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Panicum maximum leaf extract induces reproductive toxicity in adult male Wistar rats

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Abstract

Background: Many environmental chemicals are implicated in causing serious adverse health effects on the reproductive system. Some medicinal plants taken traditionally for different ailments are particularly toxic to the reproductive organs. This study was undertaken to evaluate the reproductive toxicity of the ethanol leaf extract of *Panicum maximum* on adult male rats.

Methods: The leaves were randomly collected. Ground and powdered leaves were extracted by cold maceration using ethanol. Sixteen adult male rats (130-200 g) were divided into 4 groups. The negative control group (group I) was given 10 mL/kg distilled water, while Group II-IV received 50, 100, and 200 mg/kg of *Panicum maximum* leaf extract for 21 days. The animals were sacrificed and analyzed for some reproductive parameters at the end of the 21 days.

Results: There was a decrease in the body weight of the rats, as well as the testis at 200 mg/kg when compared to the control within the treatment period. Sperm analysis showed a significant decrease in normal sperm cells, sperm variability, active sperm cells, and sperm count in all groups given *Panicum maximum* leaf extract. There was a significant increase in abnormal and dead sperm cells. Hormonal analysis showed a decrease in follicle-stimulating hormone (FSH) and testosterone (TST) levels, which was significant. The histology of the testis also indicated toxicity of the extract, and it was dose-dependent.

Conclusion: Ethanol leaf extract of *Panicum maximum* is relatively toxic to the male reproductive system.

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Highlights

What is current knowledge?

The reproductive toxicity of the leaf extract was dose-dependent.

What is new here?

The extract decreased sperm count and sperm motility in a significant manner.

Introduction

Reproductive health is extremely important for all living things to survive (1). Unquestionably, reproduction is a necessary component of life and is essential to human survival (2). An important issue in reproduction is infertility, especially for couples. In about 50% of cases, male infertility disorders, in particular, play a significant role, and the vast majority of these disorders are curable (3). The rate of infertility in males in developing countries is high. Some herbal preparations are toxic, and people consume these substances for different health conditions.

Consumption of these toxic preparations can have major effects on the reproductive systems, affecting spermatogenesis and steroidogenesis, induction of histopathological abnormalities, and depletion of sperm counts, motility, and viability in males. The risks of the use and abuse of herbal preparations are high as individuals consume these preparations without an established dosage regimen. There are speculations that some of these preparations could lead to impotence, kidney failure, and issues with hearing and vision (4). Most toxicological studies are carried out to ascertain the acute and subchronic tests, which might not provide sufficient data regarding the reproductive health risks associated with the use of these plants. Reproductive toxicity should be carefully and appropriately investigated to validate or invalidate existing literature on plant extracts with medicinal properties (5).

Plants are key raw materials for the manufacturing of the majority of modern medications since they are thought to be rich sources of folk remedies (6). Plants serve as the primary source of health care for the majority of people worldwide (7). Plants are frequently used for ethnopharmacological purposes in Nigeria. Many herbal preparations are being used worldwide, especially in Africa, in recent times due to their availability and affordability. Beyond its accessibility and importance, herbal medicine's typical difficulties include its poor-quality control and safety worries, particularly in Africa (8). Contrary to widespread misconceptions that herbal plants are safe, the usage of some plant extracts may have negative health impacts on humans, animals, and/or the environment (9). An essential forage grass in tropical and semitropical areas is *Panicum maximum* Jacq. *Panicum maximum* leaves are used in ethnomedicine by the Ibibios of Akwa Ibom State, Nigeria, to cure a variety of illnesses. There have been reports of the leaf extract's effects on malarial and pain (10), diabetes (11), bacterial infections (12,13), inflammation (14), and cancer (15). Although studies have demonstrated its therapeutic effects, there are no toxicological studies on the male reproductive system conducted on *P. maximum* leaves. It is thus pertinent that studies be carried out on this herbal plant to determine possible reproductive adverse effects associated with its use in males.

Hence, the current study evaluated the toxic effects of ethanol leaf extract of *P. maximum* in adult male sprague dawley rats.

