ORIGINAL ARTICLE

SAFETY PROFILE OF MUSA PARADISIACA AQUEOUS FRUIT- EXTRACT IN TESTIS OF NORMAL RAT

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ABSTRACT

Musa paradisiaca (plantain) is one of the common staple foods in East Africa. The leaves, roots, and fruits have been a revolutionary traditional remedy in the management of male sexual infertility. This study aimed at evaluating the safety profile of plantain aqueous fruit-extract in the testis of male rats. Thirty Adult Wistar albino rats weighing about 100–150 g in body weight (bw) were used in this study. They were divided into three (3) groups of 10 animals each (n=10). Groups I received water only. Groups II and III received low (200 mg/kg bw) and high (400 mg/kg bw) doses of plantain, respectively. The animals were allowed free access to rat feed and water. The extract was given once daily for 21 days via the oral route. The histomorphology of the testis was performed using Masson's Trichrome staining technique, while the sperm profile was analyzed using a Makler counting chamber with Olympus Microscope. Plantain administration in normal rats significantly increased the body weights of the rats. Seminal analysis showed improved sperm parameters in treated rats, but rats in the high-dose group presented a high level of agglutination compared to rats in the control and low -dose groups. Histological observations showed mild-to-moderate proliferation of cells in the testicular germ layer of the treated rats, indicating enhanced spermatogenesis. Testicular morphology in the group that received high dose showed mild degenerative changes in their testicular germ layer. Plantain, at moderate consumption can improve testicular activity and reproductive health in male while at a high consumption rate it may cause mild degenerative changes in the testicular germ layer.

Keywords: *Musa paradisiaca*, Testis, Semen, Wistar rats

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INTRODUCTION

The most of the world population currently depended on medicinal herbs for their primary health needs, and most of this therapy involves the use of plant extracts, often in aqueous solutions (1). The essential oils or (volatile or ethereal oils) are aromatic oily liquids produced from plant material. Currently, Formulations based on plant products have been used in diseases treatment and prevention since ancient time. Natural products have upper hand over synthetic drugs because they have fewer side effects and also do not alter physiological and anatomical functions of an organ. Several plants are being constantly screened in terms of pharmacological activities including antibiotic, anti-apoptotic, antioxidant, and anti-inflammatory properties (2). This study makes use of Plantain (*M. paradisiaca* L., Abbrev. Mus. par) extract on the test subject.

M. paradisiaca (plantain) is a crop in the genus Musa which has been cultivated for more than 4000 years (3), and its several varieties are staple food in the tropical regions of the world (4). M. paradisiaca promotes healthy digestion, improves affective state, helps in the retention of and serves as good sources of potassium, calcium, phosphorus and nitrogen, which build and regenerate tissues in the body, and is also a rich source of iron and vitamins, especially Vitamins C and E (3). Green plantain is also high in total dietary fibre content, especially hemicelluloses, which is higher than in most fruits and vegetables (5). The high fibre contents, particularly insoluble fibre, can lower glycaemic response by forming a physical barrier to enzymatic hydrolysis of starch. The leaves, roots and fruits of plantain have been a revolutionary breakthrough in the management of male sexual inadequacies (6).

Testicle or testis (plural testes) is the male reproductive gland or gonad in all animals, including humans. It is homologous to the female ovary. The functions of the testes are to produce both sperm and androgens, primarily testosterone. Testosterone release is controlled by the anterior pituitary luteinizing hormone, whereas sperm production is controlled both by the anterior pituitary follicle-stimulating hormone and gonadal testosterone (7).

Sperm is the male reproductive cell, or gamete, in anisogamous forms of sexual reproduction. Animals produce motile sperm with a tail known as a flagellum, which are known as spermatozoa, while some red algae and fungi produce non-motile sperm cells, known as spermatia. Flowering plants contain non-motile sperm inside pollen, while some more basal plants like ferns and some gymnosperms have motile sperm (8). Male infertility can be caused by poor penile erection, abnormal sperm quality and volume, abnormal ejaculation, among other causes. Researches into natural diets like plantain showed that its consumption by men could enhance some reproductive functions, and also alleviate certain reproductive dysfunctions (9). Oral administration of Mus. Par. at low dose caused significant increase in body weights, testosterone levels,

sperm concentration, sperm motility, sperm life and dead ratio with improved sperm morphology while oral administration of plantain at high dose caused significant decrease in body weight, testosterone levels, sperm concentration, sperm motility, sperm live and dead ratio with negative effects on sperm morphology (3, 10).

