Research Article

Cytoprotective Potential of *Spondias mombin* fractions on Sodium Arsenite-Induced Haematological, Biochemical and Genotoxic Perturbations in Rats

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ABSTRACT

Heavy metals are leading causes of environmental contamination and arsenic (As) is currently one of the most important metal contaminants particularly in the developing countries such as Nigeria. The effects of chromatographic fractions of S*pondias mombin* L. (SM) leaves on sodium arsenite-induced toxicities in male Wistar rats were evaluated. Thirty-five male rats allotted equally into seven groups (A – G): Groups A and B were treated with 0.1 mL 1% dimethylsulphoxide (DMSO) and distilled water respectively, Group C: sodium arsenite (SA) 2.5 mg/kg, Group D: 100 mg/kg ethyl acetate fraction of SM, Group E: 100 mg/kg ethyl acetate fraction of SM and 2.5 mg/kg SA, Group F: 100 mg/kg methanol fraction of SM and SA, Group G: 100 mg/kg methanol fraction. SM was administered orally for 14 days, while SA was administered as a single oral dose on the 14th day of treatment with SM. Blood was taken 24 hours after SA administration and the animals were sacrificed. Phytochemical, haematological, biochemical, histopathological analyses and micronucleated polychromatic erythrocytes (MnPCEs) induction in the bone marrow cells were evaluated at the termination of the experiment. Phytochemical screening revealed alkaloids, tannins, phenols and flavonoids in all the fractions. Ethyl acetate and methanol fractions contained cardiac glycosides and carbohydrates whereas only hexane and ethyl acetate fractions contained steroids. Results showed normocytic hypochromic anaemia, genotoxicity, kidney and perilobular hepatic damage in the group that received SA alone, which were significantly ($p<0.05$) ameliorated by the fractionated extracts. The PCV, Hb, MCV and MCH values for group G, which was treated with methanol fraction alone were significantly higher ($p<0.05$) while the differential Lymphocytes and Neutrophils counts were significantly increased ($p<0.05$) in group D. The changes in the mean values of AST, ALP and BUN for Groups D and G were significantly lower ($p<0.05$) when compared with group B. In conclusion, the methanol and ethyl acetate fractions of *S. mombin,* exhibited hepatoprotective, nephroprotective and genoprotective properties against sodium arsenite-induced toxicity in Wistar rats.

Keywords: *Spondias mombin, sodium arsenite, hepatoprotective, genoprotective activities*

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INTRODUCTION

Heavy metals rank amongst the most widespread environmental contaminants, which are introduced by natural or anthropogenic processes. Arsenic (As) is currently one of the most important metal contaminants / toxicants, particularly in the developing countries (Berge *et al.,* 2007), including Nigeria (Izah and Srivastav, 2015). Arsenic is present in the environment as a naturally occurring substance or due to contamination from human activity (Gupta *et al.,*

2005; Rana *et al*., 2010). Arsenic may be found in many foods, including grains, fruits, and vegetables, where it is present as a result of absorption from the soil and water (Yost *et al*., 2004, Arain *et al*., 2009). This study focused on inorganic arsenic, which often occurs in excess in the drinking water of millions of people worldwide, and has been previously shown to be a human carcinogen (Person *et al.,* 2015; Muenyi and Ljungman 2015). Sodium arsenite has been proven to be abundant in nature and released into the environment through human activities e.g. agricultural and industrial processes,

pesticides use, metal smelting, electronics manufacturing and mining activities (Chatterjee *et al.,* 1995; Ola-Davies *et al*., 2013). Man has been exposed to sodium arsenite through different major routes via drinking water, food, dermal and atmosphere (Waalkes *et al.,* 2003). Domestic animals are also at risk of intoxication with this environmental toxicant and carcinogen (NRC, 1999).

Arsenical compounds are environmental toxins with multiple effects in animal and human populations (Liu *et al.,* 2001, Waalkes *et al.,* 2003). The main source of environmental arsenic exposure in most populations is the drinking water in which inorganic form of arsenic predominates (Bates *et al*., 1992, Pott *et al.,* 2001). The frequent uses of arsenic as herbicides, insecticides, rodenticides, food preservatives, and by product of used fossil fuel are challenging the aquatic environment (Liu *et al*., 2017). Chronic dermal toxicity, nephrotoxicity, and skin cancer all occur with arsenic exposure (NRC 1999). Arsenic is a multi-site carcinogen in humans, causing tumors in a variety of tissues including lung, skin, and bladder (Waalkes et al., 2003, NRC 1999), arsenic is a multi-site carcinogen in humans, causing tumors in a variety of tissues including lung, skin, and bladder (Waalkes *et al.,* 2003, NRC 1999). Other studies indicate that the kidney, liver, uterus and prostate may also be target sites of arsenic carcinogenesis in humans (NRC 1999).

