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Androgenic Effects of Aqueous Extract of *Blighia sapida* (Ackee) Barks in Male Sprague- Dawley Rats

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ABSTRACT

Blighia sapida is an herbal plant cultivated in West Africa that has been observed to have multipurpose properties. To investigate the androgenic effects of *Blighia sapida* (Ackee bark) extracts in male Sprague-Dawley rats. Twenty-four rats weighing 135±15g were used for this study. They were randomly divided groups of six rats. Group A (Control) received 1 ml of DW. Group B-D received 100ml/kg, 200ml/kg and 400ml/kg of *Blighia sapida* respectively. After administration for 56 days, five animals were randomly chosen and euthanized. The testes were harvested for histology and oxidative stress, blood for hormonal milieu. There was a decrease in the values of FSH and LH when treatment groups were compared to control. Semen analysis showed decrease in parameters when treatment groups were compared to control. A significant increase was recorded when treatment groups were compared to control in MDA and significant decrease was observed in CAT, SOD and GSH when treatment groups were compared to control. A decrease was observed in testicular weight when treatment group was compared to Control. *Blighia sapida* showed deleterious effects on the male reproductive system as there was reduced secretion of testicular hormones. It also induced oxidative stress on the testis had adverse effect on seminal parameters.

Keywords: *Blighia sapida*, FSH, LH, Testosterone, Oxidative stress.

INTRODUCTION

Infertility and sexual dysfunction have been a significant clinical problem worldwide and a concern through ages. World Health Organization has documented that about 50-80 million people globally suffer from infertility ranging from primary to secondary and to unexplained infertility [1,2] and studies have shown that 20 to 70 percent of the cases occur due to male factor [3,4]It may be one or a combination of low sperm concentration, poor sperm motility or abnormal morphology [2] The incidence of sexual dysfunction resulting from hormonal imbalance is estimated to be 20-25% with hypogonadism (primary and secondary) being the most frequent cause [2]

Androgens are steroid hormones with an ambiguous role in sexuality [3]In general, they are essential for the development of the male external genitalia, the male secondary sexual characters and also in the regulation of erectile response [2] Testosterone is the most important androgen secreted by the testis in human. This hormone contributes to male fertility by influencing sperm count, morphology, and motility. Reproduction in males is a complex process that involves the testes, epididymis, vas deferens, accessory sex glands and associated hormones [1]. A reduction in the level of testosterone at the early developmental phase results in the lack of virilization, sustained height increase without closure of epiphysis, lack of pubertal growth spurt, incomplete sexual development and aspermia. In adulthood, it may result in the loss of libido and sexual activity [3]. This process of reduction can be as a result of the environment, dietary (nutrition), drugs and other factors. This study investigates possible androgenic effects of aqueous extract of the bark of *blighia sapida*.

Blighia sapida, popularly known as Ackee (English), Isin (Yoruba), Gwanje kusa (Hausa) and Okpu (Ibo) belongs to the family of Sapindaceae [5] It is a woody perennial multipurpose tree indigenous to the gold coast of west tropical Africa, and a national fruit in Jamaica[6.] It is a multipurpose herbal plant that is cultivated in countries like tropical America, India and West Indies Although Africa is where Ackee fruit originated from, yet it is not a major food crop in Africa. Ackee is eaten with salt fish in Jamaica and well liked in Jamaica; hence, it became a national fruit. The fruits consist of the pod, pulp and the aril covering the seeds . The fruit arils only become edible when the matured fruit opens up spontaneously to reveal the seeds and the fleshly arils [3]

Extracts of *Blighia sapida* are commonly employed in folk medicine to treat a wide range of disease conditions, especially in developing countries It has also been reported that extracts from the seed serves as insect repellant. Various parts of the ackee tree are used for the treatment of fever, malaria, internal hemorrhage, dysentery, yellow fever, diabetes and constipation in west Africa [7,6]. In folk medicine practice, the bark pulp is used as liniment for oedema intercostals pains in Ivory Coast. The pulp and leafy types are used as eye drop in ophthalmic and conjunctivitis. The roots are used in conjunction with *Xylopia aethiopica* to terminate unwanted pregnancy.

In Brazil, repeated small doses of an aqueous extract of the seed have been administered to expel parasites. In Colombia, the leaves and bark are considered stomachic. Although no work has been carried out to identify the effects of this bark on male reproductive system, other researches have been carried out and it has been reported that ackee bark aids digestion, lowers blood pressure, improves heart health, boosts immunity, regulates circulation. Despite the many benefits of this fruit, it is poisonous and toxic when eaten before it ripens. It can cause "Jamaican vomiting sickness" and in the most extreme cases, coma and death [8].