METHODS

Sample Collection and Extract Preparation

Mus. Par. was gotten from the local market of Okuku in Yala Local Government Area of Cross River State of Nigeria. The unripe plantain was properly washed, peeled, sliced and dried in an air-dried room temperature of about 270 c for three weeks. It was blended and kept in an air tight container.

The plantain powder was dissolved in 9000 mls of distilled water in a plastic container. The mixture was properly stirred with a stirrer and allowed to stay for twenty four (24) hours before it was filtered with a cloth-sieve. The filtrate was evaporated at about 450c with water bath to obtain the crude solid extract for three (3) weeks and the extract obtained was stored in a refrigerator at a temperature of 15°c until the commencement of the administration.

Experimental Animals

Thirty (30) adult male wistar rats were purchased from the animal house of the Department of Human Anatomy and Forensic Anthropology, University of Cross River State (UNICROSS), Okuku campus and were used for this study. The animals were randomly distributed into three groups (control, low dose and high dose) of ten (10) animals in each group (3n=10).

The animals were acclimatized for two (2) weeks, they were housed in plastic cages under controlled light (12 hours daylight cycle and 12 hours dark cycle) and were fed with standard growers vita feed and water before the start of the administration.

Experimental Design

The thirty (30) animals were allocated into three (3) groups consisting of ten animals in each group as follows:

Group A: (Control) received food and water only

Group B: (Low dose) animals received food, water and extract of plantain at a dose of about 200mg/kgbw

Group C (High dose) animals received food, water and extract of plantain at a dose of 400mg/kgbw.

Termination of Experiment

At the end of administration which lasted for twenty one days (21) the animals in all the groups were sacrificed a day after the final

administration using cervical dislocation. The testes of each animal were removed and the caudal epididymis separated from the testes and processed immediately for epididymal sperm parameters.

Morphological Studies

All the animals were weighed using sensitive weighing balance before the commencement and at the end of the administration of the extract of plantain to ascertain the morphological changes.

Tissue processing and Histological Studies

The testes were removed and preserved in a container with 10% neutral buffered formalin. This was done for seventy two (72) hours to achieve good tissue penetration and effective fixation. After this they were placed in ascending grades of ethanol for dehydration. First they were treated with two (2) changes of 70% ethanol each lasting for one hour followed by 95% ethanol and then absolute ethanol for the same duration. Following dehydration, the tissue were cleared in three changes of xylene each lasting for fifteen minutes (11).

The impregnation in molten paraffin was at 580c was carried out overnight and the following morning the tissue were embedded in wax to form blocks. This tissue blocks were trimmed and sectioned at 3 to 5µm thickness using a microtome. The sections were floated in warm water (280c) and then taken up on albuminized glass slides. They were air dried and stained using the Masson Trichrome staining method (12).

Semen Analysis

Semen was obtained from the caudal epididymis by scraping the lumen into a lumen tube pre warmed with water bath at 50-60 0C. A drop of this specimen was placed in a chamber and covered with a water glass. The following parameters; sperm motility, vitality, morphology and agglutination were determined using the Makler counting chamber with Olympus Microscope.

Statistical Analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 16 chicago Inc. one way Analysis of variance (ANOVA), followed by Bonferroni's multiple data comparison test was used to perform the analysis. Result of descriptive statistics of the experiment data was presented as mean standard error (Mean ±SEM). Paired sample T-test were considered statistically significant at $p<0.05$.

RESULTS

Morphological Observations on Body Weight

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 versus (vs) low dose, control vs high dose and low dose vs control but not observable in low dose vs high dose and high dose vs control dose. The final body weight of the control animals (115.0 ± 3.310) was significantly (P<0.05) higher than its initial body weight (91.40 ± 5.47). However, the mean final body weight of the low dose group (152.40 ± 6.72) and high dose group (160.20 ± 6.02) were significantly (P<0.05) higher than their initial body weights (133.8 ± 3.094) and (135.10 ± 6.13) respectively (Table 1).

