



Effect of the Chromatographic Fractions of *Abrus precatorius* Leaf on the Histology of Uterus and Ovary of Female Wistar Rats

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Author's contributions

This work was carried out in collaboration among all authors. Author NDA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IHO and NB managed the analyses of the study. Author NB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the effects of chromatographic fractions of *Abrus precatorius* leaf extracts on the histology of the ovary and uterus.

Study Design: *Abrus precatorius* plant contain woods in a twinning form and belong to the Fabaceae (Leguminosae) family. It has red and black seeds. *Abrus precatorius* also possess a pod which is short and stout brownish in color [1]. The plant grows in bushes and farm and sometimes in hedge. *Abrus precatorius* are said to be taken for tuberculosis and painful swellings [2]. According to Ross [3], they can be used as laxative, expectorant and aphrodisiac medicines and are sometimes used in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/leukemia and dysmenorrhea. Experiment has demonstrated that the seed have the ability to retard fertility both in male and female [4]. Studies done in the past revealed that the plant *Abrus precatorius* can kill cells or cause cell death at the same time leading to death of tumor [5].

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Extraction of the leaves of *A. precatorius* with methanol has shown through previous study to possess bronchodilatory effect and its use traditionally in the management of asthma [6]. Extracts obtained from the roots, has good antibacterial activity especially against *Staphylococcus aureus* (Prabha et al. 2015). In a study performed in Tanzania, it was confirmed that boiling the leaves of *A. precatorius* with water and taking it orally as three table spoonful in twice daily dosage regimen for the treatment of epilepsy is helpful [7].

Female wistar rats were treated with chromatographic fractions of *A. precatorius*, F1, F2, F3 and F4 (30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg) for thirty days. One hundred and ten Wistar rats were divided into twenty-two (22) groups of five rats each. All the rats were weighed before and during the experiment. Group 1 (Control) received 0.5 mls, Phosphate Buffer Solution (PBS); Group 3-7. received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F1. Group 8-12 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F2. Group 13-17 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F3 and Group 18-22 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F4 respectively. The fractions/drugs were administered orally. The rats were treated with chromatographic fractions of *A. precatorius*, F1, F2, F3 and F4 (30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg) for 30 days. The animals were sacrificed, dissected and the uterus and ovaries obtained for histology study. The study revealed histological evidence that the chromatographic fractions of *Abrus precatorius* leaf do not have any potential adverse effect on the ovary and uterus of the Wistar Albino rats.

Keywords: *Abrus precatorius*; chromatography; histology; uterus; ovary.

1. INTRODUCTION

Medicinal plants have been employed as an important tool worldwide for medications because it possesses important phytoconstituents (Sharma et al. 2014). Huge percentage of the people in the world today depends on plants and plants related products for their health challenges and diseases (Tiwari et al. 2012). They are obtained in the form of medicinal teas, decoctions and crude tablets used in traditional medicine to concentrated, standardized extracts produced in modern pharmaceutical facilities. *Abrus precatorius* plant contain woods in a twinning form and belong to the Fabaceae (Leguminosae) family. It has red and black seeds. *Abrus precatorius* also possess a pod which is short and stout brownish in color [1]. The plant grows in bushes and farm and sometimes in hedge.

1.1 Ethnomedicinal Uses

Abrus precatorius are said to be taken for tuberculosis and painful swellings [2]. According to Ross [3], they can be used as laxative, expectorant and aphrodisiac medicines and are sometimes used in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/leukemia and dysmenorrhea. Experiment has demonstrated that the seed have the ability to retard fertility both in male and female [4]. Studies that were done in the past revealed that the plant *Abrus precatorius* can kill

cells or cause cell death at the same time leading to death of tumor [5]. Extraction of the leaves of *A. precatorius* with methanol has shown through previous study to possess bronchodilatory effect and its use traditionally in the management of asthma [6]. Extracts obtained from the roots, has good antibacterial activity especially against *Staphylococcus aureus* (Prabha et al. 2015). In a study performed in Tanzania, it was confirmed that boiling the leaves of *A. precatorius* with water and taking it orally as three table spoonsful in twice daily dosage regimen for the treatment of epilepsy is very helpful [7]. Histology assay of crude *Abrus precatorius* leaf extract on female reproductive organs has been discovered to be have an enhanced folliculogenesis and ovulation with no defect in treated rats [8]. Hence, the essence of this study which is to determine the effect of chromatographic fractions of *Abrus precatorius* extract on uterus and ovary of female albino Wistar Rats.

