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In Vivo Evaluation of Methanol and Ethanol Leaf Extracts of *Spondias mombin*



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ABSTRACT

This study evaluated the phytochemical constituents of *Spondias mombin* leaves and investigated its methanol and ethanol leaf extracts *in vivo* in rats against sodium arsenite induced toxicity. Sodium arsenite was administered intraperitoneally at 2.5 mg/kg body weight. Cyclophosphamide (positive control) was administered at 1.5 mg/kg body weight. Ethanol and methanol leaf extracts of *Spondias mombin* were administered orally at 200 mg/kg body weight. The serum enzyme activities γ GT, ALP, AST and ALT; micronucleus, and haematological parameters were used to evaluate the level of toxicities induced by sodium arsenite, and possible attenuation of these toxicities by ethanol and methanol leaf extracts of *Spondias mombin*. The phytochemical analysis revealed that leaves of *Spondias mombin* contain total phenol (0.508), tannins (0.048), saponins (0.077) and flavonoids (0.192) all in ppm. Sodium arsenite and cyclophosphamide significantly induced micronuclei in mPCEs in bone marrow when compared to negative control ($p < 0.05$). Ethanol leaf extracts lowered sodium arsenite induced micronuclei in co-administration. Sodium arsenite and cyclophosphamide showed high percentage increases of ALT, AST and ALP activities with a decreased activity in γ -GT. The increased activity of some of the enzymes in co-administration of sodium arsenite with the leaf extracts indicates sodium arsenite injurious effect to liver could not be completely suppressed by the extracts.

INTRODUCTION

Spondias mombin (Hog plum) which belongs to Anacardiaceae family is a tropical fruit tree that grows to about 20 meters tall; the leaves are about 20-30 cm long and hairy underneath. This produces an abundant crop of small fragrant white flowers in panicles that starts out green and then turns to a light golden yellow upon ripening. It is a common sight in the low lands moist forest of tropical America (Leon and Shaw, 1990), especially Brazil. This naturalized around villages in West Africa; the trees are widely spread and common in farmland and secondary vegetation of the sudanian and the guineanian savannahs, it grows very easily from stake to make live fences and enclosures. In Nigeria, *Spondias mombin* is known with various names (*Iyeye* in Yoruba, *Ijikara* in Igbo and *Tsardarmasar* in Hausa). The plant has been found to be useful medicinally among many countries of the world where it commonly grows (Burkil, 1985). The leaves of *Spondias mombin* are purgative in effect, in infusion they are a common remedy for cough and laxative given in fever and constipation. In many parts of the world, the leaves are widely used for female reproductive tracts issues in traditional medicine. They are also a common remedy for various digestive problems including stomach-ache, diarrhoea, dyspepsia, gastralgia and constipation; also they are considered to be antiviral, antibacterial, antiseptic and are used in numerous microbial problems including colds, flu, cystitis, gonorrhoea etc. They are excellent vermifuge and anthelmintic and are used often for intestinal worms (Burkil, 1985).

The leaves of *Spondias mombin* have been shown to contain many compounds that are of great medicinal values which give an insight to understanding its common use in traditional medicine for various purposes. The leaf extracts contain a series of 6-alkenyl-salicylic acid (Corthout *et al.*, 1994), an anacardic acid derivative that possessed β - lactase inhibitory activity (Coates *et al.*, 1994); two caffeoyl esters, 2- O caffeoyl- +- allohydroxycitric acid and chlorogenic butyl ester (Corthout *et al.*, 1992). Ellagitannins, geraniin and galloylgeraniin were reported by Courthout *et al.*, (1991). Phytochemical analyses have revealed the presences of alkaloids, proanthocyanidin, condensed tannins, saponins, flavonoids, phenols, glycosides, cardiac glycosides, resins, protein, triterpenes and phlobatannins (Edeoga and Eriata, 2001; Ayoka *et al.*, 2005; Nworu *et al.*, 2007; Igwe *et al.*, 2008.). Adediwura *et al.*,(2009) reported the presence of 3- β -olean-12-en-3yl (9z) hexadec- 9-enoate in *Spondias mombin* methanolic leaf extract.