MATERIALS AND METHODS

Experimental Animals: A total of 24 healthy male Sprague-Dawley rats, 120-150g body weight (135±15) were obtained from the animal house of the physiology department of Bowen University, Nigeria and were identified by a Zoologist for this study. They were housed in groups (six rats per cage), in a standard well ventilated plastic cages in the Animal room of the Department of Anatomy, College of Health Sciences, Bowen University under standard room temperature ranging between 26ºC-28ºC and relative humidity of 50-55%. The animals were exposed to twelve Hours light and twelve Hours dark cycle and were left to acclimatize for a period of two weeks before the commencement of the experiment. The rats were fed with standard rat feed ad libitum and water. Wood shavings were used as bedding for the animals in the plastic cage, and this was changed frequently to ensure proper sanitation.

Experimental Design: The animals were divided into four groups of six animals each as follows:

Group A (control) - received rat chow and water only.

Group B (Low Dose) - received 100ml concentration of the aqueous extract of *Blighia sapida*

Group C (Medium Dose) – received 200ml concentration of the aqueous extract of *Blighia sapida*

Group D (High Dose) - received 400ml concentration of the aqueous extract of *Blighia sapida*.

Blighia sapida (Ackee plant) was crushed into powder using mortar and pestle and blender was used until a fine, smooth texture was obtained. The powder was weighed and 1000g of it was soaked in 3litres of water and stirred at intervals for the three days. The evaporation was done using a rotary evaporator with a regulated temperature of 90ºC. Paste-like extracts was obtained and weighed using the sensitive weighing scale and administered orally with the use of a feeding tube for 56 days after administration. The body weight of animals was taken and recorded weekly. At the end of the administration, the animals were weighed and euthanized 24hrs after the last administration. The testes were harvested and then weighed with a sensitive weighing scale. The harvested testicular tissues were dissected transversely. One halves were immediately stored in a plain bottle filled with phosphate buffer and put inside ice park for biochemical assay and the other halves were fixed in formalin(fixative) for histological assay. The epididymal was also removed for epididymal sperm count and morphology.

Histological procedures: The testes were transferred into a universal bottle containing 10% formal saline for 72 hours. 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

Statistical Analysis: Data were analyzed using Graphpad Prism 9.0 (GraphPad Software Inc., California, USA) at $p<0.05$ and Excel 2016 (Microsoft Corporation, USA). Data were expressed as Mean ± SEM. Means were compared using ANOVA (Analysis of Variance).

RESULTS

Effect on body Weight: There was a significant increase in the body weights of the rats in control and treatment groups (Table 1).

Effect on testicular weights: A decrease was observed in the testicular weight when treatment groups were compared to control

in a dose-dependent manner. A decrease was observed when Low-dose was compared to Medium-dose and High-dose, with similar pattern observed when Medium-dose was compared with High-dose (Table 2).

Effect on Hormonal Milieu: A dose-dependent decrease was observed in the values of FSH and LH when treatment groups were compared to control, with significance seen in FSH. A decrease was observed when Low-dose was compared to Medium and High doses, with significance seen in FSH. Similar decrease was seen when Medium-dose was compared to High-dose with significance seen in FSH. An irregular pattern was observed in Testosterone value as an increase was seen when Low-dose was compared to control and significant decrease was seen when Medium and High doses were compared to control. When Low-dose was compared to Medium and High doses, a significant decrease was noticed. Also, a decrease was seen when Medium-dose was compared to High-dose (Table 3).

Effect on Biochemical Markers: A significant decrease was observed in dose-dependent manner when treatment groups were compared to control, significant decrease was also noticed when Low-dose was compared to Medium and High doses, similar pattern of decrease was recorded when Medium-dose was compared to High-dose in the values of CAT, SOD and GSH. A dose-dependent significant increase was however noticed when treatment groups were compared to control, significant increase was also noticed when Low-dose was compared to Medium and High doses, similar pattern of increase was recorded when Medium-dose was compared to High-dose in the value of MDA (Table 4).

Effect of *Blighia sapida* on Sperm count and morphology of Adult Sprague-Dawley Rats: A decrease was observed when treatment groups were compared to control was compared to treatment groups for the values of normal morphology and sperm concentration in a dose dependent manner. Decrease was also noticed when Low-dose was compared to Medium and treatment group, as similar pattern was observed when Medium-dose was compared to High-dose (Table 5).

