Tropical Veterinarian

ISSN 0794-4845

Volume 31 (1) 2013

Trop. Vet. Vol. 31(1) 30-41 (2013)

MODULATORY EFFECTS OF ETHANOL EXTRACT OF *SPONDIAS MOMBIN* LEAVES ON SODIUM ARSENITE INDUCED TOXICITY

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Keywords: Sodium arsenite, *S. mombin*, Haematology, Micronucleated polychromatic erythrocytes.

Abstract

This study evaluated the ameliorative potential of aqueous extract of *Spondias mombin* against arsenic-induced toxicity in the rat brain. Forty-five albino rats were randomly divided into nine groups of 5 rats each. Groups A, C and E were administered *S. mombin* leaf extract alone in graded doses of 100, 200 and 300 mg/kg body weight respectively for 7 days. Groups B, D and F were administered the extract at 100, 200 and 300 mg/kg respectively for 7 consecutive days in a single oral dose by gavaging before oral administration of sodium arsenite ($NaAsO_2$) (2.5mg/kg) on day 7. Groups G and H served as the negative control groups and received 0.2 ml diluted propylene glycol (vehicle for the extract) and distilled water respectively. Group I received distilled water for 7 consecutive days and 2.5mg/kg $NaAsO_2$ as a single oral dose on the 7th day. Heamatological (packed cell volume, red blood cell count, haemoglobin, white blood cell count, platelets, and neutrophils count) and biochemical parameters (serum alkaline phosphatase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase) urea and creatinine were evaluated in all animals. Clastogenecity activity was evaluated by studying micronuclei formation in polychromatic erythrocyte cells in bone marrow. Pretreatment with *S. mombin* significantly reduced the elevated serum levels of liver enzymes and reduced the frequency of micronucleated polychromatic erythrocytes (MnPCEs) in rat bone marrow intoxicated with arsenic. Histological examinations showed that the extract at tested dosages protected against $NaAsO_2$-induced liver damage. Our findings suggest that the leaf extract of *S. mombin* possesses a remarkable ameliorative effect against sodium arsenite induced toxicity in albino rats.

Introduction

Arsenic is a naturally occurring carcinogen present in food, air, soil and water. It is released into the environment via natural and man-made processes (Sawyer *et al.,* 2003). It is the second most important environmental pollutant after lead. As a sulfhydryl reactive metal, acute arsenic exposure has been found to cause extensive damage to organs such as the liver, kidney, intestine, reproductive organs and brain (Roy *et al.*, 2008). It has also been found to be a potent clastogen, causing DNA damage leading to both benign and malignant tumors (Hei, 2001). Chronic exposure of human and animals to arsenic contaminated environment is indicated by its higher levels in hair, nail, hoof, and urine (Roy *et al.*, 2008; Mudhoo *et al.*, 2011). Arsenic affects mitochondrial enzymes, impairs cellular respiration, and causes cellular toxicity. It can also substitute phosphate intermediates, which could theoretically slow down the rate of metabolism and interrupt the production of energy (Sarkar *et al.,* 2003). Soluble arsenic salts such as sodium arsenite are well absorbed following ingestion and inhalation. Percutaneous absorption is clinically significant only after heavy exposure to arsenic reagent (Katzung, 2001).

Amelioration of toxic effect of arsenic by using herbal agents is a recent concept. Medicinal plants have been identified from indigenous pharmacopeias that have significant healing power (Holets *et al.*, 2003; Kayode and Kayode, 2011). *Spondias mombin* is a native to the tropical Americas including the West Indes. The tree has been naturalized in part of Africa, India, Sirlirka and Indonesia. In Nigeria the fruit is called "yeye" (Yoruba) (Ayoka *et al.,* 2005), "ngulungwu" (Igbo) and 'isada' (Hausa) (Ayeloja *et al.*; 2006). *S. mombin* is known to have a wide range of medicinal values. Reports have revealed the anxiolytic, sedative, antiepileptic and antipsychotic effects of the leaves extract in mice and rats (Ayoka *et al.*, 2005; 2006; 2008). The extracts of the plant have also shown antimicrobial (Rodrigues and Hasse *al.*, 2000), contraceptive (Uchendu and Isek, 2008; Asuquo *et al.,* 2012) activities.

