ISSN: 2754-477X

Journal of Food Technology & Nutrition Sciences

SCIENTIFIC Research and Community

Research Article

Open Access

Synergetic Effect of Ethanolic Extracts of Cyperus Esculentus (Tiger Nut) and Ripe Musa Acuminata (Banana) Peel on an Arsenic Induced Testicular Damage in Adult Male Rats

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ABSTRACT

Background: Arsenic is a toxic environmental contaminant that poses significant risks to human reproductive health. This study explored the comparative effect of ethanolic extract of tiger nut and ripe banana peel on arsenic-induced testicular toxicity in adult male Wistar rats.

Methodology: Thirty-five adult male Wistar rats were procured, acclimatized, and grouped into seven groups. Group A served as the control, receiving standard feed and water. Group B received 500 mg/kg of tiger nut extract. Group C received 500 mg/kg of banana extract. Group D received a 10 mg/kg of arsenic acid, Group E and F received 20 mg/kg of arsenic acid for 6 days and 500 mg/kg ethanolic extract of tiger nut and banana respectively, Group G received 20 mg/kg of arsenic acid and combined 500 mg/kg of tiger nut extract and 500 mg/kg of banana extract. Oral extract administration lasted for 22 days. The study evaluated sperm parameters, testosterone levels, and histopathological analysis of the testes.

Results: The groups receiving tiger nut and banana supplements showed significant improvements in reproductive parameters. The group that received arsenic alone (Group D) exhibited the lowest sperm count, sperm motility, sperm morphology and serum testicular levels of all the test groups. Sperm count, motility, morphology and serum testosterone levels improved significantly In groups treated with either *Cyperrus esculentus* or Ripe *Musa acuminate* or a combination of both extracts after exposure to arsenic when compared to the group that was administered arsenic alone. Histopathological findings revealed enhanced spermatogenesis and testicular architecture integrity in the treated groups, with the combined diet showing the most pronounced effect, displaying fewer degenerated seminiferous tubules and increased germ cell layers.

Conclusion: The combination of tiger nut and banana supplementation improves reproductive parameters and testicular histoarchitecture in adult male Wistar rats, suggesting potential benefits for male reproductive health.

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Received: October 18, 2025; **Accepted:** October 23, 2025; **Published:** October 31, 2025

Keywords: Reproductive Health, Tiger Nut, Banana, Sperm Parameters, Testicular Histoarchitecture

Introduction

Humans and wildlife are inevitably exposed to a range of environmental and dietary chemicals throughout their lives. Among these toxic chemicals, metals and metalloids are pervasive elements that are ubiquitously present in the environment. Humans are not always exposed to a single element; instead, they are often exposed to multiple elements simultaneously. Heavy metals such as lead (Pb) and arsenic (As) have attracted significant attention from both the scientific community and the public due to their environmental impact, public health concerns and toxicity issues [1,2]. Arsenic, a metalloid that is abundant in nature, is well known for its carcinogenic properties in humans [3]. Arsenic and its compounds are released into the environment through human activities, including industrial use, agriculture, medicine, and feed additives [4]. Arsenic intoxication is associated with acute issues such as encephalopathy, neuropathy, nausea, vomiting, abdominal pain, and severe diarrhea. Chronic exposure to arsenic can lead to various cancers and metabolic syndromes, including diabetes, gastrointestinal tract problems, and cardiac and neurological issues [4]. Previously, *Reddy et al*. reported that arsenic exposure in mice resulted in reduced weights of reproductive organs, impaired steroidogenesis and spermatogenesis, and elevated arsenic levels in the testes, indicating reproductive toxicity [5]. *Sarkar et al*. also found that acute arsenic exposure caused rapid and extensive disruption of spermatogenesis [6].

Many environmental contaminants, including metals, have been reported to disrupt the pro- and antioxidant balance of cells, thereby inducing oxidative stress [7,2]. Studies have demonstrated that arsenic reduces sperm number, viability, and motility in adult animals, which has implications for male fertility [11,8]. Oxidative stress is considered the primary mechanism through which arsenic induces reproductive injuries [9,10]. Arsenic may stimulate the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), leading to lipid peroxidation, protein oxidation, and DNA damage [11,10]. While ROS are essential for regulating several physiological functions, including sperm maturation, elevated levels of ROS can damage sperm and other cytoplasmic organelle membranes through the peroxidation of phospholipids, proteins, and nucleotides, thereby altering sperm motility and fertilization capacity [12-14]. Arsenic may also affect the hypothalamic-pituitary-testicular axis in adult rats, reducing serum levels of luteinizing hormone, follicle-stimulating hormone, and testosterone by suppressing steroidogenic gene expression [15,16].

Since ancient times, people have explored nature, particularly plants, in search of new drugs. This has led to the use of numerous medicinal plants with curative properties to treat various diseases [17]. The popularity and availability of traditional remedies have raised concerns regarding their safety, efficacy, and the responsibility of practitioners using them [18]. Tiger nut and banana are examples of plants that have gained popularity in treating various ailments.