The search for specific, safe and reliable treatment for arsenic toxicity still continues. The use of plants for healing purposes is very common in developing countries especially in the rural areas; this is probably due to the perceived beneficial and lower side effects profile of natural products that are extracted from plants (Leonardo *et al*., 2000). Medicinal plants have been identified from indigenous pharmacopeias that have significant healing power (Kayode and Kayode, 2011). However, most medicinal plants are used indiscriminately without knowing their possible adverse effect.

Spondias mombin L. is a member of the family Anacardiaceae with several names. *Spondias mombin* L. synonym *Spondias lutea*, commonly known as hog plum, yellow mombin or ubos. *Spondias mombin*, locally called "atoaa" or "iyeye" in Ashanti and Yoruba respectively, is a deciduous erect tree that grows to 15 - 20 meters tall with a trunk 60-75 cm wide. The plant is found in the tropical Americas, including the West Indies, and has been naturalized in parts of Africa, including Ghana, and some parts of Asia (Burkill, 1985). It is very common in Nigeria, Brazil and several other tropical forests of the world with high genetic variability among populations (Ayoka *et al*., 2008). The leaves are among the forages given to domestic animals in South Eastern Nigeria, and the young leaves are cooked as green vegetables for human consumption (Ayoka *et al*., 2008). Medicinally, *S. mombin* leaf is known to reduce anxiety, stop convulsions, calm and sedate, relieve pain, suppress cough, aid digestion and stimulate the uterus (Ademola *et al*., 2005; Amadi *et al*., 2007; Corthout *et al*., 1988). Thus, it has been evaluated as an unconventional source of vitamins A and C (Keshinro, 1985). Other reported pharmacological activities include enzyme inhibition (Coates *et al*., 1994), anti-free radical, anti-aging and reduced glutathione synthesis (Pauly and Fleury, 2002). It has also been reported to have blood

lipid-lowering activity (Igwe *et al*., 2008). Previous studies have shown that crude extracts of *S. mombin* leaves ameliorate spermatozoal and epididymal damages associated with arsenic toxicity in Wistar rats (Ola-Davies *et al.,* 2014, Ola-Davies and Ajani 2016).

The study among other reasons is targeted to experiment the protective effects of consumption of chromatographic fractions of *Spondias mombin* in the presence and absence of sodium arsenite.

MATERIALS AND METHODS

Chemicals
Sodium arsenite (0.05 M NaAsO$_2$, Sigma–Aldrich, USA) was diluted with distilled water to concentrations of 2.5 mg/kg body weight corresponding to 1/10th of the oral LD$_{50}$ of the salt.

Plant Extraction:
Spondias mombin L. leaves were collected from the botanical garden of the University of Ibadan and authenticated at the Department of Botany, University of Ibadan, Nigeria with voucher no UIH-22424. Leaves of *S. mombin were* air-dried, ground into fine powder and defatted in hexane, and later subjected to cold extraction in 96% ethanol as described by Njar *et al.*(1993). A rotary evaporator was used to concentrate the extract, followed by the use of a temperature-controlled oven set at a temperature of 40$_o$C for complete removal of ethanol from the extract (Njar *et al.*,1993). Thereafter, the ethanol 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 ultraviolet (UV) light. Eluents with similar refractive index (Rf) were pooled and used. The extractive values (yield), colors and consistencies of the fractions were recorded.

Fluorescence characteristics of different fractions of *Spondias mombin*:
The different solvent fractions of the *Spondias mombin* leaf extract were exposed to UV light at a wavelength of 365nm. Different characteristic colours were identified and recorded.

Extract preparation:
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 (Odunola and Ola-Davies, 2004). Volumes of extract administered did not exceed 0.2 mL. Prepared suspensions were kept at room temperature in the laboratory.

