiaca* revealed that terpenes, sterols, terpenoids, alkaloids were heavily present. The extract of premature *Musa paradisiaca* altered the estrous cycle of Wistar rats. Resorption was observed in high dose group. Significant increase in FSH, LH were observed in the treatment groups. Reduction in Progesterone level was observed in the high dose treatment group.

In conclusion, the extract of *Musa paradisiaca* potentially alters fertility, inhibited implantation and had a significant effect on the reproductive hormones.

KEYWORDS: Phytochemicals, Resorption, Estrous cycle, Musa paradisiaca.

ARTICLE DETAILS

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INTRODUCTION

Studies show that more than 60% of people consume herbal medicines without consulting a medical professional with a strong belief in their safety and effectiveness (Amorha et al., 2018). Herbal medicines are the fastest-growing area of the healthcare industry. Individuals may find the impersonal healthcare system intolerable so they turn to herbal remedies as substitute (Shrivastava et al., 2014). While people consume these remedies in expectation of the beneficial effects, there are the potential of their untoward effects on body functioning, hence the need for further studies.

Contraceptives are medications that are used to reduce fertility in a reversible manner. Antifertility drugs are in high demand due to worrying population trends. In emerging countries, the mortality rate has decreased while the birth rate has increased, especially in emerging countries. There is a need to establish an alternative, more effective and cheaper contraceptives in women. Although oestrogen and progesterone-based contraceptives are effective and popular, the risk connected with the medications have prompted the development of novel compounds derived from medicinal plants. As a result, herbal oral contraceptives are the need of the hour (Shrivastava et al., 2014).

Musa paradisiaca locally known as 'plantain' is the accepted name of the hybrid between *Musa acuminata* and *Musa balbisiana*. The plant belongs to a major group of *banana* varieties (genus *Musa*), a gigantic herb that springs from an underground stem, or rhizome. The premature plantain is a useful herbal remedy for treating different diseases such as hyperglycaemia in diabetes (Ojewole et al., 2003) and peptic ulcer disease (Prabha et al., 2011). The consumption of premature plantain as herbal remedy may be associated with adverse effects on various organs for example uterus and ovaries.

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

MATERIALS AND METHOD

Fruit of premature *Musa paradisiaca* was identified by Department of Plants Science and Biotechnology, Rivers State University. A voucher specimen number of RSUPb042/ *Musa paradisiaca* fruit was assigned.

Preparation of extracts

The premature fruit of *Musa paradisiaca* 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

Twenty healthy adult albino female Wistar rats, Rattus novegicus (150 - 200g 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 dose 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: PHYTOCHEMICAL CONSTITUENTS OF PREMATURE *Musa Paradisiaca*

Parameters	Results
Alkaloids	+++
Flavonoids	++
Saponins	+
Tannins	+
Terpenes	+++
Anthraquinone	-
Sterols	+++
Terpenoids	+++
Simple sugars	++

