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Semen characteristics and sperm morphology of *Pistia stratiotes* Linn. (Araceae) protected male albino rats (Wistar strain) exposed to sodium arsenite

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Abstract

Background: Sodium arsenite has been proven to be abundant in nature and released into the environment through human activities, including agricultural and industrial processes. The objective of our study was to investigate the sperm protective potential of *Pistia stratiotes* Linn. in arsenic-treated rats.

Methods: The sperm protective potential of *P. stratiotes* Linn. (Araceae) was carried out in arsenic-exposed rats using 24 male albino rats (225 to 228 g) aged between 14 and 16 weeks old. They were grouped into 4 (A–D), each group containing 6 rats. Group A animals were orally treated with 100 mg/kg ethanol leaf extract of *P. stratiotes* Linn. daily for 14 days; group B (sodium arsenite at 2.5 mg/kg body weight; positive control); group C (*P. stratiotes* extract for 14 days and single dose of sodium arsenite on day 14; group D (0.1 mL propylene glycol; negative control/vehicle).

Results: Group B had a significantly lower ($p < 0.05$) percentage sperm motility (26.7 ± 6.67 %) while group A had a significantly ($p < 0.05$) higher mean value (63.3 ± 3.33 %) when compared across the groups. The sperm motility of rats in group D was significantly higher ($p < 0.05$) than groups B and C. This implies that *P. stratiotes* extract had no adverse effect on sperm motility. The presence of *P. stratiotes* with sodium arsenite alleviated its harmful effect on sperm motility. The mean value obtained for sperm viability, semen volume and sperm count followed a similar pattern although the difference was not significant ($p > 0.05$) for semen volume and the sperm count of rats across the groups. Total sperm abnormality was 10.44 and 14.27 % with the sodium arsenite treated group having the highest value when compared with groups A treated with *P. stratiotes* extract and D treated with propylene, although the differences were not significant ($p > 0.05$).

Conclusions: The study concluded that ethanol leaf extract of *P. stratiotes* has no negative effect on sperm motility, viability and morphology and also protected spermatozoa against arsenic-induced reproductive toxicity in Wistar strain albino rats. Therefore, it may play an important role in the protection of populations with chronic sodium arsenite exposure.

Keywords: *Pistia stratiotes*, reproductive toxicity, sodium arsenite

Introduction

Sodium arsenite, which is derived from arsenic, has been proven to be abundant in nature and released into the environment through human activities including agricultural and industrial processes [1]. Human exposure to sodium arsenite occurs through drinking water, food, and atmosphere [2].

Arsenic, as trivalent arsenite (As^{3+}) or pentavalent arsenate (As^{5+}), is naturally occurring and ubiquitously present in the environment. Arsenical compounds are environmental toxins with multiple effects in animal and human populations [2, 3]. Humans are exposed to arsenic mainly through oral or inhalation routes. Oral exposure occurs via consumption of contaminated water, food, drugs, and exposure can be lifelong. Occupational exposure, on the other hand, occurs mainly through inhalation via nonferrous ore smelting, semiconductor and glass manufacturing, or power generation by the burning of arsenic-contaminated coal [3].

The main source of environmental arsenic exposure in most populations is drinking water in which inorganic form of arsenic predominates [4, 5]. Assessing the risk from exposure to inorganic arsenic in water supplies is a key issue facing the scientific community. High levels of

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arsenic in drinking water can be found in areas within many countries including Taiwan, China, Chile, Mexico, Argentina, Thailand, Finland, Hungary and, Bangladesh [5–7], but it is becoming evident that even the low levels of arsenic typically found in India may pose a significant health risk to humans [6, 7].

The frequent uses of arsenic as herbicides, insecticides, rodenticides, food preservatives, and byproduct of used fossil fuel are challenging the aquatic environment. Although chronic dermal toxicity, nephrotoxicity, and skin cancer all occur with arsenic exposure [7], arsenic is a multi-site carcinogen in humans, causing tumors in a variety of tissues including lung, skin, and bladder [2, 7]. Other studies indicate that the kidney, liver, uterus and prostate may also be target sites of arsenic carcinogenesis [7, 8]. Recently arsenic intoxication in experimental animals has been associated with hepatic tumors [8], inhibition of ovarian steroidogenic function and gonadotrophins secretion [9]. Arsenic exposure also has been associated with an elevation of adrenocortical steroidogenesis and plasma corticosterone level [10], as well as severe metabolic disorders such as diabetes in humans [11]. Acute arsenic exposure may cause gastrointestinal tract disorders [12], whereas chronic exposure may exert degenerative, inflammatory, and neoplastic changes of the respiratory, hematopoietic, cardiovascular, and nervous systems [13]. The effect of sodium arsenite on male reproductive system has been reported [14, 15]. Arsenite intoxication is associated with spermatotoxicity [2, 16], inhibition of testicular androgenesis and reduction of the weight of the testes and accessory sex organs [17] in experimental animals. However, the actual molecular events resulting in male reproductive toxicity from exposure of inorganic arsenic remain unclear. There are several possible mechanisms for the anti-gonadal activities of different chemicals. They may exert a direct inhibitory action on the testis; they may affect pituitary causing changes in gonadotrophins concentrations and thus spermatogenic impairment.

