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Reproductive effects of *Abelmoschus esculentus* fruit methanol extract in female Wistar rats

Eunice Ogunwole [a,*], Jemimah Adoh Yakubu [b], Vivian Tally Giwa [b]

[a] Department of Physiology, University of Medical Sciences, Ondo, Ondo State, Nigeria
[b] Department of Physiology, College of Health Sciences, Bingham University, New Karu, Nasarawa, Nigeria

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ABSTRACT

Current researches aim at identifying modifiable risk factors for infertility, particularly dietary lifestyle. *Abelmoschus esculentus* is one of the important vegetables in the human diet with reported valuable nutrients but has been linked with reproductive dysfunction in males. This study investigated the reproductive effects of *Abelmoschus esculentus* fruit methanol extract in female Wistar rats. Dried *Abelmoschus esculentus* fruit was extracted with methanol. Fifteen female Wistar rats (180–200 g) grouped into three (n = 5) received 1.0 mL/kg/day distilled water (control), 70 and 200 mg/kg/day of the extract once daily for 21 days via oral gavage. The estrous cycle was assessed using Marcondes and Papanicolaou methods. The histology of the tissues was evaluated by microscopy. Serum follicle-stimulating hormone, luteinizing hormone, and estrogen levels were measured using an enzyme-linked immunosorbent assay. Tissue antioxidant activities and malondialdehyde levels were assayed by spectrophotometry. Data were analyzed using the Analysis of variance at a significance of $p < 0.05$. The estrous cycle of the *Abelmoschus esculentus* fruit methanol extracts treated rats showed normal cellular characteristics. Though *Abelmoschus esculentus* fruit methanol extract increased the antioxidant activities, it reduced the body weight and follicle-stimulating hormone level and caused severe inflammation and fibrosis of the ovary and uterus. *Abelmoschus esculentus* fruit methanol extract adversely altered the reproductive functions of female Wistar rats by disrupting the ovarian and uterine cytology and reducing hormone levels.

1. Introduction

Infertility is clinically described as the inability to conceive naturally after 12 months or more of regular unprotected sexual intercourse (Zegers-Hochschild et al., 2009, 2017). Besides being a quality-of-life issue, infertility is a common disease of the reproductive system that generates disability as an impairment of function (Zegers-Hochschild et al., 2017). It also poses a risk factor for other diseases throughout an individual's life, such as cardiovascular disease, cancer, and metabolic dysfunction among others (Solomon et al., 2002). Hence, infertility is of global concern as it affects many aspects of life for both genders. Infertility can be either primary or secondary, due to genetic defects, fallopian tube injury, hormonal turbulence, fertilization and ovulation interference (Taylor, 2003), low sperm count, deficient sperm motility, and poor sperm morphology (Jawad et al., 2018). The extent of occurrence of infertility differs globally, but its rating ranges from 5 to 30% as reported for different countries (Larsen, 2000; Abebe et al., 2020). Though assisted reproductive technologies are now used to achieve conception, infertility issues remain prevalent (Pei et al., 2017). Current research now aims at identifying modifiable risk factors for infertility, such as lifestyle choices (Hakim et al., 1998) notably, dietary lifestyle.

Okra (*Abelmoschus esculentus*), also called lady's finger, is one of the important vegetables with the best dietary value, medicinal and industrial importance (Gemede et al., 2015a; Sindhu and Puri, 2016). It is a multipurpose crop with various uses for its fresh leaves, buds, flowers, pods, stems, and seeds (Gemede et al., 2018). *Abelmoschus esculentus* pods are immature fruits widely consumed with reported antioxidant potentials (Wang et al., 2018; Arapitsas, 2008). The seed is rich in phenolic compounds, majorly flavanols derivatives and oligomeric catechins (Ansari et al., 2005). Previous studies showed that okra extract possesses an *in vitro* non-enzymatic inhibitor of lipid peroxidation in liposome effects and that its peel and seed powder contain significant *in vivo* antioxidant properties in streptozotocin-induced diabetic rats (Liao et al., 2012; Mihretu et al., 2014). *Abelmoschus esculentus* plays a vital role in the human diet and health (Petropoulos et al., 2018), it is a powerhouse of valuable nutrients, such as carbohydrates, minerals, and

* Corresponding author.
E-mail address: eogunwole@unimed.edu.ng (E. Ogunwole).

