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EFFECT OF ADMINISTRATION OF ETHANOL LEAF EXTRACT OF AFRICAN EGGPLANT AND BITTER LEAF ON TESTIS FUNCTION IN MALE WISTAR RATS

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ABSTRACT Herbal medicine is becoming increasingly popular in most African countries. Undoubtedly, the intake rates of diverse plants as herbs have some influence that is not immediately evident but should be researched. This led to our study which sought to evaluate the effects of African eggplant, bitter leaf, and its combination treatment on male reproductive function. Twenty (20) male Wistar rats grouped to 5 male Wistar rats per experimental group were used in this study. Each groups were treated as stated respectively: administration of 1000mg/100kg ethanol leaf extracts of African eggplant to test group B, 100mg/100kg ethanol extracts of bitter leaf to test group C, 1000mg/100kg each combination of ethanol leaf extracts of both African eggplant and bitter leaf to test group D and were monitored within for a period of 14 days while the control group received no treatment. According to the findings of our study, the combination of African eggplant and bitter leaf had no significant effect on the weight of the testis; each plant had a reduced sperm count, motility and disruption of morphology of the sperm when compared to the control group. Combination treatment revealed a significant increase in the level of GSH and MDA, decrease in SOD and CAT, when compared to the control group which disclosed minimal antioxidant level treatment effect on the oxidative cells of the testis. Our result showed that doses range should be of keen interest in other finding using combination treatment of both plants.

Key words: African eggplant, bitter leaf plant, male reproductive system, testis

INTRODUCTION

The subject of male reproductive system disorder must be included in the infertility discussion because it affects around 7% of all males. The male reproductive system is made up of well vascularized structures; internal structures such as the testes, epididymis, vas deferens, and prostate, as well as external structures such as the scrotum and penis. These structures have glands and ducts which promote sperm formation, storage, and its ejaculation for fertilization, more so, aid in the production of important androgens for male development (Tiwana and Leslie, 2021). Since few decades, numerous evidences in contribution to a global decline in human sperm quality (Sharma *et al*., 2013; Jurewicz *et al*., 2014; Sengupta *et al*., 2017) persist as male fertility can be influenced by a variety of factors such as environmental factor, occupational factors and lifestyle practices. This decline in sperm quality, a prominent fertility issues in male reproductive system disorders is typically caused by low testosterone levels or testosterone sensitivity, which results in low libido, failure to ejaculate, bone density loss, muscle loss, infertility, and hair loss (Lee and Tillman, 2016). Krausz, (2011), reported that etiology of impaired sperm production and function can be traced back to factors that act at the pre-testicular, post-testicular, or testicular level.

Till date, plants are natural sources been used for a variety of purposes in human, most notably as food for nutrition, medicine for disease treatment in both humans and animals (Langlois-Klassen *et al*., 2007). Herbal medications are becoming more popular in both developing and industrialized countries since they are culturally acceptable, readily available, economical, and not just natural but also safer than allopathic drugs (Mensah *et al*., 2019). Medicinal plants are a rich source of bioactive compounds, which vary within and between species (Ugboko *et al*., 2020; Dar *et al*., 2017) and have therapeutic potential as antimicrobial, antifungal, anti-inflammatory, anti-cancer, immunity-stimulating, detoxifying, and neuropharmacological agents (Ugboko *et al*., 2020; Dar *et al*., 2017).

