ORIGINAL RESEARCH

Effect of the Aqueous Bark Extract of *Blighia Sapida* on Ovaries and Uteri of Female Rats

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ABSTRACT

Background: *Blighia sapida* has its origin in sub-Saharan Africa and has been reported to have folkloric use in the management of ailments like backache, constipation, gonorrhoea, dysentery, psychosis, hernia, stomach-ache, malaria, and typhoid.
Objective: The objective of this study was to determine the effect of *Blighia sapida* on the ovaries and uteri of female rats using the Sprague-Dawley rats as models.
Materials and Methods: A total of twenty adult female Sprague-Dawley rats weighing between 150g±20g (n=5, W-Z) were used. Group W served as Control and received 1 ml of distilled water, X-Z received 100, 200 and 400 mg/kg of *Blighia sapida* respectively for a period of 4 weeks. After this study, the rats were euthanized, and their ovaries and uteri were harvested for histology and oxidative stress markers and blood for hormonal functions.
Results: Micrographs of the ovaries showed degenerated theca cells within the cortex and ovarian stroma showed vascular congestion. Significant reductions in some female hormone (FSH, Estrogen and Progesterone) values were observed. There was also a significant increase in MDA and reduction in SOD and CAT uteri values were also noticed.
Conclusion: Study showed that *Blighia sapida* could affect the ovaries and reproductive hormone, hence moderation in consumption of this extract by females should be considered, as excess consumption could lead to infertility.

Keywords: *Blighia sapida*; Female rats; Hormonal milieu; Ovaries; Uteri

INTRODUCTION

The plant *Blighia sapida* is also known as Ackee, Ankye, Achee, Akee, Ackee apple or Ayee in Jamaica and Isin in Yoruba. It a fruit of the Sapindaceae soapberry family, native to tropical West Africa and prevalent in the tropics and subtropics[1]. It has been documented to have comparable proximate composition to other known oil and legume seeds[2-4]. However, it has little or no commercial and nutritional significance in the West African sub-region. Various parts of the *Blighia sapida* tree have been documented for folkloric treatment of fever, malaria, internal haemorrhage, dysentery, yellow fever, diabetes and constipation in West Africa. The roots, bark, leaves, capsules, and seeds were identified in the treatment of 22 diseases in Benin[3]. Consumption of *Blighia sapida* roots bark extract exerted significant hypoglycaemic effect on the normoglycemic albino rats[5]. Due to the paucity of information on the benefits of the *Blighia sapida,* hence

the birth of this research on its effect on the female reproductive system vis-à-vis the ovaries and uteri using Sprague-Dawley rats as experimental animals.

MATERIALS AND METHODS

Preparation of the Extract

The bark of *Blighia sapida* was freshly collected, shade dried and pulverized into powder using mortar and pestle, and a blender was used until a fine, smooth texture was obtained. The powdered bark (1000g) was soaked in 3L of water and stirred at intervals for the three days. The evaporation was done using a rotary evaporator with a regulated temperature of 90^{o}C to obtain a paste-like extract.

Experimental Animals

A total of twenty Adult female Sprague-Dawley rats weighing between 150g± 20g were used. They were obtained from the animal house of the Department of Physiology, Bowen University, Nigeria and were identified by a Zoologist (Dr. U. U. Akpan) for this study. The animals were left in well-ventilated plastic cages to acclimatize for a period of two weeks before the commencement of the experiment and fed with standard rat feed and water *ad libitum*. The body weight of animals was taken and recorded weekly.

Experimental groupings

The animals were randomly divided into four groups of five animals each. Group W served as Control that received 1 ml of distilled water and Groups X, Y and Z received 100, 200 and 400 mg/kg, respectively, of the aqueous extract for duration of 4 weeks. Animals were euthanized 24 hours after the last administration The left ovaries were fixed in Bouin's fluid to ascertain the histo-architecture of the organ and the right ovaries and uteri were placed in sample bottles and stored at -80^{o}C to determine the oxidative stress levels. Blood was collected for hormonal functions.

