Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2023; 11(2): 97-102

Received: 04-12-2022
Accepted: 10-01-2023

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Histomorphological changes in the ovarian and uterine tissues of adult wistar rats following exposure to aqueous extract of *Aspilia africana* flowers

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Abstract
Aspilia africana is a tropical plant that has recently gained relevance and popularity due to its ability to effect changes in the reproductive system/functions both in males and females. Besides its roles in fertility, it has been reported to possess antibacterial, antimicrobial, wound healing and anti-hemorrhagic effect, the latter being the reason it is called the hemorrhagic plant. The objective of this study was to assess the effect of graded dosages of aqueous extract of *Aspilia africana* flowers on the histo-morphological profile of ovarian and uterine tissues of female Wistar rats. Twenty (20) female rats were used for this study and were divided into four (4) groups of five (5) animals each. Control group (A) was given distilled water which was used as vehicle, while groups B, C and D were administered 150mg/kg, 200mg/kg and 300mg/kg for 21 days through oral gavage. Thereafter, the ovaries and uterine tissues were excised, weighed and put through routine histological processing. The results obtained from this study revealed normal ovarian epithelium except in the group treated with 200mg of the extract, which showed some degree of distortion, patchy areas of stromal degeneration, stromal congestion, atretic follicles with pyknotic granulosa cells and degenerating follicles lacking oocyte, which was also a prominent feature of all the follicles in the group treated with 300mg/kg body weight of the extract. The uterine endometrium of extract-treated groups on the other hand showed thickening of lining epithelium, mild to moderate endometrial hyperplasia, focal areas of ulcerations, and mild infiltration of inflammatory cells. It can be concluded that the administration of aqueous flower extract of *A. africana* produced distortive changes in the ovaries with increased follicular atresia in preovulatory follicles which may impair ovulation and by extension, fertility potentials. It also caused disruptive effects on the uterine tissues which can also lead to reproductive dysfunction.

Keywords: *Aspilia africana*, ovarian tissues, uterine tissues, wistar rats, reproductive dysfunction

1. Introduction
Medicinal plants are recently being accepted globally for the ameliorative and curative effects they portend towards cushioning the effects of ailments and diseases. The terms ‘organic’ and ‘botanical’ have also taken center stage as several individuals in our society have embraced local products mostly made from plant materials to meet their healthcare, skincare, and even nutritional needs. According to WHO [1], any plant or plant material that when given to human beings or [5]plant. Medicinal plants are sources or precursors of most orthodox drugs as their active principles are extracted and used in the production of drugs that are used by people all over the world [2]. The general perception that these plant products are less hazardous and/or completely safe leads to indiscriminate use by a large population in our society without recourse to the possibilities of adverse effects and/or fatalities. It is a common practice in Nigeria that herbal products are administered over prolonged periods and by persons that have little or no knowledge of science [3]. The constituents of these recipes elicit varied physiological activities. There has been a concern over adverse effects on reproduction or systemic toxicity due to prolonged use of medicinal products [4].
Aspilia africana (Asteraceae) is a common herbaceous plant widely known for its ethnomedicinal values. It is a perennial plant with a long history of Agricultural uses as Farmers usually graze their cattle, sheep, and feed their rabbits and hares with it in most African countries, especially in West Africa.

It is also known as the iodine or hemorrhage plant as it is commonly used to stop bleeding even from large arteries. In eastern Nigeria, it is used in snail farming hence the name 'oranjila'. It is polymorphic with at least 4 varieties occurring on the wasteland of savanna & forested zones throughout Africa [5]

2. Methods

2.1 Experimental animals

Twenty (20) female Wistar rats weighing between 250-300g were utilized for this study. The study animals were purchased from the Central animal house facility of Igbinedion University, Okada and were kept in the same facility through the course the study. The animals were housed in plastic cages under good hygienic conditions, and at 55±2% humidity 24±20^{0}C room temperature, and 12/12 hours light and dark cycles. Feed and water were available to the animals ad libitum.