Various medicinal plants have been claimed to improve fertility in Africa by earlier researchers. This study, on the other hand, looked at how to use

combination therapy to combat the negative effects of plant-derived bioactive chemicals. African eggplant scientifically known as *Solanum macrocarpon* is a vegetable crop grown for its edible leaves and fruits for consumption in non-arid regions (Bukenya-Ziraba and Bonsu, 2004). *Solanum macrocarpon* L, belongs to the Solanaceae family and the plant genus - *Solanum*. Various cultures described African eggplants with their own Nigerian languages such as; in Igbo, it's called "afufa" or "anara," in Hausa, "Dauta," and in Yoruba, "Igbo.". Eggplants have a wide range of nutritional value as rich in phytochemical compounds like saponins, phenols, flavonoids, and tannins, among others (Ibiam and Nwigwe, 2013) and thus, have medicinal properties make them valuable addition to diets and an important plant in herbal medicine. Eggplant fruit is used in the management, prevention, and treatment of a variety of diseases: it lowers blood cholesterol levels, regulates high blood pressure, helps people to lose weight, and has anti-haemorrhoidal and anti-glaucoma properties (Ossamulu *et al*,. 2014).

Bitter leaf plants scientifically known as *Vernonia amygdalina* belongs to the Asteraceae family and the genus *Vernonia*. In East Africa, particularly Tanzania, bitter leaf is known as Omjunso, Onugbo in Igbo-Eastern Nigeria, and Orugbo among the Itsekiri and Urhobo tribes in Nigeria, as well as Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), and Chusa-doki Shiwaka (Hausa) (Agbogidi and Akpomorine, 2013). Bioactive compounds, sesquiterpene lactones (e.g vernodalin, vernolepin, and vernomygdin) and steroid glycosides (vernoniosides) cause the bitterness; they contain a nutrient reserve as well as a variety of phytoactive compounds such as oxalate, phylate, tannins, saponins, flavonoid, cyanogenic glycosides, alkaloids, anthraquinone, steroid and phenol (Nimenibo-Uadia, 2003). Bitter leaf plant is beneficial in the treatment in lowering and maintaining diabetics' blood sugar levels (Udochukwu *et al*,. 2015).

Medicinal plants are used in both developing and developed countries as an alternative to allopathic medicine in the management, prevention, and treatment of varieties of diseases and sickness. But little or no emphasis on combination treatment in the use of African eggplant and bitter leaf was made of effects on male and female infertility cases. This study is designed to evaluate the consequence of administration of the ethanol leaf extracts of African eggplant and bitter leaf on blood glucose, body mass index, sperm analysis, antioxidant enzymes, histology, and weight of the testis.

MATERIAL AND METHODS

ANIMAL CARE

Twenty (20) adult apparently healthy male Wistar rats weighed between 160g - 200g were used for this experiment. The rats were bred in modified cage built of spacious plastic and wire guaze cages in the animal house of the Obafemi Awolowo College of Health science, Sagamu campus, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Acclimatization of the animals was for a period of two weeks and were maintained on Growers feed from Joyful Feed and Flour Mill Ltd, Nigeria. Food and water were provided ad libitum.

ANIMAL GROUPING

The animals were randomly assigned into groups of A, B, C, and D with five rats in each group.

Group A – normal (healthy) rats only, were on water (Control group)

Group B - 1000mg / kg rats of African eggplant ethanol leaves extract only

Group C - 1000mg / kg of bitter leaf plant ethanol leaves extract only

Group D - 1000mg / kg of bitter leaf plant and 1000mg / kg Africa eggplant ethanol leaves extract

PLANTS MATERIAL

Bitter leaf plant from a growing plant germination were collected from an indigenous farm in Ikenne/Sagamu area of South West Nigeria, while freshly harvested African eggplant were bought from a local market in Ikenne/Sagamu area of South West Nigeria. These samples were identified and authenticated at the Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Ogun state, Nigeria.

PREPARATION OF THE ETHANOL EXTRACT OF AFRICAN EGGPLANT AND BITTER LEAF PLANT

Leaves of African eggplant and bitter leaf plant were air dried and powdered with the use of a blender. Weighed powdered form of 150g was soaked in 750ml of ethanol (70% ethanol and 30% water) for 3days inside a refrigerator. The resultant liquid was filtered using a funnel plugged with glass wool. After filtration

the filtrate was heated at a temperature of 40°C for 5 min to allow ethanol to evaporate.