Methods

Plant collection and extraction

Panicum maximum leaves were collected from the compound of Madonna University Elele, Rivers State, Nigeria. They were cut into small pieces, air-dried, ground into powder, and stored. Extraction of the powdered leaves was done by cold maceration using ethanol for 72 h.

The powdered plant was repeatedly washed with fresh solvent until the extract/solution became clear. The filtrate was pooled together to obtain the ethanol extract (EE) and concentrated to a dry mass by drying at 400 °C in a water bath.

Experimental animals

Male rats (130-200 g) were purchased from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Nigeria. The animals were housed in well-ventilated cages at room temperature (28-30 °C). They were acclimatized for 1 week before the experiment started, during which they were fed with animal feed and given clean distilled water. The experimental animals were then weight-matched and grouped. The study was carried out under the approval of the Animal Research Ethics Committee, Madonna University, Elele, Nigeria. The experimental procedures by the National Institutes of Health Guide for the Care and Use of Laboratory Animals were observed (16).

Evaluation of acute toxicity

The acute toxicity of the plant extract was done using the method described by Lorke (17).

Evaluation of subacute toxicity

Sixteen male rats were used for the evaluation of subacute toxicity. The rats were divided into 4 groups. Groups I to III were given 50, 100, and 200 mg/kg body weight of *P. maximum* extract, while group IV (control) was given distilled water (10 mL/kg). The oral route was used for the administration of the test substance daily for 21 days. The rats were checked for signs of toxicity before, within, and after the treatment periods. Toxicity, behavioral, and physical signs were recorded.

Body and organ weight

At the beginning of the study, weekly, and after final treatments, the body weight of rats was measured. At the end of the study, the animals were anesthetized using chloroform, and the weight of the testis was measured.

Semen analysis

The epididymis was lacerated, and the semen was pressed out and emulsified with 0.5% eosin.

The percentage of normal and abnormal cells was examined, and the same was done for sluggish cells. The method described by (18) was used to determine sperm motility, and it was done individually for each sperm.

An improved Neubauer hemocytometer was used in sperm count determination, as described by (19). The method described by (20) using an eosin nigrosin was utilized in viability (percentage of live spermatozoa) determination.

Hormonal assay

The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (TST) in the blood serum were analyzed using the AccuBind enzyme-linked immunosorbent assay (ELISA) microwells, purchased from Monobind Inc Lake Forest, California, USA (21).

Histopathological analysis

Testicular tissues were dehydrated in 95% ethanol, fixed in 10% formalin and Bouin's solution, cleaned in xylene, and then embedded in paraffin after being dehydrated. Hematoxylin and eosin dye were used to make micro sections (Approximately 4 mm), which were then viewed under a light microscope. Tissue was prepared using the method described by Geoffrey Rolls (22).

Statistical analysis

The data are presented as mean ± SEM. We used 1-way analysis of variance (ANOVA) using GraphPad Prism 5.1 (GraphPad Software, San Diego, CA, USA). Statistical significance between treatments and control was established using Dunnett's multiple-comparison post hoc test with a 95% confidence limit. $P \leqslant 0.05$ represented a significant variation between variables.

Results

Acute toxicity and lethality (LD50) test

In the first phase, physical activity was reduced, and no death was recorded in the rats after 24 h. Death was recorded in the second phase at 2900 mg/kg of the extract administered. The oral The LD50 of the EE was estimated to be 2154 mg/kg.

Effects of *Panicum maximum* leaf extract on body weight

A decreased weight of the rats was recorded, and it was dose-dependent. The lowest dose (50 mg/kg) caused weight gain, while medium and highest doses (100 and 200 mg/kg) caused a reduction in weight gain. These weight changes were not statistically significant (Table 1).

Table 1. Effect of *Panicum maximum* on body weight of rats

Weeks	Control (untreated)	50 mg/kg PM	100 mg/kg PM	200 mg/kg PM
Week 0	111.00 ±12.90	112.40± 14.81	139.70±14.94	139.20± 14.94
Week 1	141.08±12.41	133.35±8.74	121.03±4.60	135.43±15.69
Week 2	129.25±10.67	138.60±8.14	104.75±3.98	126.63±16.07
Week 3	154.18±11.29	149.95±12.07	102.70±14.30	102.13±14.97

Values are expressed as mean ± SEM; Significance, n = 4.
Abbreviation: PM, *Panicum maximum*

Effects of *Panicum maximum* leaf extract on organ weight

Weight gain of the testis was recorded in the treatment groups compared to the control, but this was only statistically significant at 200 mg/kg (Table 2).