Histological Procedures

Ovarian tissues were processed for microscopic examination using a standard protocol and 5um thick paraffin sections were made. Slides were stained with routine haemoxylin, and eosin stains and photomicrographs were made at a magnification of 100 and 400 using Olympus and Leica microscopes[6]

Measurement of LH, FSH, Progesterone and Oestrogen

Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and oestrogen were measured with a two-site chemiluminescence (sandwich) immunoassay using two antibodies specific for the intact FSH molecule. Serum LH concentrations were measured with a two-site chemiluminescent immunoassay by Bayer diagnostics. The serum progesterone assay is also a competitive chemiluminescent immunoassay[6,7].

Uterine and Ovarian Homogenate for Antioxidant Activities

The ovaries were washed in ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer (pH 7.2). The tissues were placed in a mortar and laboratory sand was added. This was crushed using a pestle. The resulting homogenate was centrifuged at 2500 rmp for 15 min. The supernatant was decanted and stored at -20 °C until analysis[6,7].

Superoxide Dismutase (SOD) was assayed by its ability to inhibit the auto-oxidation of epinephrine, determined by the increase in absorbance at 480 nm. The enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Catalase (CAT) Catalase was assayed calorimetrically at 620 nm and expressed as µmoles of H_2O_2 Consumed/min/mg/protein.

Malondialdehyde (MDA), an index of lipid peroxidation, was determined using the

method from a previous study[6]. The supernatant was removed and the absorbance was read at 532 nm. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56 × 105 $M^{-1}cm^{-1}$.

Statistics

The data obtained from all the groups were compiled and statistically analysed using ONE WAY-ANOVA using Graph pad software version 8. The results of the data were expressed as mean ± SEM (standard error of mean) where $p<0.05$ was taken as significant.

RESULTS

Effect of *Blighia sapida* on body weight of female rats.

Before administration of the extract, when treatment groups were compared to control, increase in value was recorded with significance recorded in groups administered with 100 and 200 mg/kg as seen in Table 1.

Table 1: Effect of aqueous bark extract of *Blighia sapida* on body weight of female rats.

Concentration	Body Weight (kg)		Change in Body Weight (%)
	Day 0	Day 28	
Control	159.7 ± 4.24	214.4 ± 1.40*	34.25%
100 mg/kg	160.4 ± 2.19	196.3 ± 1.61*	22.38%
200 mg/kg	164.6 ± 2.45	185.4 ± 1.38*	12.67%
400 mg/kg	176.6 ± 5.60	180.8 ± 8.34	2.38%

Values are mean standard error of mean; n=5, * <0.05 (student's T test)

Effect of *Blighia sapida* on some female hormones in rats.

Administration of aqueous extract of *Blighia sapida* for 28 days to rats caused significant, dose-dependent decreases in FSH, estrogen and progesterone but an increase in LH relative to the control group (Table 2). The effect of the highest dose of extract on all the hormones was significantly higher than those of the lower doses (100 and 200 mg/kg/day).

Table 2: The effect of aqueous bark extract of *Blighia sapida* on some female hormones in rats.

GROUPS	FSH (IU/mL)	ESTROGEN (pg/mL)	LH (IU/mL)	PROGESTERONE (IU/mL)
Control	196.24 ± 0.03	4.98 ± 0.07	46.63 ± 0.04	60.29 ± 0.06
100 mg/kg	101.11 ± 0.02 ^{x}	3.77 ± 1.12 ^{x}	52.31 ± 1.03 ^{x}	42.07 ± 0.11 ^{x}
200 mg/kg	53.24 ± 1.00 ^{xy}	3.12 ± 0.02 ^{xy}	58.10 ± 0.10 ^{xy}	34.19 ± 1.17 ^{xy}
400 mg/kg	29.13 ± 0.13 ^{xyz}	1.05 ± 0.11 ^{xyz}	109.11 ± 0.02 ^{xyz}	29.14 ± 1.00^{xyz}

Key: FSH = Follicle Stimulating Hormone; LH = Luteinizing HormoneValues are expressed as Mean ± Standard Error Mean (SEM), $^{x}p<0.05$ significant compared with control; $^{y}p<0.05$ significant compared with low dose; $^{z}p<0.05$ significant compared with medium dose.

Effect of *Blighia sapida* on oxidative stress markers in ovaries and uteri of female rats.