According to Sharma (2007), chromatographic techniques are an analytical technique commonly employed for, purification and identification of constituents of a mixture. There are many types of chromatographic fractionation e.g. liquid chromatography, gas chromatography, thin layer chromatography, column chromatography, ion-exchange chromatography, affinity chromatography, but all these employ the same basic principle. Chromatography technique for separating the components or solute of a mixture on the basis of the relative amount of each solute

distributed between a moving fluid streams, called the mobile phase and a contiguous stationary phase. The mobile phase may be either a liquid or a gas, while the stationary phase is either a solid or a liquid (Calvin and Keller, 2004). Kinetic molecular motion continuously exchanges solute molecules between the two phases. Fractionation of plant extract and purification of active principle optimizes their potencies and also found to extend the activity of the plant extracts (Okoli, 2005).

Histology studies the microscopic framework of biological material and also looks at the way the individual sections are structurally and functionally linked or connected [9]. Clinically, histology is employed as a diagnostic parameter to comprehend the developments in the body tissues. It is alternatively called microscopic anatomy because it investigates the structure and effectiveness of the human body at the microscopic level.

Uterus (womb): The uterus is a cavernous, pear-shaped organ that is the abode for a ripening fetus. It has two partitions: the cervix, being the lower part that exit into the vagina, and the main body of the uterus, known as the corpus. The corpus has the ability to dilate easily to support a growing baby. There is a channel through which the cervix permits sperm to penetrate and menstrual blood also exits [10].

Ovaries: The ovaries are small glands that have an oval-shape structure and are located on the two sides of the uterus. The ovaries releases eggs and synthesize hormones. They are in charge of the synthesis of estradiol and progesterone, female reproductive hormones [10].

Previous study made us to know that there is high rate of female infertility in Africa which has called for this current study [11]. Similarly, Chimsamy et al. [12] in a different assay showed that many women are in need of an effective indigenous African drug they can trust and take with minimal side effects, for management of infertility. Previous study has shown that *Abrus precatorius* Linn extract has beneficial effect on reproductive parameters [8]. This study seeks to determine whether further purified fractions of the *Abrus precatorius* leaf crude extract will produce different or similar changes on the ovary and uterus of the female wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Samples

2.1.1 Collection

The leaves of *A. precatorius* were collected at Orlu, Imo state and were authenticated by Dr. I. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt. A voucher specimen was then deposited in the Herbarium of the Department of Plant Science and Biotechnology with Ethical Clearance certificate /specimen number UPH/P/120.

2.1.2 Extraction

375 g of pulverized *A. precatorius* leaf were successively macerated with 70% methanol in a jar for 72 hours. The extract obtained were then concentrated in a rotary evaporator to a very small volume which was dried on a water bath. Dried methanol extract was selected and stored in an air-tight container in a cool place.

Table 1. Order of elution

Hexane	Ethylacetate	Methanol
100	-	-
75	25	-
50	50	-
25	75	-
	100	-
	75	25
	50	50
	25	75
		100

2.2 Chromatographic Fractionation

A clean glass column was plugged with cotton wool at the base to support the packing material. Silica gel was then packed into the column length with gentle tapping at intervals to compact the silica gel particles tightly. This was stopped when the packing length reached the required height. N-hexane was then run through the column to fill it. The plant extract was extract then mixed, with dry silica gel and the mixture evenly transferred to the column head. Gradient elution then commenced, powered by vacuum pump using n-hexane, n-hexane + ethyl acetate, ethyl acetate, ethyl acetate + methanol, and methanol, in that sequence, as elution solvents. Fractions were collected serially during elution and were monitored by Thin Layer Chromatography (TLC). Eluates with similar chromatographic patterns

were pooled together, concentrated and dried. This process yielded four fractions which were used for the study.

