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Original Research Article

Effects of *Cyperus esculentus* (Tiger Nut) Extract on the Irradiated Testes of Wistar Rats

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ABSTRACT

Cyperus esculentus (Tiger nut) may be a useful supplement in combating fertility challenges imposed by exogenous substances like ionizing radiation. The study investigated the usefulness of *Cyperus esculentus* as a phytoremedy supplement in reversing radiation-induced changes on the testicular microstructure of Wistar rats. The animals were exposed to ionizing radiation and treated with tiger nut aqueous extract at 750 mg/kg for 2 hours 7 days post irradiation. The animals were exposed to X-rays using exposure settings of 75 kVp and 20 mAs with a moBDiagno X-ray unit and sacrificed 24 hours after the last administration. Their testes were removed and processed for histological evaluation. The results showed that ionizing radiation caused deleterious effects on the seminiferous tubules of the rats which were reversed in animals treated with the aqueous tiger nut extract. In conclusion, *Cyperus esculentus* has the potential to reverse radiation-induced damage on the ultrastructure of the testes, which may improve radiation-related male fertility effects.

Keywords: *Cyperus esculentus*, Ionizing irradiation, Testes, Wistar rats.

Introduction

Substantial decrease in sperm count and histo-morphological alterations of the testes have been reported at low doses of radiation in experimental rats.[1] These changes are known to have adverse effects on the reproductive function of the testes. The testis is one of the most radiosensitive reproductive organs because of the highly proliferative nature of its spermatogonial cells.[1] The testis possesses germ cells at different stages of development, a process known as spermatogenesis.[1] These developing sperm cells are very sensitive to ionizing radiation and are known to affect the morphology, function and ultimately, spermatogenesis.[2] It is reported that about 15% of couples seek medical care due to infertility and about 25% of the cases are attributable to the male partner.[3] Several drugs and treatments have evolved in the past decades to tackle infertility, albeit high cost, chemical effect and poor access, in a typical low-resource setting would require an alternative approach to handling infertility.[4] Globally, there is increasing interest in harnessing plant extracts and natural products for health benefits.[5] There are numerous evidence on phytomedicinal trial of different disease processes, with results proving the efficacy of such plants insignificant number.[5,6,7] Currently, phytotherapy has received considerable acceptance in the sub-Saharan African countries and most developing economy owing to the fact that these plants were hand-down legacies from traditional practices.[7] A study has shown that plants including *Cyperus esculentus* are used by at least 50% of the world's population in both developed and developing countries.[6] *Cyperus esculentus* (Tiger nut) is a popular plant consumed in Nigeria and most parts of West Africa.[8] Studies have shown that Tiger nut has many benefits as it is rich in natural sugar, phosphorus, potassium, calcium, magnesium, iron, fibre and starch.[9-12] In addition, there are reports on the function of tiger nut in reducing low-density lipoprotein-cholesterol (LDL-C) on blood triglycerides levels, a mechanism against atherosclerosis.[8,13] Studies have assessed the deleterious effects of X-rays on the testes but the focus on possible ways of combating or reversing the damage caused by ionizing radiation on the testes using plant supplements such as *Cyperus esculentus* are poorly investigated in Nigeria. The high antioxidant content of *Cyperus esculentus* might be useful in combating fertility challenges imposed by exogenous substances like ionizing radiation.[14] The study investigated the usefulness of *Cyperus esculentus* as a phytoremedy supplement in reversing radiation-induced changes on the testicular microstructure of Wistar rats.

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Materials and Methods

Breeding of the animals

Thirty (30) adult male Wistar rats weighing 180 g to 200 g were purchased from the Department of Pharmacology animal house, University of Calabar, Calabar, Nigeria. The animals were housed in well-ventilated animal cages and kept in the animal house of the Department of Physiology, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria. The animals were maintained under standard conditions of 12 hours dark/light cycle and temperature of 28 – 30^{o}C. The house was constantly kept clean and disinfected using standard universal protocols. The animals were fed with grower mesh and had free access to distilled water and were acclimatized for 14 days. After acclimatization, the rats were randomly selected and grouped into three groups (A, B and C) of six rats.

Ethical approval

Ethical approval for the study was gotten from the Faculty Animal Research Ethics Committee, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria (FAREC/FBMS/UC/052).