Table 1: Effect of *Blighia sapida* On Body Weight of Adult Male Sprague-Dawley Rats

GROUP	PRE-ADMINISTRATION	POST-ADMINISTRATION	% WEIGHT DIFFERENCE
CONTROL	195.2±13.38	211.84±7.92*	7.85
LOW	179.16±13.02	190.85±11.99*	6.13
MEDIUM	197.12±11.97	205.86±9.10*	4.25
HIGH	207.58±18.08	212.88±16.68*	2.5

Values expressed as Mean ± Standard Error of Mean (SEM).

Table 2: Effect of *Blighia sapida* on Testicular Weight of Adult Male Sprague-Dawley Rats

GROUP	TESTICULAR WEIGHT
CONTROL	1.23±0.03
LOW	1.29±0.08
MEDIUM	1.20±0.05
HIGH	1.17±0.04

Values are expressed as Mean ± Standard Error of Mean (SEM).

Table 3: Effect of *Blighia sapida* on Hormones of Adult Male Sprague-Dawley Rats

GROUP	FSH	LH	TESTOSTERONE
CONTROL	74.43±1.00	15.96±0.09	28.74±1.04
LOW DOSE	65.12 ± 1.70^{x}	14.97±0.17	29.36±0.80
MEDIUM DOSE	54.47 ± 1.37^{xy}	13.75±0.21	19.27 ± 0.88^{xy}
HIGH DOSE	46.25 ± 1.75^{xyz}	9.8±0.51	17.12 ± 1.07^{xy}

Values are expressed as Mean± Standard Error of Mean (SEM). $^{x}p>0.05$ significant compared to Control; $^{y}p>0.05$ significant compared to Low-Dose and $^{z}p>0.05$ significant compared to Medium-dose.

Table 4: Effect of *Blighia sapida* on Oxidative Stress Markers of Adult Male Sprague-Dawley Rats

GROUP	SOD	CAT	MDA	GSH
CONTROL	181.11±0.83	480.22±2.90	409.02±7	153.06±2.51
LOW DOSE	172.06 ± 1.84^{x}	388.96 ± 5.50^{X}	470.93 ± 5.17^{x}	140.36 ± 1.37^{x}
MEDIUM DOSE	164.59 ± 0.41^{xy}	320.39 ± 1.60^{xy}	576.02 ± 11.52^{xy}	132.52 ± 3.38^{xy}
HIGH DOSE	155.67 ± 1.13^{xyz}	286.97 ± 4.38^{xyz}	632.14 ± 6.17^{xyz}	124.50 ± 1.92^{xyz}

Values are expressed as Mean ± Standard Error of Mean (SEM). $^{x}p<0.05$ significant compared to Control; $^{y}p<0.05$ significant compared to Low-dose and $^{z}p<0.05$ significant compared to Medium-dose.

Table 5: Effect of Bark of Ackee on Sperm Count and Morphology of Adult Male Sprague-Dawley Rats

GROUP	SPERM MORPHOLOGY %
CONTROL	94.28±0.85
LOW	66.25±3.25
MEDIUM	62.75±4.03
HIGH	52.25±8.13

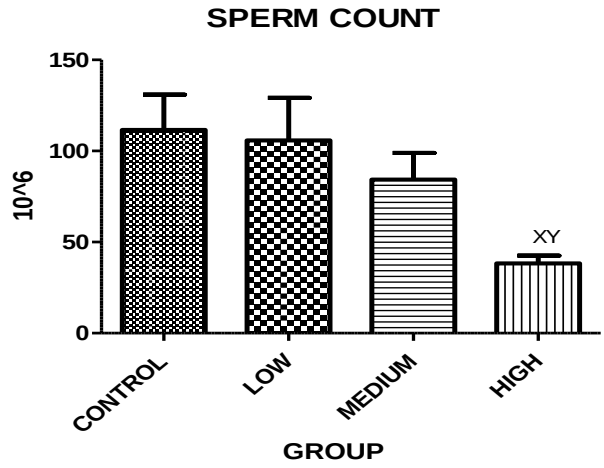
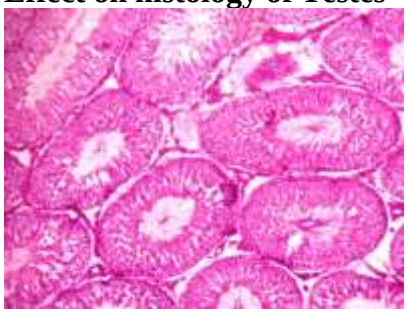
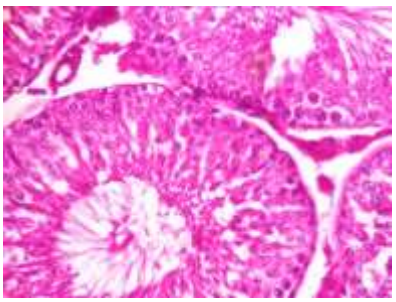