2.3 Animal Experiments

One hundred and five (105) female wistar rats were divided into twenty-one (21) groups of five rats each. All the rats in the groups were weighed every 3 days and the weight recorded [8].

Group 1 (Control) received 0.5 mls, Phosphate Buffer Solution (PBS);

Group 2-6 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F1 fraction of *A. precatorius* extract.

Group 7-11 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F2 fraction of *Abrus precatorius* extract.

Group 12-16 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F3 fraction of *A. precatorius* extract and

Group 17-21 received 30 mg/kg, 60 mg/kg, 90 mg/kg, 120 mg/kg and 150 mg/kg of F4 fraction of *A. precatorius* extract respectively.

On the Day 30 of treatment, animals were fasted and opened up under anesthesia and the animals were sacrificed, dissected and the uterus and ovaries obtained for histology.

2.4 Histopathological Procedure Drury et al. [13]

The ovary and endometrium of all the animals were fixed in 10% buffered formalin in labelled bottles, and processed routinely for histological examination. Tissues embedded in paraffin wax were sectioned 5 μ m thick, stained with H & E, mounted on glass slides and then examined under a standard light microscope.

2.5 Fixation and Washing

To preserve the tissues, formalin (10%) was utilized. A minute portion of the tissues (1- 2 cm in diameter) were sliced using a razor blade that is sharp. Small pieces of tissues that were kept in the 10% formalin and the container mixed quietly to ensure that the reagent penetrated all the tissues and also to avoid them gumming to the surfaces. At 25°C they were incubated and allow to be properly fixed. Subsequently they were washed with running water for 24 hours to wash off too much of the fixatives.

2.6 Dehydration

It was ensured that there was no water on the tissues before embedding them in paraffin. Tissues were submerged in automatic tissue processor consisting of 12 jars in other to attain the dehydration. 70, 90 and 95% absolute alcohol was introduced in the first three containers respectively. The essence of this is to get rid of the water content in the tissues. Fresh absolute alcohol was reintroduced again to make sure there was a complete removal of water. Similar procedure was done in the other jars of the automatic tissue processor.

2.7 Clearing

At his point, Xylene solutions were utilized in the clearing of the tissue sections. This procedure was indicated in the other jars of the automatic tissue processor. Xylene solution was preferred because it is miscible with both alcohol and paraffin before penetration occurs. The essence of carrying out clearing was to get rid of opacity from dehydrated tissues. The tissue stayed in the solution for 15 minutes before it was removed.

2.8 Infiltration with Paraffin

The tissues were infiltrated with paraffin wax for 50-52°C. They were moved to a bath with molten paraffin. They were incubated for 30-60 minutes in the first bath and thereafter, transferred to a fresh dish containing paraffin in fourth jars containing automatic tissue processor for the same duration of time.

2.9 Embedding (Blocking) with Paraffin

The tissues were completely soaked with paraffin and the paraffin allowed to solidify in and out of the tissues.

2.10 Paraffin Sectioning

The soaked sections of the tissues were sliced into squares and fixed in the microtome knives for partitioning and thereafter passed through the water bath.

2.11 Mounting

Thin layer of the albumen fixative was prepared on a clean glass slide. The slides were used to obtain the required section from the other partitions in the water. The partitions on the glass slides were moisturized before staining was carried out.

2.12 Staining with Hematoxylin

Series of Jars containing alcohols of reducing strength and different staining solutions were brought and the slides passed through each of them.

2.13 Microscopic Observation of Slide

Slides were made ready and viewed under the photomicroscope one after the other at 400

magnification power of the microscope. Each of the slides were photographed.

2.14 Statistical Analysis

Results were expressed as mean \pm SEM. Statistical significance was analyzed by one -way analysis of variance followed by Turkey's test with the level of significance at $p < 0.05$. Salanal computer program was used to analyze the statistical analysis, following the Bernstein et al. (1982) model.