Qualitative phytochemical analysis:
Hexane, ethylacetate and methanol fractions of *S. mombin* L. extract were screened for alkaloids, flavonoids, phenols, sterols, anthraquinones, saponins, tannins, phlobatanins and cardiac glycosides as described by Sofowora (1993). Saponin

content was evaluated using Frothing technique, alkaloid by Dragendorff and Meyer test, flavonoid by Pew's test, and phenols by Folin-Ciocalteu, while cardiac glycosides were determined by Killer-Killarnis method. Terpenes were determined by Salkowski and Libarman-Burchard's test, while anthraquinones were measured using ammonia as described by Sofowora (1993).

Experimental animals:
Thirty-five male rats (Wistar strain) (225 – 228 g) were obtained from the experimental animal house of the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The 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 ($50 \pm 15\%$) and normal photoperiod (12 hrs light and 12 hrs 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:
The thirty-five healthy male rats were grouped into 7 (A to G) in which each group contains 5 rats. Animals were acclimatized for one week before use. The treatment was administered to each group by gastric gavages as follows:
Group A: Received 0.1 mL DMSO (the vehicle) for 14 days.
Group B: Received 0.1 mL of distilled water for 14 days.
Group C: Received 2.5mg/kg body weight $NaAsO_2$ as a single oral dose equivalent to the $1/10_{th}$ LD_{50} of $NaAsO_2$ (Positive control) on the 14th day.
Group D: Received 2.5mg/kg body weight of ethyl acetate fraction for 14 days.
Group E: Received 100mg/kg body weight of ethyl acetate fraction for 14 days and 2.5 mg/kg body weight $NaAsO_2$ on the 14_{th} day.
Group F: Received 100 mg/kg body weight of methanol fraction for14 days and 2.5 mg/kg body weight $NaAsO_2$ on the 14_{th} day.
Group G: Received 100 mg/kg body weight methanol fraction for 14 days.
Twenty-four hours after the last treatment, blood samples were collected from all the animals after which they were sacrificed by cervical dislocation.

Assessment of haematological parameters:
The rats were anaesthetized using diethyl ether and blood samples collected from the retro-orbital plexus into sterile lithium heparinized sample tubes. Packed cell volume (PCV), haemoglobin concentration (Hb), Red blood cell (RBC) count, White blood cell (WBC) count, mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) as well as Platelet (PLT) counts were analyzed using automated analyser, Cell Dyne, model 331430, Abbott laboratories, IL USA.

Assessment of serum biochemical markers:
Blood was collected into sterile sample tubes without anticoagulant, allowed to clot, and separated by centrifuging at 500 rpm for 10 minutes. Supernatant serum was collected and analysed using specific kits (Randox Laboratories Ltd., United Kingdom) for activities of the enzymes - alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), using an automated analyzer, ATAC 8000 (Elan Diagnostics, CA USA). Urea and creatinine concentrations were measured using Randox laboratory reagent kits (Randox Laboratories Ltd., United Kingdom). Samples were analysed in triplicate, and the mean values were determined. Total plasma cholesterol concentration was determined according to the method of Meiathnin *et al*. (1978). Total protein plasma concentration was measured using the Biuret reaction (Lanzarot *et al*., 2005), whereas glucose and albumin concentrations were determined by spectrophotometric estimation using the Sigma Diagnostic Kit (Sigma Diagnostics, UK). Globulin concentration was obtained from the difference of total protein and albumin.

Genotoxicity assessment:
The *in vivo* micronucleus assay was employed for the evaluation of *S. mombin* L. leaf fractions on sodium arsenite-induced genotoxicity as described by Holland *et al*. (2008). Bone marrow cells from the femurs of freshly sacrificed rats were flushed with foetal calf serum, and a fine suspension was prepared in a centrifuge tube. Subsequently, the suspension was centrifuged at 1500 rpm for 10min, and a smear made by placing a small drop of the suspension on a clean slide, and air-dried. The slides were then then fixed in methanol and stained with May-Gruenwald stain, for the proper colour differentiation of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs), and with Giemsa for micronuclei staining. The smears were mounted using a mountant, dried at room temperature, cleaned and properly coded. The frequencies of micronuclei in the PCEs were estimated by scoring 1000 PCE per animal. Scoring was done by viewing the slides under a microscope at X 100 objective lens.

Histopathology:
The rats were sacrificed by cervical dislocation, and dissected to remove the liver, for preservation in 10 % buffered formalin, dehydration in ethanol (70 to 100 %), clearing in xylene, and subsequent embedding in paraffin. The liver tissue in paraffin was then sectioned, and examined under a light microscope after staining with Haematoxylin and Eosin (Lillie, 1965).