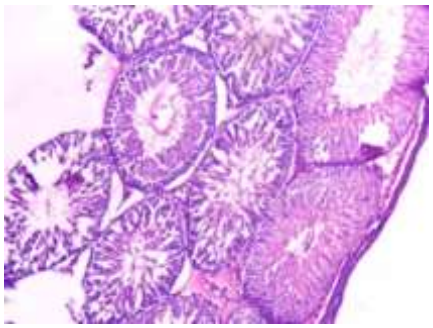
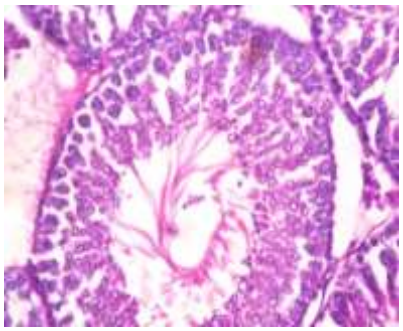
Figure 1: Values are expressed as Mean± Standard Error of Mean (SEM). ^{x}y<0.05 significant compared to Control. ^{Y}Y< 0.05 significant compared to Low-dose. ^{Z}y< 0.05 significant compared to Medium-dose.

Effect on histology of Testes

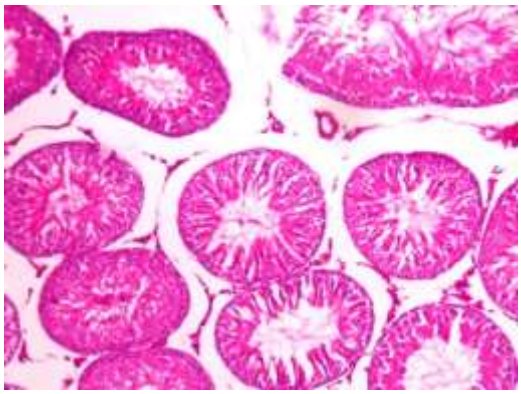
X100 x400 H&E

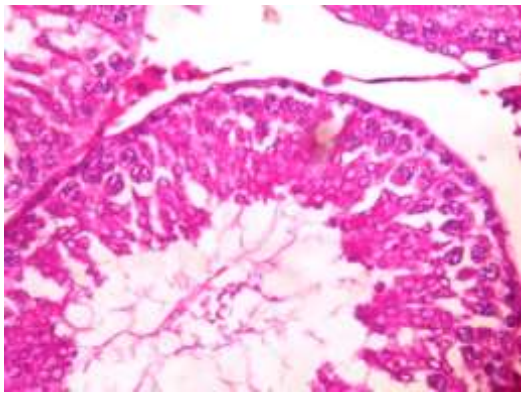
Plate 1: Photomicrograph of a Control testicular section (A) stained by Haematoxylin and Eosin showing normal testicular architecture with normal seminiferous tubules and normal maturation stages with presence of spermatozoa within their lumen(white arrow). The spermatogonia cells and the sertoli cells are normal. The interstitial spaces show normal leydig cells (slender arrow)

X100 x400 H&E

Plate 2 Photomicrograph of a low-dose testicular section (B) stained by Haematoxylin and Eosin showing normal testicular architecture with normal seminiferous tubules and normal maturation stages with presence of spermatozoa within their lumen(white arrow). The spermatogonia cells and the sertoli cells are normal. The interstitial spaces show normal leydig cells (slender arrow)