*High +++ Medium ++ Low + Absent –

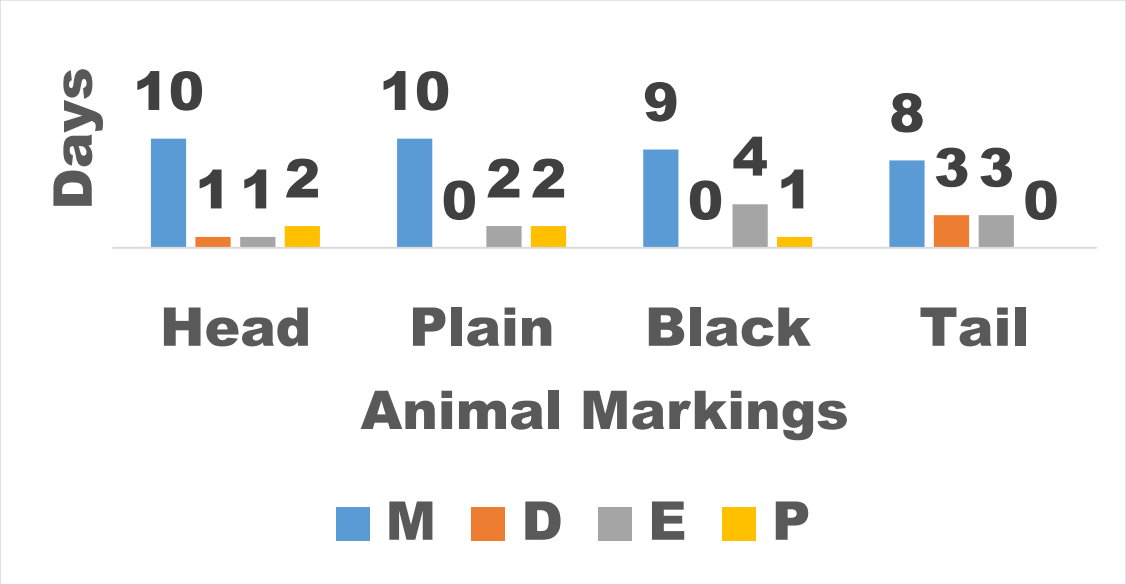


Figure I: Effect of Premature Plantain on estrous cycle (control)

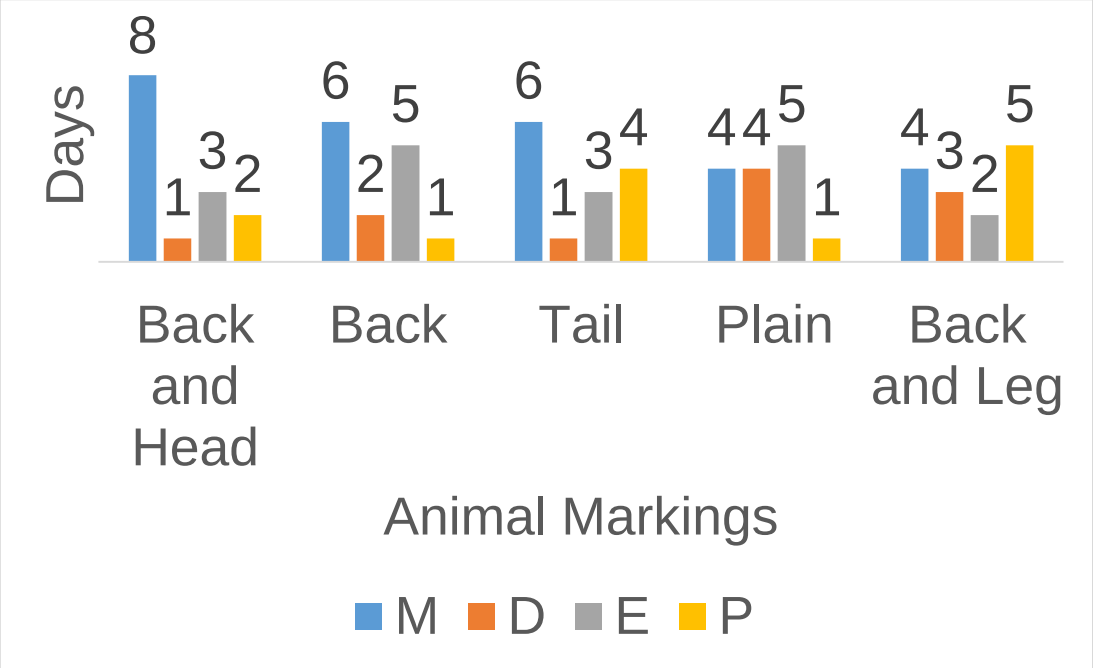


Figure II: Effect of Premature Plantain on estrous cycle (low dose)