Cyperus esculentus L. (also known as tiger nut) is a crop from the sedge family found across much of the world, including Southern Europe, Africa, the Middle East, and the Indian subcontinent [19]. In Nigeria, tiger nut is called "Ofio" by the Yorubas, "Akiausa" by the Igbos, and "Aya" by the Hausas. It is cultivated for its edible tubers, known as earth almonds or tiger nuts, used as a snack food and in the preparation of horchata de chufa, a sweet, milk-like beverage. Tiger nut can be found growing wild, as a weed, or cultivated as a crop. It is considered invasive outside its native range and is often transported accidentally. In many countries, tiger nut is regarded as a weed [20].

Despite its name, tiger nut is a tuber, but its chemical composition shares characteristics with both tubers and nuts. It is rich in energy content (Starch, Fat, Sugar, and Protein) and dietary minerals (Mainly Phosphorus and Potassium). The oil from the tuber contains 18% saturated fatty acids (Palmitic Acid and Stearic Acid) and 82% unsaturated fatty acids (Oleic Acid and Linoleic Acid) [21]. Tiger nut is a good source of calcium, iron, magnesium, phosphorus, ascorbic acid (Vitamin C), tocopherol (Vitamin E), dietary fiber, and fats like oleic acid [21]. It can be consumed raw, roasted, dried, baked, or made into a refreshing beverage called tiger nut drink. It can also be blended and strained to make Kunnu Aya (Tiger Nut Milk), which can be sweetened with honey or dates.

Musa acuminata (Banana) is a treelike perennial herb that grows 5-9 meters in height, with a tuberous rhizome and a long, hard pseudo-stem. The inflorescence is large, with a reddish-brown bract, and is eaten as a vegetable. The ripe fruits are sweet, juicy, and full of seeds, and the peel is thick [22,23]. Bananas are among the most consumed fruits in tropical and subtropical regions [24]. In Nigeria, common edible banana cultivars include *Musa paradisiaca*, *Musa acuminata* (Cavendish banana), and *Musa acuminata* (Red Dacca) [25]. In southwestern Nigeria, dried *Musa spp*. peels are often blended with yam flour as part of staple foods [23].

Banana peels, constituting up to 35% of the ripe fruit, are often discarded as household and industrial waste [26]. They can be used to treat warts and reduce swelling from mosquito bites [27]. Ripe banana peels can be made into a poultice for wounds to reduce pain and swelling [28]. The banana tree has traditional value for treating anemia and is considered a healthy food for young children as it is less likely to cause cramps or diarrhea [29]. Ripe bananas and pseudo-stems are used to treat diarrhea, and the juice from *Musa spp*. is used for abdominal pain [30,29]. Unripe banana peels are used in scientific research due to their chemical composition, which includes bioactive compounds such as alkaloids, anthocyanins, flavonoids, glycosides, phlobatannins, tannins, and terpenoids. These compounds exhibit various biological and pharmacological effects, including antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities [29]. *Musa acuminata* fruit peels are used in northern Nigeria to treat hypertension and other cardiovascular diseases [31].

Despite numerous scientific studies validating the folkloric uses of different parts of the banana, limited research has provided scientific evidence for the use of *Musa acuminata* peels in managing fertility-related diseases. Additionally, there has been limited research on the effects of the combined administration of *Musa acuminata* fruit peels and tiger nut extract on testicular parameters in experimental rat models. Therefore, this study aims to evaluate the combined effects of ethanolic extracts of Ripe *Musa acuminata* fruit peel and *Cyperus esculentus nuts* on arsenic-induced testicular damage in Wistar rats.

Materials and Methods

Collection of Plant Materials

Fresh ripe *Musa acuminate* (banana) were purchased from a banana farm at Otolo Nnewi, Anambra State. While the nuts of *Cyperus esculentus* (Tiger) were procured from Eke Amaobi market in Otolo Nnewi, Anambra State.

Preparation of Plants extract

Mature Musa acuminate fruits were bought and allowed to ripe over a period of time. The peel was removed from the fruit and allowed to dry. The dried ripe Banana peel (1000-gram) was milled into a coarse form using a local grinder. Two hundred and fifty (250) grams of the ground ripe Banana peel were macerated in 1000mls of 95% absolute ethanol (JHD Chemicals, Guangdong, china) for 48-hours. It was then filtered using a clean porcelain cloth and further filtration using Filter paper (WhatmanNo. 1 Filter paper). The filtrate obtained was concentrated using a rotatory evaporator, which was further dried using a laboratory oven at 45ºC into a gel-like form. The extract was preserved in a refrigerator for further usage.