Semen Analysis

Semen analysis was done on key seminal parameters such as sperm motility, sperm vitality, sperm morphology and agglutination. The results are reported below:

Sperm motility

Result from this study showed a significant increase in (P < 0.05) percentage sperm motility in the low dose (63.33 ± 1.67) and high dose (63.33 ± 1.67) animals when compared with the control (60.00 ± 2.89) animals (Table 2).

Sperm vitality

There was a significant increase (P < 0.05) in sperm vitality of low dose (68.33 ± 1.67) and significant decrease sperm vitality of high dose (61.67 ± 1.67) animals when compared to the parameters in control group (61.67 ± 1.67) (Table 3).

Sperm morphology

Sperm morphology is expressed as mean values in percentage. The highest percentage of sperm morphology (68.33 ± 1.67) was observed in high dose group which was significantly (P < 0.05) higher compared to the low dose and control group at (61.67 ± 1.67) and (61.67 ± 1.67) respectively (Table 4).

Sperm agglutination

There was a significant decrease (P < 0.05) in sperm agglutination of high dose (4.667 ± 2.333) when compared to the low dose (7.333 ± 0.333) and control group (7.333 ± 0.6667) respectively (Table 5).

Histological Observations

The histological result of the study on testes of control animals showed normal semeniferous tubules with intact germ cell layers (G) (Plate 1a). Histological observations on testes of treated rats administered with Musa paradisiaca at low dose showed mild proliferation in testicular germinal cell layer (G) in sections of the testes of treated animals (Plate 1b) while that of high dose showed mild to moderate germ cell layer degeneration despite displaying matured sperm cells (G) (Plate 1c).

DISCUSSION

Approximately 80% of the population of the world depends according to the World Health Organization on traditional medicine, mostly herbal remedies, for their primary health care needs (13). Interestingly, the use of herbal medicine was double to triple fold more than conventional drugs and 80% of the world population especially in developing countries still depend on plant-derived medicines for maintenance of health and treatment of disease (14), While various studies documented that Mus. par. has diverse beneficial effects in several diseased conditions, including, diabetes mellitus, hypertension, hyperlipidaemia, thyroid dysfunctions and body weight (15, 16).

Morphological studies

Findings from this present study revealed an observable significant ($P<0.05$) increase in the final mean body weight when compared with the initial body weight previously observed. The mean final body weight of rats in the low dose (152.4 ± 6.723) and high dose (160.2 ± 6.018) groups were significantly ($P<0.05$) higher than their initial body weights (133.8 ± 3.094) and (135.1 ± 6.125) respectively (Table 1) which is in affirmation with the research of Iroaganachi et al. (2015)17 where experimental rats had 13.42% gain in weight after administration of Mus. par.

Semen analysis

Specifically, sperm motility significantly increased in ($P < 0.05$) in the low dose (63.33 ± 1.667) animals and high dose (63.33 ± 1.667) animals when compared with the control (60.00 ± 2.887) animals (Table 2) which also confirmed the findings of Vinaykumar et al. (2010)18. There was also a significant increase ($P < 0.05$) in sperm vitality of low dose (68.33 ± 1.667) and decrease in high dose (61.67 ± 1.667) when compared to the control group (61.67 ± 1.667) as displayed in (Table 3) but the highest percentage of sperm morphology (68.33 ± 1.667) was observed in high dose group which was significantly ($P < 0.05$) higher compared to the low dose and control group at (61.67 ± 1.667) and (61.67 ± 1.667) respectively as seen in (Table 4).

Plantain fruits can be used as a treatment of sexual dysfunctions (19). Likely, studies conducted by Sharma et al. (2019)20 on metabolic extract of Musa paradisiaca (MEMP), fruit revealed that animal models with diabetic induced testicular disorders can have the testicular damage reversed, when given metabolic extract of Mus. par. at a moderate dosage. Hence Mus. par. has been proven to have hypoglycaemic effects in both normal and diabetic animal models, which might be a reason for its ability to facilitate improvement in male sexual functions in diabetic animals (21,22,23).