Pistia stratiotes Linn., commonly known as water lettuce or water cabbage, is an aquatic plant, stoloniferous, floating on lakes, streams, and stagnant water ponds. It is distributed in the tropical and sub-tropical region of Asia, Africa, and America [18]. Several medicinal properties have been ascribed to this plant including anthelmintic, anti-microbial and anti-fungal properties [19–21]. The anti-inflammatory and anti-pyretic activity of ethanolic extract of *P. stratiotes* has been demonstrated using carrageenan, cotton-pellet-induced granuloma model and brewer's yeast fever model [21].

P. stratiotes leaves are among the forages usually fed to domestic animals in South Eastern Nigeria but there is dearth of information on its effects on the spermatozoa characteristics and morphology. This study was therefore carried out to investigate the sperm protective potential of *P. stratiotes* Linn. in arsenic-treated rats.

Materials and methods

Chemicals

Sodium arsenite (Ioba, Chemie Co. India) was dissolved in glass distilled water. A dose of 2.5 mg/kg body weight was administered according to the guidelines for *in vivo* assays in rats [22]. Freshly prepared stock solution was used for the experiments.

Collection and extraction of plants

P. stratiotes Linn. leaves were collected from the botanical garden of the University of Ibadan and authenticated at the Department of Botany, University of Ibadan, Ibadan, Nigeria. The specimen voucher of the leaf (Voucher No. UIH-22422) was deposited in the herbarium of the Department. Leaves of *P. stratiotes* were washed with clean water and air-dried under shade. Dried leaves were ground into fine powder and defatted in hexane. Cold extraction was performed by soaking the defatted ground leaves in 96 % ethanol for 72 h with constant shaking. Extract was collected and concentrated using rotary evaporator (Bűchi 011, USA) under reduced pressure at a temperature of 40 °C to obtain the ethanol extract used in this study.

Extract suspensions were freshly prepared in Propylene glycol, which served as the vehicle and negative control. Suspensions were administered orally to the rats using cannula at a dose of 100 mg/kg body weight. Volumes of extract administered did not exceed 0.2 mL. Prepared suspensions were kept at room temperature in the Laboratory. All reagents were of analytical grade and purchased from Sigma Chemical Co. (USA).

Phytochemical screening

The extract was screened for detection of different components according to the methods previously described by Evans [23] and Sofowora [24]. The metabolites tested for were tannins, saponins, cardiac glycosides, flavonoids, steroids and alkaloids.

Experimental animals

Twenty-four apparently healthy male albino rats weighing between 225 and 228 g were used for this study. Animals were obtained from the experimental animal house of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Animals were kept in steel laboratory cages (60 × 60 × 50 cm). All animals were kept under controlled conditions of temperature (25 ± 2 °C), relative humidity (50 ± 15 %) and normal photoperiod (12 h light and 12 h dark). The rats were allowed to acclimatize for

a period of two weeks before the commencement of the experiment. They had access to rat diet (commercial pellet diet from Kesmac Feed Industry, Ibadan, Oyo State, Nigeria) and water *ad libitum*. This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan.

Experimental protocol

Twenty-four clinically healthy male albino rats were grouped into 4 (A–D) with 6 rats in each group. The treatment groups were as follows: group A was treated with 100 mg/kg body weight leaf extract of *P. stratiotes* for 14 days, B (single dose of SA 2.5 mg/kg body weight – positive control), C (ethanol extract of *P. stratiotes* for 14 days and SA added on the 14th day) and D (0.1 mL Propylene glycol for 14 days – negative control/vehicle).

Animals in groups B and D served as positive and negative controls respectively. Samples were collected from all the animals twenty-four hours after the last treatment, after which they were sacrificed by cervical dislocation.

Semen collection and analysis

The rats were anaesthetized with diethyl ether before being euthanized, the mid caudoventral abdominal incision was made with sterilized scissors, permitting instant access to the testis once pushed upward from the scrotum. The testes were then separated from the epididymis. The right and left epididymis were trimmed off the body of the testes and semen sample was collected from the tail of the epididymis through an incision made with a scalpel blade [25]. The semen was dropped on warm glass slide and stained using warm Wells and Awa stains for morphological studies and staining for live/dead ratio was done using Eosin-Nigrosin stain. Also, percentage motility was carried out using 2 to 3 drops of 2.9% warm buffered sodium citrate kept at body temperature as described by Zemjanis [26].

