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Effect of Aqueous Extract of *Abelmoschus Esculentus* on the Microsturcture of the Epididymis and Body Weight of Male Adult Wistar Rats.

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Abstract
Background :*This study is on the effect of aqueous extract of Abelmoschusesculentus on the epididymis of male adult Wister rats on the body weight.* ***Materials and Methods*** *:Twenty one adult male wistar rats weighing 100-120g were assigned into three groups (A, B and C). Group A for control received standard grower feed and water, Group B for low dose received food, water and aqueous of Abelmoschusesculentus at a dose of 1.0mg/KgBw and Group C for high dose received food, water and aqueous extract of Abelmoschusesculentus at a dose of 3.0mg/kgBw. Administration was done orally for two weeks. At the end of the administration, the body weight was recorded using sensitive weighing balance and the animals were sacrificed using cervical dislocation and the epididymis was harvested and preserved in 10% buffered formalin (NBF) followed by H&E staining. Result shows an observable significant (p<0.05) increase in the final mean body weight when compared with the initial body weight observable in control vs low dose, control vs high dose and low dose vs control but not observable in low dose vs high does and high dose vs control dose.* ***Results*** *:Histological observation shows the result of the study in control group of animals which received only standard growers feed and water revealed numerous tubules (T) and connective tissue layer (CT) that appears normal. However, animals in the low dose group received food, water and 1.0mg/kgBW of Abelmoschusesculentus shows marked tubule and connective tissue depletion and the animals in the high dose group which received food, water and 3.0mg/kgBW of Abelmoschusesculentus shows numerous tubules (T) and interstitial connective tissue (CT) appearing normal. No pathology seen.* ***Conclusion*** *:Abelmoschusesculentus showed a dose dependent distortion of the normal cytoarchitecture of the organ. This result is positive evidence that A. esculentus might be used to improve epididymal sperm vitality, mortility and morphology and increase in body weight especially at lower dose of 1.0mg/kgBW. This study also revealed that continuous administration of Abelmoschusesculentus for a long period of time without proper or recommended dosage may affect proper sperm storage in males.*

Keywords: Epididymis, Abelmoschusesculentus, wistar rats, tubule, tissue, weight

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I. Introduction

Okra (*Abelmoschusesculentus*) is a vegetable crop that belongs to Malvaceae family and is the only essential member (vegetable crop) in the family. The crop is widely distributed globally but grown in some countries especially in Africa, Asian and southern Europe [1]. Okra is also known as okro, ochro, lady's finger, bamyah, gumbo, and bhindi. It is a greenish capsule of about 7-18 cm long, slightly curved and contains many seeds [2]. For about three centuries, okra has been used for many reasons. Nutritionally, it is an important plant (vegetable) containing carbohydrates, proteins, vitamins, oils, and biologically essential elements [3]. Medicinally, it is used for remedy of many diseases such as diabetes, hyperlipidemia , asthma, ulcer, depression, cancer and renal function improvement [4]. Furthermore, it is used as antioxidant, antimicrobial, anti-inflammatory, antiviral, reduces the risk of Alzheimer's disease and other neurodegenerative diseases due to oxidative stress [5].

The seeds of okra were reported to contain a toxic compound called gossypol or a gossypol-like compound which stimulates infertility [6]. Daily consumption of gossypol can stimulate infertility in many animals including men by irreversibly blocking spermatogenesis [7]. Although several studies reported that the compound has no other side effect, as such it is used as a contraceptive for men [8] recommended high

consumption of okra (by women) during pregnancy because it promotes healthy pregnancy as well as reduces the rate of birth defects. In addition, okra was reported to be essential for the fetus' brain growth and development as well as neural tube formation.