Experimental design

Groups	Description	Number of animals (n)	Exposure	Dosage per body weight
A	Control	5	Distilled water	2ml
B	Treated group	5	Treated	150mg/kg
C	Treated group	5	Treated	200mg/kg
D	Treated group	5	Treated	300mg/kg

The above design was perfected by giving daily dosages of the extract at 8am via oral gavage. The animals were weighed at the start and end of the experiment and differences in the initial and final body weights recorded for each group in percentages. Administration of the extract lasted for three weeks only, after which the experimental animals were sacrificed by cervical dislocation. The vital organs (ovaries and uterine horns) were excised, weighed and fixed. Permission to use animal models was sought and granted by the Director of Animal house, Igbinedion University, Okada.

2.2 Tissue processing

The ovaries and uterine horns of the experimental animals were weighed and fixed using Bouin's fluid. Graduations of alcohol was used for dehydration, while xylene served as a clearing agent. The tissues were embedded in molten paraffin wax and the resultant blocks sectioned at a thickness of 5microns. Routine Haematoxylin and Eosin were used for staining, the slides so made were viewed using a microscope with camera and photomicrographs were taken.

2.3 Statistical analysis

Statistical analysis was carried out by collating all the respective data and inputting it into the IBM statistical analysis package for social science version 20. The results obtained were expressed as Mean ± standard error of mean (SEM). The relevant statistical significance was obtained through the analysis of variance (ANOVA). Finally the Duncan Post-hoc test was carried out to determine the significant differences between the groups at $p<0.05$.

3. Results

3.1 Weight changes

Percentage body weight changes of the experimental animals were assessed and are shown in figure 1, while the relative organ weights of the ovaries and uterine horns are shown in figure 2.

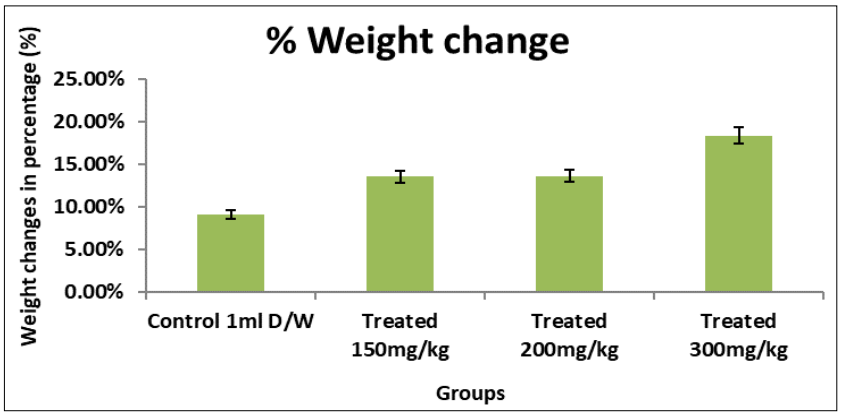

Fig 1: Percentage body weight changes of experimental animals in group A (control), groups B- D (treatment groups) showing a dose dependent significant ($p<0.05$) decreases in the treated groups relative to the control group