Scoring and Statistical analysis:
Criteria for scoring followed Mac Gregor *et al*., 1987 and OECD guideline 474. One thousand polychromatic erythrocytes (PCEs) were screened for the presence of micronuclei (MnPCEs) at high magnification. The values were expressed as Mean $\pm$ SEM (standard error of mean) and the difference of the means were considered significant at $p<0.05$. 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, 2007) were used to carry out all procedures

RESULTS

Quantitative phytochemical screening: Table 1 gives Fluorescence under UV Light (365 nm), whereas Table 2 gives colour, consistency and yield of different fractions. Table 3 shows the qualitative phytochemical analysis of various fractions of *S. Mombin* L. leaves, under UV light at 365 nm

Haematology and plasma biochemistry: Table 4 shows the haematological values of PCV, Hb, RBC, WBC, Platelets, Lymphocytes, Neutrophils, Monocytes, Eosinophil, MCV, MCH and MCHC in treated and control rats after the administration of *Spondias mombin* Linn fractions (ethyl acetate / methanol fraction) and sodium arsenite alone or simultaneously. There was no significant difference p>0.05 in PCV, Hb, RBC, WBC, Platelet, Lymphocytes, Neutrophils, Monocytes, Eosinophil, MCV, MCH and MCHC, between the control rats (Groups A and B), as well as those rats treated with sodium arsenite in Groups C, E and F. However, the PCV, Hb, MCV and MCH values for group G, which was treated with methanol fraction alone were significantly higher (p<0.05) while the differential Lymphocyte counts were significantly decreased (p<0.05) in group D.

The serum biochemical values for Total protein (TP), Albumin, Globulin, Albumin: Globulin ratio, AST, ALT, ALP, BUN, Creatinine, Cholesterol, and GGT concentrations are presented in Table 5. There was significant increase between the mean values of TP, globulin, A: G ratio, ALP and BUN (p<0.05) between Group C (Arsenic fed) and group B (control), as shown in Table 5, although there was no significant difference between the mean values of AST, ALT, creatinine, cholesterol and GGT (p>0.05) for the two groups (Table 5). The changes in the mean values of Globulin, AST and ALP for Group D was significantly higher (p<0.05) when compared with groups A and B. Group G was also significantly different (p<0.05) in its mean values for Albumin, A:G ratio, ALT and BUN concentrations when compared to group B.

Genotoxicity: The *in vivo* micronucleus assay was employed for the evaluation of the effects of *S. mombin* L. leaf fractions on sodium arsenite-induced genotoxicity as described by Holland et al. (2008). Genotoxicity, which was observed as a function of the frequency of Micronucleus observed are shown in Table 6. Exposure to sodium arsenite resulted in a significant increase in the frequency of micronucleated polychromatic erythrocytes (MnPCEs) observed in the rats compared with the control (35.83 ± 1.54 vs 1.40 ± 0.51). There were significant reductions (p <0.05) in MnPCEs levels in rats exposed to ethyl acetate and methanol fractions as compared with the sodium arsenite exposed group. This study also showed that the mean values of the MnPCEs induced for fractions administered rats in groups D and G was not significant (p>0.05) as compared with the control group. However, when sodium arsenite was co-administered with the ethylacetate and methanol fraction in rats of groups E and F, the MnPCEs induction was significantly reduced (p<0.05).

Table 1:
Fluorescence of *Spondias mombin* L. fractions under ultraviolet light

Fractions	Fluorescence Under UV light (365 nm)
Hexane Fraction	Brick Red
Ethyl Acetate Fraction	Brown
Methanol Fraction	Green

Table 2:
Colour, consistency and yield of *Spondias mombin* L. fractions

Fraction	Colour	Consistency	Yield (%)
Hexane	Green	Pasty	6.28
Ethyl acetate	Black	Dry	10.52
Methanol	Brown	Pasty	8.82

Table 3:
Qualitative Phytochemical analysis of various fractions of *Spondias mombin* L. leaves

Tests	Hexane fraction	Ethyl acetate fraction	Methanol fraction
Alkaloids	+ve	+ve	+ve
Saponins	-ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve
Cardiac glycosides	-ve	+ve	+ve
Tannins	+ve	+ve	+ve
Phenols	+ve	+ve	+ve
Steroids	+ve	+ve	-ve
Flavonoids	+ve	+ve	+ve
Carbohydrates	-ve	+ve	+ve
Proteins& amino acids	-ve	-ve	-ve