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vitamins (Adelakun et al., 2009; Chowdhury et al., 2019). (Husen et al., 2019) stated that *Abelmoschus esculentus* is used medicinally for plasma replacement or expanding blood volume, also, its soluble fiber in the form of gums and pectins lowers serum cholesterol and the risk of heart diseases (Husen et al., 2020). affirmed that okra pod extract has anti-diabetic properties against streptozotocin-induced diabetic mice and therapeutic effects as it improved open wound healing in diabetic mice (Liao et al., 2019). A polysaccharide extracted from okra was shown to reduce liver fibrosis in Type 2 Diabetes Mellitus induced mice (Lee and Joo, 2021). (Gemede et al., 2015b) reported that raw and multimethod cooked okra possess anti-inflammatory and antioxidant effects. Previous studies reported that mucilage of *Abelmoschus esculentus* binds cholesterol and bile acid carrying toxins dumped into it by the liver (Gemede et al., 2015b; Roy et al., 2014). *Abelmoschus esculentus* seed oil is also a rich source of linoleic acid and a polyunsaturated fatty acid essential for human nutrition (Husen et al., 2019). The findings of (Gaskins and Chavarro, 2018) showed that nutrition plays an important role in altering fertility-related outcomes in both men and women. They also reported a relationship between dietary consumption habits and infertility. Previous studies have linked *Abelmoschus esculentus* with reproductive system dysfunction (Olatunji -Bello, 2009; Uchenna et al., 2014). Chronic consumption of *Abelmoschus esculentus* was noted to reduce the motility, viability, count, and concentration of the spermatozoa and mitochondrial damage (El- Sharaky et al., 2010; Haris et al., 2018). While *Abelmoschus esculentus* was reported to reduce the incidence of follicular apoptosis related to diabetes (Erfani Majd et al., 2019), there is a paucity of evidence correlating *Abelmoschus esculentus* and female reproductive functions. Hence, this study investigates the effect of *Abelmoschus esculentus* fruit methanol extract on reproductive functions of female Wistar rats.

2. Materials and methods

2.1. Preparation of methanol extract of Abelmoschus esculentus fruits

Abelmoschus esculentus fruits were obtained from Karu, Nasarawa State, Nigeria. The fruit (Collector's Specimen No. YGH 163) was voucher at the herbarium of the Department of Biological Sciences, Bingham University, Karu, and given an identification number (Herbarium Specimen No: 123). The fruit was chopped into small pieces and air-dried under normal room temperature (25 °C) for 5 days. The dried *Abelmoschus esculentus* fruit was pulverized and extracted by soaking in methanol for 72 h. The filtrate was concentrated using low heat in a water bath (Azwanida., 2015). Phytochemical screening of the extract was performed using standard procedures (Sofowora, 1993).

2.2. Experimental animals

All procedures involving the use of animals were by the EU Directive 2010/63/EU for animal experiments and the study conformed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guide-line (2010) and ethical standards of the Department of Physiology, Bingham University for animal experiment. Fifteen female Wistar rats (180–200 g) obtained from Bingham University Animal Care Unit, were kept in the animal house of Bingham University, Karu. The animals received feed and water *ad libitum* and were acclimatized for two weeks before the experiment commenced. They were randomly divided into three groups ($n = 5$) to receive distilled water (1.0 mL/kg/day, control), 70 and 200 mg/kg/day of methanol extract of *Abelmoschus esculentus* fruit, respectively, once daily for 21 days via oral gavage. The dosage administration was according to the Organization for Economic Co-operation and Development method (OECD Test Guideline 425, 2001). The body weights of the animals were recorded using an electronic weighing scale (EK5055, China) once a week and on the day of sacrifice.

2.3. Estrous cycle

The estrous cycle was assessed pre and post-treatment using Marcondes and Papanicolaou principles. At the beginning of the experiment, the estrous cycle of the rats was assessed for 3 weeks before treatment between the hours of 7:00–8:00 a.m. every morning, in line with (Marcondes et al., 2002). Vaginal smeared slides were observed under the microscope and further stained using Papanicolaou's technique (Papanicolaou, 1942; Asthana and Singh, 2014) and the cell morphology was microscopically assessed using ×40 magnification. Treatment commenced afterward. When the two experimental groups received their designated doses of *Abelmoschus esculentus* fruit methanol extract, the control group received distilled water for three weeks, during which the estrous cycle was also assessed. The outcome of the study of the pre and post-treatment of the estrous cycle were compared.

2.4. Blood collection and serum preparation

At the end of the third week of administration, the rats were sacrificed and bled into EDTA and plain serum bottles via cardiac puncture. The blood was allowed to clot for at least 45 min and then centrifuged at 10,000 rpm for 15 min. The supernatant was aspirated from the centrifuged blood and stored at −20 °C for assay of hormones (follicle-stimulating hormone, luteinizing hormone, and estrogen) using Enzyme-Linked Immunosorbent Assay (ELISA) kits (Fortress diagnostics, UK).

2.5. Organ collection

Rats were sacrificed under thiopental anaesthesia (40 mg/kg, i.p) (Pereda et al., 2006). They were cut open along the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart and the organs. The ovary, uterus, liver, and kidney were harvested, freed from adherent tissues, and weighed immediately with a digital electronic scale (model EHA501, China). The ovary and uterus were fixed in Bouin's fluid for histological assessment.