II. Materials And Method

Study Design : Fresh pods of *Abelmoschusesculentus*(okra) were gotten from the local market of okuku in Yala Local Government Area of Cross River State, Nigeria. The okra pods were washed to clean up debris, sliced and dried in room temperature of about 27ºC. It was blended and kept in an air-tight container from where it was used for extraction. The okra powder was dispensed in a 10000mg of distilled water in a plastic container. The mixture was vigorously stirred intermittently with a stick and allowed to stand for 24hours before it was filtered with a cloth-sieve. The filt was evaporated at 50ºC with water both to obtain the crude solid extract for one month and the extract obtained was stored in a refrigerator until the commencement of the administration.

Twenty-one (21) adult male wistar rats were purchased from the animal house of the Department of Human Anatomy, University of Cross River State (UNICROSS) Okuku Campus and were used for this study. The animal were distributed into three (3) groups, seven (7) animal for each group.

The animal were housed in plastic cages under controlled light schedule (12 hours light and 12 hours dark cycle) and were fed with standard growers food and water before the commencement of administration. They were weighed prior to the experiment.

At the end of the two (2) weeks period, animals in all groups were sacrificed a day after the end of the administration by cervical dislocation. The epididymis of these animal was removed, preserved in 10% formal saline and evaluated to ascertain the effect of the extract administered

Study location: Department of Human Anatomy, University of Cross River State (UNICROSS) Okuku Campus, Cross River State, Nigeria.

Study Duration:15thJuly 2020 –30th July, 2020

Sample size: 21 (twenty one) adult male wistar rats.

Sample size calculation: Twenty-one (21) adult male rats were grouped into three (3) groups of control, low and high dose according to their weight respectively.Group A (control) animal received water and food only.Group B (low dose) animal received water, food and aqueous extract of *Abelmoschuseesculentus* at a dose of 1.0mg/kgBW. Group C (High dose) animal received food, water and aqueous extract of *Abelmoschusesculentus* at a dose of 3.0mg/kgBW.

Procedure methodology:

*Abelmoschuseesculentus*was administeredorally with the use of gastric tube from day one to dayfourteen to wistar rats in groups B (1.0mg/kgBW) and C (3.0mg/kgBW) while animals in group A received feed asnd water only. Prior to commencement of administration, the initial body weight of animal were taken with a sensitive weighing balance and was also taken at the 14th day which was the end of administrative procedure.

Group A – Food and water

Group B – 1.0mg/kgBW

Group C – 3.0mg/kgBW

Tissue blocks were sectioned 5µ-6µ with a rotator microtome then was dewaxed in xylene for two (2) minutes per two changes. Xylene was cleared in 95% alcohol for one minute per two changes and then 70% alcohol for another minute. The sections were then hydrated in running tap water until sections turned blue.

There were thereafter counterstained with 1% alcohol-eosin for one minute, followed by rapid dehydration through ascending grades of alcohol cleared in xylene and mounted in DPX mounting. Stain were viewed under a light microscope and photographed.All the animals were weighed using a sensitive weighing balance before the commencement and at the end of the administration of *Abelmoschusesculentus* to ascertain for any variations in body weight of animals across groups.

The epididymis was removed and preserved in a container with 10% neutral buffer formalin. There were done for 72 hours to achieve good tissue penetration and effective fixation. After this, they were placed in ascending grade of ethanol for dehydration. First they were treated with two changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute alcohol for the same duration. Following dehydration, the tissues were cleared in three changes of xylene each lasting for fifteen minutes. Then impregnation in molten paraffin wax at 58°C was carried out overnight and following morning, the tissues were embedded in wax to form blocks. These tissues block was trimmed and sectioned at 3 to 5µm thickness using a microtome. The sections were floated in warm water (28°C) and then taken up on aluminized glass slide. They were air-dried and stained using the hematoxylin and eosin staining methods [9].

Statistical analysis

Statistical analysis was done using Statistical Package for Social Science (SPSS) version 23 Chicago Inc. One way ANOVA, followed by bonifferoni's multiple Data Comparison Test was used for collation and analysis of data generated from this study. Result of descriptive statistics of the experimental data was presented as mean standard error of the mean (mean+SEM). Paired simple T-Test were considered statistically significant at $P<0.05$.