Fresh nuts (Tiger nuts) were rinsed to remove sand and other debris and allowed to dry. The dried Tiger nut (1000-gram) was milled into a coarse powdered using a local grinder. Two hundred and fifty (250) grams of the dried Tiger nuts were macerated in 1000mls of 95% absolute ethanol (JHD Chemicals, Guangdong, china) for 48-hours. It was then filtered using a clean porcelain cloth and further filtration using Filter paper (Whatman No. 1 Filter paper). The filtrate obtained was concentrated using a rotatory evaporator, which was further dried using a laboratory oven at 45ºC into a gel-like form. The extract was preserved in a refrigerator for further usage.

Ethical Approval

Ethical approval for this study was sort and obtained from the Ethical Committee of the Faculty of Basic Medical Sciences, College of Health Science, NnamdiAzikiwe University, Nnewi Campus, Okofia, Anambra State.

Animal Procurement, Care and Treatment

Thirty five (35) adult male wistar rats weighing 120-260g were purchased from Mr Austin's farm in Okofia, Nnewi South L.G.A, Anambra State. The animals were kept in the research section of the Animal House of the College of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus. They were allowed to acclimatize over a period of two (2) weeks. The animals were housed in well ventilated stainless steel cages under normal temperature (27-31°C) and were ventilated throughout the course of the experiment. They were fed with standard diet (rat chow) (Top feed, Nigeria Ltd), and distilled water ad libitum during the period of acclimatization and throughout the duration of the experiment.

Experimental Protocol/Design

The rats were divided into seven experimental groups labelled Group A—G as shown in table 3.1.

Table 3.1: Showing Experimental Groups, Materials Administered and Duration

Group	Materials administered (including feed and water *ad libitum* to all groups	Duration (days)
A	Water +feed only	28days
B	500mg/kg of Ethanolic extract of *Cyperusesculentus*	28 days
C	500mg/kg of Ethanolic extract of ripe *Musa acuminatapeel* extract	28 days
D	10mg/kg of Arsenic acid	28 days
E	20mg/kg of Arsenic was administered for 6 days and withdrawn and rats administered with 500mg/kg of Ethanolic extract of *Cyperusesculentus* for 22days	28 days
F	20mg/kg of Arsenic acid was administered for 6 days and withdrawn and then Rats were given 500mg/kg of Ethanolic extract of ripe Musa acuminata peel for 22 days	28 days
G	20mg/kg of Arsenic acid was given for 6days and Rats were then treated with co-administration of 500mg/kg of Ethanolic extract of *Cyperusesculentus and ripe Musa acuminata* peel for 22days	28 days

Animal Sacrifice and Sample Collection

The experiment lasted 28 days. Twenty-four hours after the final administration, animals were weighed, anesthetized with chloroform, and blood samples collected via ocular puncture. Serum was extracted from the blood for testosterone assay. Testes were harvested, cleaned, weighed, and trimmed to 3mm x 3mm. The samples were fixed in freshly prepared Bouin's fluid for four hours before undergoing histological and histochemical analysis.

Spermatological Studies

Semen Collection

The epididymis was separated, after retrieval of testis from the scrotum. The epididymal fluid was collected from the caudal part for the assessment of sperm count, motility and morphology which were assessed as described by *Saalu et al*,(2008).

Sperm Count Assessment

Epididymal sperm count was assessed using a modified method. The epididymis was minced in saline, rocked for 10 minutes, and incubated for 2 minutes. The supernatant was diluted 1:100 with sodium bicarbonate and formalin solution. Total sperm count was determined using a Neubauer counting chamber. Approximately 10 µL of the diluted sperm suspension was placed in the chamber, observed under a microscope, and sperm counted in five squares, then multiplied by 10^5.

Assessment of Sperm Morphology

Sperm cells were evaluated using method with a light microscope at ×400 magnification. Caudal sperm were diluted 1:20 with 10% neutral buffered formalin, and 500 sperm were scored for morphological abnormalities. Abnormalities included rudimentary tail, round head, and detached head, expressed as a percentage of normal sperm.

Sperm Motility

Immediately after dissection, a sterile pin punctured the epididymis. Semen was smeared on a slide with normal saline. Two drops of sperm suspension were placed on a slide with a cover slip. The number of progressively motile sperm cells was counted and divided by the total spermatozoa under 40x magnification, expressed as a percentage [32].

Hormonal Assay

Serum samples collected were assayed for testosterone level using the Microwell enzyme liked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc, USA).

Tissue Processing and Photomicrography

The fixed testis was processed at the Histology Laboratory, International Center for Research and Cancer Diagnosis, Abakiliki, Ebonyi State. This Histological method of processing tissue involved various ways of preparing, cutting, staining and examination slides for histological report.

Data Analysis

Differences between the mean sperm count, sperm morphology (Sperm Head Abnormality) and serum testosterone levels of the control and treatment groups were compared for significant using the Analysis of Variance (ANOVA) test and values were considered significant at $P \leq 0.05$. Post Hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD). With results presented as Mean ± S.E.M.

Results

Phytochemical Analysis of Cyperusesculentus (Tiger nut) and Ripe Musaacuminata (Banana) Peel

The screening of *Cyperusesculentus* revealed the presence of several beneficial compounds: carbohydrates, flavonoids, tannins, reducing sugars, saponins, and alkaloids. However, steroids, phenols, and terpenoids were absent (Table 1).

