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Full Length Research Paper

Effects of *Ageratum Conyzoides* on Semen Characteristics and Sperm Morphology in Rats Exposed to Sodium Arsenite

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ABSTRACT

Arsenite is a major environmental toxicant that is well known to cause reproductive injury. The sperm protective potential of *Ageratum conyzoides* Linn in arsenic-treated rats was carried out in this study taking advantage of the antioxidant constituents and its androgenic activities. Twenty-four male albino rats aged 16 weeks, weighing 225 to 228g were used. They were grouped into 4(A-Da) with each group containing 6 rats. Group A was orally treated with 100mg/kg ethanol leaf extract of *Ageratum conyzoides* L., daily for 14 days, group B (single oral dose of sodium arsenite 2.5 mg/kg body weight), C (*Ageratum conyzoides* extract daily for 14 days and sodium arsenite (SA) given on the 14th day) and group D (Propylene glycol as negative control). It was observed that group B had a more lower ($p<0.05$) percentage motility (26.7±6.67%) when compared across the groups while group A had a significantly higher ($p<0.05$) mean value (63.3±3.33%). The sperm motility of rats in group D was significantly higher ($p<0.05$) than groups B and C. This implies that *A. conyzoides* extract had no adverse effect on the sperm motility of the rats and also ameliorates the adverse effect of arsenite on sperm motility. The mean value obtained for sperm liveability, semen volume and Sperm concentration followed a similar pattern although, the differences were not significant ($p>0.05$) for semen volume and the Sperm concentration of rats across the groups. 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, although, the differences were not significant ($P>0.05$). The study concluded that ethanol leaf extract of *Ageratum conyzoides* has no negative effect on sperm motility, liveability characteristics and morphology and also protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats..

Keywords: *Ageratum conyzoides*, sperm abnormality, Sperm concentration, sodium arsenite

INTRODUCTION

Arsenicals are used as herbicides, fungicides and rodenticides and may cause air, soil and water pollution. Arsenical exposure through drinking water is common in many areas animals are raised (Borzsonyl *et al.*, 1992, Chatterjee *et al.*, 1993) Exposure to arsenic is associated with metabolic disorders, hypertrophy of adrenal glands (Biswas *et al.*, 1994), adverse effects on reproductive organs (Vijaya *et al.*, 2010, Vijaya *et al.*, 2011, Vijaya *et al.*, 2012) and anemia (Sarkar *et al.*, 1992). A number of sulfhydryl containing proteins and enzyme systems have been found to be altered by exposure to arsenite (Robert and Judd, 1986).

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Other studies indicate that the kidney, liver, uterus and prostate may also be target sites of arsenic carcinogenesis in humans (Waalkes *et al.,* 2000). Recently arsenic intoxication in experimental animals has been associated with hepatic tumors (Waalkes *et al.,* 2000), inhibition of ovarian steroidogenic function and gonadotrophins secretion (Chattopadhyay *et al.,* 1999). Arsenic exposure also has been associated with an elevation of adrenocortical steroidogenesis and plasma corticosterone level (Ghosh *et al.,* 1999), as well as severe metabolic disorders such as diabetes in humans (Tseng *et al.,* 2002). Acute arsenic exposure may cause gastrointestinal tract disorders (Goebl *et al.,* 1990), whereas chronic exposure may exert degenerative, inflammatory, and neoplastic changes of the respiratory, hematopoietic, cardiovascular, and nervous systems (Neiger and Osweiler, 1989). The effect of sodium arsenite on male reproductive system is not well established, although there are some reports in which arsenite intoxication is associated with spermatotoxicity (Pant *et al.,* 2004), inhibition of testicular androgenesis and reduction of the weight of the testes and accessory sex organs (Sarker *et al.,* 2003) in experimental animals. However, the actual molecular events resulting in male reproductive toxicity from exposure of inorganic arsenic remain unclear.