This study was carried out to explore the possible ways by which the leaf extract of *Spondias mombin* can modulate the toxicity induced by sodium arsenite using heamatological, serum biochemical, histological parameters and *in vivo* micronucleus assay in Wistar rats.

Materials and Methods

Preparation of extract: Fresh leaves of *Spondias mombin* were obtained within the premises of the University of Ibadan, Ibadan, Nigeria and authenticated at the Botany Department of the institution. The leaves were washed thoroughly, shade dried, and blended (Blender/ Miller III, model MS-223, Taiwan) to form a fine powder. Then, 250g of dry powder was defatted with hexane and the product extracted using a soxhlet extractor at low temperature (40^0C) with 80% ethanol. The extract was then air-dried. The percent yield of the ethanol extract was 12%. The testing samples were reconstituted using dilute propylene glycol as the vehicle. All reagents were of analytical grade and purchased from Sigma Chemical Co. (USA).

Experimental animal and management: Forty-five male Wistar rats (8 weeks old) ranging from 160-170 g were used for this study. They were obtained and housed in cages at the Experimental Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, and were

provided with standard laboratory animal feed and water *ad libitum*. After 14 days of acclimatization, the rats were randomly divided into nine groups of five rats each. The study was approved by the Animal Ethics Committee of the University of Ibadan.

Preparation of the clastogen solution: Sodium arsenite (5mg) (Ioba, Chemie Co. India) was dissolved in 10ml distilled water. A dose of 2.5mg/kg body weight was administered according to the guidelines for *in vivo* assays in rats (Preston *et al.*, 1987).

Experimental procedure: The experiment lasted for a period of 7 days; the animals were sacrificed 24h after administration of sodium arsenite by cervical dislocation. Group treatments were as follows after the extracts were administered by gavage:

Group A: Received 100mg/kg body weight of extract for 7 days by gavaging

Group B: 100mg/kg of extract for 7 days and 2.5mg/kg $NaAsO_2$ on the 7th day

Group C: 200mg/kg of extract for 7 days

Group D: 200mg/kg of extract for 7 days and 2.5mg/kg $NaAsO_2$ on the 7th day

Group E: 300mg/kg of extract for 7 days

Group F: 300mg/kg of extract for 7 days and 2.5mg/kg $NaAsO_2$ on the 7th day

Group G: Negative control, received 0.2 ml of diluted propylene glycol (the vehicle) for 7 days

Group H: Negative control, received 0.2 ml of distilled water for7 days

Group I: Positive control, 2.5mg/kg $NaAsO_2$ as a single oral dose equivalent to the 1/10th LD_{50} of $NaAsO_2$

Blood analysis: Blood samples were collected via the periorbital sinus into lithium heparinized bottles. The packed cell volume (PCV) was estimated by the microhaematocrit method and the haemoglobin (Hb) concentration by the cyanmethaemoglobin method. Red blood cell (RBC) and white blood cell (WBC) count were determined using the haemocytometer. Fresh smear of each blood sample was fixed with methanol and stained with Giemsa for differential leukocyte counts (Jain, 1986).

Biochemical evaluation: Commercially available kits were used according to the respective manufacture's protocol for the measurement of serum liver enzyme activity. Serum ALP activity was determined by a kit from BioSystems, SA. Spain. Serum AST, ALT and GGT activities, urea and creatinine levels were measured using RANDOX® laboratory reagent kits obtained from RANDOX Laboratories Ltd., Ardmore, United Kingdom. All samples were analysed in triplicate, and then mean values were determined. Serum cholesterol and triglyceride levels were determined by Ecoline CHOD-PAP and Ecoline 25 GPO-PAP assay kits (1.14856.0001, Merck KGaA, Darmstadt, Germany) respectively.

Micronucleus test: Clastogenic effects were evaluated using the bone marrow micronucleus test described by Holland *et al.* (2008), with some modifications. Bone marrow from both femurs were flushed into a centrifuge tube containing fetal calf serum and a fine suspension was prepared. The cell suspension was centrifuged at 1500 rpm for 10min and the pellet suspended. A small drop of the suspension was placed over a clean coded slide and the smear was prepared and air-dried and fixed with methanol. The slides were stained with May-Gruenwald and Giemsa. Slides were mounted using DPX mountant, dried (20-30°C), cleaned and properly coded. The frequencies of micronuclei (MN) in polychromatic

erythrocytes (PCE) were estimated by scoring 1000 PCE per animal (Zaizuhana *et al.*, 2003).