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Cyperus esculentus (tiger nut) extract preparation

Dried *Cyperus esculentus* tubers were bought from Watt market, Calabar, Cross River State, Nigeria. The tiger nut tubers were identified, authenticated and registered by a Botanist Mr Effa A. Effa (voucher number: HERB/BOT/UCC/132) in the Department of Botany, University of Calabar, Calabar, Nigeria. About 2.5 kg of the dried *Cyperus esculentus* was used. They were cleaned, washed, oven-dried and crushed into smooth fine powder using an electric blender. The powdered tiger nut (1 kg) was macerated in 1500 mL of water, stirred vigorously, left unperturbed for 24 hours at room temperature, and stirred at intervals. After 48 hours, the dissolved tiger nut in water was filtered using a Whatman No.1 filter paper. The filtrate was collected, concentrated to dryness using a mermmert oven (Type UNB 500 F. – Nr.: 0508.2070). The tiger nut aqueous extract obtained was collected with the aid of a spatula into a container. The extract was stored in an air-tight container and kept in the refrigerator at 4^{o}C until use.

Determination of LD_{50}

The LD_{50} of aqueous extract of *Cyperus esculentus* was established using Lorke's method. The dosage of aqueous extract administration was determined using 25% (750 mg/kg) of the established LD_{50} (3000 mg/kg body weight of aqueous *Cyperus esculentus* extract).

Extract administration

Group A served as the negative control, received animal feed and water *ad libitum*; group B served as the positive control and was exposed to radiation for seven days only and group C was also exposed to radiation for seven days and treated with 750 mg/kg body weight of aqueous extract of tiger nut post irradiation.

Exposure to X-rays

The animals in the experimental groups (B and C) were exposed to X-rays using exposure settings of 75 kVp and 20 mAs with a moBDiagno X-ray Unit in the Department of Radiography and Radiological Science, University of Calabar, Calabar, Nigeria.

Tissue processing

Twenty four hours after the last administration, the animals were sacrificed and their testes removed and fixed in a 10% buffered formaldehyde and processed histologically. The testes were stained using Haematoxylin and Eosin stains. The paraffin wax was removed from the sections in two changes of xylene for 5 minutes each, rehydrated through descending grades of alcohol, and rinsed under tap water. Sections were later stained with haematoxylin for 15 minutes and rinsed under tap water for 5 minutes. Sections were differentiated in acid alcohol for a minute, blued and counter-stained with eosin for a minute. Sections were rinsed in tap water, dehydrated in ascending grades of alcohol and cleared in xylene. Sections were allowed to dry and few drops of dibutylphthalate polystyrene xylene (DPX) was placed on the surface of the slide, cover-slipped and observed under light microscope.

Quantitative analysis

Quantitative analysis was done using the Johnsen's score (Table 1 and 2). The Johnsen's score is an important index for quantifying spermatogenesis with respect to the number of cells along the seminiferous tubules.

Statistical analysis

Data were analysed using the Statistical Package for Social Science (SPSS) and results expressed as Mean ± Standard deviation. Statistical comparisons were performed using the one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare the mean of the different groups at $p < 0.05$ taken as the level of significance.

Results and Discussion

Results of the histological evaluation are shown in plate 1. The control slides not exposed to X-rays show prominent seminiferous tubules with intact basement membrane containing proliferating spermatogonia cells at various stages of maturation. The cells were 4 to 5 cell layer thick consisting of early spermatogonia and spermatocytes closed to the basement membrane and late spermatid and spermatozoa cells closed to the lumen. The cells had deeply stained nuclei. The intertubular interstitium contained 3 to 4 cells cluster of Leydig cells.

The slides of the testes of animals exposed to X-rays without treatment with the extract are shown in plate 2. They showed widely spaced seminiferous tubules with thickened basement and sparsely populated spermatogonia cells and faintly stained nuclei. Some of the tubules contained degenerated and atrophied cells. The intertubularinterstitium were hyalinised with absent of Leydig cells. These observations are consistent with acute radiation injury with the destruction of the Leydig cells.

The irradiated group treated with aqueous *Cyperus esculentus* extract slides are shown in plate 3. The slide present with prominent and widely separated seminiferous tubules with an intact thickened basement containing proliferating spermatogonia cells at various stages of maturation. The cells were 3 to 5 cell layer thick consisting of early spermatogonia and spermatocytes closed to the basement membrane and late spermatid and spermatozoa cells closed to the lumen. The cells have deeply stained nuclei. The intertubularinterstitium was hyalinised and contained atrophied Leydig cells with numerous healthy tubules filled with mature spermatozoa.