Spondias mombin leaf extracts have been shown to possess many medicinal activities; Abo *et al.*, (1999) reported its antibacterial effect comparable to those of ampicillin and gentamycin; also its phenolic acid extract possessed pronounced antibacterial effect against *Basillus leveus*, *Streptococcus pyogenes* and *Mycobacterium cortuitum*; molluscicidal effect against snail *Biomphalaria glabrata*, an intermediate host in schistosomiasis (Corthout, *et al.*, 1994). It has antiviral activity against coxsackie, and herpes simplex viruses (Corthout *et al.*, 1991, 1992) and presents with antibiotic action (Ajao *et al.*, 1985), the dichloromethane extract has a positive lethality against shrimp larvae; other polar and non-polar extracts also have shown moderate antibacterial activity (Uchendu *et al.*, 2003).

Some other research findings have reported that the leaf extracts possess anxiolytic effect (Ayoka *et al.*, 2005), general lipid lowering effect (Igwe *et al.*, 2008), anthelmintic effects in livestock (Ademola *et al.*, 2005), abortifacient effect (Offiah and Anyanwu, 1989; Nworu *et al.*, 2007), anti-anaemic activity (Adeyemi and Gbolade, 2006) and, sedative and antidopaminergic effects (Ayoka *et al.*, 2006). It has also found use in treatment of diabetes mellitus (Adediwura and Abo, 2009).

Arsenic is a general protoplasmic poison; that is cumulative and affects the body systems and tissues on exposure (Liebscher and Smith 1968; Ho and Lee, 1999; Neiger and Osweiler 1992). Trivalent arsenicals react with sulfhydryl group in cells and inhibit sulfhydryl containing enzyme systems essential to cellular metabolism. Sodium arsenite, a trivalent arsenic compound that is a known carcinogen (Wang *et al.*, 1996; IARC, 1980; Lewis, 1991) is widely distributed in ground drinking water (Saha, 1995; Mahata *et al.*, 2004), insecticides and pesticides; and has its consumption associated with a lot of toxicities in man. These toxicities include embryo toxicities in acute exposure (Mirke and Little, 1998); liver, bladder, kidney, lungs and skin cancers in humans (IARC, 1980, Chen *et al.*, 1992; Chow *et al.* 1997), has suppressing effect on spermatogenesis, gonadotropin, and testosterone release (Manitoshi *et al.*, 2003). It induces many molecular alterations both in humans and animals (Jha *et al.* 1992; Vega *et al.* 1995; Mirke and Little 1998), manifests dose dependent chromosomal breaks and alterations (Barns *et al.*, 2002). Evidence exists that it is immunotoxic (Savabieastahani *et al.*, 1998), and has clastogenic effects in both animal and humans (Basu *et al.* 2001, Gonsebatt, 1997), mutagenic and teratogenic effects, enhanced sister chromatid exchanges in human lymphocytes (Jha *et al.*, 1992) and, induces oxidative stress by increasing GSH level and superoxide dismutase activity (Ho and Lee, 1999).

In spite of *Spondias mombin* wide use in traditional medicine, its influence over various heavy metal/metalloid-induced alterations in biochemical and haematological processes are lacking. This paucity of information on protective role of the plant leaves against some known toxic chemicals e.g. sodium arsenite has necessitated the need to evaluate various solvent extracts of the plant on this metal, with understanding that many plant extracts have shown activity against sodium arsenite induced toxicity, for example, Rouchouhury *et al.*, in 1993 showed that crude extract of garlic reduced the cytotoxicity of different doses of sodium arsenite. Odunola (2003) reported the reduction of clastogenic effects of sodium arsenite by some local condiments; *Hibiscus sabdariffa* fruit was shown by Adetutu *et al.*, (2004) to have significant reduction of micronuclei in PCEs; *Embllica officinalis* also was effective against sodium arsenite toxicity (Biswas *et al.*, 1999). With these findings, it is expected that *Spondias mombin* leaves as a known medicinal plant could contain substances of medicinal importance that could ameliorate or suppress toxic effects of sodium arsenite. This study, therefore, sought to evaluate, methanol and ethanol extracts of *Spondias mombin* on sodium arsenite induced toxic effects in albino rats.