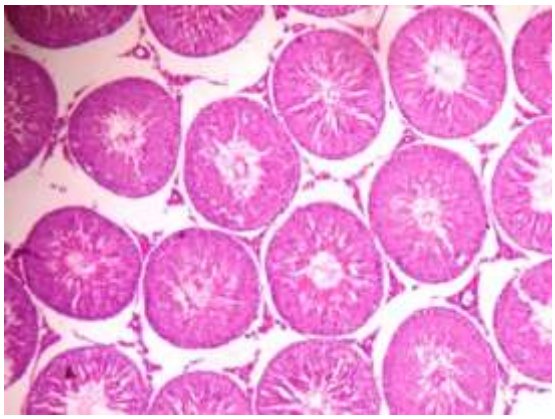


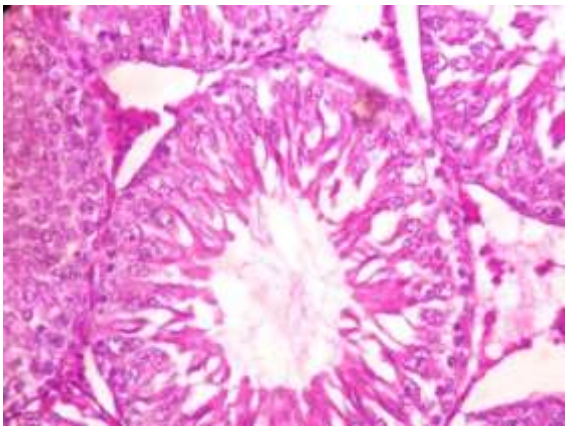


X100 x400 H&E

Plate 3 Photomicrograph of a Medium-dose testicular section (C) stained by Haematoxylin and Eosin showing normal testicular architecture with normal seminiferous tubules and normal maturation stages with presence of spermatozoa within their lumen(white arrow). The spermatogonia cells and the sertoli cells are normal. The interstitial spaces show normal leydig cells (slender arrow)

Plate 4 T esticular Section of High-Dose group (D)





X100 x400 H&E

Plate 4: Photomicrograph of a High-dose testicular section stained by Haematoxylin and Eosin showing normal testicular architecture with normal seminiferous tubules and normal maturation stages with presence of spermatozoa within their lumen(white arrow). The spermatogonia cells and the sertoli cells are normal. The interstitial spaces show normal leydig cells (slender arrow). There is mild disintegration of the interstitial leydig tissues.

DISCUSSION

Medicinal plants are been used in the management of varying ailment and to boost mental and physical wellbeing and reports have showed that about 80% of the global populace had relied and is still relying on the herbal medicine for basic medical need. Folklorically, they are being said to have little or no side or adverse effect, scientific documents have showed that some are toxic to human system(s). The various mechanisms of actions of some herbal plants in males are via effects on sex hormones particularly for suppressing fertility. Other form of effects on males could be anti-adrogenic, anti-spermatogenic, post-testicular damage and spermicidal.

Blighia sapida have been reported to be used in folkloric medicine for the treatment of fever, malaria, internal hemorrhage, dysentery, yellow fever, diabetes and constipation in West Africa [7,6] . To ascertain the safety of this medicinal plant for consumption in humans most especially in males, we carried out this study using Sprague-Dawley rats as experimental models. The administration of this extract did not result in any change in the consumption of feed, as there was no significant change in the body weight of the treatment groups to control as we recorded increases in their respective body weights. The plant extract did not produce a generalized adverse or toxic effect to the S-D rats; rather the toxicity was probably organ specific.

This study showed a reduction in the testicular weight. A reduction in the weight of the testes has been said to be direct and significant pointer of toxicity upon the exposure to certain toxic substances. Also, John[8] has reported that a reduction in testicular content could contribute to spermatogenic arrest and inhibit steroid biosynthesis of leydig cells. The alteration in sperm parameters could be attributed to direct effect on testicular tissue which leads to reproductive dysfunction such as reduced sperm count, motility and morphology. From this study, we noticed a reduction in the count and an increase in abnormal morphology. Sperm parameters are key indices of male fertility as they are the prime markers in testicular spermatogenensis and epididymal maturation. The sperm count and percentage of sperm mortality are the most commonly assessed sperm parameters used in infertility evaluation. However, the fertility of animals is still related to the morphological features of its spermatozoa.

In this study, the plasma concentrations of LH, FSH and testosterone of male rats by oral administration of *Blighia sapida* in various concentrations decreased and it may be as a result of *Blighia sapida* acting as a GnRH inhibitor or antagonist to promptly suppress pituitary gonadotropin hormones by binding to pituitary receptors (GnRH-receptor competition) leading to a decrease in LH and FSH secretions. It may also be as a result of a possible effect exerted by Ackee on the hypothalamus which leads to a decrease in gonadotropin-releasing hormone secretion thereby reducing the secretion of LH and FSH. Ackee plant has been reported to contain compounds like steroids[9]. This can cause GnRH release which normally acts on the gonadotrophs of the anterior pituitary via the binding and activation of a G protein receptor, which stimulates the anterior pituitary through inositol 1,4,5-triphosphate (IP3) activation (which increases intracellular calcium) to release FSH and LH[10]. The decrease in the level of testosterone may be as a result of a disruption in the Hypothalamic-Pituitary-Gonadal axis (HPG-axis) where GnRH, LH and FSH leads to low secretions of testosterone low.