Table 2. Effects of *Panicum maximum* leaf extract on organ weights

Treatment groups	Weight of testis
Control	1.35±0.05
50 mg/kg	1.70±0.18
100 mg/kg	1.35±0.22
200 mg/kg	1.90±0.06*

Values are expressed as mean ± SEM; significance compared with the control, n = 4.
*P < 0.05 using analysis of variance (ANOVA), post hoc-Dunnett's test

Effect of *Panicum maximum* on male hormones

The FSH level was significantly reduced (P<0.001) in all the treatment groups compared with the control group. There was a decrease in the LH levels of rats given 100 and 200 mg/kg, but this decrease was not significant. The TST levels of rats given 200 mg/kg of the extract significantly decreased (P < 0.001) compared to the control group (Table 3).

Table 3. Effect of *Panicum maximum* on the hormone levels of male rats

Hormones	Control	50 mg/kg PM	100 mg/kg PM	200 mg/kg PM
FSH (mIU/mL)	1.57 ±0.0	1.27± 0.03***	1.14±0.02***	0.77± 0.01***
LH (mIU/mL)	0.31±0.02	0.31±0.00	0.12±0.02	1.10±0.04
TST (ng/dL)	2.26±0.04	2.12±0.08	2.22±0.05	1.85±0.01***

Values are expressed as mean ± SEM; Significance ***P < 0.001 using analysis of variance (ANOVA), post hoc-Dunnett's the test compared with the control, n=4.
Abbreviations: PM, *Panicum maximum*; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; TST, Testosterone.

Effect of *Panicum maximum* leaf extract on sperm parameters

There was a dose-dependent significant decrease (P < 0.001) in sperm viability. Normal and active sperm cells with the highest decrease were recorded at 200 mg/kg. A significant increase (P < 0.001) in the abnormal and dead sperm cells in all treatment groups was observed, with the highest increase seen in rats given 200 mg/kg compared to the control group (Table 4). The significant decrease (P < 0.001) in sperm count was also dose-dependent across the treatment groups (Figure 1).

Table 4. Effect of *Panicum maximum* on some sperm parameters

Sperm parameters (%)	Control (Untreated)	50 mg/kg PM	100 mg/kg PM	200 mg/kg PM
Viability	86.25±2.39	66.25±6.58***	52.50±1.44***	50.50±0.50***
Normal cells	60.25±2.39	56.00±2.89***	52.50±1.44***	52.50±1.44***
Abnormal cells	13.00±1.23	45.00±2.04***	50.00±2.04***	53.00±1.08***
Active cells	83.75±2.39	53.75±2.39***	47.50±1.44***	42.50±1.43***
Dead cells	8.25±1.18	40.00±3.54***	42.50±1.44***	43.75±2.39***

Values are expressed as mean ± SEM; significance ***P < 0.001 using analysis of variance (ANOVA), post hoc-Dunnett's test compared with the control, n=4.
Abbreviation: PM, *Panicum maximum*

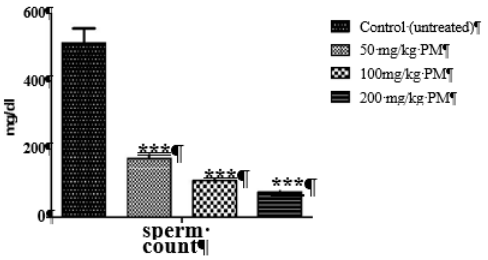

Figure 1. Effect of PM on the sperm count of rats.
Values are expressed as mean ± SEM; significance ***P < 0.001 using analysis of variance (ANOVA), post hoc-Dunnett's test compared with the control, n=4.