ADMINSTRATION OF PLANT EXTRACT

Ethanol extract of the leaves of African eggplant and bitter leaf plant (1000mg/kg) were administered orally to the rats using an oral cannula. Treatment was done in the morning every day before the animals were fed and the in the afternoon for the combination treatment for over a period of two weeks (14 days).

DETERMINATION OF PERCENTAGE YIELD OF AFRICAN EGGPLANT AND BITTER LEAF PLANT

The percentage of African eggplant extract was determined by calculating the percentage of the weight of the extract to the original weight before drying the sample, using the formula;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of African eggplant= 150g
Weight of dried shaft of African eggplant= 73.4g
Weight of extract = 150g — 73.4g= 76.6g

$$Percentage\ yield = \frac{76.6g}{150g} \times \frac{100}{1} = 51.0\%$$

The percentage yield for African eggplant is 51.0%

The percentage yield for bitter leaf plant was also calculated using the same formula stated above;

$$percentage\ yield = \frac{weight\ of\ extract}{weight\ of\ sample} \times \frac{100}{1}$$

Weight of bitter leaf plant – 150g
Weight of dried shaft of bitter leaf plant- 70.10g
Weight of extract = 150g—70.10g= 79.9g

$$percentage\ yield = \frac{79.9g}{150g} \times \frac{100}{1} = 53.3\%$$

The percentage yield for bitter leaf is 53.3g

MEASUREMENT OF FASTING BLOOD SUGAR

Fasting blood glucose was determined from a drop of blood from the tail using a glucometer (ACCU-CHECK, Roche, Germany), after an overnight fast of 14 hours every 48 hours.

DETERMINATION OF BODY MASS INDEX

BMI was calculated by dividing the rats' weight in gram by the square of their height in centimeters. The weights of the rats were determined by using analytical weighing scale (kerro BL20001), while the lengths were determined by using a meter rule for later analysis and to adjust the weights of administered extract according to the body weights. The weights and length of the rats were taken every 48 hours starting from the day of arrival till the end of the experiment. The value being given in unit of g/cm^2

DETERMINATION OF WEIGHT OF THE TESTIS AND SPERM ANALYSIS

The animals were sacrificed by cervical dislocation 24 hours after the end of administration of extract. The testis was excised following midline abdominal incision and carefully brought out. The excised testis was weighed by using a weighing scale (Kerro BL20001).

The tunica vaginalis was incised to expose the testicle, and the cauda epididymis was harvested. Each experimental group's cauda epididymis was removed and minced thoroughly in a specimen bottle containing normal saline for a few minutes to allow the sperm to become motile and swim out of the cauda epididymis (Saalu *et al*., 2008).

The semen was taken with a 1ml pipette, dropped on a clean slide, and covered with cover slips after 5-minute incubation at 37°C (with 5% CO_2). The slides were examined for sperm motility under light microscopy (Saalu *et al*., 2008), and the spermatozoa were counted under the light microscope using a Neubauer hemocytometer counting chamber.

SPERM MORPHOLOGY: This was carried out according to Saalu *et al*., (2013). A light microscope with a magnification of x400 was used to examine the sperm morphology. The caudal sperm were taken from the original motility dilution and diluted 1:20, in 10% neutral buffered formalin. In wet preparations using phase contrast optics, the spermatozoa were categorized according to Adelakun *et al*., (2018).

