

Ethanollic Extract of *Blighia sapida* Improved Male Wistar Rats Reproductive Variables

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Abstract: The effect of ethanolic extract of *Blighia sapida* on male reproductive hormones, semen parameters, testicular morphometrics, serum biochemical indices and prostate specific antigen in male wistar rats was studied. Twenty-four male wistar rats (200-220 g, n = 6) were randomly selected into four study groups, A, B, C and D. Animals in group A (control) received 1 mL of distilled water (vehicle) and test groups B, C and D received (orally) graded doses of 100, 200 and 300 mg kg⁻¹ BW of ethanolic extract of *Blighia sapida* respectively for 21 days after which they were sacrificed by cervical dislocation. Blood samples were collected via cardiac puncture for determination of serum testosterone and luteinizing hormones, cholesterol, triglycerides, prostate specific antigen and glutamate pyruvate transaminase levels. The testis was isolated for its morphometry and the caudal epididymis separated for semen analysis. The testosterone level, testicular length and width, percentage of motile sperm, sperm with normal morphology and active sperm significantly (p<0.05) increased in group C compared with the other groups. The total sperm count, Serum glutamate transaminase, cholesterol and triglyceride levels increased in a dose dependent pattern in the treatment groups and Prostate specific antigen was least in group C. It can be concluded that *Blighia sapida* may have the potential of stimulating male reproduction. It may therefore be recommended that a dose of 200 mg kg⁻¹ BW of the extract be considered in the management of male reproductive dysfunction.

Key words: *Blighia sapida*, semen analysis, testicular morphometrics, prostate specific antigen, testosterone, luteinizing hormone

INTRODUCTION

In Africa, especially South of the Sahara, the use of plants and its extract for the treatment and management of diseases has been in existence since ancient times. A larger number of these tropical plants and their extract have shown beneficial therapeutic effects including fertility enhancing and contraceptive compounds, anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and aphrodisiac (Raji *et al.*, 2006).

Blighia sapida of the family Sapindaceae is one of them and it is both known for its nutritional values and poisonous properties (Morton, 1987). The unripe or inedible portion of the fruit contains the toxins hypoglycin A and B which is responsible for the substantial death in West Africa (Meda *et al.*, 1999) *B. sapida* is also a natural source of carboxycyclopropylglycine used in pharmacy (Ekue *et al.*, 2010).

In Nigeria, it is called Isin (Yoruba). It is an ever green tree with a dense crown. It is cultivated in India, West Indies and tropical America (Gledhill, 1972). *B. sapida* is useful in African traditional medicine. The bark pulp is

used as a liniment for oedema intercostal pains in Ivory Coast. The pulp and leafy types are used as eye drops in conjunctivitis (Irvin, 1961). The ashes of the dried husks and the seeds are used in the preparation of a type of soap (Irvin, 1965).

Various preparation and combination of the extract have been made for the treatment of diseases such as dysentery, epilepsy, yellow fever (Kean and Hare, 1980) and diabetics (Gbolade, 2009). The plant has been reported to be effective against cold and pain when applied. It is as well acaricidal and insecticidal (Mitchell and Ahmad, 2006). As reported by Ekue *et al.*, (2010), twenty-two diseases have been recognized to be healed with ackee. Dental decay, fever, malaria, internal haemorrhage, dysentery, burns, eyes inflammation, yellow fever, constipation, cutaneous infections, whitlow and head lice are the most common. All parts (bark, capsules, seeds, roots, leaves) are involved in the composition of drugs. The bark is useful in the treatment of 13 different diseases followed in decreasing order by leaves, capsules (IPGRI, 1999) and seeds (Ekue *et al.*, 2004).

Table 1: Nutritional composition of raw Akee aril

Contents	Quantity/100 g
Moisture	57.60 g
Fat	18.78 g
Carbohydrates	9.55 g
Protein	8.75 g
Fiber	3.45 g
Ash	1.87 g
Phosphorus	98 mg
Calcium	83 mg
Ascorbic acid	65 mg
Iron	5.52 mg
Niacin	3.74 mg
Riboflavin	0.18 mg
Thiamine	0.10 mg
Carotene	-

Morton (1987)

There is dearth of information on the medicinal use of its fruit aril which is edible when fully ripped which can also be cooked with other food (Moya, 2001). Table 1 shows the nutritional composition per 100 g of the raw arils (Morton, 1987).