2.6. Biochemical analysis

Tissue (ovary and uterus) lipid peroxidation was determined by measuring Thiobarbituric acid reactive substances (Malondialdehyde) produced during lipid peroxidation in line with the method (Buege and Aust, 1978). Reduced glutathione level was measured using a spectrophotometric assay kit (Oxford Biomedical Research, USA). Tissue catalase and superoxide dismutase activities were assessed as described by (Sinha, 1972; Misra and Fridovich, 1976) respectively.

2.7. Histology of the ovary and uterus

The ovary and uterus were fixed in Bouin's fluid and processed for microscopic examination. The tissues were embedded in paraffin and sectioned to obtain a 4-5 μm-thickness with a microtome. The dewaxed sections were stained with hematoxylin and eosin and the slides were viewed under a light microscope at 400 × magnification.

2.8. Statistical analysis

Data was collected and calculated using version 5.0 Graph Pad Prism Statistics software (USA) and presented as the mean ± standard error of the mean (mean ± SEM). The level of significance was at $p < 0.05$.

3. Results

3.1. Phytochemical screening of Abelmoschus esculentus fruit methanol extract

The results show the presence of saponins, steroids, alkaloids, phenols, terpenoids, tannins, flavonoids, anthraquinones, and cardiac glycosides and the absence of resin (Table 1).

3.2. Effect of Abelmoschus esculentus methanol extract on the percentage change in body weight

The result shows a significant increase ($p < 0.05$) in the percentage change in body weight of the 70 mg/kg/day treated rats compared to control, and a significant decrease ($p < 0.05$) in the percentage change in body weight of the 200 mg/kg/day treated rats as compared to both the control and 70 mg/kg/day treated rats (Fig. 1).

3.3. Effect of Abelmoschus esculentus methanol extract on the relative weight of organs

Table 2 below shows a significant increase ($p < 0.05$) in the relative weight of the liver in the treated rats compared to control, but the relative weight of the ovary and kidney both decreased significantly ($p < 0.05$) compared to the control and 200 mg/kg/day treated rats.

3.4. Effect of Abelmoschus esculentus methanol extract on malondialdehyde level and antioxidant enzymes in ovary and uterus

There were no significant differences in the malondialdehyde level of the organs. There were significant increases ($p < 0.05$) in the catalase activity and levels of reduced glutathione of the ovary of the 200 mg/kg/day treated rats when compared with the control and 70 mg/kg/day treated rats. Also, the superoxide dismutase activity of the ovary of the 70 mg/kg/day treated rats increased significantly ($p < 0.05$) compared to the control, and concurrently there was a significant decrease in catalase activity of the same group. The superoxide dismutase activity of the uterus significantly increased ($p < 0.05$) in the 200 mg/kg/day treated rats compared to the control and the 70 mg/kg/day treated rats. Meanwhile, the uterine reduced glutathione level of both treated groups was significantly decreased ($p < 0.05$) as related to the control (Table 3).

3.5. Effect of Abelmoschus esculentus methanol extract on the number of times of occurrence of phases of the estrous cycle during pretreatment and treatment

There was no significant difference in the number of times the various phases of the estrous cycle occurred during the experiment (Table 4).

Table 1
Phytochemical constituents of *Abelmoschus esculentus* fruit methanol extract.

Constituent	Observation
Tannin	+++
Steroid	++
Flavonoids	+++
Saponins	+
Alkaloid	++
Anthraquinones	+++
Phenols	++
Resin	–
Terpenoid	++
Cardiac Glycosides	+++

Key: + = low, ++ = Moderate, +++ = High, - = absent.

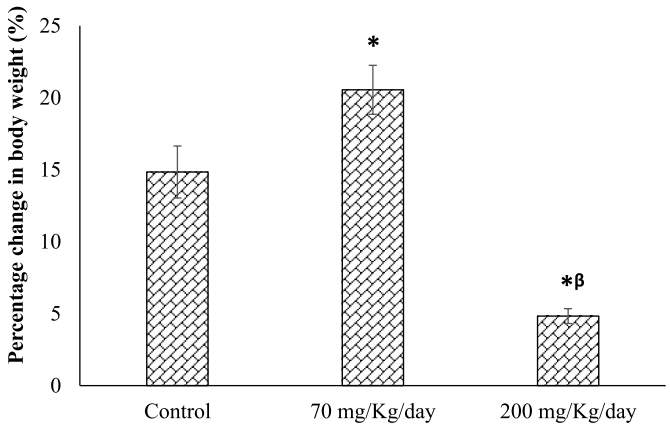

Fig. 1. Effect of *Abelmoschus esculentus* methanol extract on the percentage change in body weight of rats. Columns represent mean ± standard error of mean, *$p < 0.05$ compared to control, $^{\beta}p < 0.05$ compared to 70 mg/kg/day, n = 5.