Despite the ameliorative potential of plantain Adnan and Noory (2017)24 observed the spermatozoa had some of the abnormal morphological features listed below: Tail defects (short, irregular coiled or multiple tails); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (round head, small or large size, double or detached head). These findings negatively impact the normal sexual functions of the animals, thereby reducing their ability to fertilize an ovum successfully (3,25). At a higher dose of plantain fruit, the percentage motility and Life/Death ratio was significantly low with reduction in the forward directional movement of spermatozoa when compared with the control.

Histological studies

Histological observations revealed that when mature aqueous extract of Mus. Par. was administered at low dose there was mild proliferation in testicular germ cell layer (G) which suggests positive effect of Mus. par. on spermatogenesis which can play a vital role in the treatment of sexual dysfunctions in males (Plate 1a). These observations were in consonance with the findings of Alabi et al. (2017)26 and Nejati and Kanshi (2014) 27 reporting that Mus. par. has reproductive enhancing potentials when consumed moderately and increases the spermatogenesis in infertile diabetic patients, respectively. This effect was undoubtedly evident as an improvement in the quantity and quality of spermatozoa in treated adult male wistar rats especially at low dose.

CONCLUSIONS

At a low dose, Musa paradisiaca showed positive effects on body weight, semen parameters (sperm motility, vitality, morphology and agglutination), testicular histomorphology and spermatogenesis but can cause degenerative changes if taken at a high dose for a long time. Consumption should be at a moderate and controlled quantity in order to enhance optimal improvement in male reproductive health.

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Table 1: Morphological observation of body weight (Values are presented as Mean ± SEM)

	BODY WEIGHTS	
GROUPS	**INITIAL**	**FINAL**
Control	91.40 ± 5.467	115.0 ± 3.310

Low Dose	133.8 ± 3.094	152.4 ± 6.723
High Dose	135.1 ± 6.125	160.2 ± 6.018

Table 2: Showing the effect of *Musa Paradisiacal* extract on sperm motility between control and the treatment groups of Wistar Rats

GROUPS	MEAN	STANDARD ERROR
Control	60.00	2.887
Low Dose	63.33	1.667
High Dose	63.33	1.667

Table 3: Showing the effects of *Musa Paradisiacal* extract on sperm vitality between control and the treatment groups of wistar rats.

GROUPS	MEAN	STANDARD ERROR
CONTROL	61.67	1.667
LOW DOSE	68.33	1.667
HIGH DOSE	61.67	1.667

Table 4: Showing the effects of *Musa Paradisiacal* extract on sperm morphology between control and the treatment groups.

GROUPS	MEAN	STANDARD ERROR
CONTROL	61.67	1.667
LOW DOSE	61.67	1.667
HIGH DOSE	68.33	1.667

Table 5: Showing the effects of *Musa Paradisiacal* extract on sperm Agglutination between control and treatment groups.

GROUPS	**MEAN**	**STANDARD ERROR**

CONTROL	7.333	0.6667
LOW DOSE	7.333	0.3333
HIGH DOSE	4.667	2.333

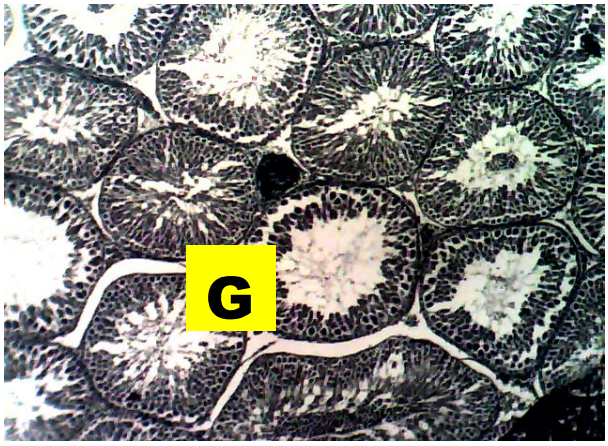

Plate 1a

Plate 1b

Plate 1c

Plate 1(a-c): Histology of rat testis administered with *Musa Paradisiacal* aqueous fruit-extract
Plate 1a (Control of testis showing seminiferous tubules with normal cells in testicular germ cell layers (G). MT. X 40 (G).MT. X 40),
Plate 1b(Low dose of testis showing mild proliferation of cells in testicular germ layer (G).MT. X 40), Plate 1c(High dose of testis showing mild to moderate proliferation of cells with some degenerative changes in testicular germ layer).