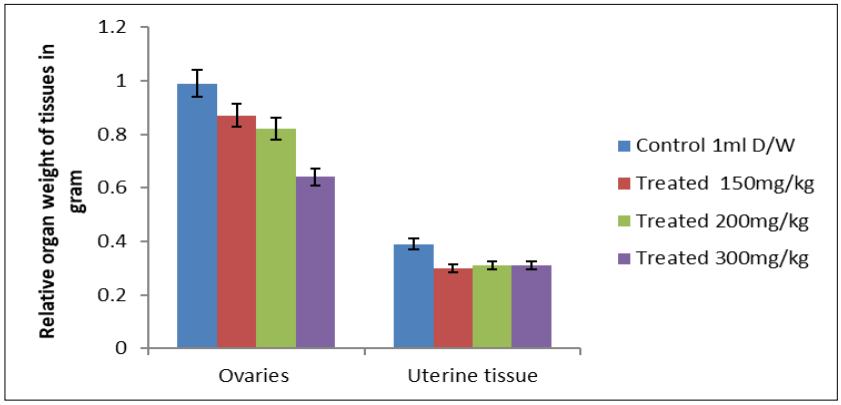

Fig 2: Relative organ weights of the ovarian and uterine tissues of Group A (control), groups B-D (treatment groups) showing significant ($p<0.05$) reduction in both ovarian and uterine weights compared to the control

3.2 Histological studies

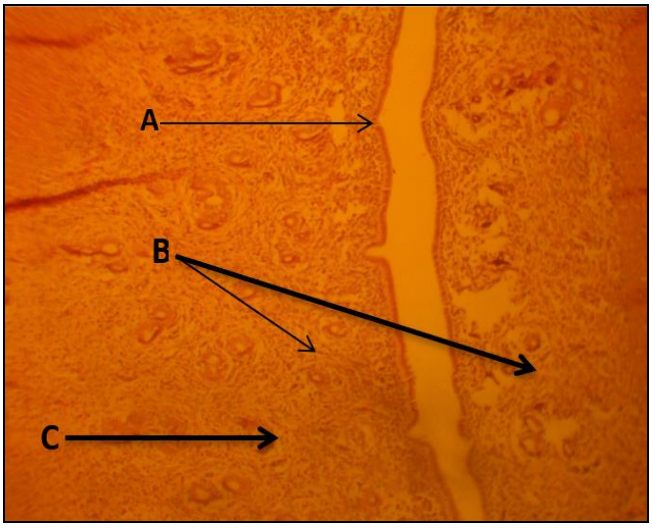

Plate 1: Control uterus showing, endometrial lining-A, endometrial glands-B and normal endometrial stroma-C (H & E × 100).

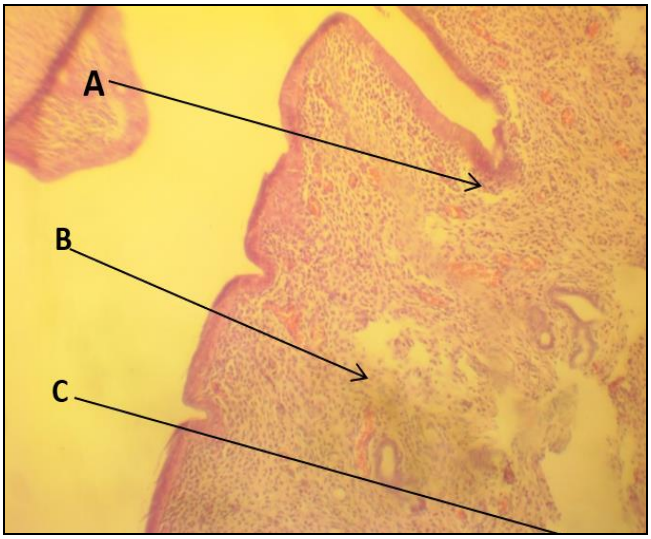

Plate 2: Rat uterus given 150mg/kg *A. africana*, showing moderate thickening of the lining epithelia-A, and patchy endometrial ulceration-B and mild distortion of the myometrium-C. (H&E × 100)