Table 2
Effect of *Abelmoschus esculentus* methanol extract on the relative weight of organs.

Group	Control	70 mg/kg/day	200 mg/kg/day
Ovary	0.0542 ± 0.001	0.0480 ± 0.002$^{*\#}$	0.0536 ± 0.002
Uterus	0.0468 ± 0.003	0.0486 ± 0.003	0.0526 ± 0.002
Liver	2.7459 ± 0.058	2.9477 ± 0.051*	3.4068 ± 0.038*
Kidney	0.3200 ± 0.009	0.2682 ± 0.006$^{*\#}$	0.3024 ± 0.011

Data are presented as mean ± standard error of mean, *$p < 0.05$ compared to control, $^{\#}p < 0.05$ compared to 200 mg/kg/day, n = 5.

Table 3
Effect of *Abelmoschus esculentus* methanol extract on malondialdehyde level and antioxidant enzymes in ovary and Uterus.

Ovary			
	Control	70 mg/kg/day	200 mg/kg/day
MDA (U/mg)	7.44 ± 1.69	4.64 ± 0.78	4.23 ± 0.80
SOD (U/mg)	0.17 ± 0.03	0.37 ± 0.04*	0.25 ± 0.03
Catalase (IU/L)	12.96 ± 2.04	3.39 ± 0.27	23.25 ± 2.15$^{*\beta}$
GSH (uM/mg)	3.63 ± 0.13	3.68 ± 0.09	4.18 ± 0.18$^{*\beta}$
Uterus			
	Control	**70 mg/kg/day**	**200 mg/kg/day**
MDA (U/mg)	4.99 ± 0.57	3.64 ± 0.34	8.19 ± 1.76
SOD (U/mg)	0.23 ± 0.04	0.22 ± 0.04	0.46 ± 0.04$^{*\beta}$
Catalase (IU/L)	13.0 ± 14.04	19.26 ± 3.19	15.32 ± 0.81
GSH (uM/mg)	3.71 ± 0.04	3.02 ± 0.19*	3.19 ± 0.14$^{*\beta}$

Data are presented as mean ± standard error of mean, *$p < 0.05$ compared to control, $^{\beta}p < 0.05$ compared to 70 mg/kg/day n = 5, MDA = Malondialdehyde, SOD = Superoxide dismutase, GSH = Reduced glutathione.

3.6. Effect of Abelmoschus esculentus methanol extract on the length and number of estrous cycles within 21 days

There were no significant differences in the length and number of estrous cycles that occurred during pretreatment and treatment periods. (Table 5).

3.7. Effect of Abelmoschus esculentus methanol extract on serum hormone levels

The result shows significant decreases ($p<0.05$) in follicle-stimulating hormone levels of the treated rats. There were no significant differences in the luteinizing hormone and estrogen hormone levels of the treated rats (Figs. 2 and 3).

Table 4
Effect of *Abelmoschus esculentus* methanol extract on the number of times of occurrence of phases of the estrous cycle during pretreatment and treatment.

Phase	Proestrus		Estrus		Metestrus		Diestrus	
Group	Pre-treated	Treated	Pre-treated	Treated	Pre-treated	Treated	Pre-treated	Treated
Control	3.2 ± 1.0	6 ± 0.8	7.6 ± 0.8	5.2 ± 0.4	8 ± 0.3	8.4 ± 0.8	2.2 ± 0.5	1.4 ± 0.4
70 mg/kg/day	5 ± 0.6	6.2 ± 0.7	6 ± 0.8	7.0 ± 0.6	6.2 ± 0.8	6.6 ± 0.8	3.4 ± 1.0	1.0 ± 0.5
200 mg/kg/day	4.6 ± 1.0	5 ± 0.8	5.6 ± 0.5	8.2 ± 1.1	7.4 ± 1.1	5.8 ± 0.8	3.4 ± 0.9	1.0 ± 0.4

Data are presented as mean ± standard error of mean, n = 5.

Table 5
Effect of *Abelmoschus esculentus* methanol extract on the length and number of estrous cycles within 21 days.

GROUP	Estrous cycle length (days)		Number of cycles within 21 days	
	Pre-treatment	Treatment	Pre-treatment	Treatment
Control	4.90 ± 0.12	5.47 ± 0.35	3.8 ± 0.2	3.6 ± 0.24
70 mg/kg/day	5.03 ± 0.30	5.56 ± 0.41	3.0 ± 0.32	3.2 ± 0.2
200 mg/kg/day	4.78 ± 0.21	5.34 ± 0.36	3.6 ± 0.24	3.4 ± 0.24

Data are presented as mean ± standard error of mean, n = 5.