Effect of *Panicum maximum* on the histology of the testis

Group I showed the photomicrography of the testes, indicating seminiferous tubules containing spermatids, spermatocytes, and spermatogonia. Also seen are the Sertoli cells and Leydig cells consistent with normal histology of the organs. Group II showed normal histology with minimum reduction in sperm cell motility at the lumen. Group III showed extensive exudation and interstitial necrosis, indicating toxicity. Group IV showed a gross reduction in sperm motility at the lumen and extensive interstitial necrosis (Figure 2).

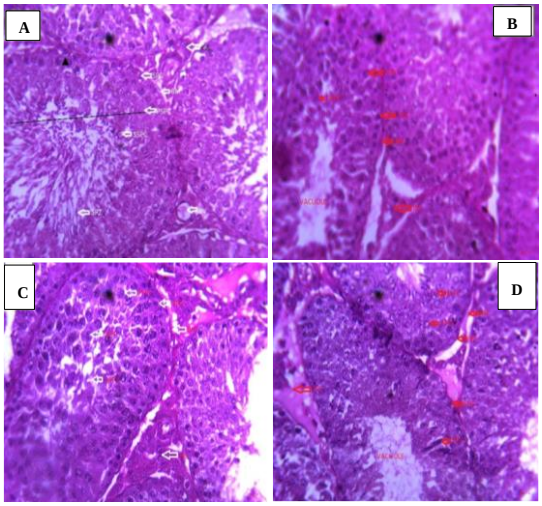

Figure 2. The histology of the testis. (A) Control group, (B) 50 mg/kg, (C) 100 mg/kg, (D) 200 mg/kg.

Discussion

Panicum maximum leaf extracts have been used traditionally for the management of diabetes, inflammation, and immunomodulation, and these effects have been reported scientifically (11,14,15).

Currently, there is little information in the literature about the toxicity of the plant to the male reproductive organ. Hence, this study was conducted to ascertain the toxicity of the ethanol leaf extract of P. maximum in the reproductive system of adult male rats.

Compared to the negative control, the leaf extract did not produce any significant change in the body weight of the animals. However, changes in weight were dose-dependent, as the lowest dose (50 mg/kg) caused increased weight gain in the rats, while the medium and highest doses used (100 and 200 mg/kg) caused decreased weight gain. Many phytochemicals present in plants hurt weight changes in animals, as reported in the literature (23,24). Tannins are present in *P. maximum* (11) and have the ability to antagonize digestive enzymes (25), resulting in decreased weight gain.

In toxicological research, determining the weight of the organs is one of the key aspects of the investigation. Testicular size or weight typically indicates whether testes are normal (26). The weight of the testis was significantly decreased at the highest dose (400 mg/kg) administered compared to the control. The significant reduction in testicular weight may be a result of inhibited steroidogenesis and spermatogenesis. The amount of differentiated spermatogenic cells determines a significant portion of the testicular weight. Hence, a decrease in testicular weight may be an indication of damage to the germ cells (27).

Reduced testosterone levels, sperm count, and sperm motility are necessary for the characterization of harmful substances that might affect a patient's ability to conceive, especially in the male reproductive system (28). *Panicum maximum* disrupted reproductive parameters in rats as sperm count and sperm viability. Normal and active sperm cells were significantly ($P < 0.001$) decreased. Abnormal and dead sperm cells in all treatment groups were significantly increased ($P < 0.001$). Thus, sperm motility function is the ability of sperm to fertilize eggs (29), as fertility will be greatly affected if there is any harmful effect on sperm motility (30).

Gonadal secretion of sex steroids, testosterone, is stimulated by LH, whereas FSH functions in gonadal development, steroidogenesis, and spermatogenesis during fertile life (31). Levels of testosterone, FSH, and LH were reduced in the extract treatment groups. This might be a result of testicular interference with anterior pituitary activity. The effects of FSH and androgen in rodents, primates, and other mammals seem to be comparable; therefore, the impact of various treatments on hormones can be extended to include humans. Because the hypothalamus' gonadotropin-releasing hormone (GnRH) controls the release of LH and FSH. A significant decrease ($P < 0.001$) in FSH in all the treatment groups and a significant decrease ($P < 0.001$) in testosterone at 200 mg/kg could potentially be attributed to the inhibitory effect of the extract on the hypothalamic-pituitary-gonadal axis, which reduced their secretion. The reduced hormone level will likely affect spermatogenesis and the quality of semen (32,33). The biological effects of FSH are mediated via G protein-coupled receptors found in the testes. Different experimental approaches and animal models have been obtained because of the LH's crucial function in starting and maintaining spermatogenesis (34). Modulation of post-receptor events within Sertoli cells is likely the mechanism through which FSH, LH, and testosterone work together to encourage quantitative spermatogenesis (32,33). Testosterone and FSH has been reported in some studies to promote spermatogenesis by encouraging round spermatid attachment to Sertoli cells (35,36).