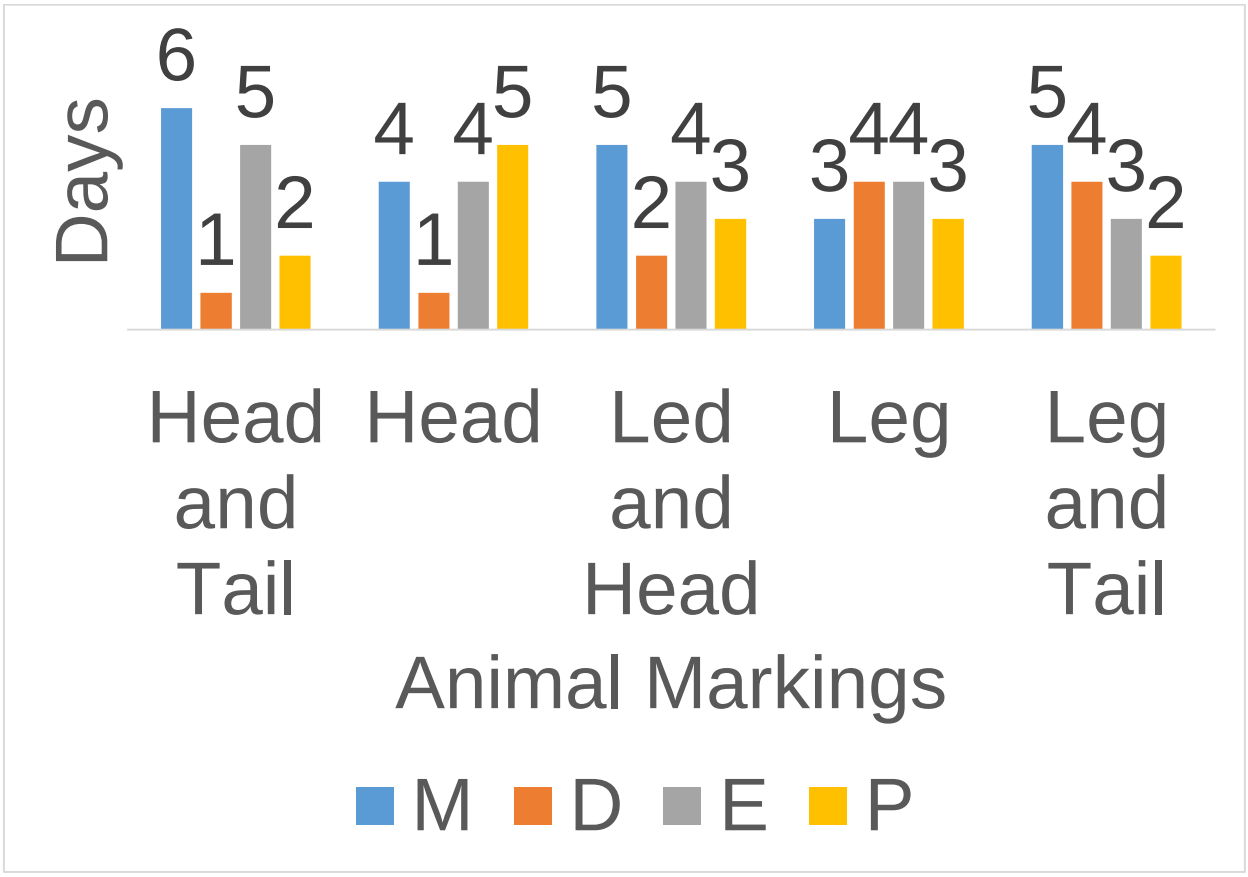


Figure III: Effect of Premature Plantain on estrous cycle (middle dose)