The world over, there is high interest in harnessing plant extracts and natural products for health benefits.[5] This has received much acceptance in the sub-Saharan African countries and most developing economies.[5] Histologically, section of the testis in Group A (control), revealed no pathological alterations including prominence of seminiferous tubules with intact basement containing proliferating spermatogonia cells at various stages of maturation. This is indicative of normal histology of the testis (plate 1). However, histological changes were observed in group B that was exposed (irradiated) without the tiger nut extract. In this case, the seminiferous tubules appeared widely spaced with thickened basement membrane and sparsely populated spermatogonia cells (plate 2). These alterations are highly suggestive of the damaging effects of ionizing radiation on the testes of the rats consistent with a similar report.[15] The implication of such changes are a decline in the reproductive ability of the testes in this group (B), possibly due to the oxidative effects of reactive oxygen species (ROS) produced as X-rays interact with the testicular tissue, resulting to reproductive failure.[15]

Photomicrograph of the treated irradiated group with *Cyperus esculentus* extract revealed recovery of the irradiated testis as mildly thickened basement containing proliferating spermatogonia cells at various stages of maturation. In addition, the cells were 3 to 5 cell layer thick consisting of early spermatogonia with cells having deeply stained nuclei. Intertubularinstertitium was hyalinised. However, the presence of atrophied Leydig cells on the group C, explains some reduced levels of oxidative stress. Since this group had *Cyperus esculentus* extract post-irradiation, it can be said that this supplement may have acted on the ROS, mopping off these radicals to restore the normal cellular activity of the testis in group C (plate 3). This result is in consonant with findings from studies conducted by Achoribo and Ong, Trinidad and Mallilin who reported that *Cyperus esculentus* improves sperm viability and fertility in male Wistar rats.[9,16]

Quantitative histological parameters like the Johnsen's score (Table 1) and the Germinal Epithelial Height (GEH) (Table 2) were compared across the groups. The mean Johnsen's spermatogenesis scores for each group were estimated at 8.30±0.88, 6.59±1.78 and 7.27±1.11 for control, irradiated and the treated group respectively. One-way ANOVA with the post hoc test revealed a higher Johnsen's score for the control compared to the experimental groups. However, the irradiated group treated with the *Cyperus esculentus* extract, recorded higher Johnsen's scores than the radiation only group ($p < 0.05$). The Johnsen's score is an important index for quantifying spermatogenesis

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with respect to the number of cells along the seminiferous tubules. It indicates the amount of spermatogenesis activity and determines the fertility status of a male subject.[17] The GEH for the control (30.10 ± 4.56) was significantly ($p < 0.05$) thicker than those of the experimental groups (group B =17.13±3.24 and group C = 18.82 ± 4.14). This means that histo-pathological processes and alterations may have taken place before the anti-oxidative function of the tiger nut extract on the damaged testicular microstructure occurred. This effect may have accounted for the lower values of Johnsen's scores and GEH in the treated group compared to the control. In addition, the time taken to combat ROS and fully restore the cellular activities that promote spermatogenesis and reproductive function may have contributed to this statistical difference between the control and the group treated with tiger nut extract post-irradiation. The GEH values were however, comparable between the experimental groups. Since the histology of the treated group revealed appreciably favourable cellular activities, this means that the tiger nut extract may have initiated some repair mechanisms capable of boosting cell renewal ability and antioxidant defence mechanism of the testis. Findings in this study suggest that *Cyperus esculentus* extract may be useful to repair radiation-induced injuries and improve fertility chances. The results obtained from the present study confirm the therapeutic effect of *Cyperus esculentus* on the germ cells and suggest that this extract could modulate the oxidative status of cell environment thereby preventing lipid peroxidation and improving antioxidant defence mechanism of the testis.[18]

Table 1: Mean Johnsen's score for control and experimental groups

GROUP	N	Mean Johnsen's score
A	6	8.30 ± 0.88[a]
B	6	6.59 ± 1.78[c]
C	6	7.27 ± 1.11[b]

The mean Johnsen's score for control and experimental groups: [a] means that control (A) is significantly higher than both experimental groups (B and C).[b] values shows that experimental group C is statistically higher than experimental group B. [c] value for B is statistically lower than control and group C. ANOVA with a Turkey test . A (control group); B (irradiated group without treatment); C (irradiated with treatment).