3. HISTOLOGY RESULTS

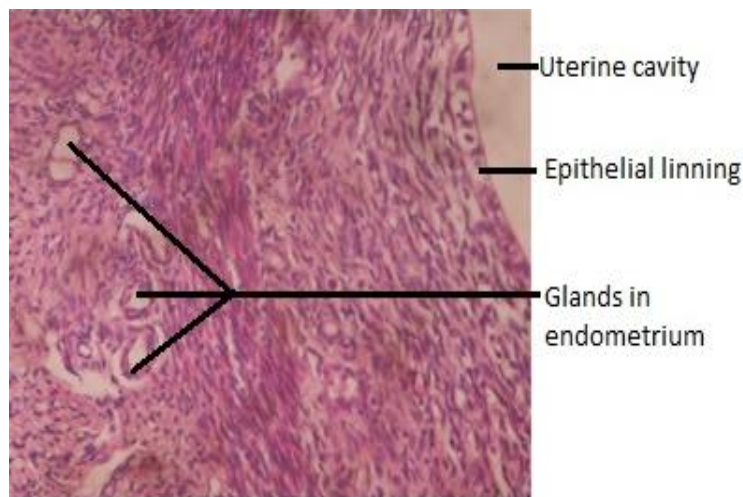


Fig. 1. Uterus showing single layer of epithelial cells lining the luminal border of control of fraction 1 at 30 mg/kg (x400, H&E). No damage or defect

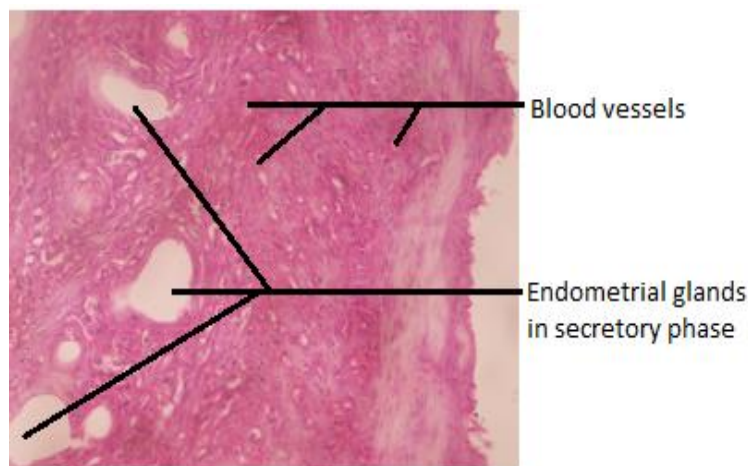


Fig. 2. Uterus showing blood vessels, and endometrial glands in the secretory phase of fraction 1 at 90 mg/kg (X400, H&E). No damage or defect

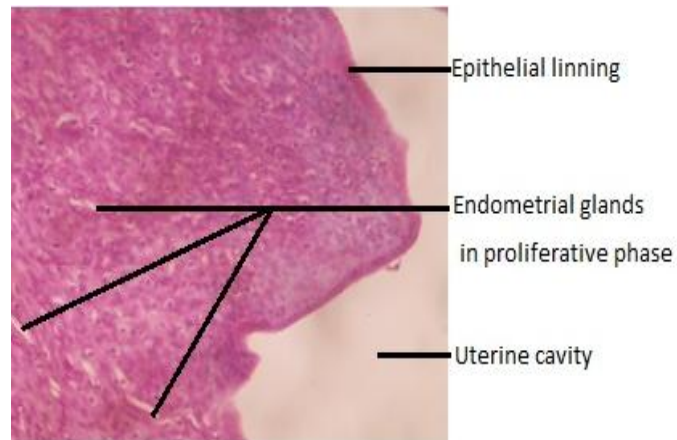


Fig. 3. Uterus showing endometrial glands in the proliferative phase of fraction 1 at 150 mg/kg (X400, H&E). There is absence of damage or defect