Similarly, the ripe peel of *Musa acuminata* was found to have carbohydrates, flavonoids, tannins, reducing sugars, saponins, and alkaloids, but lacked steroids, phenols, and terpenoids (Table 2).

Table 1: Qualitative Phytochemical Screening of *Cyperusesculentus*

Chemical constituents	Remark
Carbohydrates	+
Flavoids	+
Tannins	+
Reducing Sugar	+
Saponins	+
Steroids	-
Phenols	-
Alkaloids	+
Terpenoids	-

Table 2: Qualitative Phytochemical Screening of Ripe *Musaacuminata* (Banana) Peel

Chemical Constituents	Remark
Carbohydrates	+
Flavoids	+
Tannins	+
Reducing Sugar	+
Saponins	+
Steroids	-
Phenols	-
Alkaloids	+
Terpenoids	-

Effect of Arsenic Acid Exposure and Extract Administration on Body Weight

Treatment with ethanolic extracts of *Cyperusesculentus* and Musa acuminata significantly boosted body weights in the test groups compared to the control group. Group E (treated with *Cyperusesculentus* extract) and Group C (treated with *Musa acuminata* extract) had final average body weights of 228.25 g and 219.25 g respectively compared to the control group with 186.52 g (Table 3 and Figure 1).

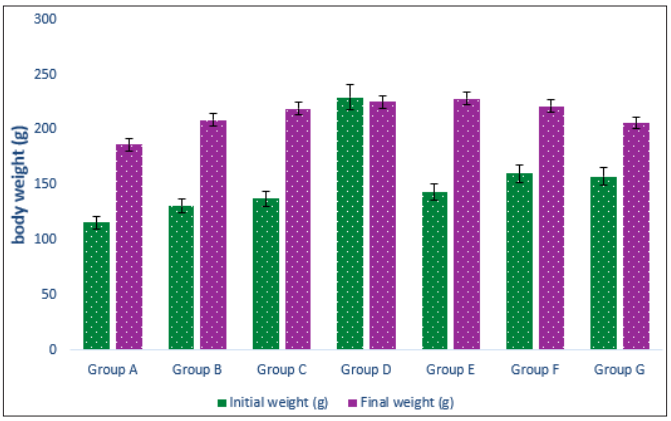


Figure 1: Effect of Ethanolic Extract of Cyperusesculenta and Ethanolic Extract of Musa Acuminatapeel on Body Weight Following Arsenic Acid Toxicity

Table 3: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of *Musa Acuminataon* Body Weight Following Arsenic Acid Toxicity

	Initial body weight (g)	Final body weight (g)	p-value	t-value
	MEAN±SEM	MEAN±SEM		
Group A	115.90±1.62	186.52±16.89	0.022*	-4.390
Group B	130.70±2.61	209.00±17.00	0.012*	-4.388
Group C	137.65±1.30	219.25±8.02	0.003*	-8.753
Group D	229.53±11.98	225.00±15.57	0.837#	0.216
Group E	143.23±1.80	228.25±8.87	0.001*	-11.950
Group F	160.50±3.20	221.50±14.85	0.039*	-3.502
Group G	157.55±0.75	206.00±28.00	0.326#	-1.778

Data was analysed using paired t-test and values were considered significant at p≤0.05, **SEM: Standard Error of Mean, Ema: Ethanolic Extract of Musa Acuminata, Ece: Ethanolic Extract of *Cyperusesculenta*, *: Significant, #: Not Significant.**

Relative Testicular Weight

Testicular weights were notably lower in the groups exposed to arsenic acid alone and with the extracts. Group D (arsenic acid only) had the highest mean relative weight of 0.76 g. In contrast, Group B (*Cyperusesculentus* extract) and Group C (*Musa acuminata* extract) had lower weights of 0.52 g and 0.54 g, respectively (Figure 2).

Table 4: Effect of Ethanolic Extract Of Cyperusesculenta and Ethanolic Extract of Musa Acuminata on Relative Testicular Weight Following Arsenic Acid Toxicity

	Relative testicular weight (g)
	MEAN±SEM
Group A	0.64±0.01
Group B	0.52±0.01* [a]
Group C	0.54±0.01# [a]
Group D	0.76±0.01#
Group E	0.61±0.07# [a]
Group F	0.63±0.07# [b, d]
Group G	0.57±0.09# [a]
F-ratio	3.600

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at p≤0.05. **SEM: standard error of mean, EMA: ethanolic extract of Musa acuminata, ECE: ethanolic extract of *Cyperusesculenta*, *: significant, #: not significant when comparison was made to group A, and a: significant, b: not significant when comparison was made to group D; c: significant and d: not significant when comparison was done between E and F.**