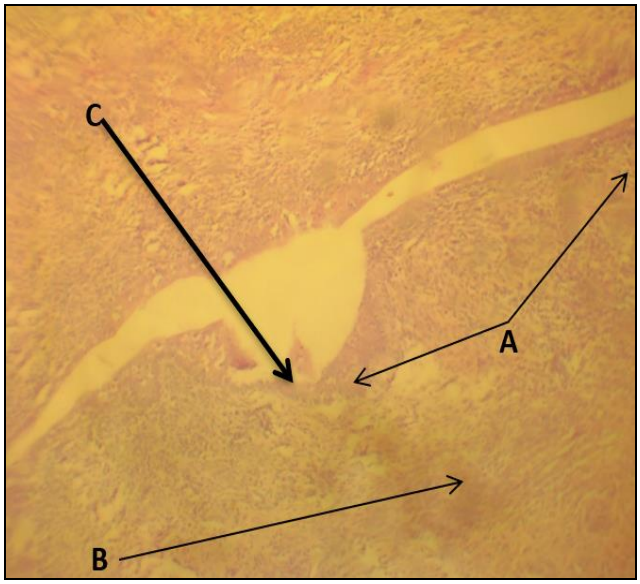

Plate 3: Rat uterus given 200mg/kg of *A. africana*, showing mild endometrial lining hyperplasia-A, mild stromal infilitrates of inflammatory cells -B and focal area of ulceration-C (H & E × 100)

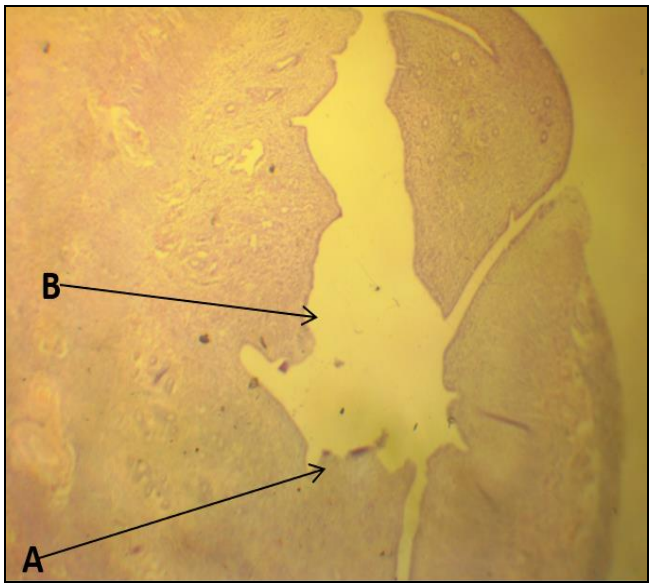

Plate 4: Rat uterus given 300mg/kg of *A. africana*, showing focal endometrial lining hyperplasia-A, and ulceration-B (H & E × 40).

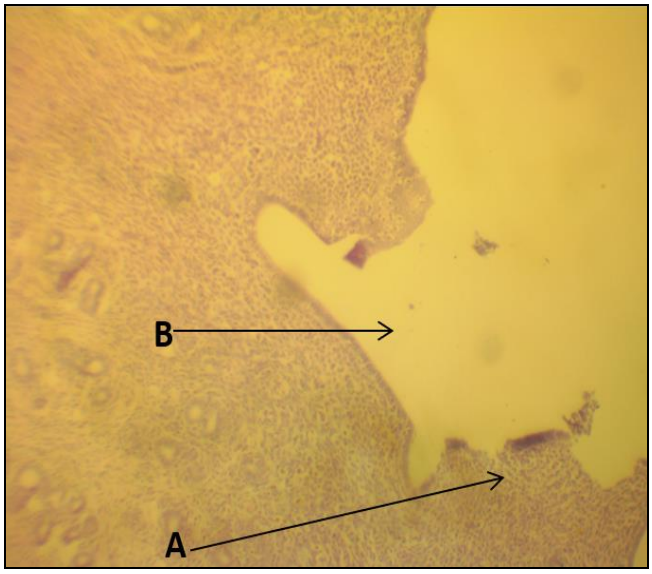

Plate 5: Rat uterus given 300mg/kg of *A. africana*, showing focal endometrial lining hyperplasia-A, and ulceration-B (H & E × 100)