+ve = Present.., -ve = absent

Table 4:
Haematological parameters of control and rats exposed to sodium arsenite and *Spondias mombin* fraction*s*

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G
PCV (%)	45.00±0.63	44.60±1.03a	46.60±0.51	45.40±1.91	43.40±0.0	45.00±0.55	47.60±0.51a
HB (g/dl)	14.60±0.40	14.52±0.45a	15.20±0.20	14.50±0.77	14.22±0.30	14.92±0.33	15.92±0.13a
RBC (x 10⁶/µL)	7.39±0.07	7.41±0.17	7.72±0.10	7.68±0.31	7.32±0.18	7.48±0.15	7.41±0.17
MCV (fl)	60.92±0.91	60.16±0.30a	60.38±0.11	59.12±0.26	59.32±0.76	60.18±0.76	62.90±1.15a
MCH (ρg)	19.72±0.55	19.58±0.28a	19.66±0.07	18.86±0.31	19.42±0.16	19.94±0.45	21.08±0.40a
MCHC (g/dl)	29.40±3.18	32.52±0.33	32.56±0.20	31.88±0.43	32.76±0.27	33.14±0.36	32.52±0.33
Platelet (x10⁶/µL)	1.41±0.27	1.46±0.11	2.43±0.491	1.24±0.25	1.53±0.14	1.14±0.89	1.46.±0.11
WBC (x 10₃/µL)	4.95±0.64	7.33±1.87	10.37±1.37	8.86±1.30	9.39±1.22	7.12± 0.93	5.62±0.46
Lymphocytes (%)	73.20±4.93	76.60±3.96a	77.00±1.76	60.20±6.80a	82.20±2.42	75.80±5.78	66.20±2.75
Neutrophils (%)	22.0±5.32	19.8±4.13	19.2±1.46	37.8±6.80	15.8±2.42	21.4±5.47	29.20±2.15
Monocytes (%)	2.00±0.63	1.60±0.25	2.00±0.55	0.80±0.37	1.20±0.20	1.60±0.40	1.80±0.58
Eosinophils (%)	2.80±0.37	2.00±0.32	1.80±0.37	0.80±0.37	0.80±0.37	1.20±0.20	2.80±1.24

a-cTest values carrying the same superscript as the control for each parameter are significantly different (p<0.05)

Table 5:

Mean Serum Biochemical Parameters of Experimental Animals

Parameters	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Total Protein(g/dl)	7.50±0.10	6.86±0.17ab	8.14±0.19b	6.64±0.29	6.62±0.12	6.30±0.19c	6.98±0.27
Albumin (g/dl)	4.58±0.07	4.22±0.14a	4.42±0.14	4.44±0.14	4.42±0.09	4.18±0.06	4.58±0.17b
Globulin (g/dl)	2.92±0.15	2.72±0.06abc	3.92±0.10a	2.20±0.20b	2.42±0.15	2.00±0.20c	2.40±0.13
A:G ratio	0.60±0.04	0.60±0.04ab	1.10±0.04a	0.48±0.05	0.54±0.04	0.48±0.05	1.88±0.07b
AST (i.u/l)	44.60±1.25	44.40±0.87a	43.6±1.60	38.00±0.95a	44.80±1.07	44.40±0.93	44.60±0.93
ALT (i.u/l)	30.00±0.95	28.00±1.48a	26.40±1.91	26.00±2.12	28.00±0.95	29.40±1.12	32.80±0.49a
ALP (i.u/l)	117.00±4.76	122.60±1.03abc	78.00±2.92a	90.20±5.64b	110.40±4.65	96.2±7.84c	115.40±4.80
GGT (i.u/dl)	2.02±0.19	1.90±0.12a	2.16±0.18	1.54±0.08	1.34±0.11	1.16±0.16a	2.22±0.54
BUN (mg/dl)	12.40±0.75	11.60±0.51abc	16.60±0.40a	12.60±0.25	12.00±0.32	13.20±0.49b	16.40±0.25c
Creatinine (µg/dl)	0.62±006	0.58±0.04	0.76±0.04a	0.46±0.04abc	0.50±0.05	0.56±0.07	0.54±0.02
Cholesterol (mg/dl)	70.80±8.13	60.40±12.32	57.00±7.37	52.20±2.63	49.6±3.66	45.80±1.32	71.80±8.35

a-d Test values carrying the same superscript as the control for each parameter are significantly different (P<0.05)