Blighia sapida reduced SOD, CAT and GSH levels in regular patterns in the treatment group when compared with Control while MDA levels were increased also in regular pattern in the treatment group when compared with Control. This suggests that *Blighia sapida* might have induced oxidative stress on testicular tissues of the rats. Oxidative stress in the testes has been said to be one of the major factors inducing

germ cell apoptosis. It has been suggested that many of the clinical and pathological manifestations result from an imbalance between free radical defense system and free radical production. The increase in free radical production has increased the potential for free radical-mediated tissue damage. Although, it has been reported in few articles that leaf extracts of *Blighia sapida* acted as an antioxidant on organs like liver and kidney [11] but the bark extracts of this part has not proven a similar outcome on testis.

ETHICAL CONSIDERATION

The experimental procedure was carried out in conformity with the rules guiding animal handling and care in biomedical researches and ethical clearance was obtained from the Bowen university, Iwo, Osun State.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONCLUSION

In conclusion, this study suggested that *Blighia sapida* may have adverse effects on the secretion of LH, FSH and Testosterone. It also induced oxidative stress on the testis, with a negative effect on sperm count and morphology.

REFERENCES

1. Briceag, I., Costache, A., Purcarea, V, *et al*. Fallopian tubes-literature review of anatomy and etiology in female infertility. *J Med Life* (2015). 8(2): p129.
2. Kumar, N. and Singh, A. K. Trends of male factor infertility, an important cause of infertility: a review of literature. *J Hum Reprod Sci.* (2015). 8(4): p191
3. Masoumi, S. Z., Parsa, P., Darvish, N., Mokhtari, S., Yavangi, M. and Roshanaei, G. An epidemiologic surveyon the causes of infertility in patients referred to infertility center in Fatemieh Hospital in Hamadan. *Iran J Reprod Med.* (2015).13(8): p513
4. Adebajo, A., Gbotolorun, S. and Oremosu, A. The effect of chronic use of Dextromethorphan on fertility in male Sprague-Dawley rats: a pilot study. *Journal of Basic Medical Sciences* (2017).; 5(**10**)
5. Adekola, M. B., Areola, J.O., Omisore, N.O., Asaolu, F.T., Ogunleye, S.G., Apaolu, O.E. and Babalola, O.O. Sub-chronic toxicity study of ethanol stem-bark extract of Blighia sapida (Sapindaceae) in wistar rats (2020). *Elsevier*
6. Ekué, M.R. M., Sinsin, B., Eyog-Matig, O. & Finkeldey, R. Uses, traditional management, perception of variation and preferences in ackee (Blighia sapida K.D. Koenig) fruit traits in Benin: Implications for domestication and conservation. *Journal of Ethnobiology and Ethnomedicine*, (2010). 6(12), 1-14.
7. Dossou VM, Agbenorhevi JK, Combey S, Afi-Koryoe S. Ackee (Blighia sapida) fruit arils: Nutritional, phytochemicals and antioxidant properties. Int J Nutr Food Sci. 2014 N Dossou VM, Agbenorhevi JK, Combey S, Afi-Koryoe S. Ackee (Blighia sapida) fruit arils: Nutritional, phytochemicals and antioxidant properties. Int J Nutr Food Sci. 2014 Nov 10; 3:534-7.;3:534-7.
8. John Staughton. 7 wonderful benefits of ackee fruit. *Organic Facts* (2020).
9. Sinmisola A, Oluwasesan BM, Chukwuemeka AP. Blighia sapida KD Koenig: A review on its phytochemistry, pharmacological and nutritional properties. *Journal of ethnopharmacology*. 2019 May 10; 235:446-59.
10. Gurung P, Yetiskul E, Jialal I. Physiology, male reproductive system. *InStatPearls [Internet]* (2021) May 9. StatPearls Publishing.

11. Amira, Philip. O & Oloyede, Hussein O. B. Antioxidant Activity of Aqueous Extract of Blighia sapida Stem Bark in Alloxan-induced Diabetic Rats. *Global Journals Inc.* (2017). (USA), 17(2).