Percentage viability

This was done according to Zemjanis [26] by staining one drop of semen and a drop of warm Eosin-Nigrosin stain on a warm slide. A thin smear was then made of mixture of semen and stain. The smear was air dried and observed under the microscope. The ratio of the *in vitro* dead sperm cells was observed and it was based on the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repelled the stain.

Percentage motility

This was evaluated using a drop of semen with drop of 2.9% buffered sodium citrate on a warm glass slide covered with a glass slip and viewed at a magnification of ×40. Only sperm cells moving in a unidirectional motion were included in the motility rating, while sperm cells moving in circles, in backward direction or pendulating movement were excluded [26].

Data analysis

The data generated was expressed in mean ± SEM and analyzed using one way analysis of variance (ANOVA). SPSS Version 15 for Windows (SPSS Inc., 2006) and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were used to carry out all procedures. The values for $p < 0.05$ were considered to be statistically significant.

Results

Phytochemical screening of ethanol extract of *P. stratiotes* revealed the presence of tannins, saponins, cardiac glycosides, flavonoids and the absence of steroids and alkaloids (Table 1).

Table 1: Qualitative phytochemical screening of ethanol extract of *P. stratiotes* Linn. (PSE).

	Constituents	Qualitative tests	Inference
1	Tannins	Ferric chloride	+ve
2	Saponins	Frothing test	+ve
3	Cardiac glycosides	Killer-killanins	+ve
4	Flavonoids	Ferric chloride	+ve
5	Steroids	Salkowski's	–ve
6	Alkaloids	Dragendorff's	–ve

Note: +ve = present, –ve = absent.

It was observed that the animals treated with sodium arsenite (group B) had the lowest mean percentage motility (26.7 ± 6.67%) when compared across all the groups and the difference was significant ($p < 0.05$) (Figure 1). This indicates that exposure to arsenite had an adverse effect on sperm motility in albino rats. On the other hand, animals treated with leaf extract of *P. stratiotes* (group A) had the highest mean value of motility (63.3 ± 3.33%) when compared across the groups and the differences were significant ($p < 0.05$) as shown in Figure 1.

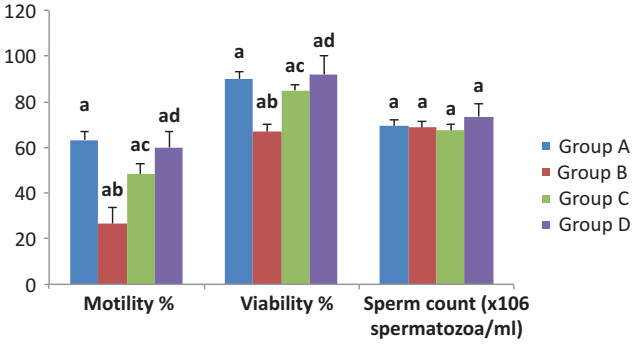

Figure 1: Mean values for percentage motility, viability and sperm count of albino rats in different treatment groups.

It was also observed that the mean percentage sperm progressive motility of rats in group D (treated with propylene glycol) was significantly higher ($p < 0.05$) than groups B (treated with sodium arsenite) and C (treated with *P. stratiotes* extract and sodium arsenite). This indicates that *P. stratiotes* extract had no adverse effect on the sperm motility of rats and it also ameliorates the adverse effect of arsenite on motility.

The mean values obtained for sperm volume for groups A, B, C and D were 5.13 ± 0.33, 5.15 ± 0.33, 5.18 ± 0.02 and 5.18 ± 0.03, respectively. The mean value obtained for sperm viability, semen volume and sperm count followed a similar pattern, although the difference was not significant ($p > 0.05$) for semen volume and the sperm count of rats across the groups.

The mean total percentage abnormality in various parts of the sperm cells ranges between 10.44 and 14.27 % with arsenite-treated group B having the highest number of abnormal sperm cells when compared across the groups. Group C (treated with *P. stratiotes* extract for 14 days and sodium arsenite on the 14th day) had the lowest mean percentage abnormality although the differences across the groups were not significant ($p < 0.05$). This implies that sodium arsenite can affect sperm cells structurally or morphologically. Co-treatment with *P. stratiotes* Linn. leaf extract was found to protect against adverse effects of sodium arsenite (Table 2).

Discussion

The protective effects of ethanol leaf extract of *P. stratiotes* Linn. on testicular toxicity of sodium arsenite were investigated in male Wistar rats.