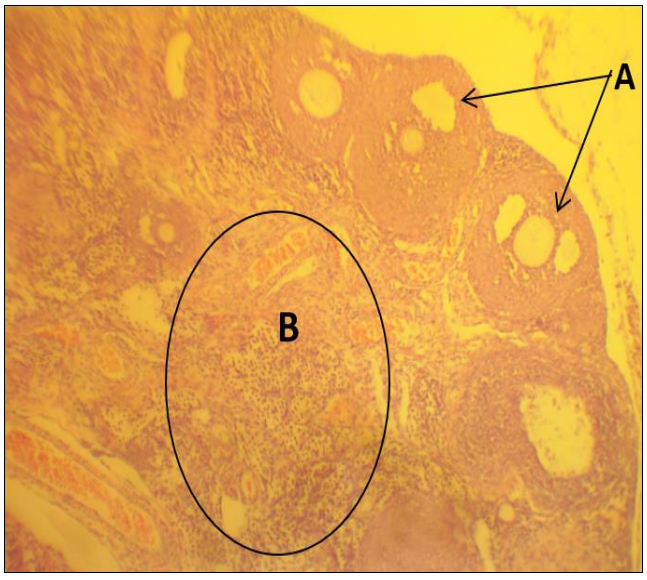

Plate 6: Control ovary showing ovarian follicles at different stages of maturation-A, and supported by a luteinized stroma- B (H& E × 100)

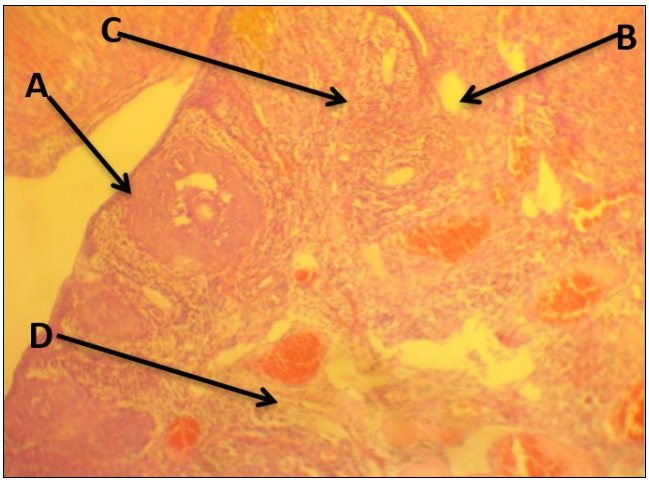

Plate 7: Rat ovary given 150 mg/kg showing distorted mature follicles-A, patchy areas of stromal degeneration- B, corpus atreticum- C and moderate stromal congestion-D (H & E × 100)

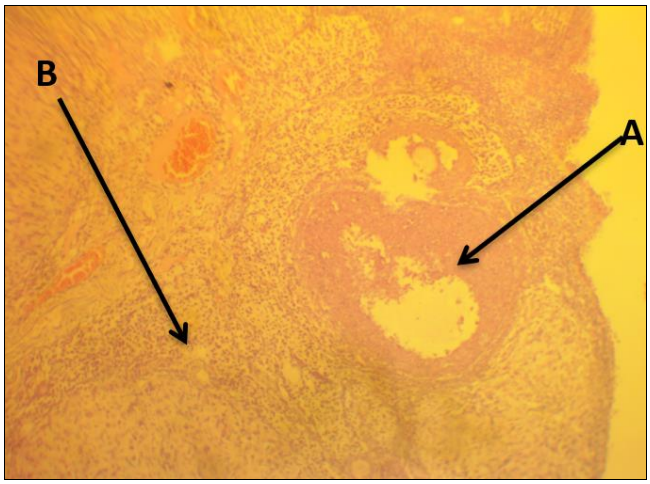

Plate 8: Rat ovary given 200mg/kg of *A. africana* showing follicles at different stages of development, all degenerating with pyknotic granulosa cells-A, and stromal degeneration-B (H & E × 100)

