

Testicular and Epididymal Parameters of *Spondia mombin*. L. (*Anacardiaceae*) Protected Male Wistar Rats Exposed to Sodium Arsenite

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Abstract: The testicular and epididymal protective potential of *Spondias mombin*. L in arsenic-treated rats was carried out. Thirty-five male albino rats (225-228g) were used and grouped into 7 (A to G), each group containing 5 rats. Group A was treated with 0.1ml Dimethylsulphoxide (DMSO), B (0.1ml of Distilled water), C (Sodium arsenite; SA-2.5mg/kg body weight), D (Ethylacetate fraction), E (Ethylacetate fraction for 7 days and Sodium arsenite the 7th day), F (Methanolic fraction for 7days and Sodium arsenite the 7th day) and G (Methanolic fraction for 7 days). The results showed significant decrease ($P>0.05$) in the mean value of both the right and left testes length for group C (treated with Sodium arsenite only) compared to the remaining groups. It was also observed that Group G (treated with Methanolic fraction) had the lowest mean value ($P>0.05$) for the left and right testes weight and the left and right testicular diameter when compared to other groups. It was also observed that Group G (treated with Methanolic fraction) had the lowest mean values for all the epididymal parameters in this study. There was a significant decrease in the mean value of left epididymal weight, right and left epididymal length of group G compared to group B (treated with distilled water) and group A (treated with DMSO). It was concluded that the Methanolic fraction of *Spondias mombin*. L has no protective effect on the male albino rats exposed to sodium arsenite as it caused a reduction in epididymal parameters and testicular weight and diameter which may trigger testicular degeneration leading to infertility.

Key words: *Spondias mombin* · Wistar Rats · Testicular Parameters · Epididymal Parameters

INTRODUCTION

A great deal of attention has been paid to some plant-derived chemicals that influence endocrine activities in both humans and animals due to their possible beneficial and sometimes their adverse effects. Some of these plants possess fertility and anti-fertility effects through their actions on hypothalamo-gonadal axis or direct hormonal effects through their action on hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs [1].

Spondias mombin L. (*Anacardiaceae*), synonym *Spondias lutea*, commonly known as hog plum, yellow mombin or ubos. Locally called 'atoaa' in Ashanti, is a

delicious erect tree, which grows to 15-20 meters tall with a trunk 60-75cm wide. It has a grayish bark, slightly buttressed, thick, coarse trunk [2].

Spondias mombin L. is traditionally known 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 young leaves are also cooked as green vegetables for human consumption [3]. 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 intestinal 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 [4].

The bark is reported to reduce inflammation, relieve pain, reduce spasms, kill fungi, kill bacteria, heal rashes, heal wound and stop bleeding. It has also been used as a contraceptive [5]. The leaves bark and fruit juices of the plant have been widely used for both medicinal and non-medicinal purposes. The tree is used in some community as living fences, in farmlands and shelter by artisans.

The study of Raji *et al.* [6] reported the antifertility action of aqueous *Spondias mombin* bark extract. The study reported a marked dose dependent reduction in epididymal sperm progressive motility, sperm count, viability and a dose-dependent increase in percentage abnormal spermatozoa. However, cessation of treatment with the extract resulted in full recovery within four weeks.

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 [7]. 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 [7, 8]. Ingestion of the metalloid like arsenic in drinking water presents the greatest hazard. Efforts to prevent and treat arsenic toxicity by therapeutic measures had only limited success [9].

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 [10]. Exposure to arsenite has been linked to diverse effects in both experimental animals and humans [11, 12].

Arsenic has been claimed to be of clinical utility in the treatment of syphilis, amoebiasis and certain other diseases [13] and also has been used in Fowler solution in the treatment of arthritis [13]. However, arsenic intoxication in experimental animals has been associated with hepatic tumors [12], the inhibition of testicular steroidogenic function, [14] and spermatogenesis, [12] as well as with severe metabolic disorders such as diabetes in humans [15, 16]. The application of plants products as drugs is as important to humans as their dietary source of nutrients [17].

Spondias 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

testicular and epididymal parameters of wistar strain albino rats exposed to arsenic toxicity.