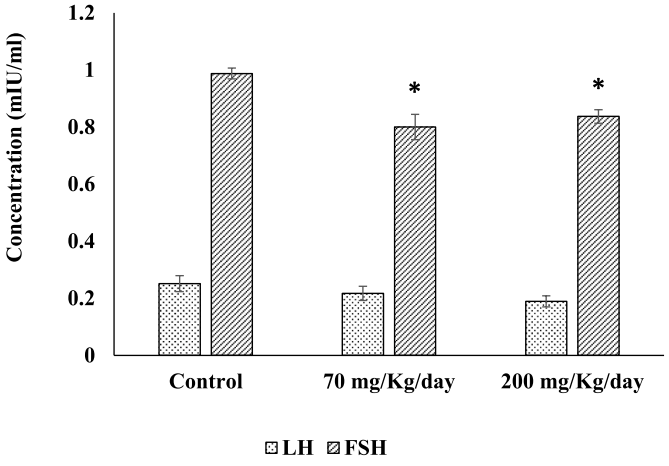

Fig. 2. Effect of *Abelmoschus esculentus* methanol extract on serum level of follicle-stimulating hormone and luteinizing hormones during treatment. Columns represent mean ± standard error mean, *p< 0.05 compared to control, n = 5.

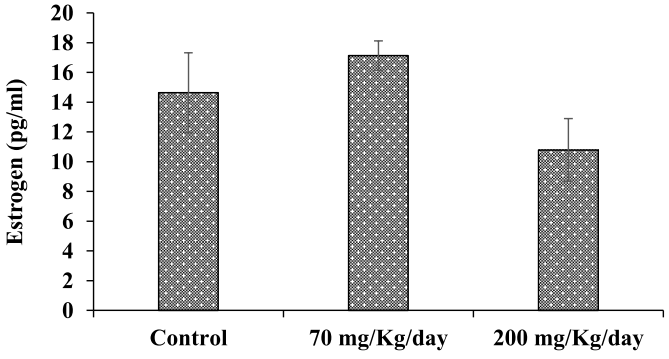

Fig. 3. Effect of *Abelmoschus esculentus* methanol extract on serum level of estrogen hormone. Columns represent mean ± standard error mean, n = 5.

3.8. Effect of Abelmoschus esculentus methanol extract on vaginal epithelial cells

The characteristics of vagina epithelial cells in each phase of the estrous cycle in all groups appeared normal as observed in the unstained and the Papanicolaou stained cells (Fig. 4a and b).

3.9. Effect of Abelmoschus esculentus fruit methanol extract on histology of the ovary and uterus

The ovarian cytology showed infiltration of inflammatory cells and severe fibrosis in the groups that received *Abelmoschus esculentus* fruit methanol extract compared with the control (Fig. 5a). The histology of the uterus revealed thickened endometrium epithelial layer, cell inflammation, and fibrosis in groups treated with the *Abelmoschus esculentus* fruit methanol extract as compared with the control (Fig. 5b).

4. Discussion

This study showed that *Abelmoschus esculentus* fruit methanol extract contains beneficial phytochemicals in line with previous evidence which revealed that it contains secondary metabolites responsible for its therapeutic activities (Sakakibara et al., 2003; Sindhu and Puri, 2016). (Williamson et al., 2018) also reported that the seeds of immature okra pods contain large amounts of polyphenols. The oral administration of *Abelmoschus esculentus* fruit methanol extracts altered the percentage change in body weight of the female rats. The lowest dose increased the body weight affirming the study of (Gaskins and Chavarro, 2018), who noted a gain in body weight of male rats that received the same extract at the same dose level. Also (Olatunji -Bello, 2009) reported a corresponding increase in body weight due to a stepwise increase in the administered doses of the same extract. Contrariwise, the highest dose of *Abelmoschus esculentus* fruit methanol extract reduced the body weight of the rats in agreement with (Das et al., 2019) *who stated that Abelmoschus esculentus* fruit consumption along with a healthy eating habit and lifestyle can help curtail weight gain. This fruit is low in calories but high in dietary fibers like pectin and guar gum, which allow satiety without excessive eating (Das et al., 2019). *Abelmoschus esculentus* fruit methanol extract also reduced the weights of the ovary and kidney in support of previous evidence that it caused a decrease in testicular weight (Roy et al., 2014; Gaskins and Chavarro, 2018). However, the extract increased the weight of the liver, these reported changes in organ weight may be associated with the activities of the organ.