III. Results

Body weight observations

Morphological observation from the study shows an observable significant ($p<0.05$) increase in the final mean body weight when compared with the initial body weight observable in control vs low dose, control vs high dose and low dose vs control but not observable in low dose vs high does and high dose vs control dose. The final body weight of the control animals (133.9±7.058) was significantly ($p<0.05$) higher than its initial body weight (111.4±8.162). However, the mean final body weight of the low dose group (142.3±4.716) and high dose group (145.7±4.786) were significantly ($p<0.05$) higher than their initial body weights (128.0±6.856) and (130.3±6.157) respectively.

Table no 1 :Initial and final body weight of animals in various experimental groups

BODY WEIGHT		
GROUPS	**INITIAL**	**FINAL**
CONTROL	111.4 ± 8.162	133.9 ± 7.058
LOW DOSE	128.0 ± 6.856	142.3 ± 4.716
HIGH DOSE	130.3 ± 6.157	145.7 ± 4.786

Values are presented as Mean± SEM

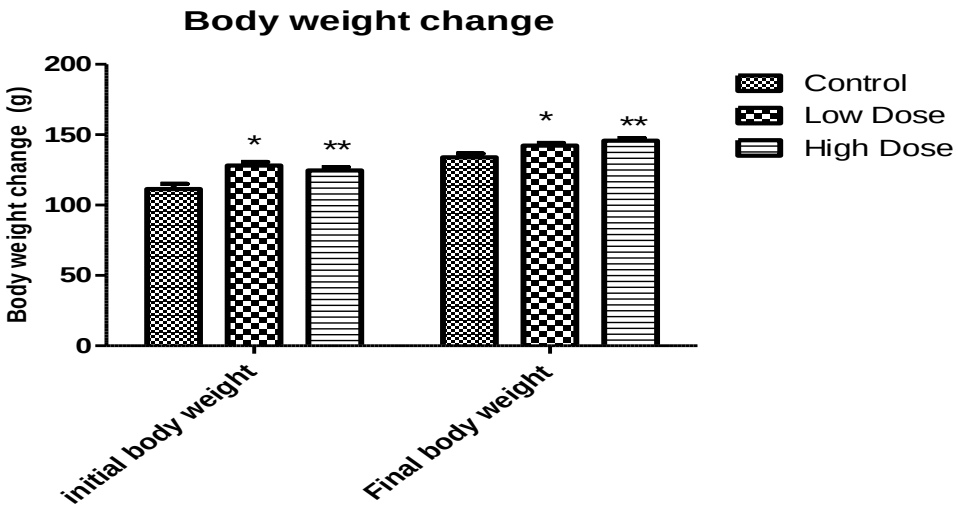

Figure no 1. showing the effect of daily administration of *Abelmoshusesculentus.* Values are expressed in mean +SEM, n=7, P,0.05. **P<0.05 vs control

Histological observation of the epididymis

Histological observation shows the result of the study in control group of animals which received only standard growers feed and water revealed numerous tubules (T) and connective tissue layer (CT) that appears normal. However, animals in the low dose group received food, water and 1.0mg/kgBW of *Abelmoschusesculentus* shows marked tubule and connective tissue depletion and the animals in the high dose group which received food, water and 3.0mg/kgBW of *Abelmoschusesculentus* shows numerous tubules (T) and interstitial connective tissue (CT) appearing normal. No pathology was seen.