This study was therefore carried out to investigate the effects of the chromatographic fractions of *Spondias mombin* on the testicular and epididymal parameters of male Wistar rats exposed to Sodium arsenite.

MATERIALS AND METHODS

Chemicals and Plant Materials: Sodium arsenite (0.05 M NaAsO₂, Sigma-Aldrich, USA) was diluted with glass-distilled water to concentrations 2.5mg/kg body weight corresponding to 1/10th of the oral LD₅₀ of the salt. Freshly prepared solution was used for each experiment.

Spondias mombin L (*Anacardiaceae*) leaves were collected in July 2010 from the University of Ibadan botanical garden and authenticated at the Department of Botany, University of Ibadan, Ibadan, Nigeria. Leaves of *Spondias mombin* were washed with clean water and air-dried. Leaves were grinded into fine powder. Cold extraction was done by soaking the ground leaves in 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 GF₂₅₄ (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 for further work. Extract suspensions were freshly prepared in Dimethylsulfoxide (DMSO), which served as vehicle and negative control. Suspensions were administered orally at a dose of 100mg/kg body weight. Volumes of extract administered did not exceed 0.2ml regardless of the body weight of the animal. Prepared suspensions were kept at room temperature.

Experimental Animals: Thirty five adult male albino rats (Wistar strain) (125-228 g) were used. Animals were obtained from the Experimental Animal house of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. Animals were examined to be clinically healthy and kept in steel laboratory cages (60 X 60 X 50cm). All animals were kept under controlled conditions of temperature (25 ± 2°C), Relative humidity (50 ± 15%) and normal photoperiod (12 hr light and 12 hr 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 (225-228g) were grouped into 7 (A to G) in which each group contains 5 rats. Animals were acclimatized before use and the treatments were as follows-Group A was treated with 0.1ml Dimethylsulfoxide (DMSO), B (0.1ml of Distilled water), C (Sodium arsenite (SA) 2.5mg/kg body weight), D (Ethyl acetate fraction (FB), E (Ethyl acetate fraction for 7 days with Sodium arsenite one the 7th day), F (Methanolic fraction for 7days and Sodium arsenite the 7th day) and G (Methanolic fraction for 7 days (FC). Animals in group A and B served as negative controls.

Sample Collection: Twenty four hours after the last administration of Sodium arsenite (SA) / extract, the rats were anaesthetized with diethylether before they were sacrificed, 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 then the weight, length and the diameters of each were determined.

RESULTS

Table 1 showed the mean values for testicular parameters of the albino rats.

The values of left testes length of group C (1.74±0.11cm) and right testes length of the same group (1.80±0.10cm) were significantly lower ($P>0.05$) than groups A, B, D, E, F and G.

Group G (treated with methanolic fraction) had the lowest mean value ($P>0.05$) for the left and right testes weight and the left and right testicular diameter when compared to the rest of the groups

Table 2 showed the mean values for epididymal parameters of the albino rats.

The values of the left epididymal weight in group E (0.39±0.04g) was significantly higher ($P<0.05$) than group G (0.23±0.04g) which in turn was significantly lower ($P>0.05$) than the values of groups A, B, C, D and F.

The mean values of both left epididymal length and right epididymal length in groups C and D were significantly higher ($P<0.05$) than groups A, B, E, F and G.

Group G (treated with methanolic fraction) had the lowest mean values for all the epididymal parameters in this study. There was a significant decrease ($P<0.05$) in the mean value of left epididymal weight, right and left epididymal length of group G compared to group B (treated with distilled water) and group A (treated with DMSO).