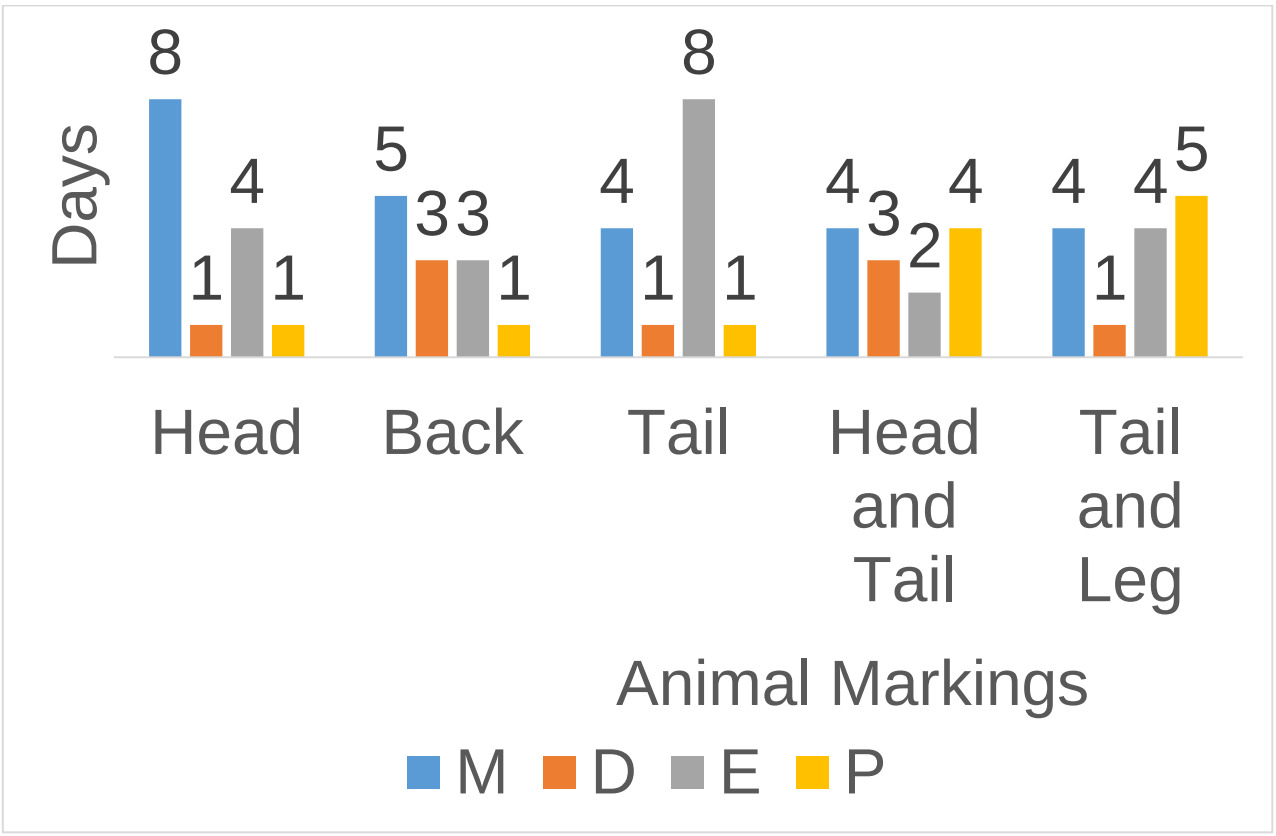


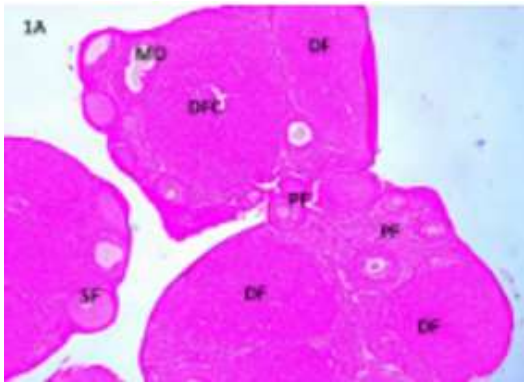
Figure IV: Effect of Premature Plantain on estrous cycle (high dose)

Table 2: Effect Of Ethanolic Extract Of Premature *Musa Paradisiaca* On Reproductive Hormone Levels

Hormone	Control Mean±SD	Low Dose Mean±SD	Middle Dose Mean±SD	High Dose Mean±SD	F-Value	P-Value
FSH	0.210±0.014	0.235±0.021	0.685±0.035	0.445±0.021	166.149	0.000
LH	0.470±0.056	0.595±0.050	1.315±0.049	0.786±0.035	118.822	0.000
ESTROGEN	61.000±5.657	57.350±65.973	56.500±0.707	95.000±1.414	0.622	0.697
ROGESTERONE	9.45±0.212	36.50±2.121	32.000±1.414	13.750±0.353	212.851	0.000

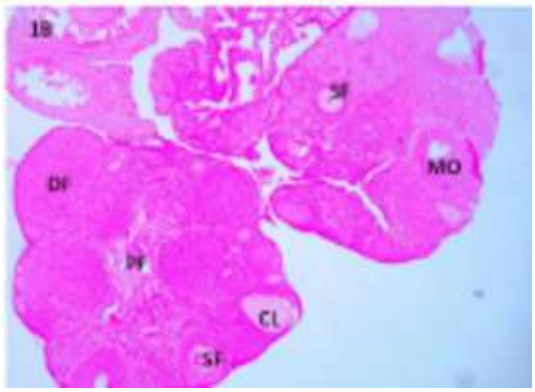
HISTOLOGICAL PLATES SHOWING THE EFFECT OF PREMATURE *Musa paradisiaca* ON THE OVARY