Considering the enormous uses of this plant and nutritional values of the aril, it becomes imperative to investigate the implication of the administration of its aril on the reproductive system, this study aims at investigating the effect of *Blighia sapida* arils on reproductive hormones, semen parameters, testicular morphometry and some serum biochemical variables in adult male wistar rats.

MATERIALS AND METHODS

Plant materials: The whole fruits of *Blighia sapida* were harvested from Baptist theological seminary, Ogbomoso. *Blighia sapida* (Ackee) fruit was botanically identified and authenticated by Dr. A.T. Ogunkunle, a plant taxonomist in the department of Pure and Applied Biology, Faculty of Pure and Applied Sciences of LAUTECH Ogbomoso. The sample of the plant collected was stored in the herbarium with voucher number LHO 234.

Preparation of ethanolic extract of *Blighia sapida*: The arils (fleshly edible) were separated from the seeds; air dried for a month, blended using Phillip Electric blender made by Phillip Electric Plc, Japan. 746.2 g of the powdered *Blighia sapida* was well macerated with 2 L of ethanol at temperature of 35°C as solvent and left for 72 h. The juice was filtered with whartmanns' filter paper and the filtrate was then concentrated using rotary evaporator (RE 52-3) which was stored in a refrigerator at temperature of 4°C until use. Calculations were done to determine the dosage of the filtrate that will be administered:

$$\text{Volume} = \frac{\text{Weight of the animal} \times \text{Dosage}}{1000 \times \text{Stocks solution}}$$

Phytochemical screening: Phytochemical screening was carried out on the extracts and on the powdered specimens using standard procedures to identify the phytochemical constituents (Odebiyi and Sofowora, 1978; Trease and Evans, 1989). This test was carried out at the Laboratory, Department of Pharmacognosy, Faculty of Pharmacy and University of Ibadan, Nigeria.

Experimental animals: Twenty four adult male wistar rats, weighing between 200 and 220 g were purchased from the animal house of University of Ibadan, Nigeria. The animals were allowed to acclimatize to the laboratory condition (temperature 24-27°C and 12 h light-dark cycle) for 2 weeks before commencement of the experiment with free access to solid pellet rat diet and water *ad libitum* throughout the study. All animals received humane care in compliance with the institution's guideline and criteria for humane care as outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Experimental design: The animals were randomly allocated into four groups, each group consisting of six rats each. Group A served as the control and were given distilled water (vehicle for the extract). Groups B, C and D were given ethanolic extract of *Blighia sapida* at the doses of 100 mg kg⁻¹, 200 and 300 mg kg⁻¹ BW, respectively. The vehicle and extract were given orally for 21 days after which blood was collected by cardiac puncture into labeled sterilized plain bottles and allowed to clot at room temperature for 1 h and then centrifuged at 3500 g for 15 min. The supernatant (serum) was isolated and stored at -30°C until required for analysis after which the rats were euthanized to remove testes.

Testicular morphometry: The rats' scrotums were incised and the testes located and removed. The length and width measured using venier caliper, the weight of each testis was measured with electronic weighing scale and then recorded.

Semen kollection: The testes were removed along with the epididymides. The caudal epididymides were separated from the testes, blotted with filter papers and lacerated to collect the semen.

Semen analysis

Progressive sperm motility: This was done immediately after the semen collection. Semen was squeezed from the

caudal epididymis onto a pre-warmed microscope slide (27°C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using 400X magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labeled as either motile or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e., 100) (Mohammad-Reza *et al.*, 2005).

Sperm morphology: This was done by adding two drops of warm Walls and Eosin stain to the semen on a pre-warmed slide, a uniform smear was then made and air dried; the stained slide was immediately examined under the microscope using 40X magnification (Laing, 1979). Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa was expressed as a percentage of the total number of spermatozoa.

Sperm viability: Two drops of warm Eosin stain was added to the semen on a pre-warmed slide; a uniform smear was then made and dried with air the stained slide was immediately examined under microscope using 400X magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated (Laing, 1979).

Sperm count: Epididymal sperm was obtained by mincing the epididymis in normal saline and filtered through a nylon mesh (80 µm pore size). Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers (Freund and Carol, 1964).

Hormonal assay: The levels of hormones were measured in serum by ELISA testosterone and LH standard kits (Biocheck, Inc. Foster City CA, USA). The procedure described in the hormone assay kits was used according to the principle highlighted by Tietz (1995) for testosterone while the method of Uotila *et al.* (1981) was used for luteinizing hormones.