HISTOLOGICAL EXAMINATION

The testis tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 µm thick sections were prepared and stained with haematoxylin and eosin using standard procedures. The slides were viewed under light microscope and photomicrographs were taken (400×)

PROCEDURE FOR DETERMINATION OF ANTIOXIDANT ENZYMES

The testis tissues to be assessed for oxidative studies were homogenized in phosphate buffer in ratio four to one. Superoxide dismutase (SOD) activities, glutathione reductase (GSH) activities, catalase (CAT) activities and malondialdehyde (marker of lipid peroxidation (MDA) were determined. Superoxide

dismutase activities were determined according to the method of Valerino and McCormack (1971). Increased absorbance was monitored with a UV spectrophotometer at 480 nm every 60 seconds for 180 seconds. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during one minute. The activity of SOD was expressed as µg/mg protein. Reduced glutathaione was determined using the method the methods of Sedlak and Lindsay (1968), Jollow *et al*., (1974). The absorbance of the yellow color formed upon the addition of Ellman's reagent was read within 5 minutes at 412 nm with a UV spectrophotometer. A plot of absorbance versus concentration of reduced GSH was then obtained from a serial dilution of the stock GSH prepared by adding 1.5 mL of phosphate buffer and 1.5mL of Ellman's reagent. The amount of GSH was expressed as µg/mg protein. Catalase activity was determined with the method described by Shina (1972). Proper dilution of the serum samples was done at ratio one to ten dilution in series. Catalase was expressed as mmoles of H_2O_2 consumed per minute per mg protein. It used the principle that dichromate in acetic acid as an unstable intermediate. The chromic acetate produced was measured colorimetrically at 570 nm.

Malondialdehyde (MDA) was determined spectrophotometrically from the pink color product of thiobarbituric acid (TBA) reactive substances complex. 0.1mL of the test sample was mixed with 0.5mL of 10% TCA and 0.5mL of 75% TBA was added to it. The mixture was then placed in a water bath at 80oC for 45 minutes. The resulting pink solution's absorbance was measured against a reference blank of distilled water at 532 nm. The test sample was calibrated using the MDA as a standard and the result was expressed as the amount of free MDA produced. The MDA level was calculated according to Adam-Vizi and Sergi, (1982) The Lipid peroxidation was expressed as µg/mg protein.

STATISTICAL ANALYSIS

The statistical analysis of data collected from twenty (20) male Wistar rats was done using the SPSS-25.0 statistical software package for analysis of data. The data was presented as Mean± Standard Error of Mean (SEM) and statistical analysis was carried out using the student t-test and analysis of variance (ANOVA). Values considered to be statistically significant were expressed using $p<0.05$.

RESULTS AND DISCUSSION

Administration of Ethanol Leaf Extract of African Eggplant and Bitter Leaf on Some Sperm Parameters, Fasting Blood Sugar, and BMI in Male Wistar Rats

Table 1 shows the results of the administration of ethanol leaf extract of African eggplant and bitter leaf plant on sperm parameters, fasting blood sugar and body mass index.

Testis Weight: Observation from the weight of the testis indicates an increase that was not significant ($p>0.05$) in test group B (1.40± 0.47) and group C (1.40±0.25) when compared to group A (1.36 ±0.27)g, there was no significant difference in test group D (1.36±0.25) when compared to group A(1.36 ±0.27), and group B (1.40± 0.47) when compared to group C (1.40±0.25). Test group D (1.36±0.25) showed a decrease that was not significant ($p>0.05$) when compared to group B (1.40± 0.47) and group C (1.40±0.25).

Tijani *et al.,* (2014), reported that the weight of testis is correlated with fertility. Small or larger than average testis causes less sperm density, and this causes infertility. Evidenced in the study was an increase that was not significant in the weight of testis in the groups of rats treated with *Solanum macrocarpon* and *Vernonia amygdalina*. However, the group that received a combination of both plants' treatments showed not only no significance difference, but weight of testis slightly deviated from the control weight value with minimal of 0.01kg difference. These findings suggest that combined treatment at 1000mg/kg ethanol leaves extract of *Solanum macrocarpon* and *Vernonia amygdalina* has no effect on the weight of testis in Wistar rats.