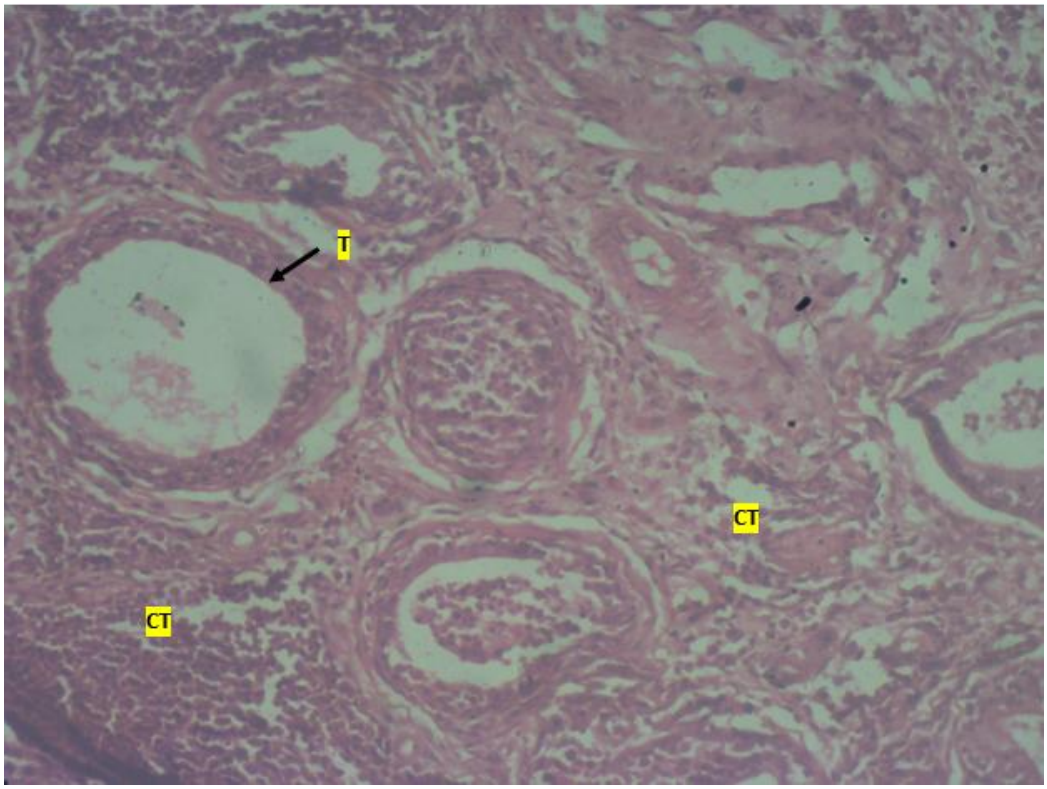

Plate 1. Control showing numerous tubules (t) and connective tissue layer (ct) that appears normal. H & E. X100