There was a dose-dependent increase in MDA, SOD and CAT of ovaries from extract-treated rats, compared to control, with the highest effect seen at the dose of 200 mg/kg (Table 3) These effects were not significant except in CAT at the two higher doses (200 and 400 mg/kg).

In the uteri, pre-treatment of animals with the extract (100, 200 and 400 mg/kg) caused significant dose-dependent increases in MDA but reductions in SOD and CAT levels relative to control (Table 3)

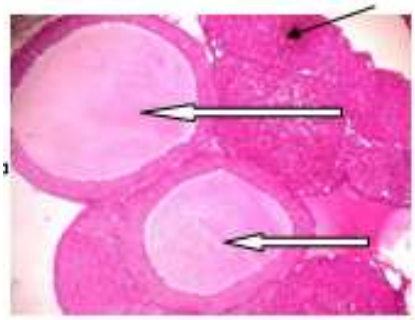
Table 3: The effect of aqueous bark extract of *Blighia sapida* on oxidative stress markers in ovaries and uteri of female rats.

ORGANS		OVARIES				UTERI		
GROUP	MDA	SOD	CAT	MDA	SOD	CAT		
Control	412.13 ± 0.17	324.11 ± 3.06	206.44 ± 0.26	416.32 ± 0.09	320.11 ± 6.02	536.02 ± 0.01		
100 mg/kg	420. 04 ± 0.03	331.29 ± 0.06	210.10 ± 0.04	418.26 ± 1.03	315.02 ± 4.33	530.17 ± 1.00		
200 mg/kg	424.43 ± 1.03	335.18 ± 0.10	239.20 ± 0.04 ^{xy}	438.04 ± 0.19 ^{xy}	302.10 ± 2.94 ^{xy}	526.03 ± 1.42 ^{xy}		
500 mg/kg	419.78 ± 1.04	326.40 ± 3.00	219.03 ± 0.39 ^{xz}	459.04 ± 0.34 ^{xy}	296.42 ± 1.98 ^{xy}	503.07 ± 0.26^{xy}		

Key: SOD = Superoxide Dismutase; CAT = Catalase; MDA = Malondialdehyde. Values are expressed as Mean ± Standard Error Mean (SEM), $^{x}p<0.05$ significant compared with control; $^{y}p<0.05$ significant compared with low dose; $^{z}p<0.05$ significant compared with medium dose.

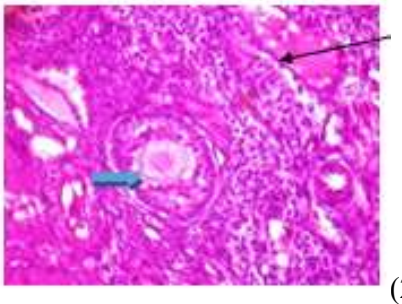
Photomicrographs of the Ovaries

Section of control group (0 mg/kg) appeared normal, showing normal antral and Graafian follicle (white arrow) with normal theca cells within the ovarian cortex. The ovarian stroma appears normal with connective tissues and Luteinized stroma cells (slender arrow). Sections of 100 mg/kg group (low dose) also appear normal, showing normal antral and Graafian follicle (white arrow) with normal theca cells within the ovarian cortex. The ovarian stroma shows normal connective tissues and Luteinized stroma cells. Section of 200 mg/kg group (medium dose) showing normal antral with mild degenerated theca cells (blue arrow) within the ovarian cortex. The ovarian stroma shows mild vascular congestion (slender arrow) and Luteinized stroma cells. The section of 400 mg/kg group (high dose) appears to have mild to moderate vascular congestion.

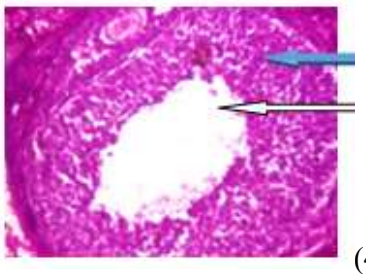


(0mg/kg)

(100mg/kg)



(200mg/kg)



(400mg/kg)

Figure 1: Micrographs of sections of ovaries

DISCUSSION

Medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Various parts of plants, such as leaves, roots, barks, stems, flowers, stalks, buds, and seeds have been documented to be of value to the humans. These plants play a vital role in the development of human cultures around the whole world. Moreover, some plants are considered as important source of nutrition, hence they are recommended for their therapeutic values.