Table 6:

Frequency of micronucleated polychromatic erythrocytes (MnPCE) per 1000PCE of control and treated rats

Group	MnPCEs /1000PCE
Group A	2.00 ± 0.52a
Group B	1.40 ± 0.51b
Group C	35.83 ± 1.54abcdef
Group D	0.33 ± 0.21c
Group E	3.00 ± 0.26d
Group F	5.67 ± 0.33e
Group G	2.33 ± 0.21f

a-f test values carrying the same superscript for the parameter are significantly different (p<0.05)

Histopathology

Histological examination of the liver section of rats in the control and treated groups are shown (Plates 1: A – G). The liver section of rats in groups A and B showed no visible histological changes (Plate 1A&B). The liver of rats administered sodium arsenite showed severe disseminated congestion of vessels and sinusoids, mild periportal inflammation, focal areas of macrovesicular steatosis, disseminated cytoplasmic vacuolation and karyorrhexis of hepatocytes (Plates 1C$_1$ &1C$_2$).

DISCUSSION

Exposure to environmental pollutants constitutes a major threat to animal and human survival in the ever-increasing industrialized world. Arsenic contamination of drinking water from various sources has been reported in many parts of the world including developed and developing countries (Smith and Smith, 2004). Analysis of blood parameters is believed to be relevant indicators in risk evaluation and response to therapy as changes in the haematological system have high predictive value (Olson *et al.*, 2000).

The methanol fraction of *S. mombin* when administered alone induced desirable alterations in the packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) counts, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), as well as platelet (PLT) counts of the treated animals.
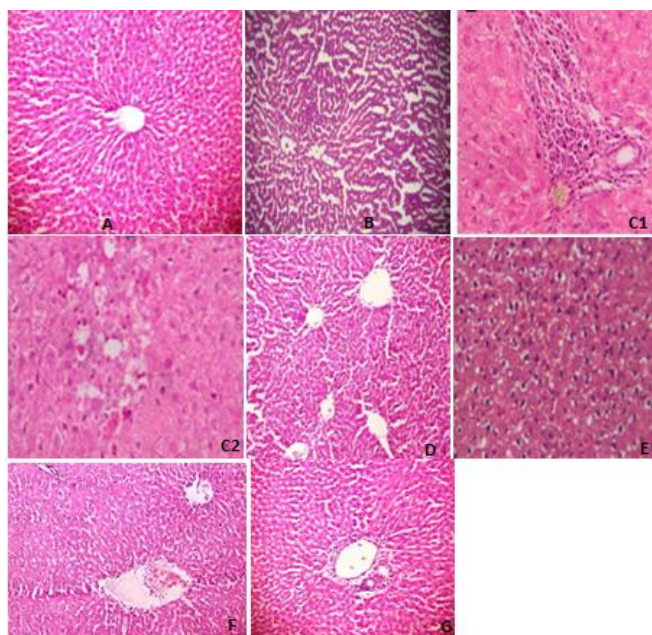

Plate 1

A: Photomicrograph of the liver of rats exposed to DMSO showing no visible lesion (X400)

B: Photomicrograph of the liver of rats exposed to Distilled H$_2$O showing no visible lesion (X400)

B: Photomicrograph of the liver of rats exposed to NaAsO$_2$ showing severe disseminated congestion of vessels and sinusoids(X400)

C$_2$: Photomicrograph of liver of rats exposed to NaAsO$_2$ showing mild periportal inflammation, focal areas of macrovesicular steatosis, disseminated cytoplasmic vacuolation and karyorrhexis of hepatocytes (X400)

D: Photomicrograph of liver of rats exposed to Ethyl acetate fraction showing no visible lesion (X400)

E: Photomicrograph of liver of rats exposed to Ethyl acetate fraction +NaAsO$_2$ showing lymphocytic infiltration. (X400)

F: Photomicrograph of liver of rats exposed to methanol fraction of *S mombin* showing no lesion (X400)

G: Photomicrograph of liver of rats exposed to methanol fraction of *S mombin* + NaAsO$_2$ (X400) showing normal central venules, few portal tracts with mild congestion of the portal vein, morphology of the hepatocytes and the sinusoids appear normal and not infiltrated, no pathological lesion seen. (Haematoxylin and Eosin stain).