The estrous cycle is known to be disrupted by genetic, environmental, age, endocrine, and nutritional factors (Polito et al., 2011). Marconde's method was used in this study to determine the estrous cycle phases in unstained vaginal smears considering its simplicity (Marcondes et al., 2002). The result showed the presence of the nucleated epithelial cells, leukocytes, and anucleated cornified cells consistent with the findings of (Marcondes et al., 2002). Vaginal cells were also visualized in the smear stained with Papanicolaou stain, and it presented a clearer view of nucleated parabasal cells (prevalent in the diestrus phase), anucleated superficial cells (prevalent in the estrus phase), small and large intermediate nucleated cells (prevalent during all phases of the cycle except estrus) following the findings of (Paccola et al., 2013), who noted that Papanicolaou staining method is of better advantage compared to Marconde's method in terms of color changes during different stage of the estrous cycle and cell morphology.

Antioxidants scavenge free radicals and help in maintaining a balanced oxidative status (Atawodi et al., 2009). Previous studies reported that immature okra pods have antioxidant activity as they

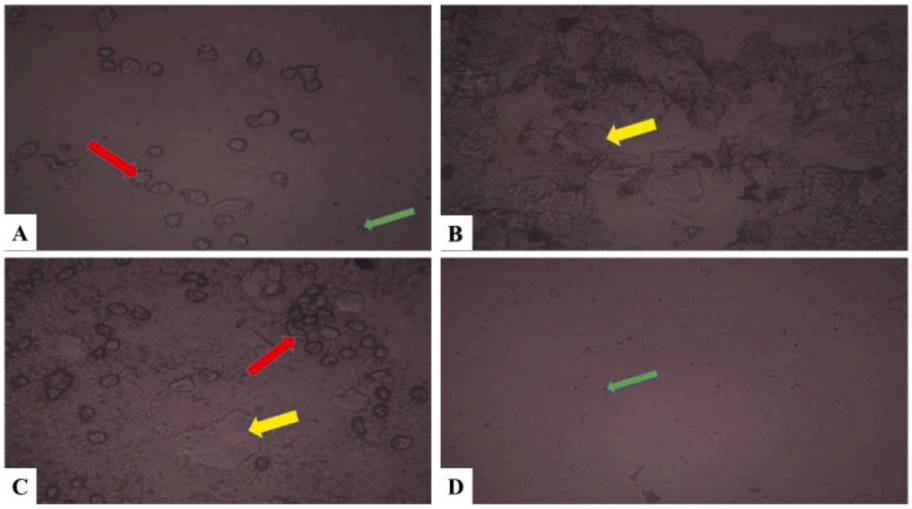

Fig. 4a. Photomicrograph of unstained vaginal epithelial cells. **A** Proestrus phase **B** Estrus phase **C** Metestrus phase **D** Diestrus phase. **Note:** Nucleated epithelial cells (red arrow). Leukocytes (Green arrow) Anucleated cornified cells (Yellow arrow). Magnification: x400. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

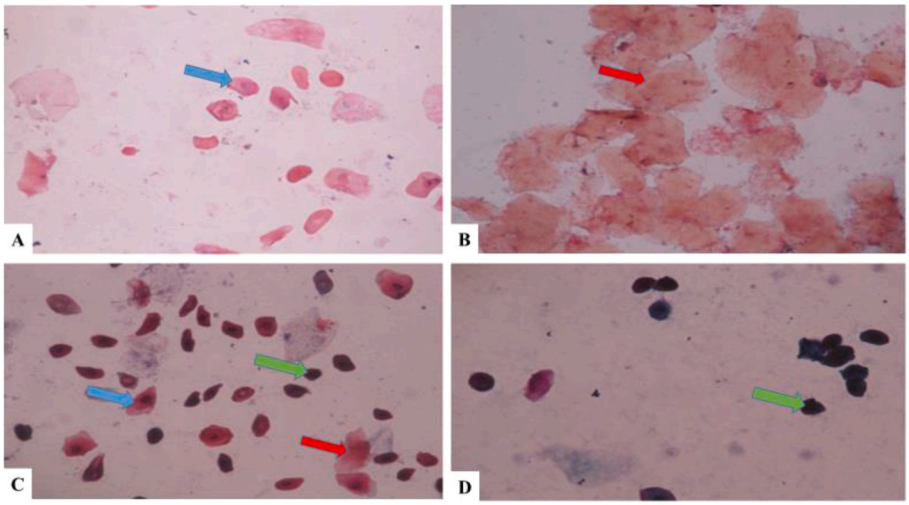

Fig. 4b. Photomicrograph of vaginal epithelial cells. **A** Proestrus phase **B** Estrus phase **C** Metestrus phase **D** Diestrus phase. **Note** Parabasal epithelial cells (blue arrows). Anucleated superficial cells (red arrows). Intermediate cells (green arrow). Papanicolaou stain. Magnification: x400. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