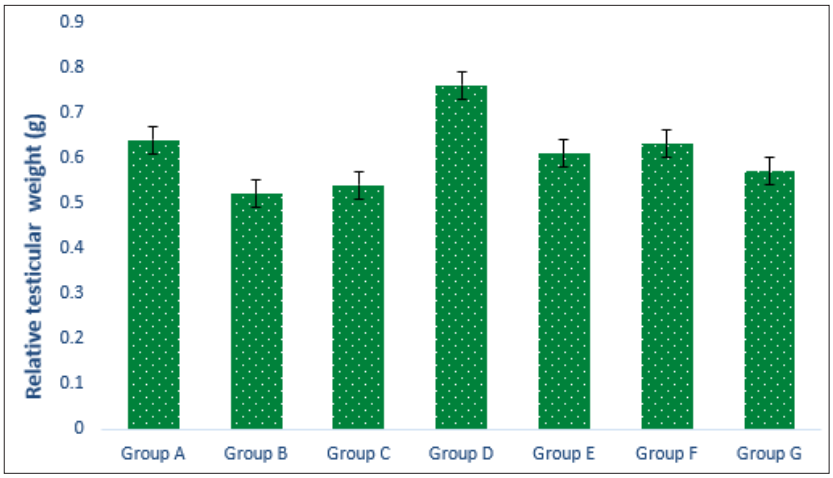


Figure 2: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of Musa Acuminata Peel on Relative Testicular Weight Following Arsenic Acid Toxicity

Effect of Arsenic Acid Exposure and Extract Administration on Total Sperm Count and Sperm Motility

Groups treated with the extracts showed the highest percentage of active motile sperm at 92.66% (Groups B and C), compared to 90% in the control group. Total sperm count was highest in Group C (85.46 x10^6/ml) and lowest in Group D (58.35 x10^6/ml), highlighting the protective benefits of the extracts (Table 5 and Figure 3).

Table 5: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of *Musa Acuminata* on Sperm Motility and Total Sperm Count

	Active motile sperm (%)	Non-motile sperm (%)	Total sperm count (x10^6/ml)
	MEAN±SEM	MEAN±SEM	MEAN±SEM
Group A	90.00±2.88	10.00±2.88	69.43±0.69
Group B	92.66±1.45#[a]	7.33±1.45*, [a]	77.96±2.79*, [a]
Group C	92.66±1.45# [a]	7.33±1.45*, [a]	85.46±0.93*, [a]
Group D	75.00±0.00*	25.00±0.00*	58.35±1.25*
Group E	92.50±0.50# [a]	7.50±0.50*, [a]	71.90±0.10*, [a]
Group F	82.50±2.50*, [c]	17.50±2.50*, [a, c]	67.25±3.85*, [a, d]
Group G	82.55±2.50* [a]	17.45±2.50*, [a]	66.20±2.80*, [a]
F-ratio	10.590	10.590	18.615

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p \leq 0.05$.

SEM: standard error of mean, EMA: ethanolic extract of Musa acuminata, ECE: ethanolic extract of Cyperusesculenta, *: significant, #: not significant when comparison was made to group A, and a: significant, b: not significant when comparison was made to group D; c: significant and d: not significant when comparison was done between E and F.

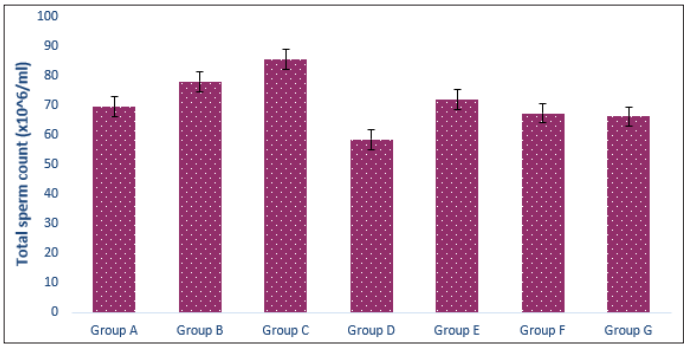


Figure 3a: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of *Musa Acuminata* on Total Sperm Count

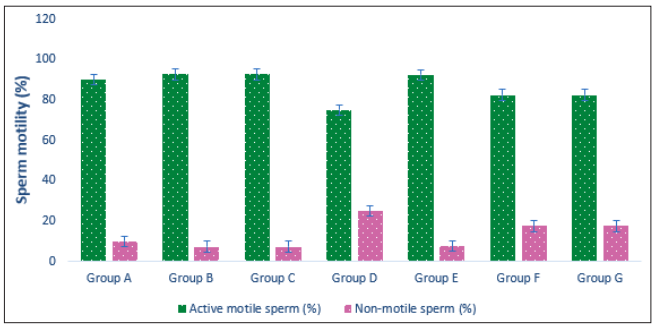


Figure 3b: Effect of Ethanolic Extract of *Cyperusesculenta* and Methanolic Extract of *Musa acuminata* on Sperm Motility

Effect of Arsenic Acid Exposure and Extract Administrationon Spermmorphology

Normal sperm cell percentages were highest in Group E (94.50%) and Group A (94%). Group D (arsenic acid only) had the lowest percentage of normal sperm cells at 77%, and the highest percentage of abnormal sperm cells at 23%, indicating arsenic acid's harmful effects without the extracts (Table 6; Figure 4).

