academicJournals

Vol. 6(2), pp. 63-66, February, 2014
DOI: 10.5897/JVMAH2013.0245

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Spermatozoa morphology and characteristics of *Spondias mombin* L. (*Anacardiaceae*) protected male Wistar rats exposed to sodium arsenite

Olufunke Ola-Davies[1], Ajani O. S.[2]* and Oyeyemi M. O.[2]

[1]Department of Veterinary Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
[2]Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

Accepted 30 December, 2013

The sperm protective potential of *Spondias mombin* L in arsenic-treated rats was carried out. Thirty-five male albino rats (225 to 228 g) were used and grouped into 7 (A to G), each group containing 5 rats. Group A was treated with 0.1 ml dimethyl sulphoxide (DMSO), B (0.1 ml of distilled water), C (sodium arsenite (SA-2.5 mg/kg body weight), D (ethyl acetate fraction), E (ethyl acetate fraction for 7 days and sodium arsenite the 7th day), F (methanolic fraction for 7 days and sodium arsenite the 7th day) and G (methanolic fraction for 7 days). It was observed that groups G and E had the lowest percentage motility 25.00±15.00 and 38.00±13.90%, respectively which were significantly lower (P<0.05) than groups A and B (89.00±2.45 and 85.00±3.16%), respectively. Percentage motility of groups A to D was similar but higher (P<0.05) than groups E to G. The morphological characteristics ranged between 10.88 and 12.74% in all the groups. In this study, motility of the sperm cells in groups A to D and F were above 60%. This indicated that the sperm motility in these groups was not affected by the treatment. However, groups E and G showed a reduction in percentage motility and viability. The study concluded that *S. mombin* fractions did not affect sperm cells structurally but treatment with ethyl acetate and methanolic fraction caused significant reduction (P<0.05) in percentage motility and viability, thus may precipitate infertility.

Key words: *Spondias mombin*, Wistar rats, spermatozoa characteristics, sperm morphology, sodium arsenite.

INTRODUCTION

Spondias mombin L. (Anarcardiaeae), synonym *Spondias lutea*, is commonly known as hog plum, yellow mombin or ubos. Locally called 'atoaa' in Ashanti, is a delicious erect tree which grows to 15 to 20 m tall with a trunk of 60 to 75 cm wide. It has a grayish bark, slightly buttressed, thick, coarse trunk (Burkill, 1985).

It is traditionally known widely for the treatment of a variety of disease conditions. Its bark, leaves, roots and fruits are used in various ways. *S. mombin* leaves are among the forages usually fed to domestic animals in South Eastern Nigeria. The juvenile leaves are also cooked as green vegetables (Ayoka et al., 2008). The leaves are also being used in the treatment of bacterial infections, the prevention and inhibition of the progression of viral infections, treatment of candida infections, and expelling parasites such as intestinal worms. It is also known to reduce anxiety, stop convulsions, calm and sedate, relieve pain and suppress cough. It has been reported that it aids digestion and stimulates the uterus (Corthout et al., 1988; Caceres et al., 1995; Ademola et al., 2005; Amadi et al., 2007). The bark is reported to reduce inflammation, relieve pain, reduce spasms, kill fungi

*Corresponding author. E-mail: drgoforth09@yahoo.com or drgoforth09@gmail.com. Tel: +2348038087625.

fungi and bacteria, heal rashes and wound and stop bleeding. It has also been used as a contraceptive (Villegas et al., 1997; Uchendu et al., 2008). The stem bark and fruit juices of the plant have been widely used for both medicinal and non-medicinal purposes. The tree is commonly used for living fences, in farmlands and shelter by artisans.

Millions of persons in the world especially in the developing countries are exposed to inorganic arsenic compounds through drinking water and are suffering from its chronic or acute toxic effects. Arsenic compound is widely distributed in nature in many forms and its compounds are used extensively as components of herbicides, insecticides, rodenticides, food preservatives, and drugs (Baxley et al., 1981; Mustafa et al., 2010). Ingestion of the metalloid in drinking water presents the greatest hazard. Efforts to prevent and treat arsenic toxicity by therapeutic measures had only limited success (Yousef et al., 2008). A positive correlation between dietary supplementation with certain vegetables and plants and the reduction of toxic effects of various toxicants, environmental agents including heavy metals has been established (Nandi et al., 1997).

Arsenic, a well-documented human carcinogen, is a naturally occurring metalloid present in food, soil and water. This is released in the environment via natural and man-made processes (Tchounwou et al., 1999). Exposure to arsenite has been linked to diverse effects in both experimental animals and humans (Prasad and Pandey, 1984; Waalkes et al., 2003).

S. mombin 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 of Wistar strain albino rats exposed to arsenic toxicity.

This study was therefore carried out to investigate the effects of the chromatographic fractions of *S. mombin* on the semen characteristics and morphology of male Wistar rats exposed to sodium arsenite (SA).