contain polyphenols (Gemede et al., 2015c; Shen et al., 2019). This fact is confirmed by the phytochemical screening result of this present study as it also shows that A*belmoschus esculentus* fruit contains phenolic compounds (Mishra et al., 2013). Mishra et al., 2013 stated that there are several mechanisms involved in the antioxidant capacity of polyphenols which include suppression of reactive oxygen species (ROS) formation by either inhibition of enzymes involved in their production, or scavenging of ROS, or upregulation or protection of antioxidant defenses. The insignificant level of malondialdehyde, a well-known biomarker of lipid peroxidation (Xia et al., 2015) observed in the ovary showed that A*belmoschus esculentus* fruit methanol extract probably acted via inhibiting the enzymes responsible for the production of malondialdehyde and concurrently upregulated its antioxidant defenses as observed by the increased activities of superoxide dismutase, catalase, and reduced glutathione in the ovaries. Furthermore, this study showed that the production of malondialdehyde in the uterus was insignificant, and there was a higher superoxide dismutase activity (Hussain et al., 2016).

Follicle-stimulating hormone aids the maturation of the primary follicle to a Graafian follicle which then secrets estrogen needed for the proliferative phase of the uterine cycle (Schoenwolf et al., 2015). Nonetheless, this study revealed that *Abelmoschus esculentus* fruit methanol extract reduced follicle-stimulating hormone levels and its highest dose caused a decrease in estrogen level (though not significant), resulting in the insignificant increase in the length of the estrous cycle that was observed. This implied that *Abelmoschus esculentus* fruit methanol extracts probably acted by adversely altering the hypothalamic-pituitary-gonadal axis at the level of secretion of the gonadotropin-releasing hormone in the hypothalamus, which should have stimulated the production of follicle-stimulating hormone in the pituitary gland (to begin the development of the follicle in the ovary) that would have led to a rise in the level of estrogen (Raju et al., 2013) but the reverse was the case in this study. Hence, it is plausible that the extract may eventually cause poor ovarian function that can result in a prolonged estrous cycle during a longer period of administration.

(Willcox et al., 2004) reported that excess production of reactive oxygen species can cause tissue injury that may lead to the inflammatory process. This could be the cause of the severe inflammation and fibrosis

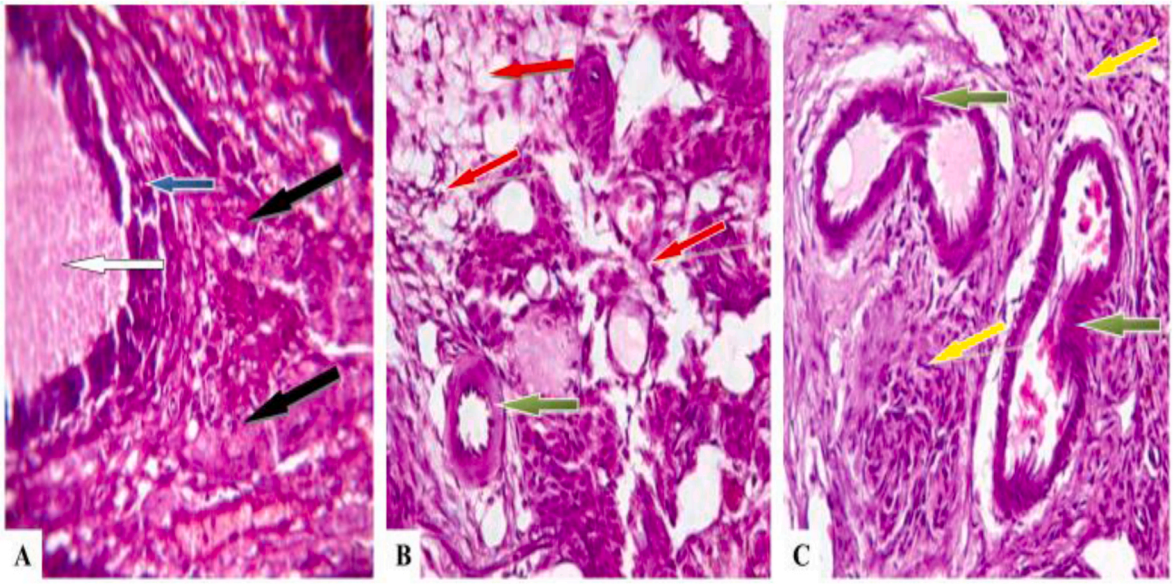

Fig. 5a. Photomicrograph of ovarian sections of control rat and *Abelmoschus esculentus* fruit methanol extract treated rats. **A** Control **B** 70 mg/kg/day **C** 200 mg/kg/day. **Note** Normal Graafian follicle (white arrow), stroma with normal connective tissues (black arrow), normal theca cells (blue arrow). Thick-walled vessels with severe vascularization (green arrow), severe fibrosis (red arrows), and mild infiltration of inflammatory cells (yellow arrows). Stained by H&E. Magnification: x400. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