Table 6: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of Musa Acuminata on Sperm Morphology

	Normal sperm cells(%)	Abnormal sperm cells (%)
	MEAN±SEM	MEAN±SEM
Group A	94.00±2.08	6.00±2.08
Group B	91.67±1.20#[a]	8.33±1.20*,[a]
Group C	91.00±0.57* [a]	9.00±0.57*,[a]
Group D	77.00±3.00*	23.00±3.00*
Group E	94.50±0.50# [a]	5.50±0.50*,[a]
Group F	82.50±2.50#,[b,d]	17.50±2.50*,[b, d]
Group G	85.50±0.00# [a]	15.00±0.00*,[a]
F-ratio	14.085	13.316

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p \leq 0.05$.

SEM: standard error of mean, EMA: ethanolic extract of Musa acuminata, ECE: ethanolic extract of Cyperusesculenta, *: significant, #: not significant when comparison was made to group A, and a: significant, b: not significant when comparison was made to group D; c: significant and d: not significant when comparison was done between E and F.

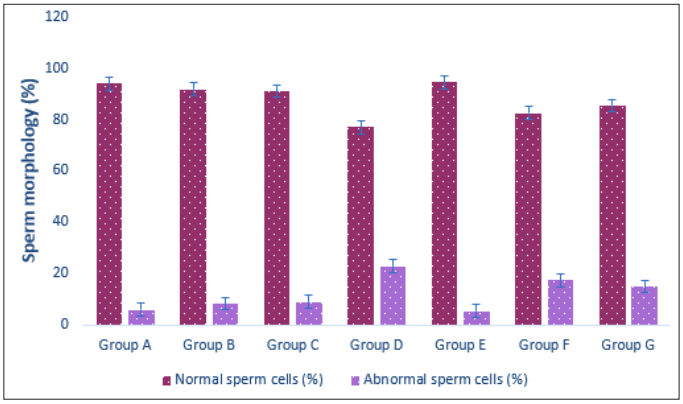


Figure 4: Effect of Ethanolic Extract of *Cyperusesculenta* and Methanolic Extract of *Musa acuminata* on Sperm Morphology

Effect of Arsenic Acid Exposure and Extract Administration Onserum Testosterone Level

The highest testosterone levels were found in Group C (3.49 ng/ml) and Group B (3.08 ng/ml), compared to the control group (2.12 ng/ml). Group D (arsenic acid only) had the lowest level at 0.31 ng/ml, demonstrating the protective effect of the extracts against arsenic-induced toxicity (Table 7; Figure 5).

Table 7: Effect of Ethanolic Extract of *Cyperusesculenta* and Ethanolic Extract of *Musa Acuminata* on Serum Testosterone Level

	Testosterone (ng/ml)
	MEAN±SEM
Group A	2.12±0.42
Group B	3.08±0.59#,[a]
Group C	3.49±0.63*,[a]
Group D	0.31±0.01*
Group E	2.02±0.01#,[a]
Group F	1.95±0.06#,[a, d]
Group G	2.07±0.06#,[a]
F-ratio	4.350

Data was analysed using ANOVA followed by post Hoc LSD multiple comparison and values were considered significant at $p \leq 0.05$, **SEM: standard error of mean, EMA: ethanolic extract of *Musa acuminata*, ECE: ethanolic extract of *Cyperusesculenta*, *: significant, #: not significant when comparison was made to group A, and a: significant, b: not significant when comparison was made to group D; c: significant and d: not significant when comparison was done between E and F.**