Histological study: The liver samples were collected in 10% buttered formalin for histopathology. The organ tissues were processed and embedded in paraffin wax and sections were made of about 5µm. After staining with haematoxylin and eosin (H&E), slides were examined under the microscope (Olympus, Japan) for histopathological changes and photographed.

***Statistical analysis*:** The values were expressed as mean ± SEM (standard error of mean). The homogeneity of data was analyzed by one-way analysis of variance (ANOVA) and the Bonferroni's Multiple Comparison Test was used as post-hoc test for comparison between means using Graph-Pad Prism (version 4.00 for Windows, Graph-Pad Software, San Diego, California, USA). P values <0.05 were considered significant.

Results

Packed Cell Volume (PCV), red blood cell (RBC) count, white blood cell, platelets, neutrophils and Hb concentrations were significantly ($P<0.05$) decreased in group I when compared to the other test groups (Table 1). The values of these parameters in Groups B, D and F were not significantly different from the negative control groups (group G and H). Group C rats had a statistically significant increase in PCV.

In group I, a significant ($p<0.05$) elevation was noticed in levels of liver enzymes (AST, ALT, ALP and GGT) with respect to the negative control animals. The increase in levels of these liver enzymes in groups B, D and F were not significantly ($p>0.05$) different from the negative control. Groups A, C and E did not show any significant alteration in the activity of these enzymes. There were significant increases ($p<0.05$) in urea concentration in group I when compared with the other test groups.

The *in vivo* micronucleus test showed that administration with varying doses of *S. mombim (SM)* extract in Groups A, C and E resulted in no statistically significant increase in the number of micronuclei polychromatic erythrocytes (MnPCE)/1000PCE compared with the negative control groups (Fig. 1). The proliferation index of micronuclei increased in all the groups that were pretreated with the extract before administration of arsenite, however these increases were not statistically significant ($p>0.05$). Group I (administered arsenite only) showed statistically significant ($p<0.05$) increase in the frequency of micronuclei.

No visible lesions were observed in the histology of liver sections from Groups A, C and the negative control groups (Fig. 2A). Mild periportal infiltration by macrophages was observed in Group B. Groups D and F showed moderately congested central veins and mild lymphocytic infiltration. Treatment with arsenite in group I resulted in marked congestion of the portal vessels, diffuse necrosis and moderate around the central veins lymphocytic infiltration (Fig. 2B).

Table 1: Heamatological parameters of experimental rats administered with test substance

Groups	PCV	RBC	Hb	WBC ($x10^6/mm^3$)	Platelets ($x10^6/mm^3$)	Neutrophil	Lymphocyte	Monocyte
A	33.20 ± 1.88[a]	8.09 ± 0.52 [a]	10.88 ± 0.65 [a]	15.08 ± 1093.34[a]	482.40 ± 22.13 [c]	29.00 ± 12.66[a]	58.40 ± 12.60 [a]	12.80 ± 1.96 [a]
B	37.75 ± 1.03[a]	7.92 ± 0.21 [a]	12.06 ± 0.36 [a]	15.48 ± 2908.14 [a]	437.00 ± 76.60 [a]	21.76 ± 4.09 [a]	64.75 ± 6.20 [a]	16.00 ± 4.43 [c]
C	42.40 ± 2.38[b]	9.09 ± 0.38 [a]	13.30 ±0.72[a]	15.92 ± 1756.82 [a]	301.40 ± 26.07 [b]	12.60 ± 1.21 [b]	76.40 ± 2.25 [b]	11.00 ± 1.87 [a]
D	37.00 ± 2.08[a]	8.01 ± 0.95 [a]	12.33 ± 0.88 [a]	12.78 ± 2208.46 [a]	358.00 ± 53.82 [b]	12.75 ± 2.87 [b]	72.00 ± 7.16 [b]	15.25 ± 9.68 [c]
E	37.00 ± 1.61[a]	8.97 ± 0.62 [a]	11.80 ± 1.04 [a]	14.24 ± 2247.80[a]	467.84 ± 62.46 [a]	31.00 ± 11.51 [a]	58.20 ± 11.33 [a]	11.80 ± 1.11 [a]
F	34.80 ± 1.83[a]	8.01 ± 0.74 [a]	11.56 ± 0.59 [a]	15.16 ± 1299.85[a]	499.60 ± 43.21 [c]	29.80 ± 8.53[a]	61.00 ± 9.04 [a]	9.00 ± 1.00 [b]
G	35.33 ± 1.33[a]	8.32 ± 0.12 [a]	11.57 ± 0.52 [a]	12.78 ± 1572.35 [a]	370.15 ± 12.28[b]	26.50 ± 4.92 [a]	62.00 ± 6.10 [a]	11.50 ± 2.02 [a]
H	39.75 ± 0.63[a]	8.49 ± 0.12 [a]	13.20 ± 0.20[a]	12.30 ± 3077.34[a]	412.00 ± 23.53 [a]	27.50 ± 4.05 [a]	72.25 ± 5.78 [b]	10.25 ± 1.89 [a]
I	26.00 ± 2.08[c]	7.07 ± 0.32 [b]	8.80 ± 0.76 [b]	9.03 ± 3319.30[b]	514.67 ± 65.09[c]	14.33 ± 8.41 [b]	77.00 ± 10.54 [b]	8.67 ± 3.19 [b]