Table 1: Mean values for testicular parameters of albino rats in different treatment groups

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F	GROUP G
Left testes weight (g)	1.12±0.04	1.07±0.03	1.06±0.03	1.15±0.07	1.11±0.07	0.91±0.14	0.90±0.10
Right testes weight (g)	1.18±0.07	1.09±0.04	1.08±0.04	1.11±0.06	1.07±0.08	0.89±0.12	0.89±0.10
Left testes length (cm)	2.14±0.02	2.10±0.04 ^{bcd}	1.74±0.11 ^a	2.14±0.02 ^{cd}	2.08±0.12 ^{de}	2.12±0.07	2.22±0.08 ^e
Right testes length (cm)	2.26±0.06	2.10±0.03	1.80±0.10 ^a	2.26±0.04 ^b	2.22±0.07 ^{bc}	2.14±0.07 ^{bcd}	2.20±0.08 ^{bcd}
Left testes diameter (cm)	3.44±0.02	3.20±0.03	3.12±0.26	3.40±0.08	3.30±0.07	3.44±0.09	2.98±0.12
Right testes diameter (cm)	3.38±0.05	3.20±0.03	3.30±0.09	3.36±0.12	3.32±0.06	3.20±0.05	3.08±0.13

^{a,b,c,d,e}Means with same superscripts are not significantly different at 0.05 level along the row

Table 2: Mean values for epididymal parameters of albino rats in different treatment groups

PARAMETERS	GROUP B						
	GROUP A	(Control)	GROUP C	GROUP D	GROUP E	GROUP F	GROUP G
Left Epid weight (g)	0.36±0.02	0.34±0.02	0.32±0.01	0.35±0.03	0.39±0.04 ^a	0.33±0.02	0.23±0.04 ^b
Right Epid weight (g)	0.36±0.01	0.34±0.02	0.33±0.02	0.35±0.03	0.33±0.03	0.33±0.03	0.24±0.04
Left Epid length (cm)	4.50±0.07 ^b	4.50±0.08 ^{bc}	4.66±0.13 ^a	4.66±0.13 ^a	4.40±0.13	4.52±0.12	3.90±0.17 ^{bcd}
Right Epid length (cm)	4.44±0.07	4.56±0.10 ^b	4.64±0.11 ^a	4.66±0.14 ^a	4.22±0.06	4.46±0.04 ^{bcd}	3.82±0.29 ^{bc}
Left Epid diameter (cm)	1.62±0.15	1.62±0.07	1.60±0.07	1.60±0.09	1.62±0.05	1.62±0.12	1.42±0.06

^{a,b,c,d,e} Means with same superscripts are not significantly different at 0.05 level along the row

DISCUSSION

The analysis of the testicular and epididymal parameters of *Spondias mombin*. L. (*anacardiaceae*) protected male Wistar rats exposed to sodium arsenite in this study shows that there was significant decrease ($P<0.05$) in the mean values of both the right and left testes length for group C (treated with Sodium arsenite only) compared to the remaining groups. This indicates that arsenite was toxic and will interfere with testicular size and functions. This finding support the report of Prasad and Pandey [11] that arsenite causes inhibition of testicular androgenesis and reduction of weight of the testes and accessory sex organs in experimental animals.

It was also observed that Group G (treated with methanolic fraction) had the lowest mean value ($P>0.05$) for the left and right testicular weight and the left and right testicular diameter when compared to the rest of the groups. This implies that the methanolic fraction slightly affected the testes and may trigger testicular degeneration thereby reducing sperm production which may lead to the problem of infertility. This is similar to the report of Olayemi *et al.* [18] that methanolic root extract of *Cnestis ferruginea* caused degeneration and necrosis of seminiferous epithelium in Wistar strain albino rat.

Finally, it was observed that Group G (treated with methanolic fraction) had the lowest mean values for all the epididymal parameters in this study. There was a significant decrease ($P<0.05$) in the mean value of left epididymal weight, right and left epididymal length of group G compared to group B (treated with distilled water) and group A (treated with DMSO). This indicates that methanolic fraction of *Spondias mombin* will adversely affect the epididymis thereby interfering with the sperm storage, transport and maturation and this may lead to infertility. This finding supports the report of Simmon *et al.* [19] and Olayemi *et al.* [18] in *Cnestis ferruginea* extract treated male albino rats.

In conclusion, the methanolic fraction of *Spondias mombin*. L has no protective effect on the male Wistar rats exposed to sodium arsenite as it caused a reduction in epididymal parameters and testicular weight and diameter which may trigger testicular degeneration leading to infertility in the male albino rat.

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