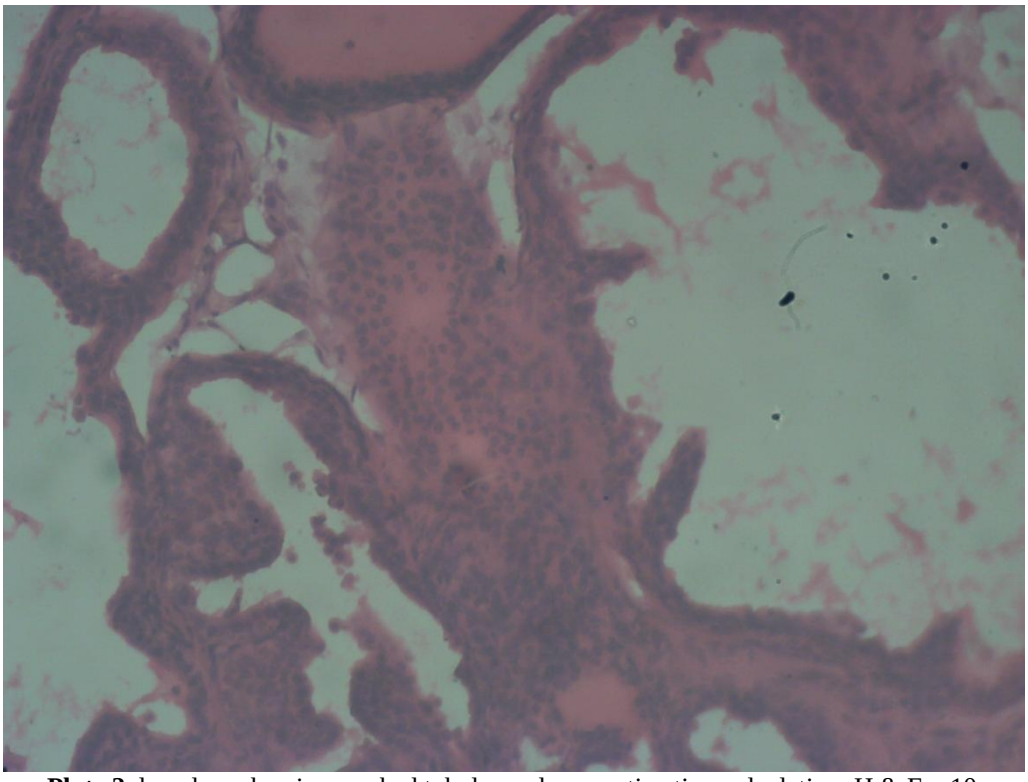

Plate 2. low dose showing marked tubular and connective tissue depletion. H & E. x10

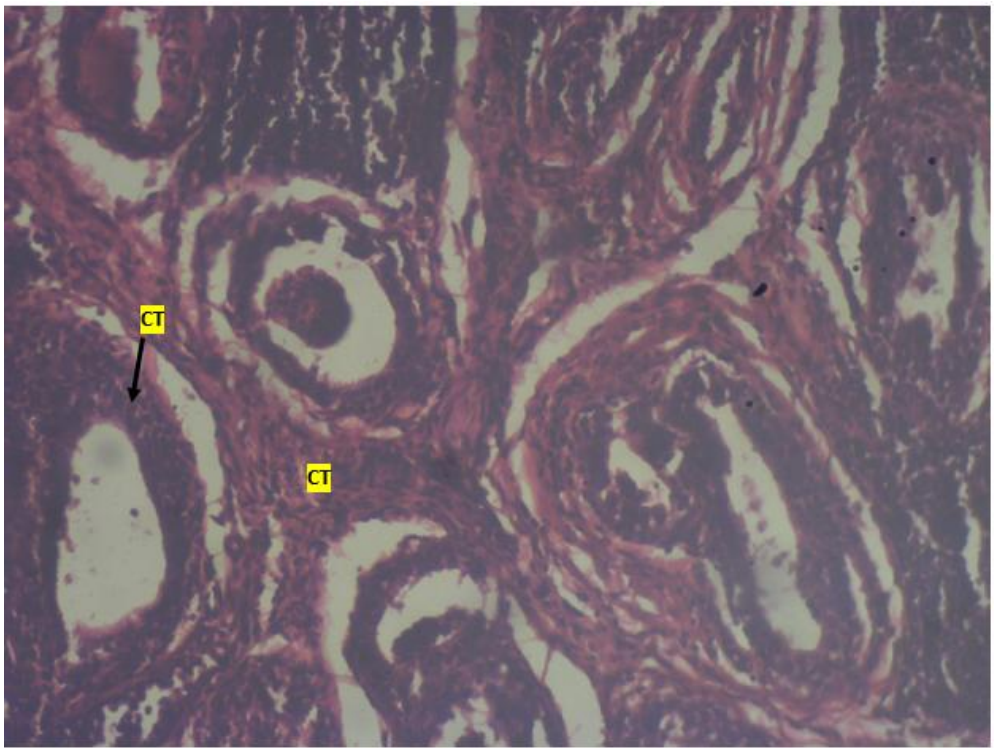

Plate 3. High dose showing numerous tubules (t) and interstitial connective tissue (ct) appearing normal. no pathology seen. H & E. x10

IV. Discussion

Since ancient times, plants have been used in medicine and are still used today [10]. The use of these plants has been gradually refined over the generations, and this has become known in many contexts as traditional medicine. The therapeutic properties of plants gave rise to medicinal drugs made from certain plants with these benefits [11]

The epididymis is a tube that connects the testicle to the vas deferens in the male reproductive system. It is present in all male reptiles, birds, and mammals [12]. Spermatozoa formed in the testis enter the caput epididymis, progress to the corpus, and finally reach the caudal region, where they are stored. Sperm entering the caput epididymis are incomplete; they lack the ability to swim forward (motility) and to fertilize an egg. It stores the sperm for 2–3 months. During their transit in the epididymis, sperm undergo maturation processes necessary for them to acquire these functions.