The increase as shown in this study, revealed the probable capability of the methanol fraction of *S. mombin* to improve anaemic conditions in animals (Blood, 1989). Similarly, the increase in MCV shown agrees with the incapability of the

fractions to induce anaemic condition in animals (Blood, 1989). These beneficial effects of the methanol fraction of *Spondias mombin* L. on the haematological profile may be attributed to the presence of flavonoids, tannins, vitamin C, iron and other substances with antioxidant and erythropoietic stimulatory properties in the plant (Igwe *et al*., 2010, Njoku *et al*., 2007), which may help in red cell membrane stabilization (James *et al*., 2008). The presence of these antioxidant molecules could possibly explain why the red blood cell integrity was generally unaffected by sodium arsenite in the presence of the fraction administration.

Leucocytes are known to increase sharply when inflammation occurs, as one of the first line of defense of the body against infection and stress (Rehman *et al*., 2016). The oral administration of sodium arsenite caused a significant increase in the lymphocyte counts when compared to the control. The increase in lymphocyte counts following administration of methanol fraction of *Spondia mombin* L. (Group G) depicts the anti-inflammatory and anti-stress properties of the fraction. This result corroborates the report of Swenson and Reece (1993) who reported that toxic plants do not produce a direct effect on WBCs and its functional indices. The percentage increase in neutrophils may be adduced to the ability of the animals to ingest foreign agents. A similar effect was reported on the use of drugs, for example histamine (Sacher and McPherson, 1991). This study is in agreement with earlier findings of Tang *et al.,* 1997 who showed that treatment with garlic extract improved the activation of natural killer cells T-lymphocytes and also that of Sumiyoshi (1997) who showed that garlic extract stimulates immune functions.

Monitoring of the activities of some clinical chemistry parameters like total protein, albumin, globulin, ALT, AST, ALP and GGT are among the most sensitive tests employed in the detection of acute liver damage, and some herbal medicines have been reported to possess hepatotoxic effect due to elevation of these indices (Ranjna *et al*., 1999). Total serum protein and albumin are good criteria to assess the function and secretory capacity of the liver (Yakubu *et al*., 2005). This study showed that there was a significant increase in the albumin, albumin:globulin ratio, and ALT activities when the methanol fraction of *S. Mombin* L. was administered alone compared with distilled water administered group (Group B). However, when administered with sodium arsenite, *S. mombin* caused a significant reduction in these parameters when compared to the control. Several medicinal plants and plant products have been reported to ameliorate sodium arsenite-induced hepatotoxicity. For instance, quercetin, a polyphenolic flavonoidal compound found in large amount in vegetables, fruits and medicinal herbs has been reported to prevent sodium arsenite induced fibrosis in the liver by inhibiting lipid peroxidation, improving antioxidant status and decreasing the activity of ALT and ALP in the serum (Mandal *et al.,* 2006). There are growing evidences that sodium arsenite intoxication can compromise the integrity of the liver in mouse, rat, fish, and goat (Sharma *et al*., 2009, Yousef *et al*., 2008, Roy *et al*., 2009, Srinavas *et al*., 2007). Moreover, exposure to low level arsenic in drinking water can result in physiological disturbances and hepatocellular carcinoma in man (Hua *et al*., 2004).

The kidney is the major route for the excretion of arsenic and its metabolites from the body and a major site for the biotransformation of arsenic. As a result, the kidney is highly prone to arsenic toxicity. Increased concentrations of urea and creatinine are considered for investigating drug-induced nephrotoxicity in man and animals (Ali *et al.,* 2001). In this study, arsenite treatment interfered with kidney functions as seen by elevation of urea and creatinine. Urea is a waste product of protein catabolism that can rise when the kidney is defective. In renal disease, serum urea accumulates because the rate of urea production exceeds the rate of its clearance. Exposure to arsenic or its various forms like Arsenic hydride (AsH_3) can lead to induced nephrotoxicity in experimental animals (Kimura *et al*., 2006; Ayala-Fierro *et al*., 2000).