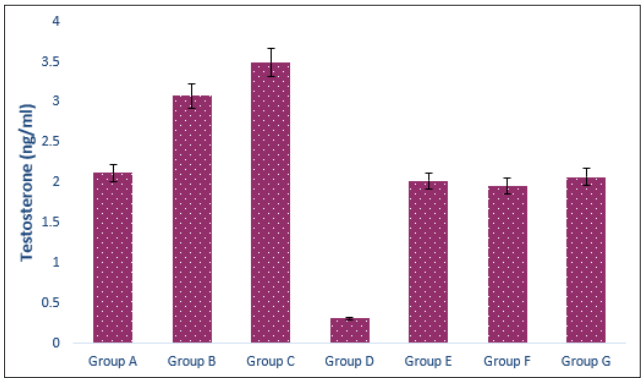
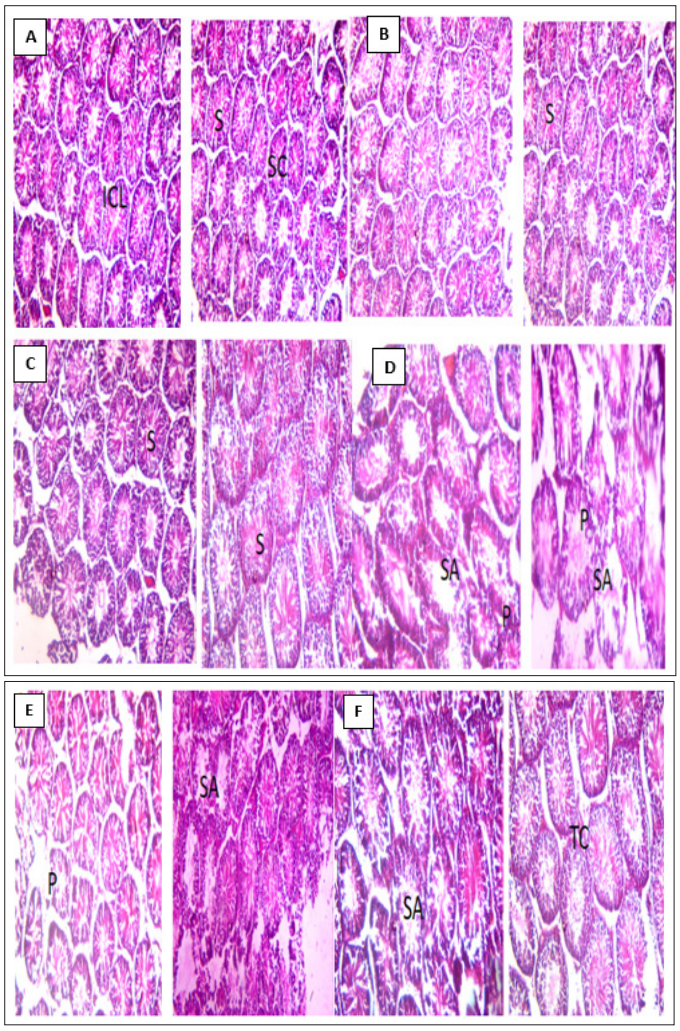


Figure 5: Effect of Ethanolic Extract of *Cyperusesculenta* and Methanolic Extract of *Musa acuminata* on Testosterone Level

Histological Findings

Arsenic-treated animal testes showed significant degeneration of the seminiferous tubules with severe spermatogenic arrest and pyknotic testicular cells (Figure 6). Treatment with extract alone and both with arsenic intoxication showed structurally and functionally active seminiferous tubules somewhat similar to those of the normal control.



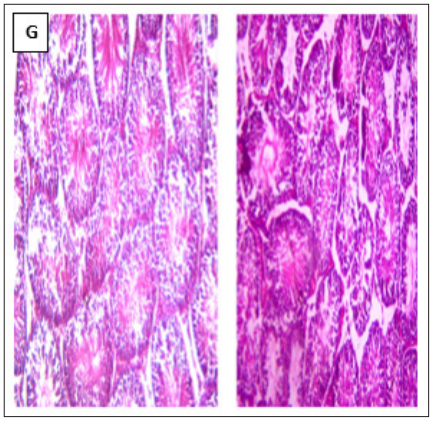


Figure 6: Hematoxylin and eosin stained testes section of (A) normal mice testes (x400), (B) extract only (ECE) and (C) extract only (EMA) shows seminiferous tubule active testicular cell and well enhanced spermatogenesis (x400), (D) toxins (As) group, shows marked severe degeneration of seminiferous tubules with severe spermatogenic arrest and pyknotic testicular cells (x400), (E) As + ECE pretreated, (F) As + EMA post-treated revealed moderate degeneration of seminiferous tubules with moderate spermatogenic arrest and pyknotic testicular cells (x400), (G) As + ECE + EMA testes section shows moderate degeneration of seminiferous tubules with moderate spermatogenic arrest and pyknotic testicular cells (x400).

ICL: Interstitial Cells of the Leydig, SC: Sertoli Cell, S: Spermatogenesis, SA: Spermatogenic Arrest, P: Pyknotic Testicular Cells, TC: Testicular Cells

Discussion

This study investigated the comparative effects of ethanolic Extract of *Cyperus Esculenta* (ECE) and ethanolic Extract of *Musa Acuminata* (EMA) on testes following arsenic acid-induced toxicity in male Wistar rats. Consistent with our results show that exposure to Arsenic Acid (As) decreased the body weight of the rats, as observed in the reduced final body weight and body weight change. Arsenic acid resulted in a non-significant increase in body weight, as indicated in group D (10 mg/kg). However, both extracts, at various doses, improved the body weight of the arsenic acid–exposed rats (Table 3). Administration of ECE and EMA alone showed a significant increase in body weight post-treatment after arsenic acid induction. Specifically, groups E (receiving 20 mg/kg of arsenic and 500 mg/kg of tiger nut) and F (receiving 20 mg/kg of arsenic and 500 mg/kg of banana peel) exhibited significant increases in body weight. The increase in body weight following the ethanolic extract of Musa acuminata peel alone and post-treatment with arsenic is attributed to the flavonoids contained in the extracts, as well as their high fiber content. reported a significant increase in body weight following the intake of *C. esculentus*, which aligns with our study findings. Flavonoids are known to aid in weight gain by regulating energy and immune status through the satiety center in the hypothalamus [33].