MATERIALS AND METHODS

Chemicals and plant

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 LD_{50} of the salt. Freshly prepared solution was used for each experiment.

S. mombin L (Anacardiaceae) leaves were collected from the botanical garden of the University of Ibadan and authenticated at the Department of Botany, University of Ibadan, Nigeria. Leaves of *S. mombin* were washed with clean water and air-dried. Leaves were ground into fine powder and defatted in hexane. Cold extraction was done by soaking the defatted ground leaves using 96% ethanol. Extract was collected and concentrated using rotary evaporator under reduced pressure at a temperature of 40°C. Ethanolic extract was subjected to fractionation using Vacuum Liquid Chromatography (VLC) technique with varying graded concentrations of hexane, ethyl acetate and methanol. Eluents were collected and spotted on thin layer chromatography aluminum plate GF_{254} (TLC), subjected to a mobile phase, allowed to dry and observed under UV light. Eluents with similar refractive index (Rf) on spotting were pooled together and used.

Extract suspensions were freshly prepared in dimethyl sulfoxide (DMSO), 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 room temperature in the Laboratory.

Experimental animals

Thirty-five adult male albino (Wistar strain) (125 to 228 g) were used after which 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 ± 2°C), relative humidity (50 ± 15%) and normal photoperiod (12 h light and 12 h dark). The animals were fed on a standard rat diet (commercial pellet diet from Kesmac Feed Industry, Ibadan, Oyo State, Nigeria) and given water *ad libitum*.

Experimental protocol

Thirty-five clinically healthy male albino rats (225 to 228 g) were grouped into 7 (A to G) in which each group contains 5 rats. Animals were acclimatized before use and the treatment groups were as follows: group A was treated with 0.1 ml DMSO, B (0.1 ml of distilled water), C (SA 2.5 mg/kg body weight), D (ethyl acetate fraction (FB)), E (ethyl acetate fraction for 7 days and SA on the 7th day), F (methanolic fraction for 7days and SA on the 7th day) and G (methanolic fraction for 7 days (FC)) .

Animals in groups A and B served as negative controls. Twenty-four hours after the last treatment of SA extract, samples were collected from all the animals 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 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 epididymides 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. The semen was dropped on warm glass slide and stained using warm Wells and Awa stains for morphological studies and Eosin-Nigrosin stain was used to stain for live/dead ratio. Also, percentage motility was carried out using 2 to 3 drops of 2.9% warm buffered sodium citrate kept at body temperature.

Percentage viability

This was done by staining one drop of semen and 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

Table 1. Mean values for percentage motility and viability of albino rats in different treatment groups.

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Motility (%)	89.00±2.45	85.00±3.16*	83.20±13.31	81.25±4.27	38.00±13.90*	66.00±2.45*	25.00±15.00*
Viability (%)	97.40±0.60	96.80±0.74*	84.00± 6.03	81.50±5.61	50.00±12.65*	86.00±2.92*	57.00±11.58*

*Mean difference is significant at (P<0.05).

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

Parameter	Tailess head (%)	Headless tail (%)	Rudimentary tail (%)	Bent tail (%)	Curved tail (%)	Curved mid-piece (%)	Bent mid-piece (%)	Coiled tail (%)	Total abnormal cells (%)	Total normal cells (%)
Group A	1.19±0.14	1.04±0.15	0.39±0.09	2.09±0.11	1.99±0.11	2.08±0.12	1.98±0.11	0.39±0.09	11.17	88.83
Group B	1.04±0.14	1.19±0.14	0.49±0.11	2.38±0.19	2.24±0.08	2.38±0.16	2.23±0.13	0.49±0.16	12.45	87.55
Group C	1.03±0.14	1.03±0.15	0.44±0.09	1.87±0.12	1.92±0.12	2.07±0.19	2.07±0.12	0.43±0.12	10.88	89.12
Group D	1.13±0.33	1.00±0.25	0.37±0.13	2.72±0.04	2.47±0.03	2.47±0.22	2.59±0.09	-	12.74	87.26
Group E	1.23±0.13	1.13±0.17	0.37±0.07	2.30±0.09	2.21±0.11	2.17±0.16	2.27±0.13	0.43±0.11	12.12	87.88
Group F	1.28±0.16	1.23±0.11	0.35±0.06	2.07±0.39	2.52±0.12	2.43±0.21	2.27±0.14	0.29±0.05	12.45	87.55
Group G	1.04±0.15	1.04±0.14	0.43±0.12	2.42±0.14	2.22±0.10	2.37±0.17	2.37±0.11	0.39±0.10	12.28	87.72

*Mean difference is significant at (P<0.05).

Eosin penetrating and staining the dead autolysing sperm cells whereas viable sperm repel the stain (Zemjanis, 1977).