Sperm Count: Observations from table 1, also showed that there was decrease which was significant ($p<0.05$) in the sperm count in test group B (21.75±1.22), group C (16.40±1.14), and group D (6.00±0.71) when compared with group A (30.00±1.58). Also, there was a significant decrease ($p<0.05$) in group C (16.40±1.14) when compared to group B (21.75±1.22), and test group D (6.00±0.71) when compared to group B (21.75±1.22) and group C (16.40±1.14). The morphology of sperm was normal in all the groups, so also their motility.

Fertility is related to basic sperm parameters i.e. count, motility and morphology of sperm (Naina *et al.,* 2015). Results of the concluded experiments showed sperm count decrease that were significant ($p<0.05$) on administered *Solanum macrocarpon, Vernonia amygdalina* and a combined treatment. Results from this study suggest that *Solanum macrocarpon* caused decrease in sperm count corresponding with the findings of Ezechukwu *et al.,* (2020). The results also

showed decrease in sperm count caused by *Vernonia amygdalina* aligned with the results from a study by Saalu *et al.*, (2013) who found that groups treated with 150g of leaf extract of bitter leaf had decrease in sperm count. Their research however showed an increase in sperm count of rat groups treated with low doses of leaf extract of *Vernonia amygdalina.* This suggests that low doses of leaf extract of bitter leaf could improve sperm count while high doses cause decrease in sperm count. The sperm motility and morphology remained motile and normal respectively in all groups of rats.

Fasting Blood Sugar Level: Table 1, also shows that there was an insignificant decrease ($p>0.05$) in the percentage change of blood sugar in test group B (-7.7±8.44) and group C (8.66±2.43) when compared with group A (-3.27± 13.03), Test group D (-51.88±0.90) showed a significant decrease ($p<0.05$) in percentage change of blood sugar when compared to test group A (-3.27± 13.03), B (-7.7±8.44), and C (8.66±2.43).

Groups treated with bitter leaf and African eggplant showed a decrease that was not significant ($p>0.05$) in the percentage change in blood sugar level when compared to the control group. The group that received combination treatment showed a decrease that was significant ($p<0.05$) in the percentage change in blood sugar level. This showed that African eggplant and bitter leaf especially in combination have anti-hyperglycemic effect. The anti-diabetic effect of African eggplant corresponds with the findings of a study done by Onuora and Okafor, (2016) and the anti-diabetic effect of bitter leaf corresponds with the findings of Nwaoguikpe (2010). Evident in past research, found that type I or type II diabetes could have detrimental effects on male fertility (Ding *et al.*, 2015). Hence, antidiabetic effect of African eggplant and bitter leaf, can be used especially via combination therapy to manage the impairments of male reproductive functioning that comes with diabetes.

Body Mass Index (BMI) Level: Observation from table 1, also shows the results of the administration of the ethanol leaf extract of African eggplant and bitter leaf plant on body mass index recorded for over a period of 14 days. It was observed that test group B (-3.34±6.09), C (1.02±1.40) and D (-3.98±1.51) showed a significant decrease ($p<0.05$) in the percentage change of BMI when compared to test group A (6.61±0.49). Test group D (-3.98±1.51) showed an insignificant decrease ($p>0.05$) in the percentage change of BMI when compared to test group B (-3.34±6.09), Test group C showed an insignificant increase ($p>0.05$) in percentage change of BMI when compared to group B (-3.34±6.09).

Groups treated with African eggplant, bitter leaf and a combination of both plants showed a decrease that was significant ($p<0.05$) in the percentage change of Body Mass Index. These findings suggest that bitter leaf, African eggplant, and a combination of both as herbs especially could effectively reduce body mass index and be used to manage obesity. The ability of African eggplant to reduce body weight is supported by a study carried out by Emiloju and Chinedu, (2016) while the ability of bitter leaf to reduce body weight corresponds with the results of a research carried out by Atangwho *et al.*, (2012). Evidence suggests that male obesity affects male reproductive potential negatively (Palmer *et al.*, 2012). These findings suggest that African eggplant and bitter leaf can be used, especially in combination to treat obesity therefore, subsequently, treating the male reproductive complications.