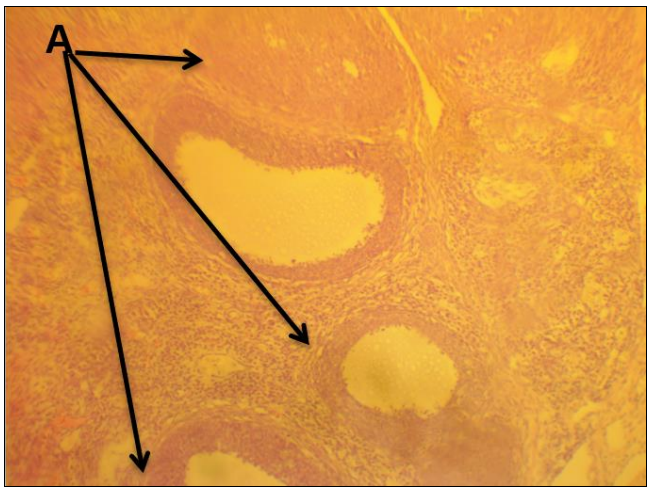

Plate 9: Rat ovary given 300mg/kg of *A. africana* showing degenerating follicles at various developmental stages-A. No oocyte (H & E × 100)

4. Discussion

Several researchers have investigated and reported the effective use of various extracts of the *Aspilia africana* plant to ameliorate the effect of some disease conditions. It has been reported to have bactericidal, anti-inflammatory, anti-fungal, gastro-protective, and wound-healing potentials [6]. The result obtained from the body weight assessment in this study is in line with the results reported by [7]. According to them, weight gain in experimental animals that is higher than that of the control group may be a result of increased food intake induced in the experimental animals by the extract. An increase in body weight may be associated with the presence of trace elements in the extract, including magnesium, zinc, and copper [8, 9]. Whilst zinc is said to improve linear growth, copper is implicated in high body weight gain. The above elements are present in the aqueous extract of *A. africana* flowers [10], and may have contributed to the weight gain recorded in this study. This significant weight gain, therefore, implies that the treatment with aqueous extract of *A. africana* flower had no negative impact on the well-being of the experimental animals.

One of the most sensitive and effective drug toxicity indicators is the organ weight which oftentimes is altered before changes in appearance, form, and shape become evident [11]. However, changes that are more or less significant can occur between the extract-treated groups and the control without any visible morphological alterations [12]. The weights of the organs recorded showed significant decreases in the treatment groups compared to the control. This is an indication that the aqueous extract of *A. africana* flowers did not have any toxic

The presence of inflammatory cells in the endometrial tissue is an indication that aqueous flower extract of *A. africana* may have been injurious to the endometrium and/or uteri as a whole, thus causing the activation of the local immune system. The above follows inflammation, which in this case may be a result of the interaction between the phytoconstituents of the extract and the body's immune-vascular system.

Endometritis is defined as inflammation of the endometrium (uterine lining), the most common symptom being abdominal distention or swelling [13], and is one of the key causes of infertility. Inflammatory diseases are associated with oxidative reactions and reduced antioxidant defense capacity, resulting in tissue damage. Endometritis has been reported to disturb fertility and hinder uterine involution [14], while uterine diseases are known to impair conception [15]. The findings of this study are in line with the report of Emtenan and colleagues, [16] who confirmed endometritis by the presence of inflammatory cells in the endometrium. Again, endometrial hyperplasia which is the abnormal or hyperproliferation of the endometrium/endometrial lining was observed in the uteri of the treated rats. Endometrial hyperplasia can either be physiological or pathological when it is a result of hormonal imbalance, especially the excessive production or secretion of estrogen in the absence of progesterone, and is known to precede uterine neoplasm in some cases. This finding is in line with the work of some researchers, [17], who reported that *Gnetum africanum* caused endometrial hyperplasia and dilation of endometrial glands in the uteri of rats. The focal areas of ulcerations that were observed in the endometrial glands, suggest the erosion of epithelial tissue. It is therefore suggestive that the aqueous flower extract of *A. africana* had injurious effects on the uterine tissue which can lead to reproductive dysfunction. The injurious effects may have been exerted by the flavonoids and saponins present in the extract as stated by previous Researchers.