Ageratum conyzoides is a common annual herbaceous weed with long history of traditional medicinal use in many countries especially in the tropical regions. Extracts and metabolites from this plant is widely utilized in traditional medicine as a purgative, analgesic and as a heart tonic (Tandon *et al.,* 1994). Herbal preparations from the leaves of *Ageratum conyzoides* has been used in the treatment of high blood pressure, fever, diabetes, pneumonia and numerous infectious diseases (Xuan *et al.,* 2004).

Ageratum conyzoides is widely utilized in traditional medicine systems wherever it grows, although applications vary by region. Traditional communities in India use this plant as a bacteriocide, antidysenteric, and antilithic (Okunade, 2002), and in Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide (Okunade, 2002). In Central Africa it is used to treat pneumonia, but the most common use of this plant is to cure wounds and burns (Okunade, 2002). *A. conyzoides* is also reported useful in treatment of fever, rheumatism, headache and colic (Okunade, 2002). In Cameroon, aqueous extracts of the leaves or the whole plant have been reported to be used as an antidiabetic (Tsabang *et al.,* 2001).

Ageratum conyzoides L. *leaves* are among the forages usually fed to domestic animals in South Eastern Nigeria (Oladejo *et al.,* 2003) but there is dearth of information on its effects on the spermatozoa characteristics and morphology of Wistar strain albino rats exposed to arsenic toxicity. This study was therefore carried out to investigate the sperm protective potential of *Ageratum conyzoides* L. in arsenic-exposed rats.

MATERIALS AND METHODS

Chemicals

Sodiun Arsenite (SA) (0.05 M $NaAsO_2$, Sigma–Aldrich, USA) was diluted with glass-distilled water to concentrations of 2.5 mg/kg body weight corresponding to 1/10th of the oral LD50 of the salt. Freshly prepared solution was used for the experiment.

Collection and Extraction of *A. conyzoides* leaves

Ageratum conyzoides L. leaves were collected from the botanical garden of the University of Ibadan and authenticated at the Department of Botany, University of Ibadan, Nigeria. The specimen voucher of the leaf (Voucher No. UIH-22423) was prepared and deposited in the herbarium of the same Department. Leaves of *Ageratum conyzoides leaves* were cleaned, air-dried, ground into fine powder and defatted in hexane. Cold extraction was done by soaking the defatted ground leaves in 96% ethanol. Extract was collected and concentrated using rotary evaporator under reduced pressure at a temperature of 40°C to obtain the ethanol extract used in this study. The yield of the extraction process was 12.10%.

Extract suspensions were freshly prepared in Propylene glycol, which served as vehicle and negative control. Suspensions were administered orally to the rats at a dose of 100 mg/kg body weight. Volumes of extract administered did not exceed 0.2 ml. Prepared suspensions were kept at 4^{O}C for use in the Laboratory.

Phytochemical screening

Ageratum conyzoides L leaf extract was subjected to the phytochemical test using Trease and Evans (Trease and Evans, 1983) and Harbourne (Harbourne, 1983) methods for Alkaloids, Saponins, Tannins, Anthraquinones, Flavonoids and Cardenolides.

Experimental animals

Twenty-four male albino rats aged 16 weeks, weighing between 225 and 228g were used after the ethical clearance had been obtained for the study. Animals were obtained from the experimental animal house of the Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Animals were healthy and kept in steel laboratory cages (60 × 60 × 50 cm). All animals were kept under controlled conditions of temperature (25 ± 02°C), relative humidity (50 ± 15%) and normal photoperiod (12 hour light and 12 hour dark). The animals were fed on a standard rat diet (commercial pellet diet from Kesmac Feed Industry, Ibadan, Oyo State, Nigeria) and water provided *ad libitum*.

Experimental protocol

Twenty-four clinically healthy male albino rats were grouped into 4 (A-D) with 6 rats in each group. Animals were acclimatized before use and the treatment groups were as follows: group A was treated with 100mg/kg body weight leaf extract of *Ageratum conyzoides*, B (SA 2.5 mg/kg body weight), C (ethanol extract of *Ageratum conyzoides* for 14 days and SA added on the 14th day), D (0.1ml Propylene glycol).