PLATE 1



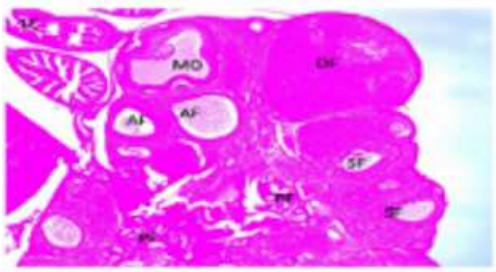
Control group: Photomicrograph section of Ovarian tissue of rats which received distilled water. Section showed primary follicle (PF), secondary follicle (SF), and mature ovum (MO) at all stages of development (DF). Section also showed degenerating follicle (DF), H&E × 40.

PLATE 2



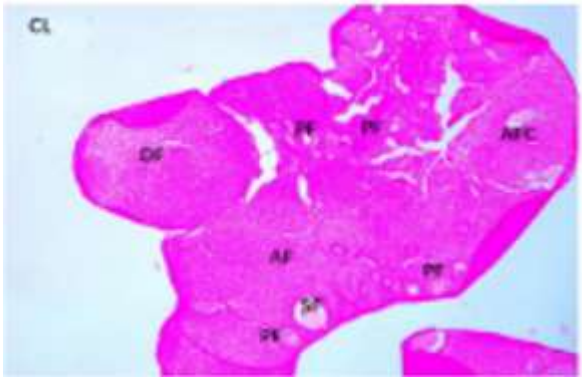
Low Dose group: Photomicrograph section of Ovarian tissue of rats which received low dose of plantain pulp. Section showed primary follicle (PF), secondary follicle (SF), and mature ovum (MO) at all stages of development (DF). Section also showed degenerating follicle (DF) and Corpus luteum (CL), H&E × 40.

PLATE 3



Medium Dose group: Photomicrograph section of Ovarian tissue of rats which received low dose of plantain pulp. Section showed primary follicle (PF), secondary follicle (SF), and mature ovum (MO) at all stages of development (DF). Section also showed degenerating follicle (DF) and atretic follicle, H&E × 40.

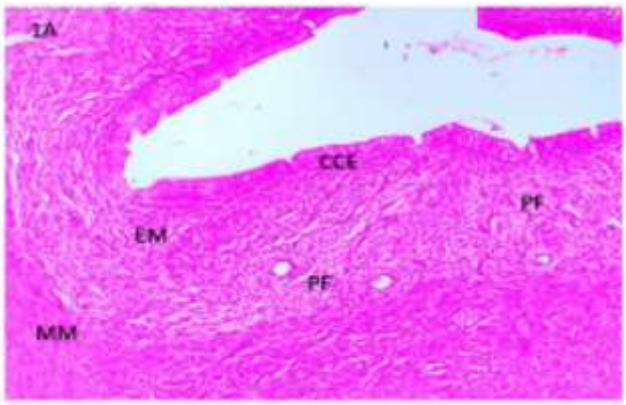
PLATE 4



High Dose group: Photomicrograph section of Ovarian tissue of rats which received low dose of plantain pulp. Section showed primary follicle (PF), secondary follicle (SF), and mature ovum (MO) at all stages of development (DF). Section also showed degenerating follicle (DF) and atretic follicle with cicatrization, H&E × 40.

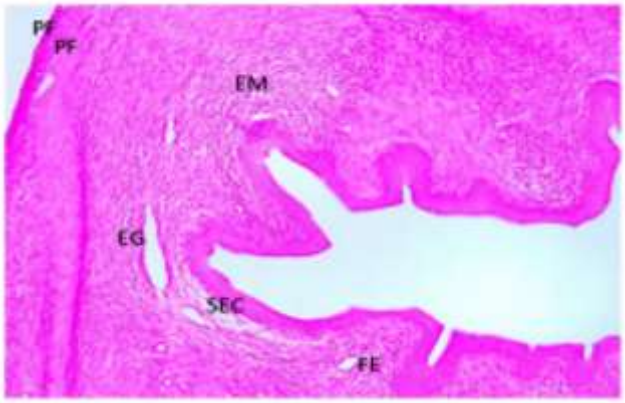
HISTOLOGICAL PLATES SHOWING THE EFFECT OF PREMATURE *Musa paradisiaca* ON THE UTERUS