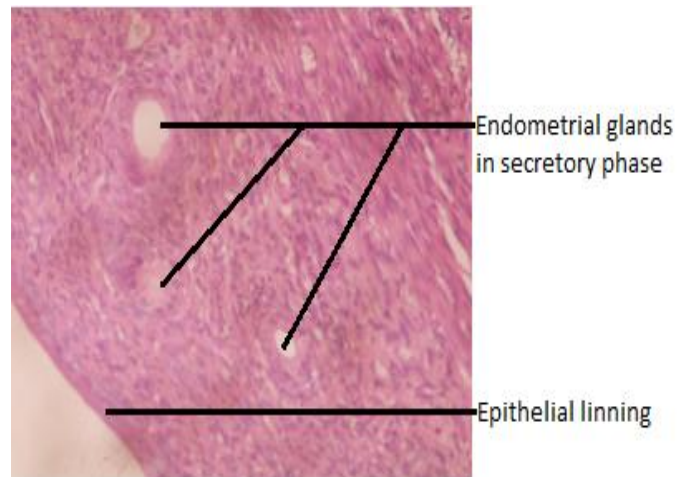


Fig. 4. Uterus showing endometrial glands in the secretory phase with single columnar epithelial lining of fraction 2 at 90 mg/kg (X400, H&E). No damage or defect

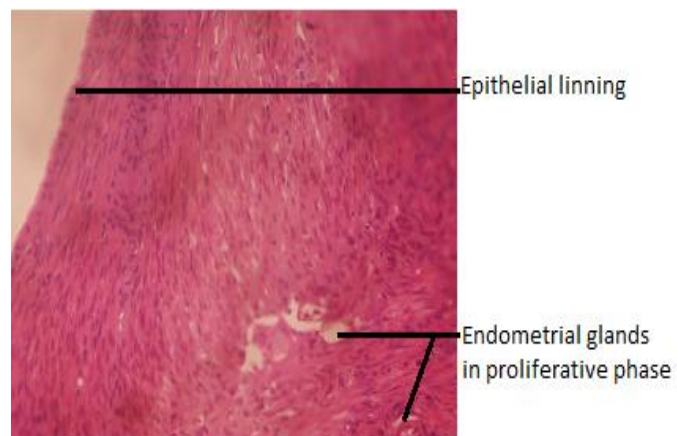


Fig. 5. Uterus showing endometrial glands in the proliferative phase fraction 2 of 150 mg/kg (X400, H&E). No damage or defect

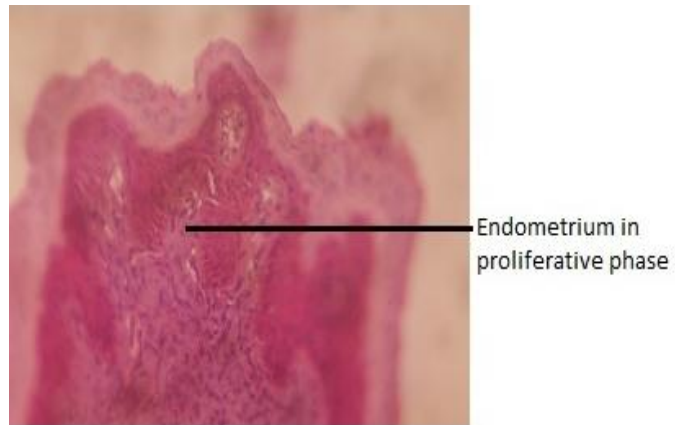


Fig. 6. Uterus showing endometrial glands and features of proliferative phase of fraction 3 at 150 mg/kg (X400, H&E). There is absence of damage or defect



Fig. 7. Uterus showing endometrial glands and features of secretory phase of fraction 4 at 120 mg/kg (X400, H&E). No damage or defect



Fig. 8. Uterus showing endometrial glands and features of secretory phase of fraction 4 at 150 mg/kg (X400, H&E). No damage or defect

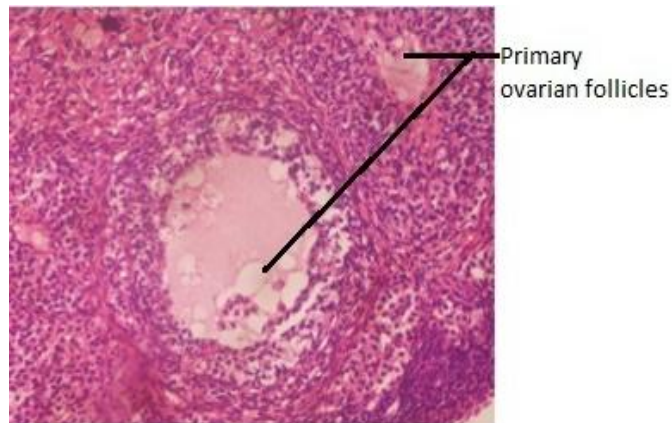


Fig. 9. Ovary showing blood vessels in the medulla and ovarian follicles in the cortical area of fraction 1 at 120 mg/kg (X400, H&E). No damage or defect seen