Table 1, Effect of administration of Ethanol leaf extract of African Eggplant and Bitter leaf on some Sperm parameters, Fasting Blood Sugar, and BMI in Wistar Rats

Grps	Treatment	Weight Of Testis	Sperm Count(10^6)	Sperm Morphology	Sperm Motility	Body mass index(%Change)	Fasting blood sugar(%Change)
A	Control	1.36 ±0.27	30.00 ±1.58	Normal	Motile	-3.27 ± 13.03	6.61 ±0.49
B	100mg / kg of African eggplant	1.40 ±0.47	21.75 ±1.22[a]	Normal	Motile	-7.70 ±8.44	-3.34 ±6.09[a]
C	100mg / kg of Bitterleaf plant	1.40 ±0.25	16.40 ±1.14[a]	Normal	Motile	8.66 ±2.43	1.02 ±1.40[a]
D	100mg / kg of bitter leaf plant and 100mg / kg Africa eggplant extract	1.36 ±0.25	6.00 ±0.71[a]	Normal	Motile	-51.88 ±0.90[a]	-3.98 ±1.51[a]

Each value is an expression of mean ± SEM. (P <0.05)

[a]*showed significance when compared with group A*

Administration of Ethanol Leaf Extract of African Eggplant and Bitter Leaf on Antioxidant enzymes activity in the testis of Wistar rats

Table 2 shows the results of the administration of ethanol leaf extract of African eggplant and bitter leaf plant on the antioxidant enzymes activities in the testis of Wistar rats. Gawel *et al.,* (2004), reported that the major antioxidant enzymes in the testis are Super oxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH), Malondialdhye (MDA) is also a marker of oxidative stress.

Glutathione: Observation from results comparison showed that there was a decrease that was not significant ($p>0.05$) in the level of Glutathione in group B (16.43±0.86) and group C (16.04±1.92) when compared to group A (17.97±6.88). There was an increase that was significant ($p<0.05$) in the level of gluthathione in group D (44.63±0.88) when compared to the group A (17.97±6.88), group B (16.43±0.86) and group C (16.04±1.92).

The amount of GSH in the group treated with African eggplant and the group treated with bitter leaf both decreased, but not significantly. The group that received both herbs experienced a substantial increase ($p< 0.05$). Low intracellular GSH levels reduce cellular antioxidant capability, whereas high GSH levels boost antioxidant capacity and oxidative stress resistance (Ballatori *et al*., 2009). This study implies that using African eggplant and bitter leaf to treat testicular cells could lower GSH levels, rendering them more vulnerable to oxidative stress. Treatment with both herbs in combination therapy, on the other hand, raises the level of GSH in testicular cells, making them more resistant to oxidative stress.

Super oxide dismutase (SOD): Also in the levels (SOD), there was a decrease that was significant ($p<0.05$) in the level of SOD in test group B (2.92±0.35), group C (10.41±0.08), and group D (4.04±0.84) when compared to group A (17.21±2.76). Test group D (4.04±0.84) when compared to group C (10.41±0.08), and test group D (4.04±0.84) when compared to group B (2.92±0.35).

Catalase: There was a decrease that was significant ($p<0.05$) in the level of Catalase in test group B (12.09±0.96), group C (11.46±1.56), and group D (11.40±0.82) compared to the control group A (15.82±1.52). There was a insignificant decrease in the level of Catalase in test group C (11.46±1.56) and group D (11.40±0.82) when compared to group B (12.09±0.96), and test group D (11.40±0.82) when compared to group C (11.46±1.56).

All treatments produced a decrease that was significant in the levels of SOD and CAT. SOD and CAT are enzymes that protect cells from free radicals' attack (Matsumoto *et al.,* 1991). If free radicals overwhelm the body's ability to regulate them, oxidative stress occurs (Lobo *et al.,* 2010). The findings of this study suggest that treatment with African eggplant and bitter leaf and their combination especially, could cause oxidative stress due to their reduction of SOD and CAT.