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 with SA, after which they were sacrificed by cervical dislocation.

Semen collection and analysis

The rats were anaesthetized with diethyl ether before sacrifice, the mid caudoventral abdominal incision was made with sterilized pair of scissors, permitting instant access to the testis once pushed upward from the scrotum with gloved hand. The testes were then separated from the epididymis. The right and left epididymides were trimmed off the body of the testes and semen sample was collected thereafter from the tail of the epididymis through an incision made with a scalpel blade. A drop of semen sample was placed on warm glass slide and stained using warm Wells and Awa stains for sperm cell morphological studies (Wells and Awa, 1970) and staining for liveability 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 (Zemjanis, 1970).

Percentage liveability

This was carried out by staining one drop of semen using one 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 is based upon the principle of Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repel the stain (Zemjanis, 1970).

Percentage motility

It was evaluated with 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.

Data analysis

The data generated was 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.

RESULTS

The yield of the extraction process was 12.10% (w/w). Phytochemical screening of ethanol leaf extract of *A. conyzoides* revealed the presence of alkaloids, saponins, tannins, anthraquinones, flavonoids cardenolides and terpenes (Table 1).

Table 1:
Phytochemical screening of ethanol extract of Ageratum conyzoides

	Constituents	Inference
1.	Alkaloids	+
2.	Saponins	+
3.	Tannins	+
4.	Anthraquinone	+
5.	Flavonoids	++
6.	Cardenolides	+
7.	Terpenes	+

+ = present ; + + = abundant

It was observed that group B treated with sodium arsenite had the lowest mean percentage motility (26.7±6.67%) when compared across the groups and the differences were significant ($p<0.05$. On the other hand, group D being the negative control (treated with propylene glycol) had the highest mean value of motility (58.00±5.83%) when compared across the groups and the differences were significant ($p<0.05$).

The mean percentage sperm motility of rats in groups A (53.33±4.94) treated with A*geratum conyzoides* extract and D (58.00±5.83) treated with propylene were significantly higher ($p<0.05$) than groups B (26.67±6.67) treated with sodium arsenite (SA) and C (48.33±4.77) treated A*geratum conyzoides* for 14 days and sodium arsenite added on the 14th day (Table 2).

Table 2:
Mean values for percentage motility, liveability, volume and Sperm concentration of albino rats in different treatment groups.

Parameters	Group A	Group B	Group C	Group D
Motility (%)	53.33± 4.94[a]	26.67±6.67[ab]	48.33±4.77[c]	58.00± 5.83[d]
Percentage liveability (%)	91.33±2.26[a]	66.67±3.33[bc]	85.00± 2.24[cd]	94.60±1.29[d]
Volume (mls)	5.18±0.02[a]	5.17±0.03[a]	5.18±0.02[a]	5.16±0.02[a]
Sperm concentration (x10^6 spermatozoa/ml)	67.33±3.09[a]	68.67±2.40[a]	67.33± 2.87[a]	69.20±2.52[a]

Values are reported as mean±SEM
Means with the same superscripts are not significantly different at (P<0.05) level of significance along rows

Table 3:
Mean values for spermatozoa morphology of albino rats in different treatment groups