n=5 Values are expressed as mean ± SEM (standard error of mean) Groups with different superscript within columns are significantly different from each other at $p<0.05$

PCV – Packed cell volume
Hb – Haemoglobin concentration
WBC – White blood cell counts
RBC – Red blood cell counts

Table 2: Mean serum biochemical values of experimental rats administered test substances

Groups	ALP (IU)	AST (IU)	ALT (IU)	GGT (IU)	UREA (mg/dl)	Creatinine (mg/dl)
A	156.50 ± 19.45 ^{c}	104.00 ± 12.12 ^{a}	75.50 ± 3.77 ^{a}	3.50 ± 1.55 ^{a}	37.50 ± 2.96 ^{a}	0.15 ± 0.03 ^{a}
B	209. 75 ± 33.33 ^{b}	183.50 ± 11.29 ^{c}	61.75 ± 1.55 ^{a}	15.75 ± 4.42 ^{b}	42.50 ± 1.44 ^{a}	0.48 ± 0.05 ^{b}
C	293.60 ± 71.91 ^{b}	195.20 ± 55.86 ^{c}	73.00 ± 18.99 ^{a}	2.80 ± 0.66 ^{a}	36.40 ± 9.74 ^{a}	0.32 ± 0.06 ^{b}
D	258.75 ± 21.59 ^{b}	214.50 ± 16.29 ^{b}	73.50 ± 6.18 ^{a}	7.25 ± 3.20 ^{c}	42.50 ± 3.28 ^{a}	0.35 ± 0.05 ^{b}
E	365.25 ± 21.59 ^{a}	184.50 ± 19.07 ^{c}	96.75 ± 6.49 ^{b}	2.75 ± 0.75 ^{a}	42.50 ± 2.53 ^{a}	0.30 ± 0.04 ^{b}
F	192.00 ± 27.99 ^{c}	171.25 ± 10.59 ^{b}	65.00 ± 13.21 ^{a}	3.75 ± 11.30 ^{a}	57.25 ± 9.51 ^{b}	0.40 ± 0.04 ^{b}
G	283.00 ± 67.18 ^{b}	205.20 ± 18.55 ^{b}	93.40 ± 15.16 ^{b}	3.60 ± 1.50 ^{a}	53.40 ± 3.83 ^{b}	0.36 ± 0.02 ^{b}
H	164.00 ± 86.03 ^{c}	101.75 ± 54.93 ^{a}	56.75 ± 30.85 ^{c}	2.50 ± 0.29 ^{a}	20.75 ± 9.72 ^{c}	0.20 ± 0.04 ^{a}
I	375.20 ± 48.41 ^{a}	186.20 ± 13.98 ^{c}	93.40 ± 8.33 ^{b}	33.20 ± 18.90 ^{c}	58.80 ± 3.81 ^{b}	0.40 ± 0.03 ^{b}