Moreover, treatment with arsenic acid resulted in increased relative testis weights compared to the normal control rats. The extracts themselves exhibited a tendency to increase testicular weights, and this effect was significant compared to normal control rats but not compared to lead-treated rats (Table 4). previously reported increased testicular weight following *C. esculentus* administration [32]. The relative testicular weights of the arsenic-treated rats that received 500 mg/kg of both extracts

were comparable to those of the normal control rats. However, the significant increase in relative testicular weight following arsenic toxicity could result from inflammation caused by free radical species of arsenic metabolites during its metabolism. reported a significant reduction in relative testicular weight following arsenic intake, which is inconsistent with our study's findings[34].

Consistent with previous studies, all sperm parameters evaluated in this study were negatively affected following administration of arsenic acid (As), implying that As impairs sperm function [46,35,36]. AS has been previously reported to decrease sperm motility, viability, and functional maturity [37]. Studies have also reported reduced sperm count in mice and disruption of spermatogenesis in rats following As administration [35,38]. Our study showed that arsenic intake in experimental rats resulted in a significant decrease in active sperm motility and an increase in non-motile sperm. The decreased total sperm count and motility, along with increased abnormal morphology of sperm in As-treated rats, may indicate oxidative stress. Increased ROS concentration in tissues has been postulated as a primary cause of disorders related to arsenic exposure [47,39]. Consistent with previous studies, rats administered *C. esculentus* extract alone had significantly increased sperm count and motility, and decreased abnormal sperm morphology compared to normal control rats, indicating the extract's ability to improve sperm function [32,40]. A recent study by on the aqueous extract of unripe *Musa paradisiaca* revealed enhanced sperm count and motility relative to the untreated control, which aligns with our findings. Both extracts, at various doses, were able to improve these sperm parameters in rats administered As [41]. The mechanism behind the significantly improved levels of total sperm count is linked to the scavenging of Reactive Oxygen Species (ROS) formation following arsenic intake by flavonoids and alkaloids, consistent with previous studies [40,42]. The ability of the extracts to reduce sperm abnormal morphology in As-treated rats showed a significant difference compared to both the normal control and arsenic groups (Table 6).

Exposure of rats to arsenic resulted in a marked decrease in testosterone levels (Fig. 5). A decrease in this hormone indicates impairment in spermatogenesis. Testosterone, secreted by the Leydig cells, is essential for the growth and division of the testicular germinal cells (the first stage in sperm formation) [40]. A decrease in testosterone production is evidence of male reproductive toxicity [43]. The decreased serum testosterone level in the rats administered As could explain the reduction in sperm count observed in group D, as low testosterone levels could not facilitate spermatogenesis. This study supports the work of [38,9]. Consistent with previous reports, rats administered the extracts alone had significantly increased serum testosterone levels compared to normal control rats (Table 7) [40,42]. The ability of the extracts to increase testosterone levels could be due to the presence of antioxidants like flavonoids and alkaloids in *C. esculentus* and *M. acuminata* [44,45]. These phytochemicals have powerful antioxidant properties and have been reported to be associated with improved serum testosterone levels in male rats and mice [40,42].

Furthermore, this study revealed that arsenic ingestion in experimental rats led to severe degeneration of seminiferous tubules, spermatogenic arrest, and pyknotic testicular cells (Fig. 6). The structural and morphological changes in the testes of mice in our study may be due to the accumulation of arsenic. It is well known that arsenic induces adverse toxic effects on the testes, such as reduced sperm production by decreasing the number of germinal epithelial layers in the testes [35]. Increased accumulation of arsenic in the testes in our study might also be associated with increased oxidative stress. This study supports the findings of, which indicate alterations in testicular architecture [46,39]. However, administration of ECE and EMA improved testicular architecture, leading to the regeneration of sperm cells, an increase in spermatogonia, and improved spermatogenesis.

Conclusion

This study demonstrates that both the Ethanolic Extracts of *Cyperusesculenta* (ECE) and *Musa Acuminata* (EMA) exhibit beneficial effects on male reproductive health compromised by arsenic acid toxicity in Wistar rats. These extracts significantly counteracted the negative impacts of arsenic on body weight, sperm parameters, and testicular architecture. ECE and EMA improved sperm count, motility, and morphology while enhancing testosterone levels, potentially due to their antioxidant properties. Despite arsenic's adverse effects, including testicular degeneration and reduced sperm production, treatment with these extracts facilitated significant recovery in reproductive metrics. This suggests that ECE and EMA have potential as therapeutic agents for mitigating arsenic-induced reproductive toxicity, highlighting their effectiveness in promoting testicular health and sperm quality. Consumption of both concurrently also provided a soothing effect against arsenic-induced testicular damage in Wistar rat model

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