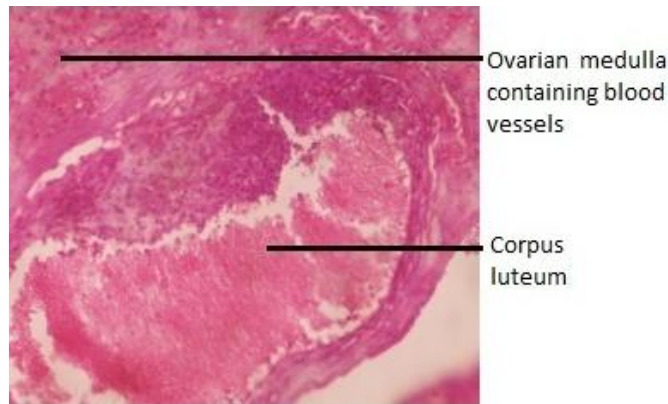


Fig. 10. Showing ovary with corpus luteum in the cortical region of the uterus of fraction 1 at 150 mg/kg (X400, H&E). No damage or defect seen



Fig. 11. Ovary showing primary ovarian follicles in the cortical region of fraction 2 at 120 mg/kg (X400, H&E). No damage or defect

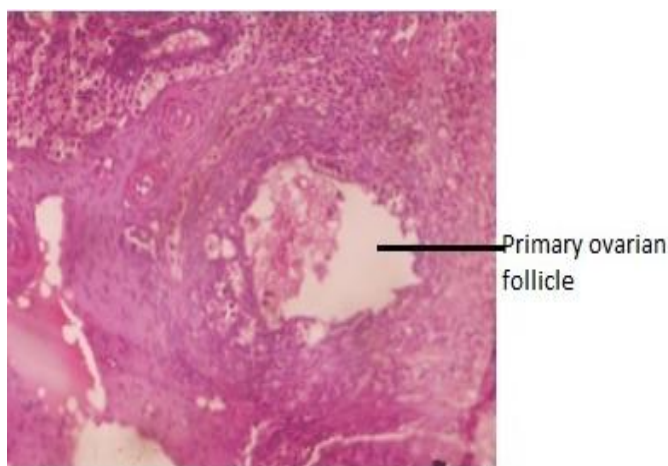


Fig. 12. Ovary showing primary ovarian follicles of fraction 2 at 150 mg/kg (X400, H&E). No damage or defect

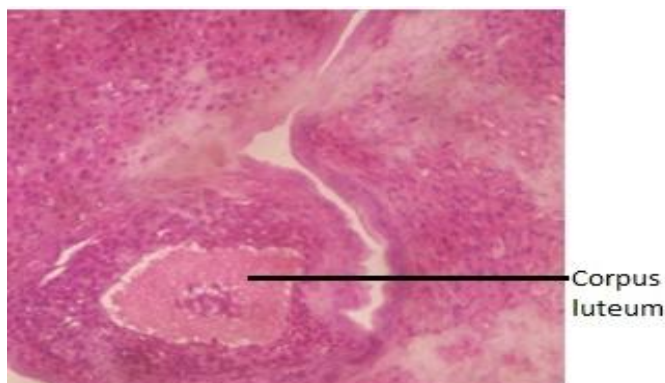


Fig. 13. Ovary showing corpus luteum of fraction 4 at 150 mg/kg (X400, H&E). No damage/defect

4. DISCUSSION

The Preliminary Phytochemical investigation of *Abrus precatorius* extract revealed that the leaf contains alkaloids, Triterpenes, glycosides, tannins, flavonoids, carbohydrates, steroids, oils and saponin. Tannins, Saponins, Flavonoids, Steroids and Terpenoids have menstrual regulation potentials and also used in management of infertility (Bussmann and Glenn, 2010; Koch et al. 2015). The uterus composed of the uterine cavity, epithelial lining, the glands in the endometrium or endometrial glands and blood vessels.