Assay for serum total cholesterol: The serum level of Total Cholesterol (TC) was quantified after enzymatic

hydrolysis and oxidation of the sample as described by method by Stein (1987). Total 1000 µL of the reagent was added to each of the sample and standard. This was incubated for 10 min at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 min at 546 nm. The value of TC present in serum was expressed in the unit of mg/dL.

Assay for serum triglyceride: The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of (Tietz, 1990). The 1000 µL of the reagent was added to each of the sample and standard. This was incubated for 10 min at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 min at 546 nm. The value of triglyceride present in the serum was expressed in the unit of mg/dL.

SGPT: Serum Glutamate Pyruvate Transaminase (SGPT) was measured spectrophotometrically by using the method of Reitman and Frankel (1957).

Prostate specific antigen: Serum samples were processed and refrigerated within 3 h of blood draw, the specimen was stored at 2-8°C (Woodrum *et al.*, 1996). Anti-PSA monoclonal antibodies are added to serum (or plasma) and incubated using standard methods and standard conditions (RCPI, 2006). Anti-PSA monoclonal antibodies bound to PSA are detected.

Statistical analysis: Results obtained were expressed as the Mean±SD means were analyzed using one way analysis of variance, followed by the Duncan Multiple Range test to determine significant differences between pairs. The $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of extracts of *Blighia sapida* shows the presence of some groups of phytochemicals such as saponins, reducing sugar, phytosterols and Polyphenol.

The results of the Effect of Ethanolic Extract of *Blighia Sapida* (EEBS) on some serum biochemical variables, testicular morphometric, semen analysis, testosterone level and luteinizing hormone level after 21 days of treatment are summarized in Table 2-4 as well as Fig. 1 and 2, respectively.

Table 2: Showing the effect of *Blighia sapida* on some serum biochemical variables

Parameters	Group A (0.1 mLs of DW)	Group B (100 mg kg ⁻¹ BW EEBS)	Group C (200 mg kg ⁻¹ BW EEBS)	Group D (300 mg kg ⁻¹ BW EEBS)
SGPT (IU L ⁻¹)	167.50±1.730 ^a	143.75±2.630 ^a	159.50±11.96 ^a	238.25±20.86 ^b
Cholesterol (mg dL ⁻¹)	30.00±1.630 ^a	31.25±2.630 ^a	33.50±1.73 ^a	52.73±2.50 ^b
Triglyceride (mg dL ⁻¹)	46.25±0.960 ^a	42.00±1.830 ^b	47.50±1.91 ^a	63.75±2.99 ^c
PSA (ng mL ⁻¹)	0.94±0.025 ^a	0.83±0.026 ^b	0.72±0.06 ^c	1.05±0.04 ^d

SGPT: Serum Glutamate Pyruvate Transaminase; DW: Distilled Water; EEBS: Ethanolic Extract of *Blighia Sapida*; BW: Body Weight; PSA: Prostate Specific Antigen; a-d Values with different super script are statistically significant at p<0.05

Table 3: Showing values of testicular morphometrics after the administration of EEBS

Parameters	Group A (0.1mLs of DW)	Group B (100mg kg ⁻¹ BW EEBS)	Group C (200 mg kg ⁻¹ BW EEBS)	Group D (300 mg kg ⁻¹ BW EEBS)
Weight (g)	1.82±0.02 ^a	1.84±0.04 ^a	1.88±0.03 ^a	1.77±0.01 ^b
Length (cm)	1.67±0.05 ^a	1.79±0.37 ^b	1.81±0.02 ^b	1.69±0.01 ^a
Width (cm)	0.71±0.01 ^a	0.74±0.01 ^b	0.83±0.01 ^c	0.66±0.22 ^d

DW: Distilled Water; BW: Body Weight; EEBS: Ethanolic Extract of *Blighia Sapida*; a-d: Values with different super script are statistically significant at p<0.05; Width (cm)