Parameters (%)	Group A	Group B	Group C	Group D
Tailess head	1.15±.0.13[a]	1.49±0.14[a]	1.44±0.07[ac]	1.04±0.16[ad]
Headless tail	1.32±.0.12[a]	1.08±0.08[a]	0.94±0.19[a]	1.29±0.09[a]
Rudimentary tail	0.37±0.05[a]	0.41±0.08[a]	0.29±0.07[ac]	0.49±0.08[ad]
Bent tail	2.09±0.28[a]	2.90±0.29[ab]	1.85±0.33[ac]	2.54±0.20[d]
Curved tail	2.59±0.19[a]	2.57±0.36[a]	2.14±0.45[a]	2.79±0.16[a]
Curved midpiece	2.43±0.15[a]	2.99±0.01[ab]	1.82±0.31[a]	2.73±0.07[ad]
Bent midpiece	2.51±0.21[a]	2.49±0.24[a]	1.93±0.37[ac]	2.74±0.11[ad]
Lopped tail	0.37±0.054[a]	0.33±0.080[a]	0.25±0.088[a]	0.35±0.059[a]
Total abnormal cells	12.84±0.38[a]	14.27±0.88[a]	10.44±1.86[ac]	14.18±0.47[ad]
Total normal cells	87.16±0.38[a]	85.73±0.88[bc]	89.89±1.86[cd]	85.82±0.47[d]
Total cells	405.00±1.83[a]	401.67±1.67[a]	1003.33±599.34[a]	583.25±179.83[a]

Values are reported as mean±SEM .
Means with the same superscripts are not significantly different at (P<0.05) level of significance along rows

The mean value obtained for percentage sperm liveability, semen volume and Sperm concentration followed a similar pattern although, the difference was not significant for semen volume and the Sperm concentration of rats across the groups (Table 2).
The mean total percentage abnormality of sperm cells in all the groups ranged 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 had the lowest mean percentage abnormality although the difference across the groups was not significant (p<0.05) (Table 3). This implies that sodium arsenite can affect sperm cells structurally or morphologically.

DISCUSSION

The semen characteristics obtained in this study showed a significant low (p<0.05) 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% (Reece, 1997) for successful breeding to occur. This indicated that sodium arsenite (SA) had an adverse effect on sperm motility and 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*. (2003) and Pant *et al*. (2004). It is also in agreement with Sarkar *et al.* (2003) in which arsenite intoxication is associated with inhibition of

testicular androgenesis and reduction of the weight of the testes and accessory sex organs in experimental rats. It was observed that the sperm liveability was significantly lower ($p<0.05$) in groups B and C when compared across the groups, although the values were higher than 60% requirement for qualification as potential breeder. However, these changes were significant ($p<0.05$) and therefore imply that a further administration of SA will negatively affect the sperm liveability and reduce the fertility potential of male albino rats. The rats in groups A and D treated with leaf extract of *Ageratum conyzoides* had significant increase ($p<0.05$) in their sperm motility and liveability. This implies that the extract has a protective effect against arsenic toxicity and therefore will boost the sperm fertility potentials of male albino rat possibly due to their intrinsic antioxidant properties as revealed in the phytochemical screening. Previous reports by Yosiki *et al*. (1998) and Kahkonen *et al.* (1999) alluded to this fact. This finding is similar to a report by Balamurugan *et al.* (2013) in which ethanol leaf extract of *Melastoma malabathricum* enhanced the sperm motility of male albino rat.

There were no significant changes ($P>0.05$) in the semen volume obtained across the groups. However, the Sperm concentration in group A rats treated with *Ageratum conyzoides* extract was the highest (69.33 ± 2.60 $x10^6$cells/ml) when compared with the other groups even though the difference was not significant ($P>0.05$). This is another indication that the plant extract has a potential to boost Sperm concentration and this agrees with the report of Balamurugan *et al.* (2013) in which ethanol leaf extract of *Melastoma malabathricum* enhanced the Sperm concentration of male albino rat but is at variance to the report of Singh *et al.* (2014) in which ethanol extract of *Pistia stratiotes* caused a significant decrease ($p<0.05$) in Sperm concentration of experimental mice.

The total sperm abnormality obtained across the groups ranged 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, although, the differences were not significant ($P>0.05$). This implies that arsenite has a negative effect by increasing the structural or morphological abnormalities of spermatozoa but can be ameliorated when the same rats are treated with *Ageratum conyzoides* ethanol leaf extract.

In conclusion, the ethanol leaf extract of *Ageratum conyzoides* has no negative effect on sperm motility, liveability and morphology and also moderately protected spermatozoa against arsenic reproductive toxicity in wistar strain albino rats.

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