Sperm parameters such as motility, counts and morphology are vital indices of male fertility as these are markers in testicular spermatogenesis. The result of this study showed a significant decrease ($p < 0.05$) in the mean percentage motility of rats in groups B and C (26.70 and 48.33 %) respectively. These values were below the minimum required value of 60 % [27] for successful breeding to occur. This indicated that sodium arsenite had an adverse effect on sperm motility and livability, therefore will reduce fertility potential of albino rats. The observed effect may be due to the spermatotoxic property of arsenite as reported by Waalkes et al. [2] and Pant et al. [16]. It is also in agreement with another report that arsenite intoxication is associated with inhibition of testicular androgenesis and reduction of the weight of the testes and accessory sex organs [17] in experimental rats.

The sperm viability was significantly reduced ($p < 0.05$) in groups B and C rats when compared with other treatment groups, although the values were higher than 60 % required for an animal to be classified as satisfactory potential breeder. However, these changes were significant ($p < 0.05$) and therefore imply that a further administration of SA will negatively affect the sperm viability and also reduce the fertility potential of male albino rats. The rats in groups A treated with leaf extract of *P. stratiotes* and D treated with propylene glycol had significant increase ($p < 0.05$) in their sperm motility and viability. This implies that the extract has a protective effect against arsenic toxicity and therefore will boost the sperm fertility potentials of male albino rat. This finding is similar to a report by Balamurugan et al. [28] in which ethanol leaf extract of *Melastoma malabathricum* enhanced sperm motility of male albino rat but is contrary to the report of Singh et al. [29] that

Table 2: Mean values for spermatozoa morphology of albino rats in different treatment groups.

Parameters (%)	Group A	Group B	Group C	Group D
Tailless head	1.24 ± 0.14^{a}	1.49 ± 0.14^{a}	1.44 ± 0.07^{a}	1.12 ± 0.16^{a}
Headless tail	1.15 ± 0.21^{a}	1.08 ± 0.08^{a}	0.94 ± 0.19^{a}	1.30 ± 0.12^{a}
Rudimentary tail	0.50 ± 0.15^{a}	0.41 ± 0.08^{a}	0.29 ± 0.07^{a}	0.49 ± 0.09^{a}
Bent tail	2.23 ± 0.23^{a}	2.90 ± 0.29^{ab}	1.85 ± 0.33^{ac}	2.54 ± 0.28^{d}
Curved tail	2.56 ± 0.23^{a}	2.57 ± 0.36^{a}	2.14 ± 0.45^{a}	2.36 ± 0.08^{a}
Curved midpiece	2.64 ± 0.27^{a}	2.99 ± 0.01^{ab}	1.82 ± 0.31^{ac}	2.41 ± 0.18^{d}
Bent midpiece	2.48 ± 0.16^{a}	2.49 ± 0.24^{a}	1.93 ± 0.37^{a}	2.35 ± 0.07^{a}
Lopped tail	0.33 ± 0.08^{a}	0.33 ± 0.08^{a}	0.25 ± 0.09^{a}	0.31 ± 0.06^{a}
Total abnormal cells	13.13 ± 0.41^{a}	14.27 ± 0.88^{a}	10.44 ± 1.86^{a}	12.88 ± 0.57^{a}
Total normal cells	86.86 ± 0.41^{a}	85.73 ± 0.88^{a}	89.89 ± 1.86^{a}	87.12 ± 0.57^{a}

Note: Values are reported as mean ± SEM. Means with the same superscripts are not significantly different at ($p > 0.05$) value of significance along rows.

ethanol extract of *P. stratiotes* caused a significant decrease in sperm viability of mice.

There were no significant changes ($p > 0.05$) in the semen volume obtained across the groups. However, the sperm count in group A rats treated with *P. stratiotes* extract was the highest ($69.33 \pm 2.60 \times 10^6$ spermatozoa/mL) when compared with the other groups even though the difference was not significant ($p > 0.05$). This observation is a further indication that the plant extract has a property that can boost sperm count and this was supported by the report of Balamurugan et al. [28].

The total sperm abnormality obtained across the groups ranges between 10.44 and 14.27 % with group B treated with sodium arsenite (SA) having the highest value when compared with groups A and D treated with *P. stratiotes* extract. This implies that arsenite has adverse effect of increasing the structural or morphological abnormalities of rat's spermatozoa but can be attenuated by co-treatment with *P. stratiotes* leaf extract.

In conclusion, this study showed that ethanol leaf extract of *P. stratiotes* has no negative effect on sperm motility, viability and morphology. The protective effects of *P. stratiotes* against arsenite reproductive toxicity in Wistar strain albino rats have also been shown as evidenced by a clear attenuation of arsenite-induced damage sperm functions and reproductive indices. This plant extract may potentially play an important role in the protection of populations chronically exposed to arsenite.

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