Malondialdhye (MDA): The level of malondialdhye in test group B (1.42±0.33) showed there was a decrease that was significant ($p<0.05$) when compared to the group A (2.77±1.29). There was an increase that was significant ($p<0.05$) in malondialdhye level in group C (4.03±0.22) and group D (4.44±0.72) when compared to group A (2.77±1.29), test group C (4.03±0.22) and group D (4.44±0.72) when compared to group B (1.42±0.33). There was an increase that was not significant in Malondialdhye level in test group D (4.44±0.72) when compared to group C (4.03±0.22).

The group treated with bitter leaf showed a decrease that was significant ($p<0.05$) in the level of MDA. The groups treated with African eggplant and the combination of both herbs however, showed an increase that was significant ($p<0.05$) in the level of MDA. MDA is toxic at higher levels and high level of MDA indicates the occurrence of oxidative stress and lipid peroxidation (Ayman *et al.,* 2006). The findings of this study suggest that treatment with African eggplant, bitter leaf and especially a combination of them could lead to oxidative stress and lipid peroxidation.

Table 2: Effect of the administration of ethanol leaf extract of African eggplant and Bitter leaf on antioxidant activities in the testis of wistar rats

Grps	Treatment	Glutathione (µmol/ml)	Super oxide dismutase (µmol/ml/min /mg pro)	Catalase (µmol/ml/min/mg pro)	Malondialdhye (µmol/ml)
A	Control	17.97±6.88	17.21±2.76	15.82±1.52	2.77±1.29
B	100mg/100g of African eggplant	16.43±0.86	12.92±0.35[a]	12.09±0.96 [a]	1.42±0.33 [a]
C	100mg/100g of Bitterleaf plant	16.04±1.92	10.41±0.08 [a]	11.46±1.56 [a]	4.03±0.22[a,b]
D	100mg/100g of bitter leaf and African eggplant	44.63±0.88[a,b,c]	4.04±0.84 [a]	11.40±0.82 [a]	4.44±0.72 [a,b]

Each value is an expression of mean ± SEM. (P<0.05)
[a]-Values were significant when compared to A
[b]-Values were significant when compared to B
[c]-Values were significant when compared to C

Histological study of the testis of male wistar rats during the oral administration of Ethanol Leaf Extract of African Eggplant and Bitter Leaf

Figure 1 shows the effect of oral administration of ethanol leaf extract of African eggplant and bitter leaf on the histology of testis in male Wistar rats. Observation from results showed that the control group A; testicular tissue showing normal and well differentiated spermatogonia cells (black circle), leydig cells at the interstitial layer (black thin arrow), sertoli cells (red thin arrow) and the lumen (L) housing lots of late spermatids.