The increasing demand for the inclusion of plants extracts and other herbal preparations, in both human and animal health programmes, necessitates the assessment of their inherent toxicity levels and the effect of acute overdose. This is to safeguard the safety limits of their use in both animal and man. However, nature cannot be said to be totally safe as some studies have documented that some plants with beneficial properties can also be toxic or have adverse effects on some vital viscera or organs[7].

Dose-dependent decreases in body weight values of extract treated animals compared to control group, is an indication of toxicity as had been reported. This reduction in body weight could be directly attributed to the observed hypocholesterolaemic property of the extract[8], because cholesterol significantly contributes to the total fat content of a person[9], hence, suppressing appetite as seen in the treatment groups.

Decrease in female reproductive hormones, like the findings from our study, has been reported to cause irregular pattern and complete seizure of the oestrous cycle, otherwise termed amenorrhoea of female rats at dioestrus phase. The oestrous cycle is the reproductive cycle that happens in sexually mature females and otherwise gives rise to the functionality of the ovaries and uteri[10,11]. The reduction in these hormones, in the extract-treated groups suggests that the extract may cause the hypothalamus to release glucocorticoid hormones in response to the stress. Continuous increase in serum glucocorticoid concentration leads to production of free radicals, and also a decline in the concentration of GnRH leading to reduced synthesis and secretion of FSH. Progesterone is produced majorly by the corpus luteum whose formation is triggered by a surge of LH; hence significant decrease in the progesterone level of the treatment group compared to control can be attributed to the reduced synthesis and secretion of FSH. Estrogen has antioxidant properties, but with suppressed generation of FSH and elevation of glucocorticoids, its synthesis and secretion are highly reduced. This leads to excessive oxidative stress which can subdue or hinder folliculogenesis and the entire oestrous cycle[10-13].

This present work showed an increase in MDA activity, with significant reduction in SOD and CAT activities in the uteri. An increase in the level of MDA will increase the generation of free radicals and increase the cell's susceptibility to oxidative stress which will invariably alter cell membrane integrity, permeability, and function of the reproductive organ. SOD and CAT are said to be considered as the first line of defence against toxic effect of reactive oxygen species and have been reported to exhibit synergism in their functioning. Superoxide radicals are converted to H_2O_2 and O_2 that are deleterious to polyunsaturated fatty acids and proteins by SOD which is subsequently converted into water and oxygen by the action of CAT. From this study, SOD and CAT were observed to be significantly reduced in the uteri when treatment group was compared to control. It can be inferred, therefore, that overproduction of ROS overwhelmed the antioxidant defence system provided by SOD thereby resulting in oxidative stress. A reduction in SOD activity usually leads to an increased level of superoxide anion, which can subsequently inactivate CAT activity. Conversely, the failure of CAT to prevent H_2O_2 accumulation in the cell could lead to the inactivation of SOD[14]. The reduction in CAT activity is therefore suggestive of the inactivation of the enzyme and its inability to eliminate H_2O_2 produced by SOD resulting to the

accumulation of this free radical in the organ[14]. H_2O_2 is a potent oxidant capable of inducing oxidative damage in macromolecules, and its increased production may further aggravate the oxidative damage in the reproductive organs.

The photomicrograph of the ovaries showed distortion within the cyto-architecture of some of the treated rats having mild degenerated theca cells within the ovarian cortex and mild to moderate vascular congestion. The histopathological changes might be due to the presence of bioactive compounds (saponins, alkaloids, tannins, oxalate)[15,16]. The presence of oxalate in food has been reported to be associated with toxicity and acidity[16].

CONCLUSION

With the present review, we conclude that bark of Ackee plant (*Blighia sapida*) could affect the ovaries and alter the reproductive hormonal milieu in female. Based on the findings of this study, moderation in consumption of this extract by females is advised, as excess consumption could lead to infertility.

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The authors declare no conflict of interest.

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