PLATE 5



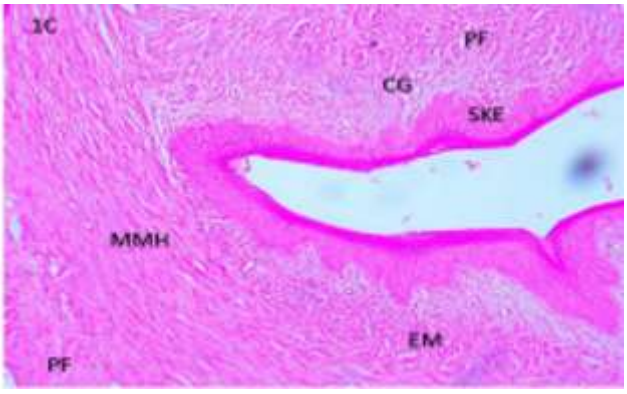
Control: Photomicrograph section of uterine tissue from control rat received distill water. Section showed normal myometrium (MM) and endometrium (EM). There are coiled (CG) and tortous tubular (TG) endometrial glands. The lamina propria and numerous blood vessels are seen with surface simple columnar epithelium (SCE). H&E 100.

PLATE 6



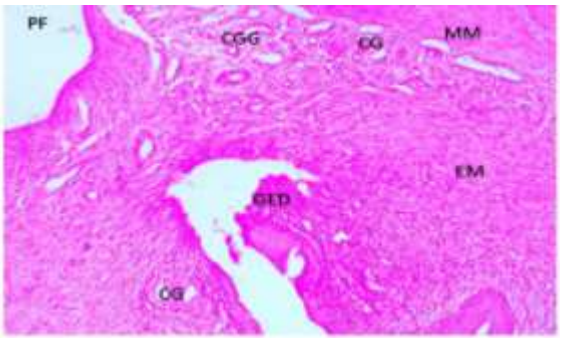
Low dose: Photomicrograph section of uterine tissue from rats which received low dose. Section showed normal myometrium (MM) and endometrium (EM). There are very few coiled (CG) and enlarged tubular (TG) endometrial glands. Surface epithelium showed stratified and keratinized epithelium (SCE). H&E 40.

PLATE 7



Medium dose: Photomicrograph section of uterine tissue from rats which received medium dose. Section showed myometrial hypertrophy (MMH) and reduced endometrial layer (EM). There are few coiled (CG) and few blood vessels. The surface epithelium showed stratified and keratinized epithelium (SCE). H&E 100.

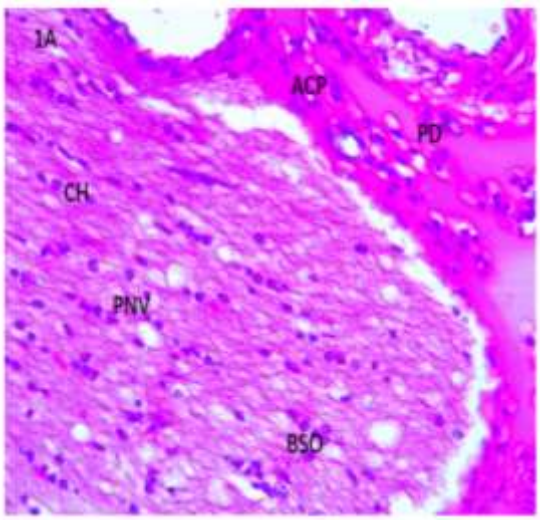
PLATE 8



High dose: Photomicrograph section of uterine tissue from rats that received high dose. Section showed normal myometrium (MM) and endometrium (EM). There are many coiled (CG), cystic coiled gland (CCG). The surface epithelium showed glandular epithelial damage (GED). H&E 100.