N = 5 Values are expressed as mean ± SEM (standard error of mean). Groups with different superscript within columns are significantly different from each other at $p<0.05$

ALT – Alanine aminotransferase
AST – Aspartate aminotransferase
GGT – Gamma Glutamyl Transferase
ALP – Alkaline Phosphatase

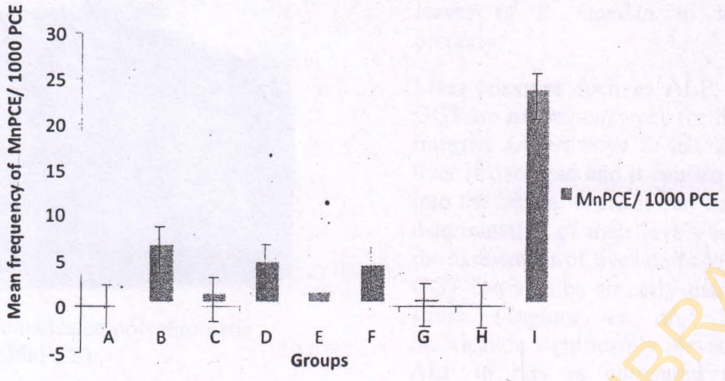

Fig. 1: Mean MnPCE/ 1000 PCE in bone marrow of rats administered with varying doses of *Spondias mombin.*

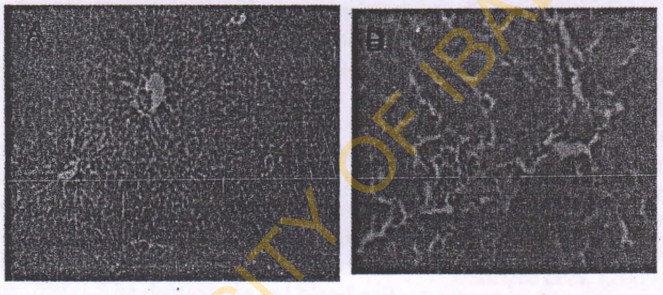

Fig. 2: Photomicrograph of liver sections of rats treatcd with:
(A) extract of *Spondias mombin* (100 mg/kg) showi ıg no visible lesion (H &E x 250).
(B) SA (2.5 mg/kg) showing marked congestion c f the portal vessels and lymphocytic infiltration (x 400).

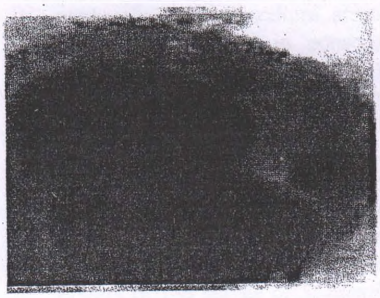

Fig. 3: Micronucleated polychromatic erythrocyte (MnPCE)

Discussion

Exposure to arsenic is considered as major public health issue (Roy *et al.*, 2013). The present study showed the ameliorative effect of *Spondias mombin (SM)* on sodium arsenite (SA) induced toxicity considering various parameters including haematology, serum biochemistry and frequency of micronucleus formation in the bone marrow.

The administration of $NaAsO_2$ as a single oral dose resulted in a significant decrease in PCV. This has been attributed to a direct haemolytic or cytotoxic effect on the blood cells (Nuno *et a.,* 2009) and a suppression of erythropoiesis (Ratnaike, 2003). The decrease in red blood cell count and Hb concentration in the arsenite group might be as a result of a reduction in erythropoiesis and haemoglobin synthesis respectively. This corroborates the findings of Tripathi *et al.* (2003), that acute intoxication with arsenic caused bone marrow depression and hemolysis may develop. However, pre-treatment with varying doses of *S. mombin* before administration of arsenite did not cause any significant change in the values of PCV, lymphocyte, neutrophil, and haemoglobin. Asuquo *et al.* (2013), had earlier reported the leaves of *S. mombin* to have haematinic property.