Table 4: Showing values of semen analysis after the administration of EEBS

Parameters	Group A (0.1mLs of DW)	Group B (100 mg/BW EEBS)	Group C (200 mg/BW EEBS)	Group D (300 mg/BW EEBS)
Sperm motility (%)				
Motile	55.00±3.66 ^a	58.75±2.18 ^a	65.00±2.10 ^b	60.50±1.50 ^a
Non-Motile	45.00±3.66 ^a	41.25±2.18 ^a	35.00±2.00 ^b	39.50±1.50 ^a
Sperm morphology (%)				
Normal	48.75±3.26 ^a	31.25±1.15 ^c	55.00±4.46 ^a	42.50±2.29 ^b
Abnormal	51.25±3.26 ^a	68.75±1.15 ^c	45.00±4.46 ^a	57.50±2.29 ^b
Sperm viability (%)				
Live	40.00±2.08 ^a	32.50±3.50 ^a	40.00±5.07 ^a	37.50±5.50 ^a
Dead	60.00±2.08 ^a	67.50±3.50 ^a	60.00±5.07 ^a	62.50±5.50 ^a
Sperm Count (×10 ⁶)	41.25±0.25 ^b	38.75±2.15 ^b	43.75±1.73 ^a	45.75±2.17 ^a

DW: Distilled Water; BW: Body Weight; EEBS: Ethanolic Extract of *Blighia Sapida*; a-d: Values with different super script are statistically significant at p<0.05

Serum Glutamate Pyruvate Transaminase (SGPT), cholesterol and triglyceride showed dose dependent increases with group D (300 mg kg⁻¹ BW EEBS) having a significantly (p<0.05) higher values compared to the control group. Also prostate specific antigen significantly (p<0.05) increased in group D (300 mg kg⁻¹ BW EEBS) compared with control group (Table 2). The testicular weight was not significantly different in groups; A (0 mg/kg BW EEBS), B (100 mg kg⁻¹ BW EEBS) and C (200 mg kg⁻¹ BW EEBS) but the testicular length and width were significantly (p<0.05) higher in group C (200 mg kg⁻¹ BW EEBS) compared with control group (Table 3). The percentage motile sperm and sperm with normal morphology were significantly (p<0.05) higher in group C (200 mg kg⁻¹ BW EEBS) compared with control group while there was no significant difference in sperm viability but sperm count showed a dose dependent increase with rats in group D (300 mg kg⁻¹ BW EEBS) having the highest sperm count compared with control group (Table 4).

Figure 1 showed that testosterone level was significantly (p<0.05) higher in group C (200 mg kg⁻¹ BW EEBS) compared to the control group. While Fig. 2 showed that the luteinizing hormone level was significantly (p<0.05) reduced in a dose dependent pattern.

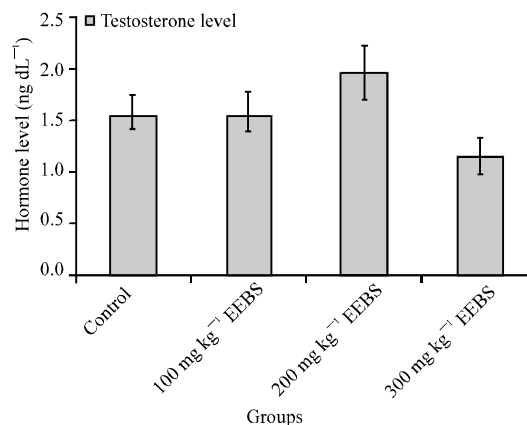


Fig. 1: Testosterone levels following administration of ethanolic extract of *Bilighia sapida*; Bars carrying letters are statistically different at p<0.05

The result of this study showed that Serum Glutamate Pyruvate Transaminase (SGPT), Cholesterol and Triglyceride showed a dose dependent increase, suggesting that increase in the dosage of EEBS increases its toxicity.

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases (Dhuley *et al.*, 1999). High serum levels of triglycerides and the LDLs

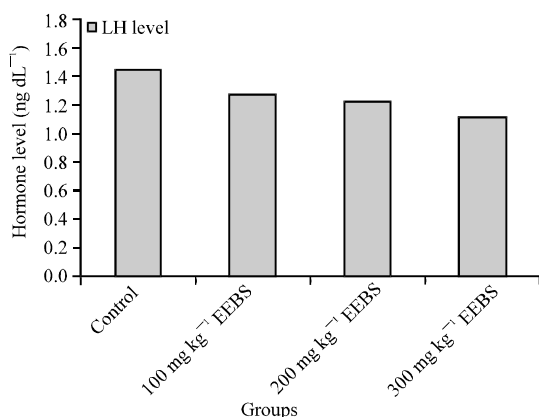


Fig. 2: Leutinizing hormone levels following administration of ethanolic extract of *Blighia sapida*, Bars carrying letters are statistically different at $p < 0.05$

are associated with coronary artery disease (Hornstra, 1988; Sundram *et al.*, 1995; Eisenhower *et al.*, 1998). The results of this study showed that EEBS at doses not $> 200 \text{ mg kg}^{-1} \text{ BW}$ has significant ($p < 0.05$) serum lipid lowering effect on the level of total cholesterol and triglyceride. The observed result may be attributed to the effect of saponins. Saponins are known antinutritional factors which reduce the uptake of certain nutrition especially cholesterol at the gut through intraluminal physiochemical interactions.