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

It was observed that groups G and E had the lowest percentage motility (25.00±15.00 and 38.00±13.90%), respectively and significantly lower (P<0.05) than groups A and B (89.00±2.45 and 85.00±3.16%), respectively which were negative controls. Percentage motility of groups A to D was similar but higher (P<0.05) than groups E to G (Table 1).

Group C treated with SA and group D of fraction B had 83.20±13.31 and 81.25±4.27% of motility, respectively. This indicated that the treatment did not affect the percentage motility negatively. Group E motility was 66.00±2.45%, though significantly less than the negative control groups A and B, the percentage were still within normal range for insemination.

The percentage abnormality in various parts of the sperm cells ranges between 10.88 and 12.74% in both control and treatment groups. It was also observed that spermatozoa of both treated and untreated groups were affected structurally or morphologically (Table 2).

DISCUSSION

For animals to be classified satisfactorily in terms of breeding soundness examination, the percentage sperm motility and livability must not be less than 60% (Zemjanis, 1977). In this study, motility of the sperm cells in groups A to D and F were above

60%. This showed that both the sperm percentage motility and livability in these groups were not affected by the treatment.

It was observed that group E, treated with B (Ethyl acetate fraction) and G treated with fraction C (methanolic fraction) were adversely affected. The percentage motility in these groups was 38.00±13.90 and 25.00±15.00%, while the percentage livability was 50.00±12.65 and 57.00±11.58%. These can precipitate infertility since the sperm percentage motility and percentage livability were low. The findings of Hafez (1993) support our observations which revealed that high percentage motility and livability will result in high fertility potential.

It was also believed that fractions B and C with or without SA proved to be anti-fertility since the sperm cells died easily and percentage motility was negatively affected. The morphological characteristics were observed in all the groups to be within the normal range of 10 to 20% (Zemjanis, 1977; Hafez, 1993).

The observation of the result in all the groups indicated that the plant extract and SA did not affect spermatozoa structurally.

Conclusion

In conclusion, the *S. mombin* fractions did not affect sperm cells structurally, but treatment with ethyl acetate fraction and methanolic fraction caused significant reduction ($P<0.05$) in percentage motility and viability, thus may precipitate infertility. This implies that the two fractions have no protective effect against arsenic toxicity in Wistar strain albino rats.

REFERENCES

Ayoka AO, Akomolafe RO, Akinsomisoye OS, Ukponmwan OE (2008): Medicinal and economic value of *Spondias monbin.* Afr. J. Biomed. Res. Vol. 11 (2008):129-136.

Baxley MN, Hood RD, Vedel GC, Harison WT, Szczech GM (1981). "Prenatal toxicity of Orally Administered Sodium Arsenite in Mice". Bull. Environ. Contam. Toxicol. 26:749–756.

Burkill HM (1985), The useful plants of West tropical Africa, (Families A-D). 2nd Ed. Royal Botanic Gardens, London

Hafez ESE (1993). Reproduction in farm animals, 6th edition.lea and Philadelphia. 405-423

Mustafa TA, Kadriye O, Saygi A, Isa KB, Mustafa S (2010). "Selective speciation and determination of inorganic arsenic in water, food and biological samples". Food Chem. Toxicol. 48:41–46.

Nandi P, Talukder G, Sharma A (1997). Dietary supplementation with leaf extract of *Beta vulgaris* L. var. *benghalensis* Hort. in modifying cytotoxicity of lead subacetate in mouse *in- vivo.* Phytother. Res. 11:273–76.

Prasad SJ, Pandey K (1984). Impaired spermatogenesis in arsenic treated freshwater fish, *Colisa fasciatus* (Bl. and Sch.). Toxicol. Lett. 21:191-5.

Tchounwou PB, Wilson BA, Ishaque A (1999). Important considerations in the development of public health advisories for arsenic and arsenic containing compounds in drinking water. Rev. Environ. Health 14:1–19.

Uchendu CN, Choudhary MI (2004). The in vitro effects of butanolic leaf extract of *Spondias mombin* on rat uterine muscle. Niger. J. Exp. Appl. Biol. (1):1109-113.

Waalkes MP, Ward JM, Liu J, Diwan BA (2003). Transplacental carcinogenicity of Inorganic arsenic in the drinking water: Induction of hepatic, ovarian, pulmonary and adrenal tumors in mice. Toxicol. Appl. Pharmacol. 186:7-17.

Yousef M, El-Demerdash F, Radwan F (2008). "Sodium Arsenite Induced Biochemical Perturbations in Rats: Ameliorating Effect of Curcumin". Food Chem. Toxicol. 46:3506-3511.

Zemjanis R (1977). Collection and evaluation of semen. In: Diagnostic and therapeutic technique in animal production, 3rd Ed. The Williams and Wilkins company, Baltimore. pp. 139-180.