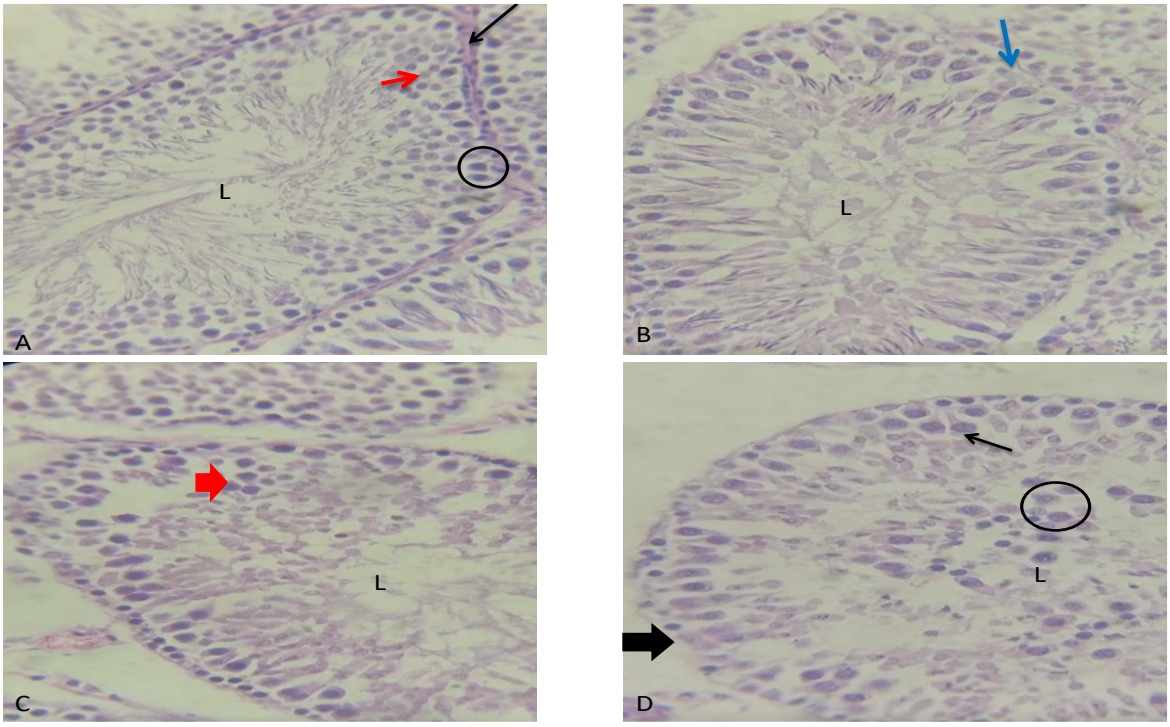
Group B treated with 1000mg/ kg of the ethanol extract of African eggplant showed little degenerative changes at the interstitial layer (blue arrow), well differentiated lumen(L), and loss of function.

Group C treated with 1000mg/ kg of the ethanol extract of bitter leaf showed little degenerative changes at the interstitial layer with loss of leydig cells, loss of spermatogonia cells and sertoli cells (red thick arrow) with dilated lumen(L)

Group D which was treated with a combination of 1000mg/ kg of the ethanol extract of African eggplant and bitter leaf showed severe degeneration and loss of morphological appearance of the spermatogonia cells(black thin arrow), infiltration of cells into the lumen(black circle), degenerative interstitial layer with loss of leydig cells(black thick arrow).

For normal spermatogenesis to occur, the integrity of the histology of testis is necessary (Layla *et al*., 2011; Weinbauer *et al*, 2010). The group treated with African eggplant showed little degenerative changes and loss of function. The group treated with bitter leaf also showed degenerative changes and loss of important testicular cells. The group treated with a combination of both bitter leaf and African eggplant showed severe degeneration, loss of morphological appearance and loss of leydig cells. The findings of this study suggest that African eggplant, bitter leaf and particularly their combination, compromise the histo-morphological structure of the testis, thereby hindering normal spermatogenesis.

Figure 1: Effect of oral administration of ethanol leaf extract of African eggplant and bitter leaf on histology of testis in male Wistar rats.



Photomicrograph of testicular tissue – Testes Function

CONCLUSION

This study shows that African eggplant, bitter leaf and their combination showed a drastic reduction in sperm count, when African eggplants and bitter leaf treatment has effect on the weight of testis, however, no changes was seen in the combination treatment using both plants as herbs. African Eggplants, bitter leaf and its combination treatment as herbs acts as anti-glycemic agent for treatment of body glucose level as well as obesity. Distinct use of African eggplant or bitter leaf only is not advisable for use in the treatment of testicular disorders/functions as increase oxidative stress in testicular cells, caused degenerative changes in the histology of testis. More importantly, its combination therapy increased all antioxidant level which could be more effective in treatment of testis and ultimately, testis function. Previous studies have reported many health benefits associated with the leaf's consumption; therefore, further study should be carried out to determine the safer doses.

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