Hence, saponins have been reported to have hypocholesterolic effect (Price *et al.*, 1987). Presence of saponins has been reported in the plant extract (Antwi *et al.*, 2009) and this study also report the presence of saponin in the arils, this saponin may explain the antilipidemic effect observed in this study. Similar results were obtained on the aqueous and ethanolic extract of leaves of *Blighia sapida* in earlier study by Owolabi *et al.* (2010).

The increase in the enzyme SGPT indicates that the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby, causing leakage of the enzymes leading to the increased serum level of the enzyme (Dortman and Lawhorn, 1978). Therefore, the result of SGPT indicate that liver damage may occur at higher doses of the extract, also the dosage of EEBS at $200 \text{ mg kg}^{-1} \text{ BW}$ and below may not be harmful to the liver.

The result of the study showed that EEBS improved the testicular length and width significantly in group C ($200 \text{ mg kg}^{-1} \text{ BW}$ EEBS) compared with that of the other groups, similar result was shown by Al-Sa'aidi *et al.*

(2009) who reported that use of alcoholic extract of *Nigella sativa* resulted in an increase in the testes size. Another study has shown that a decrease or increase in the testes size was most likely attributed to the testosterone level (Simanainen *et al.*, 2008). This showed that the administration of the extract improved testicular morphometry at a dose of $200 \text{ mg kg}^{-1} \text{ BW}$.

The percentage motile sperm and sperm with normal morphology were significantly ($p < 0.05$) higher in group C ($200 \text{ mg kg}^{-1} \text{ BW}$ EEBS) compared to the control group, while sperm count showed a dose dependent increase with rats in group D ($300 \text{ mg kg}^{-1} \text{ BW}$ EEBS) having the highest sperm count compared to the control group.

Testosterone is specifically produced by Leydig cells containing enzyme 11β -hydroxysteroid dehydrogenase (Ge *et al.*, 2005). Following the plant extract treatment, there was a significant increase in the testosterone level at a dose of $200 \text{ mg kg}^{-1} \text{ BW}$ of the extract compared to that of the control. The fact that LH reduced is indicative of the negative feedback effect of testosterone on LH (McLachlan and Allan, 2003). Saponin one of the phytochemical in the extract has the potency to increase the levels of testosterone (Hadley, 2000). The increment in the sperm concentration was mainly due to the increase in testosterone levels in the testicular tissue. Testosterone is not only important in maturation of sperms but also is the main hormone responsible for spermatogenesis and spermatogenesis in seminiferous tubules (Sakamoto *et al.*, 2008). Similar report was made by Al-Sa'aidi *et al.* (2009) who reported that use of alcoholic extract of *N. Sativa* had resulted in an increase in spermatogonia cells (Al-Sa'aidi *et al.*, 2009).

Prostate Specific Antigen (PSA) is widely used as a disease biomarker for diagnosis and monitoring of Prostate Cancer (PCa) (Papsidero *et al.*, 1980). Serial serum PSA measurements are the most specific and reliable indicator to monitor response to therapy and to signal residual and recurrent disease (Laing *et al.*, 1979; Stamey *et al.*, 1987; Oesterling, 1991). Therefore, the fact that PSA decreases significantly at a dose of $200 \text{ mg kg}^{-1} \text{ BW}$ of EEBS suggests that *Blighia sapida* may have anticancer effects at this dosage. Phytochemicals such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to elicit antioxidant activities (Heber, 2004; Kaur and Kapoor, 2002). These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer (Steinmetz and Potter, 1991; Caragay, 1992).

CONCLUSION

It can be concluded that *Blighia sapida* may have the potential of stimulating male reproduction at a dose of 200 mg kg⁻¹ BW. It may therefore be recommended that a dose of 200 mg kg⁻¹ BW of the extract be considered in the management of male reproductive dysfunction.

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