MATERIALS AND METHODS

Syringes, Oral intubation/cannula, Electric Osterizer (USA), Water bath, Rotary evaporator, Roche/Hitachi Auto analyser, EDTA tubes, Plain tubes, Thermostat, Muslin-cloth, Metra balance, Vacuum pump, Needles, Counting chamber, Microscope, Capillary tubes, Slides, Hand Gloves, Desiccators, Conical flasks/Beakers, Sodium arsenite, Cyclophosphamide, Ethanol, Methanol, Distilled water(HPLC grade), haematoxylin and eosin , Normal saline, Serum sample, Sodium arsenite (Na_2AsO_2) [Mol wt. 129.9, As 57.6% (As No. 778 4-46-5)] from Sigma Chemical Co., St. Louis, MO. All other chemicals used were of analytical grade and were also obtained from Sigma chemical Co. Louis Mo. USA. Gamma-GlutamylTransferase (-GT), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) kits were obtained from Randox Laboratories Ltd., United Kingdom and were used according to specifications. Fresh leaves of *Spondias mombin* were obtained from a *Spondias mombin* tree at Okpara Avenue in Enugu, Nigeria and were properly identified by Dept. of Plant Science and Biotechnology, University of Nigeria Nsukka with a voucher No. UNH No. 241^a.

Plants extract preparation

The freshly cut leaves of *Spondias mombin* were dried at room temperature. The dried leaves were pulverised with electric Osterizer (USA). Part of the pulverised leaves was used for phytochemical analysis. Ethanol and methanol were used for the cold extraction individually. In 250 ml of each solvent, 150 g portion of pulverised leaves was soaked and allowed to stand for 24 hrs before separation with muslin-cloth. The filtrates were concentrated with Rotary evaporator before the water bath drying at 40°C.

Animal treatment protocol

Animals were handled according to ethics and animal welfare committee of University of Nigeria Enugu campus. The procedures followed in this study were in accordance to their guidelines on handling animals for experiments.

35 male Wistar albino rats of 100-130 g from animal House, Department of Physiology University of Nigeria Enugu Campus were used for the study, the animals were grouped into Seven (7) of Five (5) per group according to substance administered.

Group I [Sodium arsenite alone]

Group II [Positive Control – Cyclophosphamide]

Group III [Negative control – Distilled water]

Group IV [Ethanol extract alone]

Group V [Methanol extract alone]

Group VI [Ethanol extract and sodium arsenite]

Group VII [Methanol extract and sodium arsenite]

The animals were feed with high grade animal feed, water was given ad libitum, and 12 hr light and 12 hr dark cycle were observed throughout the experiment. Experimental animals were treated according to recommended animal welfare guide.

The substances were administered to the rats in ratio to their body weights. Sodium arsenite was administered intraperitoneally twice, each time at the start of the week at 2.5 mg/Kg

body weight, Cyclophosphamide was also administered intraperitoneally twice at 1.5 mg/kg body weight. The plant extracts were administered every day orally for 14 days at 200 mg/kg body weight; 2/5 of its oral LD₅₀ (Ayoka *et al.*, 2005).

Sample collection protocols

At the end of the two weeks; blood was obtained via ocular vein with capillary tubes from the rats into plain sample tubes and allowed to stand for 2 hrs and thereafter centrifuged at 3000 g for 10mins to separate the serum from the blood cells and were stored at -20°C until required. The serum was used to assay for ALT, AST, ALP and γ -GT. The serum activities of the enzymes were estimated using the Roche/Hitachi Autoanalyzer that uses IFCC (International Federation of clinical chemistry and laboratory medicine) primary reference procedures for measurement of catalytic activity concentrations of enzymes. Blood samples were also collected into heparinised sample tubes for haematological analysis, after which the rats were sacrificed and their femurs were used for micronucleus analysis.