Increased urea and creatinine levels observed in the arsenite-exposed group may be an indication of nephrotoxicity by sodium arsenite (Anwar *et al.,* 1999). This is in consonance with the findings of Ola-Davies *et al*. (2013) that arsenite toxicity induces several metabolic disorders including urea and creatinine elevation following proximal tubule damage and glomerular injury respectively. However, pre-treatment with *S. mombin* L. fractions had reversal effects on these parameters. Pre-treatment with ethyl acetate fraction in sodium arsenite exposed rats significantly reduced the elevated urea concentration to near normal in the combined group. Also, co-administration with methanol fraction of *S. mombin* L. significantly decreased ($p < 0.05$) serum urea and creatinine concentrations. This corroborates the reported diuretic potential of the plant in ethno medical practice (Akubue *et al.,* 1983) and the earlier report by Igwe *et al*., 2011 that ethanol extract of S. *mombin* significantly reduced serum urea and creatinine concentrations. The present study showed the nephroprotective effects of the fractions of *S. mombin* L. leaves in arsenite-induced toxicity although the ethylacetate fraction seems to possess more nephroprotective potentials than the methanol fraction.

Exposure to sodium arsenite resulted in severe disseminated congestion of vessels and sinusoids, mild periportal inflammation, focal areas of macrovesicular steatosis, disseminated cytoplasmic vacuolation and karyorrhexis of hepatocytes (Plates $1C_1$ & $1C_2$). This is in support of the findings of Jaeschke *et al*. (2002) that observed foci or non-specific hepatitis with scattered foci of cell necrosis accompanying lymphocytic infiltration in pathological hepatic injury. Similar lesions of parenchyma disorganization (Pires-Das-Naves *et al.,* 2004), necrosis and fibrosis in periportal region were similarly observed in arsenic-induced toxicity in rats (Mandal *et al.,* 2006, Ola-Davies *et al*., 2013). Meanwhile, there were no serious visible lesions in the liver section of rats administered ethyl acetate and methanol fraction (Plates 1D & F). However, mild periportal and diffuse cellular infiltration by mononuclear cells were seen in the liver section of rats in group E (Plate 1E). Arsenic toxicosis is likely to cause these lesions probably due to its ability to trigger production of reactive oxygen species and glutathione depletion thereby inhibiting the activity of enzymes such as superoxide dismutase and catalase. The inhibition of these enzyme activities will lead to alteration in cells intrinsic antioxidant defenses, resulting in

oxidative stress and architecture disruption (Nordenson and Beckman, 1991).

Micronuclei formation has been reported as a predictive index for evaluating the carcinogenic potential of environmental and occupational chemical exposure (Celik*et al.,* 2005). Sodium arsenite has been reported by several authors to induce chromosomal damages (Celik *et al.,* 2005; Ola-Davies *et al.,* 2013). This study shows that sodium arsenite significantly ($p<0.05$) induces the formation of micronuclei in the bone marrow cells of the experimental animal model (Table 6). This observation is consistent with an earlier observation in our laboratory and those of others on genotoxic potentials of sodium arsenite in the bone marrow cells (Moore *et al.,* 1997; Vaglenov*et al.,* 2001; Valverde*et al.,* 2002; Ola-Davies *et al.*, 2013; Poma*et al.,* 2003; Celik *et al.,* 2005). This study also showed that the mean values of the MnPCEs for fractions administered rats in groups D and G were similar to the unexposed control. However, pre-treatment with the ethyl acetate and methanol fractions of *Spondias mombin* L. for 14days significantly reduced the frequencies of MnPCEs induced indicating that the fractions modulated the clastogenic effects of sodium arsenite (Table 6). This might probably be due to the presence of flavonoids and other antioxidants in the plant, which are known to reduce arsenic toxicity (Njoku, 2007). In this study, sodium arsenite exposure produced pronounced hepatic histopathology evidenced by periportal inflammation, focal areas of macrovesicular steatosis, disseminated cytoplasmic vacuolation and karyorrhexis of hepatocytes. In consonance with the present finding, Bashir et al., (2006), Ola-Davies *et al*. (2013) showed that the liver section of arsenite treated rats, revealed remarkable degenerative alterations. These pathological changes were modulated to moderate extent in pre-treated groups.

In conclusion, the result of this study shows that arsenic toxicity results in the perturbations of haematological, hepatotoxic, nephrotoxic and genotoxic indices while ethyl acetate and methanol fractions of *S. mombin* L. due to their phytochemical constituents confer antioxidative and cytoprotective activities to prevent the adverse toxicokinetics pathway of arsenic toxicosis in biological system.

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