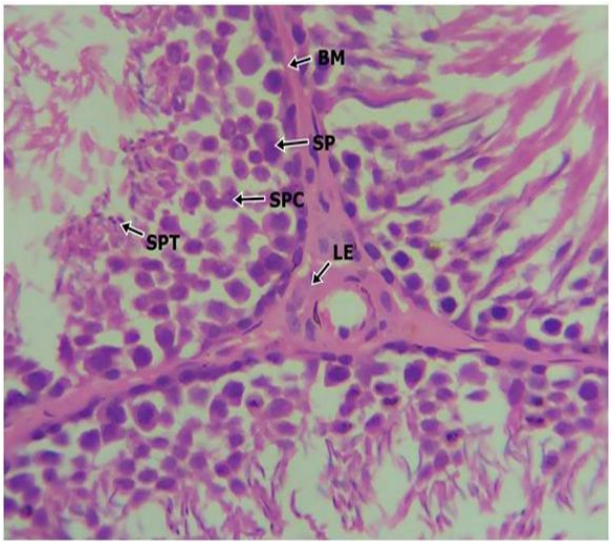

Plate 1: Photomicrograph of testis of the control (Group A) showing prominent seminiferous tubules, intact spermatogonia at various stages of development and Leydig cells. Mg X 400

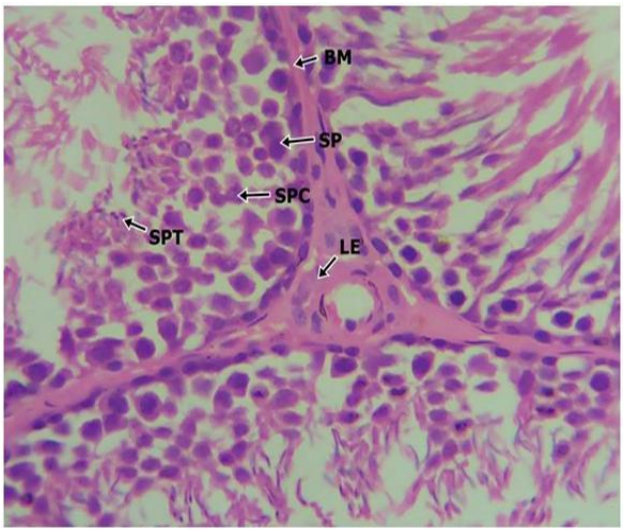

Plate 2: Photomicrograph of Group B (irradiated testis) showing widely spaced seminiferous tubules, sparsely populated, degenerated and atrophied spermatogonia and absence of Leydig cells. Mg X 400

Table 2: Mean Germinal Epithelial Height (GEH) for control and experimental groups

GROUP	N	Mean Germinal Epithelial Height
A	6	30.10 ± 4.56[a]
B	6	17.13 ± 3.24[b]
C	6	18.82 ± 4.14[b]

The mean Germinal Epithelial Height (GEH) for the control and experimental groups: [a] means that A is significantly higher than both experimental groups (B and C). [b]values are comparable for B and C using one-way ANOVA with a Turkey test. A (control group); B (irradiated group); C (irradiated with treatment).

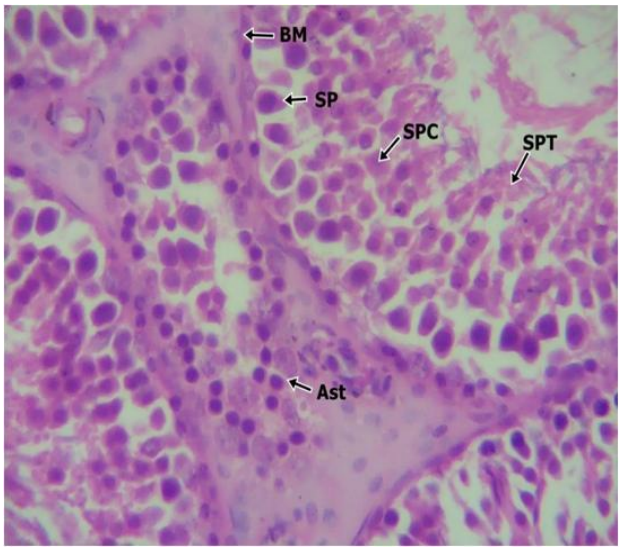

Plate 3: Photomicrograph of the testis of irradiated rat treated with 750 mg/kg body weight of tiger nut aqueous extract (GROUP C) showing prominent and widely spaced seminiferous tubules, intact spermatogonia and atrophied Leydig cells Mg x 400 BM: Basal membrane; LE: Leydig cell; SP: Spermatogonia SPT: spermatids; SPC: Sertoli cells.

Conclusion

Aqueous extract of *Cyperus esculentus* (tiger nut) has ameliorative effect and could reverse radiation-induced damages on the ultrastructure of the testes.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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