The photomicrograph of Fraction 1 uterus revealed endometrial gland in the secretory phase which tends to be larger for at 90 mg/kg but smaller at 60 mg/kg. The endometrial gland at 120 mg/kg and 150 mg/kg are smaller and at the proliferative phase. The photomicrograph of

Fraction 2 uterus at 90 mg/kg is large and at the secretory phase while the endometrial gland at 120 mg/kg and 150 mg/kg are smaller in size and at the proliferative phase. Fraction 3 photomicrograph displayed a uterus with endometrial gland at the proliferative phase at 150 mg/kg. Fraction 4 photomicrograph of the uterus at 120 mg/kg has endometrial gland at the secretory phase while at 150 mg/kg has endometrial gland of the uterus at the proliferative phase.

The ovary is composed of ovarian follicles in the cortical area and blood vessels in the medulla. Photomicrograph of Ovary of Fraction 1 at 120 mg/kg revealed the primary ovarian follicles and blood vessels in the medulla and ovarian follicles in the cortical area. Fraction 1 at 150 mg/kg displayed a photomicrograph of the ovarian medulla containing blood vessels and also corpus luteum in the cortical region of the uterus.

Photomicrograph of the Ovary of Fraction 2 at 120 mg/kg on the other hand revealed showed the primary follicles in the ovarian cortex or the primary ovarian follicles in the cortical region. Photomicrograph of the ovary of Fraction 2 and 3 at 150 mg/kg displayed the ovary with primary ovarian follicles, while Fraction 4 photomicrograph showed the corpus luteum.

In rodents, the secretory phase of the uterus begins at ovulation. In this phase, the glands become even more complexly coiled and the endometrial lining reaches its maximal thickness. secretions rich in glycogen and glycoprotein can be observed in the lumina of the glands Laurie, [14]. If fertilization does not produce Human chorionic gonadotropin (hcG), the corpus luteum degenerates. The uterine lining does not receive the progesterone causing the spiral arteries to constrict and the endometrial tissue to become ischemic Laurie, [14]. So generally, at the secretory phase, the uterine lining produce chemicals that will either help support an early pregnancy or will prepare the lining to break down and shed if pregnancy doesn't occur [15]. The proliferative phase commences from the end of the period until ovulation. At this period, the uterus builds up a thick inner lining. The ovaries are working on producing the egg-containing follicles while the uterus is responding to the estrogen synthesized by the follicles, restoring the lining that wears off during the previous period Laurie, [14]. This is called the Proliferative phase because the endometrium (the lining of the uterus) becomes thicker. The uterus does this to create a place where a potential fertilized egg can implant and grow [16]. The ovaries are made up of the outer cortex and inner medulla. The cortex consists of ovarian follicles, the interstitial gland cells and the stromal elements. Based on the stages of development, ovarian follicles can be primordial, primary, secondary and antral/mature (Charlotte, 2000). The primordial follicles are those follicles with one layer of flattened granulose cells, primary follicle possesses one layer of cuboidal granulose cells, secondary follicles present two layers of cuboidal granulose cells while the antral follicles present the antral cavity [17]. The cortex is made up of degenerated follicles which arise at later phase of follicular development (Charlotte, 2000). The medulla consists of connective tissues containing so many elastic fibers, large blood vessels, nerves and lymphatics.

Therefore, from the photomicrograph of the ovary and uterus shown above, it indicates that there

was no structural defect or damage on the ovary and the uterus after the administration of the chromatographic fractions of *Abrus precatorius* leaf on the white albino rats.

5. CONCLUSION

The study revealed histological evidence that the chromatographic fractions of *Abrus precatorius* leaf do not have any potential adverse effect on the ovary and uterus of the Wistar Albino rats. There was no damage or degenerative changes observed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained and the research subjected to the Research Ethics Committee of the University of Port Harcourt Centre from Research Management and Development. Necessary procedures to be carried out while using animals for experimentation were done in accordance with set guidelines and regulations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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