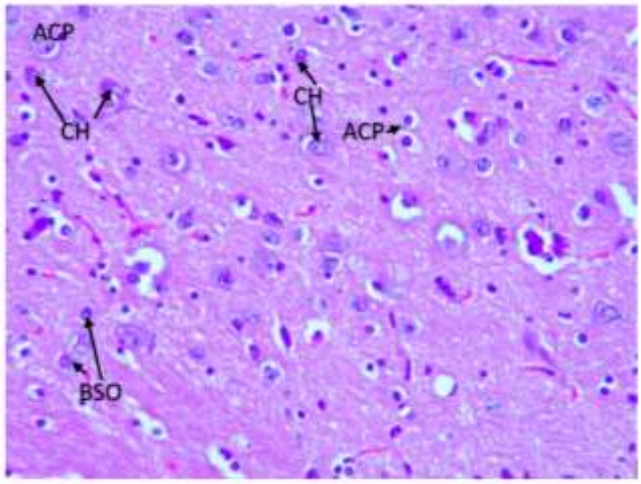
HISTOLOGICAL PLATES SHOWING THE EFFECT OF PREMATURE *Musa paradisiaca* ON THE ANTERIOR PITUITARY GLAND

PLATE 9



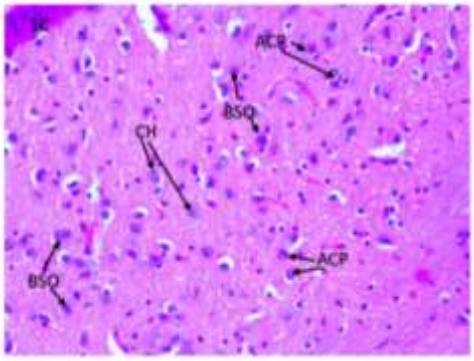
Control: Photomicrograph section of Pituitary gland showing pars nervosa (PNV) and distalis (PD) from rat tissue given distilled water. Section showed normal chromophobes (CH), Acidophils (ACP) and Basophils (BSO) cells. H&E 400

PLATE 10



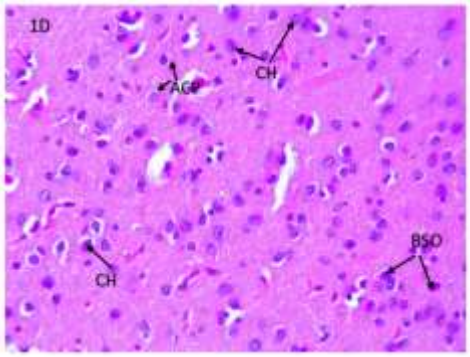
Low dose: Photomicrograph section of Pituitary gland showing adenohypophysis of rat tissue from low dose group. Section showed normal chromophobes (CH), Acidophils (ACP) and Basophils (BSO) cells. H&E 400

Plate 11



Medium dose: : Photomicrograph section of Pituitary gland showing adenohypophysis of rat tissue from medium dose group. Section showed less chromophobes (CH), high Acidophils (ACP) and Basophils (BSO) cells. H&E 400

PLATE 12



High dose: Photomicrograph section of Pituitary gland showing adenohypophysis of rat tissue from medium dose group. Section showed less chromophobes (CH), high Acidophils (ACP) and Basophils (BSO) cells. H&E 400

PHOTOGRAPHIC EVIDENCE OF IMPLANTATION AND RESORPTION

Plate 13: implantation sites are shown by red arrows; corresponding resorption sites are shown by blue arrows as shown in the fallopian tube of rats administered with premature plantain

DISCUSSION

Fertility is defined as the ability to reproduce, and fertility is defined as the concretization of reproduction, with the number of new individuals being evaluated at the end (Almeida et al., 2000). In females, reproductive capacity refers to a number of features of reproductive physiology, including the ability to produce sufficient gametes, balanced hormonal production, and morphological and biochemical changes in the oviduct and uterus (Mukherjee and Mitra, 2009). However, numerous studies have demonstrated that plants can be used as contraceptives in both rodents and people.

Phytochemical studies of ethanolic extract of *Musa paradisiaca* fruit revealed that it contains high concentration of terpenes, steroids, terpenoids, alkaloids; moderate concentration flavonoids and simple sugars and low concentration of saponin and tannin as shown in Table I. Some studies have revealed that saponin and steroidal glycosides have contraceptive activity (Kanita et al., 1996). This implies that *Musa paradisiaca* fruit is likely to have contraceptive activity. Khushalani et al., (2006) reported that non-steroidal com cccpounds with estrogenic activity for example flavonoids, alkaloids, and phenols have anti-fertility agents.