Statistical analysis

The data were recorded and statistically analyzed using one way analysis of variance (ANOVA) and where significant Dunnett test was utilized. The results were expressed as mean standard deviation ($X \pm SD$) and level of significance placed at $p < 0.05$

RESULTS

Table 1: Phytochemical compounds of pulverised leaves of *Spondias mombin*

Constituent	ppm
Total Phenols	0.508
flavonoids	0.192
Tannins	0.048
Saponins	0.077

Table 2: The mean standard deviation (mean±SD) of the enzyme activities (µkat/L)

Group	γ-GT	ALT	AST	ALP
Group I	0.063±0.02	1.20±0.15	4.68±1.53	6.98±1.42
Group II	0.087±0.03	1.22±0.11	4.10±0.78	6.40±1.23
Group III	0.097±0.04	0.76±0.27	4.05±0.90	4.59±0.40
Group IV	0.133±0.06	0.89±0.28	3.37±0.74	9.40±4.37
Group V	0.083±0.02	0.81±0.17	3.39±0.33	7.01±2.24
Group VI	0.093±0.01	1.05±0.07	3.64±0.64	6.24±1.00
Group VII	0.083±0.03	1.27±0.53	3.80±0.99	6.14±1.52

Table 3: The percentage increase/decrease in the mean values of the enzymes

Group	γ-GT	ALT	AST	ALP
Group I	35.05 ^d	57.89 ⁱ	15.56 ⁱ	52.06 ⁱ
Group II	10.31 ^d	60.52 ⁱ	1.23 ⁱ	39.43 ⁱ
Group III				
Group IV	37.4 ⁱ	17.11 ⁱ	16.78 ^d	104.79 ⁱ
Group V	14.43 ^d	6.58 ⁱ	16.29 ^d	52.72 ⁱ
Group VI	4.12 ^d	38.16 ⁱ	10.12 ^d	35.95 ⁱ
Group VII	14.43 ^d	67.11 ⁱ	6.17 ^d	33.77 ⁱ

^dpercentage decrease, ⁱpercentage increase with reference to group III (negative control)

Table 4: The Mean ±SD of Haematological analysis

Group	PCV (%)	Hb (g/dl)	WBC (mm ³)	Neutrophils (%)	Lymphocytes (%)
Group I	40±2.73	13.28±0.87	5340±581.3	32.2±3.11 ^a	65.2±2.95 ^a
Group II	37.4±2.61	12.38±0.87	4620±130.38	47.40±3.13	49.8±3.11
Group III	38±2.12	12.6±0.60	4480±258	46.0±0.71	50.8±0.84
Group IV	36.8±3.83	12.18±1.22	5920±1054	49.8±4.60	47.2±4.49
Group V	39±2.0	12.8±0.51	4740±602.5	49±3.87	48.6±4.6
Group VI	35.4±4.39	11.76±1.46	5240±1101	45.8±4.32	51.6±4.72
Group VII	36.6±2.07	11.66±1.12	6220±311 ^a	43.2±7.19	56.4±5.64

Comparisons of mean values: ^a statistical significant at p<0.05 when compared with group III (negative control) Hb(Haemoglobin); WBC (white Blood cells); PCV(Packed Cell Volume)

Table 5: Micronucleus assay: mPCEs/1000PCEs

Group	Mean±SD
Group I	22.50±6.45 ^a
Group II	35.0±4.08 ^a
Group III	6.25±2.50
Group IV	11.25±2.5
Group V	15.0±4.08
Group VI	17.5±2.88
Group VII	27±2.89 ^a

^a statistical significant at p<0.05 when compared with group III (negative control)

DISCUSSION

The fact that some plant extracts are beneficial in treatment of many human ailments has led to increase in search for more plants with medicinal effects for therapeutic purposes in recent times. The present study was undertaken to evaluate the effects of ethanol and methanol leaf extracts of *Spondias mombin* against sodium arsenite induced toxicity in rats. *Spondias mombin* is commonly used for medicinal purposes in both humans and animals (Abo *et al*, 1999; Nzegebulu and Meregini, 1999; Corthout *et al*, 1994; Burkil, 1985). Arsenic is major environmental contaminants with a diverse range of detrimental effects including cancers (Wang *et al*, 1996). Sodium arsenite a trivalent arsenic compound has been classified as a potent human carcinogen that affects millions of people mostly in prevalent areas of the world. Some food additives and plant extracts have shown to be effective in reducing some toxic effects of sodium arsenite (Odunola *et al*, 2011); in this study therefore, the effect of ethanol and methanol *Spondias mombin* leaf extracts were investigated using the enzyme assays, haematological analysis and micronucleus assay.