Morphological observation from the study shows an observable significant ($p<0.05$) increase in the final mean body weight when compared with the initial body weight observable in control vs low dose, control vs high dose and low dose vs control but not observable in low dose vs high does and high dose vs control dose. The final body weight of the control animals (133.9±7.058) was significantly ($p<0.05$) higher than its initial body weight (111.4±8.162). However, the mean final body weight of the low dose group (142.3±4.716) and high dose group (145.7±4.786) were significantly ($p<0.05$) higher than their initial body weights (128.0±6.856) and (130.3±6.157) respectively. This could be attributed to the nutritive value of Okra (*Abelmoschusesculentus*).

In the study, *Abelmoschusesculentus* had clearly demonstrated its positive influence as increase weight gain was observed after values gotten from the assay after administration were compared to values of the initial body weight of experimental animals before administration. This may be due to the presence of high nutritional component of the extract, such as proteins), which main primary function is to increase cell productivity, this study is in line with report from [13]. Results indicated that ethanolic extract of *F. parviflora* leaves have significant increase found in epididymal sperm density and percent of morphologically normal sperm in extract-treated rats. Serum testosterone levels were significantly higher in rats received 200 and 400 mg/kg/day.

Histological observation shows the result of the study in control group of animals which received only standard growers feed and water revealed numerous tubules and connective tissue layer that appears normal. However, animals in the low dose group received food, water and 1.0mg/kgBW of *Abelmoschusesculentus* shows marked tubule and connective tissue depletion and the animals in the high dose group which received food, water and 3.0mg/kgBW of *Abelmoschusesculentus* shows numerous tubules and interstitial connective tissue appearing normal. This study is in concordance with previous research [14] on Epididymal Effect on Wistar Rats Treated with EthanolicExtract of*Sidaacuta*leave which shows the histological section of epididymis

in the olive oil control group showed surface epithelium with stereocillia and lumen filled with sperm cells. The epithelial cells were supported on a basal lamina surrounded by smooth muscle cells and by loose connective tissues. There was no pathology seen (Plate 2). The animals in the low dose group which received 500mg/kgBw of Sidaacutaethanolic leaf extract, the epididymis showed surface epithelium with stereocilia surrounded by connective tissue and smooth muscle. The lumen was filled with matured sperm cells. No pathology was observed when compared with the control groups (Plate 3).The epididymis in the medium dose group that received 1000mg/kgBw of the extract showed surface epithelium with stereocilia surrounded by connective tissue and smooth muscle. The storage centers showed early stages of granulomatous response, (Plate 4). Animals in the high dose group that received 1500mg/kgBw of ethanolic leaf extract of Sidaacutathe epididymis showed surface epithelium with overlying stereocilia surrounded by connective tissue and smooth muscle. Part of the lumen showed onset of granulomatous response that destroys stored sperm cells. There was observable pathology when compared to the control and other treatment groups (Plate 5) this morphological changes is also in agreement with the findings of[15] who reported the leaf fraction of *I. suffructicosum* increased the caudaepididymal sperm vitality, testicular weight, spermatogenesis, sperm counts, lessened sperm agglutination. This study therefore supports the usage of *I. suffructicosum* in traditional medicine for infertility.

V. Conclusion

Results gotten from this study concluded that *Abelmoschusesculentus*showed a dose dependent distortion of the normal cytoarchitecture of the organ. This result is positive evidence that *A. esculentus* might be used to improve epididymal sperm vitality, mortility and morphology and increase in body weight especially at lower dose of 1.0mg/kgBW. This study also revealed that continuous administration of *Abelmoschusesculentus* for a long period of time without proper or recommended dosage may affect proper sperm storage in males.

Conflict of interest: None

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