Steroidogenesis is the process in which cholesterol is converted into testosterone. The first step is based on the conversion of cholesterol to pregnenolone, mediated by an enzyme known as the cholesterol side-chain cleavage (P450scc) enzyme (37).

The synthesis of testosterone (T) in the male organism is carried out by Leydig cells located in the testes and controlled by LH produced by the anterior pituitary. The LH specifically binds to LH receptors located in the plasma membrane of Leydig cells and stimulates the activity of intracellular signaling pathways coupled with the receptor, regulating steroidogenesis (38). Chronic stimulation by LH is required for optimal expression of steroidogenic enzymes, leading to sustainable steroid formation. Levels of testosterone, FSH, and LH were reduced in the extract treatment groups and could be a possible mechanism by which the extract caused toxicity to the male reproductive system.

The molecular steroidogenic steps within the adrenal cortex are generally similar between rats, mouse, and human, supporting the relevance of the rodent as a predictive toxicological model in vivo, but species differences do exist. For example, the dominant glucocorticosteroid in rodents is corticosterone, compared with cortisol in humans and other higher mammals, which is due to a lack of CYP17 in rodents (39).

The histology of the testis showed that sperm motility was reduced dose-dependently at the lumen and extensive interstitial necrosis of the extract. Different mechanisms (such as physiological, cytotoxic, and genetic mechanisms) can cause damage to sperm cells, suggesting that spermatozoa's sperm DNA concentration varies, and severe morphological defects may be genetically governed (40). Such anomaly in genetic theory may be attributed to spermatogenesis-related harm sustained during the pre-meiotic stages (41). The anomaly may be caused physiologically by a series of intricate and coordinated morphological and biochemical steps involved in the development of typical sperm heads during spermatogenesis (42). This is a clear indication that moderate and high doses of the extract are toxic to adult male rats, while low doses are safer.

Rats are commonly used in toxicological studies and have well-characterized reproductive processes. In general, the human male is likely at relatively greater risk from toxic agents because of differences in gonadal function. Differences in specific organ function may play a particularly significant role in the etiology of animal/human variation in reproductive risk. Although some spermatogenesis parameters are similar, human males have markedly smaller relative testis size and the lowest rate of daily sperm production per gram testis by a factor of more than 3. Moreover, the percentages of progressively motile sperm and morphologically normal sperm in human semen are lower. Hence, the duration of spermatogenesis (ie, the length of time it takes for a given stem cell to produce mature spermatozoa) corresponds to the length of approximately 4 to 4.5 cycles in laboratory animals and humans (Working, 1988) (43). The results of the study can be related to humans, even though there will always be room for variations.

Conclusion

This study has proposed a potential chronic spermatozoic effect of the ethanol leaf extract of *P. maximum*. Caution is advised against its unrestricted usage to prevent potential male infertility issues. Yet, the mechanism causing its toxicity on the male reproductive system remains undefined. Therefore, additional research is needed to explore the potential mechanisms of its reproductive toxicity, given the limited available information on this substance.

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Ethical statement

The research was conducted with the endorsement of the Animal Research Ethics Committee at Madonna University, Elele, Nigeria. The experimental protocols adhered to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Conflicts of interest

There are no competing interests. Each author has read the document and given their consent for publication.

Author contributions

U.L.I. designed the protocol for the study and analyzed the data statistically. E.B.E. wrote the manuscript's first draft. U.L.I and E.E.I managed laboratory experiments and literature searches for methodology. The final manuscript was approved by all authors.

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