Changes in the levels of serum ALT, AST, ALP and γ -GT were determined to assess the effect of sodium arsenite *in vivo* in rats and to evaluate therapeutic effects of *Spondias mombin* leaf extracts on functional integrity of the liver. The parameters are useful markers for assessment of tissue damage associated with toxic effects of pollutants in the body, and a sensitive indicator of hepatocellular damage (Owu *et al*, 1998). A rise in serum levels of these parameters is as a result of leakage of enzymes and other metabolites out of the cellular compartments into the extracellular fluid, thereby increasing their concentrations in the blood once the liver cells are damaged. The enzymes assay showed high percentage increases in

ALT, ALP and AST activities but with percentage decrease in γ -GT, though the changes in serum levels were not statistically significant to negative control ($p>0.05$)(Tables 2, 3).The decreases in γ GT activity as observed in some treated groups could be as a result of liver injury. Gupta and Flora, (2005) reported the significant decreases of AST and ALT activity following arsenic exposure suggesting possible hepatic injury. Adeniyi *et al*, (2010) reported significant reduction of ALT, AST, and ALP activity in plant extracts treated animals which were suggested to be a reflection of liver injury in the animals. Chakraborty *et al*, (2010) also reported that arsenite exposure inhibited the activities of alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase AST. Whitby *et al*, (1984) showed that hepatic injury is often associated with alterations in the serum and liver level of some enzymes notably ALT and ALP. Liver enzymes are markers for liver function, and several findings have shown that whenever there is damage to the liver this could increase serum concentrations of these enzymes (Effaraim *et al*, 2000), and it is mostly associated with necrosis (Adedapoet *al*, 2004). Though some reports have shown that liver enzymes activity could be increased without substantial necrosis (Heiberg and Svegaard 1981; Burges *et al*, 1994), the percentage increases of the enzyme activity as observed in this study (Table. 2, 3) may suggest mild liver toxicity to the animals.

In the haematological analysis, there was no significant difference between all the groups in PCV; similar observation applied to haemoglobin level in all the groups; an indication that the substances did not have much effect on these parameters. In WBC there was significant difference between the negative control group (group III) and group VII. In neutrophils group, I (treated with sodium arsenite alone) had decreased value that was significant when compared to the negative control, while in lymphocytes, significant value also exit between negative control group (group III) and group I (Table 4).

This study also shows that sodium arsenite significantly induced micronuclei in polychromic erythrocytes (PCEs) when compared to negative control (Table 5). Ethanol leaf extract of *Spondias mombin* showed reduction of sodium arsenite induction of micronuclei in mPCEs (Table 5). Some experimental findings have shown an inverse relationship between some plant extracts and clastogenic induction by sodium arsenite (Adetutu *et al.*, 2004; Odunola, 2003; Biswas *et al.*, 1999; Roychoudhury *et al.*, 1993). The methanol leaf extract of *Spondias mombin* did not show any reductive effect of the sodium arsenite induced toxicity in mPCEs (Table 5)

In conclusion, the ethanol extract of *Spondias mombin* showed some positive effect against micronuclei induction by sodium arsenite, though not statistically significant at the dose of 200 mg/kg body weight. The mild increase in enzymes activity could reflect deleterious liver injury that led to leak of the liver enzymes to the serum. The findings in this study suggest that administration of the methanol and ethanol extracts of *Spondias mombin*, especially co-administration with chemical related substance with aim of achieving therapeutic purpose should, therefore, be done with caution.

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