Liver enzymes such as ALP, AST, ALT and GGT are marker enzymes for liver function and integrity (Adaramoye *et al.*, 2008). When the liver is damaged and it can leak ALT and ALP into the serum where it can be measured; thus determination of their levels is largely used in the assessment of liver damage (Renner, 1995). GGT can also be an early marker of oxidative stress (Sugiura *et al.,* 2005). Arsenic intoxication significantly elevated the ALT and ALP in rats as compared to the animals pretreated with the extract and the negative control groups. The hepatotoxic effects of arsenic which results in increased ALT, ALP values and hepatic fibrosis have been reported by several investigators (Roy, 2006; Dalal *et al.,* 2009). The efficacy of any hepatoprotective agent depends on its capacity to either restore the normal hepatic physiology or reduce the harmful effect caused by a hepatotoxin (Palanive *et al.,* 2008). The observed significant reduction in the levels of these liver enzymes in groups pretreated with the ethanolic extracts of *Spondias mombin* prior to arsenite administration suggests that the extract may have some protective roles on the structural integrity of hepatocytes (Muthulingam, 2010). Banerjee *et al.* (2009) had observed that vitamin C; an antioxidant can combat arsenic toxicity and restore the value of some biochemical parameters like AST, ALT, and ALP.

Increased concentration of serum urea and creatinine are associated with drug-induced nephrotoxicity in animals and man (Ali *et al.,* 2001). In this study, SA treatment interfered with kidney functions as seen by elevation of sarium urea and creatinine values in rats. Urea, a waste product of protein catabolism can rise when the kidney is defective. Increased urea and creatinine levels observed in the positive

control group may be an indication of nephrotoxicity by sodium arsenite (Anwar *et al.,* 1999). This is in consonance with the findings of Nandi *et al.* (2006), that arsenite toxicity induces several metabolic disorders including urea and creatinine elevation following proximal tubule damage and glomerular injury respectively. The pre-treatment with *S. mombin* extracts had a dose dependent reversal effect on these parameters. The present study showed the nephroprotective effects of the leaves of *S. mombin* in arsenite-induced toxicity.

Evaluation of micronucleus induction *in vivo* is one of the primary clastogenicity tests recommended internationally by regulatory agencies for product safety assessment (Zaizuhana *et al.,* 2003). The present study confirms the clastogenic potential of sodium arsenite as evident from the significant ($p<0.05$) increase in frequency of micronucleated polychromatic erythrocytes (MnPCEs) induced in the bone marrow of SA treated rats. This observation is consistent with earlier obdervation on the clastogenic potentials of SA in the bone marrow (Odunola, 2003; Ola-Davies, 2004; Dahal *et al.,* 2008; Ramesh *et al.,* 2012). There was significant ($p<0.05$) increase in MnPCEs in the SA-treated group when compared with the control (Figure 1). This may be due to the fact that arsenite generates free radicals that can attack DNA leading to chromosomal damages (Zaizuhana *et al.,* 2003). However, there is, a significant decrease ($p<0.05$) in MnPCEs formation in the SA and *SM* pretreated groups when compared to the SA-only treated groups. The frequency of MnPCEs in the SM only-treated groups was similar to that of the control, indicative that *SM* on its own may not be genotoxic or result in any clastogenic activity leading to chromosomal instability and disease development.

In the present investigation, histological examinations of liver sections of treated animals showed that SA was potentially hepatotoxic evidenced by marked congestion of the portal vessels, necrosis and inflammation. This is in accordance with the findings of Das Neves *et al.* (2004), and Bashir *et al.* (2005), that the liver section of arsenite treated rats, revealed remarkable degenerative changes. Liver sections from *SM* pretreated and SA groups exhibited very mild hepatic degeneration, while those of the *SM* treated group showed no visible lesions confirming a modulatory effect of *SM* on SA-induced hepatocytes damage.

In conclusion, the results of this investigations have shown that acute exposure to arsenite causes various toxicities in the body. Pretreatment with varying doses of *Spondias mombin* modulated the hepatotoxic, nephrotoxic and genotoxic effects induced by sodium arsenite in rats, suggesting that *Spondias mombin* may serve as a hepatoprotective and anti-tumor agent. However, caution should be exercised in its use at dosages of 300 mg/kg body weight. Further studies are being carried out to identify the mechanism of actions involved in the observed pharmacological properties.

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Tropical Veterinarian
ISSN 0794-4845

Contents: Vol 31 (1) 2013