Follicle-stimulating hormone (FSH) plays a key role in the process of follicular maturation, part of which involves the regulation of estradiol secretion, development of antral follicles [18], and selection of the dominant follicle [19]. The

maturation of the ovarian follicles determines their developmental requirements. However, studies have shown that FSH is required by the different cells of the different stages of maturation [20]. The expulsion of the mature or preovulatory follicle at ovulation gives way for the other growing follicles which were not selected as the dominant follicle to begin the process of atresia, in which follicular cells and oocytes undergo necrosis and are disposed of by phagocytic cells [21]. Granulosa cell death is an early feature of atresia and the process is usually marked by the discontinuance of mitosis in the granulosa cells, detachment of granulosa cells and eventual death of oocytes alongside the granulosa cells. Granulosa cells near the antrum are sloughed off into the antrum, and their death has features more consistent with that of other cell types that undergo death as a result of apoptosis [22].

The result of the ovarian histological study is similar to that that of a group of authors, that reported disintegration of the antral follicle following treatment with *Andrographis paniculata* root extract in female albino rats [23]. Widespread follicular atresia in the ovarian histology of rats treated with amodiaquine has been reported [24]. Typically, the proestrus ovary is made up of degenerate corpora lutea, cytoplasmic vacuoles, and the proliferation of fibrous tissue in the central cavity. An atretic follicle represents degenerating follicle, irrespective of its stage of maturation. Follicles degenerate when the process of growth and differentiation is compromised. Follicular atresia is an integral part of ovarian function, but when there is an increase in the number of atretic follicles with an attendant decrease in the population of normal follicles, it then becomes pathological; as was the case in this study. The increased atresia recorded in the present study may have been due to the non-availability of the required amount of the extra ovarian regulators- the gonadotrophins [24]. The extract may have acted on the hypothalamus indirectly or on the pituitary gland; thus making the follicular receptors less responsive to the available gonadotrophins. The active principle in the extract may have also compromised the intra- ovarian and intra- follicular regulatory factors and in turn, hindered follicular growth in the ovaries. Some of the histopathological changes that oxidative stress can give rise to in the ovaries include atretic follicles, bleeding, inflammation (characterized by inflammatory cell infiltration), vascular congestion, and edema [25], [26] [27]. In the present study, some of these typical features of oxidative stress causing changes [28] were seen; therefore, it is probable that some form of oxidative stress may have been induced in the ovaries which resulted in vascular congestion and other degenerative damages recorded in the current study. Therefore, it can be concluded that the administration of aqueous flower extract of *A. africana* produced degenerative changes in the ovaries as it increased follicular atresia in preovulatory follicles which may in turn impair ovulation and fertility. The mechanism of action of the extract may be through the depletion of hormones perpetuated by the tannins in the extract as found in previous studies [29].

Conclusion

There was a clear demonstration in this study that the aqueous flower extract of *A. africana* produced disruptive and degenerative effects in the normal histology of the ovarian and uterine tissues of Wistar rats. This is indicative of its potential to impair fertility in female Wistar rats and if consumption is not checked in humans may also elicit similar effects. Investigations to elucidate the mechanisms of actions of isolated compounds of aqueous flower extract of *A. africana* should be carried out.

Acknowledgment

We like to acknowledge the contributions of the Chief Technologist of the Departments of Anatomy Igbinedion University, Okada. Our gratitude also to the staff of the histopathology laboratory of Igbinedion University Teaching Hospital, Okada for assisting with slide preparation.

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