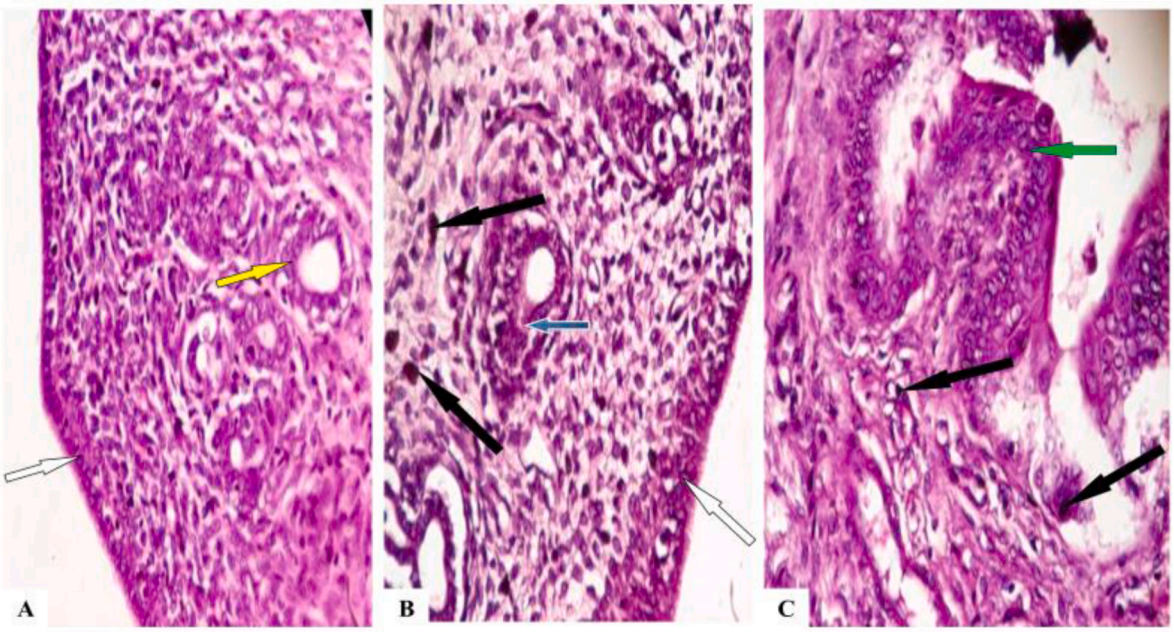

Fig. 5b. Photomicrograph of uterine sections of control rat and *Abelmoschus esculentus* fruit methanol extract treated rats. **A** Control **B** 70 mg/kg/day **C** 200 mg/kg/day. **Note:** Normal endometrium epithelial layer (white arrow) normal proliferation of endometrial gland (yellow arrow), severe infiltration of inflammatory cells in the endometrial stroma which appear fibrotic (black arrows), the mild proliferation of endometrial gland with epithelial hyperplasia (blue arrow), thickened endometrium epithelial layer (green arrow). Stained by H&E. Magnification: x400. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

noted in the cytology of the ovary and uterus. Also, the antioxidant activity of polyphenols depends on the structure and number of their functional and hydroxyl groups respectively, which determines the mechanisms of antioxidant activity (Heim et al., 2002). From the foregoing, it is plausible that the activity of the type of phenolic compound present in the *Abelmoschus esculentus* fruit methanol extract was by the above-aforementioned mechanisms (either inhibition of enzymes involved in the production of ROS and upregulation or protection of antioxidant defenses) and not via scavenging of the ROS that possibly caused the observed inflammation and fibrosis (Haris et al., 2018). reported that *Abelmoschus esculentus* adversely altered the histology of reproductive organs due to the activities of its phytochemical constituents. Similarly (Olatunji -Bello, 2009), reported that *Abelmoschus esculentus* fruit caused degeneration of the basement membrane of the testicular germ cells impacting negatively the spermatogenic process.

Limitation of the study: There was no staging of estrus at the beginning of the experiment, hence the experiment started at different phases of the estrous cycle.

5. Conclusion

This study revealed that *Abelmoschus esculentus* fruit methanol extract caused adverse alterations in reproductive functions. Hence, constant consumption of *Abelmoschus esculentus* fruit may cause harmful effects on the female reproductive functions that can lead to infertility in female rats.

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CRediT authorship contribution statement

Eunice Ogunwole: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jemimah Adoh Yakubu:** Project administration, Resources, Funding acquisition, Investigation, Validation, Visualization, Writing – original draft, editing. **Vivian Tally Giwa:** Project administration, Resources, Funding acquisition, Investigation, Validation, Visualization, Writing – original draft, editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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