In this study, it was observed that middle and high doses of ethanolic extract of premature plantain prolonged oestrous and proestrous phases as seen in figure III and IV. Low dose also prolonged dioestrous and Metaoestrous as shown in figure I. This suggests that the plant *Musa paradisiaca* caused acyclicity of the oestrous cycle, therefore may likely alter fertility. It was also observed that ethanolic extract of *Musa paradisiaca* fruit had a significant effect on the oestrous cycle of Wistar rats. This implies that long term consumption of premature *Musa paradisiaca* fruit is likely to affect the ability of an individual to reproduce. This result agreed with Monima et al., (2019), they stated that any interference that alters the normal functioning of agents of reproduction affects the ability to reproduce.

The results of this study have revealed that the consumption of ethanolic extract of premature *M. paradisiaca* fruit caused an effect on the levels of FSH, LH and Progesterone as shown in table II. Implying that the extract contains compounds capable of influencing Gonadotropin releasing hormone. This agrees with Hafez and Hafez (2000), they reported that chemical compounds of plant extracts induce release of Gonadotropin releasing hormone thus increasing FSH and LH levels. The observed increase can also be attributed to the effect of phytochemical constituents present in the plant, on Gonadotropin releasing hormone. These findings agreed with

Egba et al., (2014), they reported that plant extract can influence hormonal response in host animals.

In the present study, fluctuations in the level of Progesterone was observed treatment group. These fluctuations can also be attributed to the fact that the plant extract disrupted the reproductive hormones. This is in agreement with some authors Mclean et al., (2012) and Aritonang et al., (2017), who have reported that fluctuations in circulating levels of ovarian steroids: Oestrogen, Progesterone, FSH, and LH, affect the oestrus cycle and histological appearance of the female reproductive organs.

Histopathological findings of the anterior pituitary gland showed that secretory cells (Acidophils, Basophils, and Chromophobes) in the low-dose group were normal and consistent with the control as shown in Plates 9 and 10. In the middle dose group (Plate 11), there was an increase in secretory cells acidophils, and basophils but fewer chromophobes. In the high-dose group, there was an increase in all secretory cells, acidophils, basophils, and chromophobes. The increase in basophils (cells responsible for secreting Follicle stimulating hormone and Luteinizing hormone) as noticed in the histological slide could be the reason for the increment in the hormonal level of FSH and LH observed. This result was similar to the result of Olawuyi et al., (2019). They stated that a decrease in secretory cells leads to a decrease in FSH and LH. An increase in Gonadotropins and gonadotropin-releasing cells of the anterior pituitary gland observed in this study suggests that the extract of premature *Musa paradisiaca* pulp has an effect on the anterior pituitary gland. This finding is in accordance with Ndem et al., (2017). They stated that an increase in pituitary gland cells was necessary for a surge in necessary reproductive hormones.

Degenerating corpus luteum was observed in the ovaries. This implies that ovulation was reduced by the administered plant extract. The result was in contrast with Ernest et al., (2018). They stated that an increase in corpus luteum shows an inducing effect of ovulation by plant extract. Myometrial hypertrophy seen in the histopathological studies of the uterus could be because of the presence of flavonoids. This was in accordance with Burrow et al., (2000) who stated that members of the flavonoid class of phytochemicals possess estrogenic activity. Also, Shynlova et al., (2010) stated that estrogens cause myometrial hypertrophy.

In this study, evidence of foetal resorption was observed in pregnant animals in high dose group. This implies that ethanolic extract of *Musa paradisiaca* fruit is likely to have abortifacient effects. The reason for the foetal absorption seen in the present study could be because of the presence of flavonoids. Result from this study agreed with Robaki et al., (1988) and Iranloye et al (2010), which reported that flavonoids have antispasmodic actions, and act directly on the uterine muscles to cause foetal abortion. This study also agrees with Dabe et al., (2020), they stated that plant extract which increases the number of resorption sites are said to exhibit abortifacient effect.

In conclusion, the phytochemical constituents of premature *Musa paradisiaca* is likely to cause acyclicity. It is likely to have a negative effect on implantation.

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