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SUB-CHRONIC HEPATO-TOXICITY EVALUATION OF THE AQUEOUS- ETHANOL EXTRACT OF THE LEAF OF *SPONDIAS MOMBIN* IN RATS

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Abstract

Spondias mombin leaf used traditionally in Nigeria in the management of some chronic ailments was analysed to detect and quantify its phytochemical, anti-nutrient and heavy metal contents. Following a sub-chronic oral administration of the 80 % aqueous-ethanol leaf extract at 250 and 1000 mg/kg dose levels, for 3 weeks, to Wistar albino rats, its effect on some hepatic and haematological indices was determined. This was done by *in vivo* assay of the levels of serum aspartate transaminase (AST), serum alanine transaminase (ALT), serum alkaline phosphatase (ALP) and serum haemoglobin (Hb) level. The phytochemical screening revealed the presence of phytates, oxalates, tannins, saponins, triterpines, glycosides and resins. Among the heavy metals, iron and aluminium were dominant, while cadmium, mercury and lead were detected at micro quantities. The levels of anti-nutrient phytate, oxalate, tannins, resins and cyanide were found to be 7.85, 2.57, 2.51, 0.40, and 0.04, percent respectively. The extract induced an inconsistent but significant increase in haemoglobin (Hb) value. There was significant decrease in serum levels of AST, ALT and ALP of rats treated with 250 and 1000 mg/kg oral doses of the extract ($p < 0.05$). At the upper dose, however, ALP values were slightly higher than those of the control normal animals. The findings indicated that extract of *Spondias mombin* at the oral dose of 1000 mg/kg is not toxic in albino rats.

Keywords: *Spondias mombin*, haematological parameter, hepatic function indices, medicinal plants,

INTRODUCTION

According to the World Health Organisation (WHO) Facts Sheet on traditional medicine, about 80 % of the people in the developing countries depend on herbs for primary healthcare. Even though, the efficacies of some of the herbs have been confirmed by certain traditional methods, their toxicities are usually not organs like liver. It became imperative to assess their toxicity profiles especially as the doses and regimens of herbal preparations are not usually regulated (Chan, 1997).

Spondias mombin (Anacardiaceae), commonly known as *yellow mombin*, is a native of the tropical America, as

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well as, India and Indonesia (Wikipedia, 2008). It is widely cultivated and naturalised in tropical Africa, and adapts well to arid and humid zones (USDA, 2008). The *mombin* tree is deciduous, losing its leaves around February or March, but growing them back before the rains begin. The leaf of *yellow mombin* is commonly used for induction of labour, as an abortefacient and child birth aid (Offiah, 1989; Kramer *et al.*, 2002; Njoku and Afamefula, 2002).

The fruit juice of *Spondias mombin* is drunk as a diuretic and febrifuge; the decoction of the astringent bark serves as an emetic, a remedy for diarrhea, dysentery, heamorrhoids and treatment of gonorrhoea and leucorrhoea (Morton, 1980). Its anti-inflammatory effect has also been confirmed (Abad, 1996). *S. mombin* is also used in the management of digestive tract ailment, lower back pains, rheumatism, anginas, sore throat, malaria fever, diarrhea, urethritis, and as contraceptive (Anon, 2003). Akubue *et al*., (1983) highlighted and validated some of the ethno-medicinal uses of *yellow mombin* in Nigeria.

S. mombin leaf has been reported to contain salicylic acid derivatives, and a group of pain-relieving chemicals known as caryophyllene (Abad, 1996). It is also a significant source of chlorogenic acid – a natural phytochemical with potent antibacterial, antiviral and anti-inflammatory activities (Abad, 1996).

Our investigation aimed at evaluating the toxicological effects of *S. mombin* in Wistar albino rats. This was premised on the high incidence of its use as herbal drug, and also given the fact that toxicity and risk associated with herbal drugs are sometimes not easily detected by the common users (Miller, 1990; Huxtable, 1990).

EXPERIMENTAL

S. mombin leaves extractions

Fresh leaves of *S. mombin* were harvested on 8 June, 2008, at Nsukka, Enugu State, Nigeria, and were authenticated by Mr. Alfred Ozioko, Taxonomist, Bioresources Development and Conservation Programme (BDCP), Nsukka, Nigeria. A voucher specimen no. BDCPH/124 was deposited at the institute's herbarium. The leaves were air-dried at ambient temperature and pulverised to a coarse powder. A 500-g portion of the leaves was macerated in 1 L of 80 % ethanol/water mixture. The macerate was allowed to stand for 48 h with frequent agitation before filtration. The extract was dried *in vacuo* at less than 40 °C in a rotating evaporator to obtain amorphous deep-greyish solid in 12.47 % yield.

Animals

Nine Wistar albino rats (58.30 – 219.30 g) were obtained from the Department of Veterinary Medicine, Animal House, University of Nigeria, Nsukka. They were fed with Top Feeds starter/chick mash, of Top Feeds Nigeria Ltd, obtained commercially, and had free access to water *ad libidum*. The animals were handled in accordance with the guidelines of the University of Nigeria Ethical Committee on the use of experimental animals and humans in bioresearch (UNBEC, 2006).

Sub-chronic administration of *S. mombin*

The rats were divided into three groups of three animals per group. Groups I and II received a single daily oral dose of 250 and 1000 mg/kg, respectively, for 21 days. Group III, control, received no treatment for same period but had full access to feed and water. On the 21st day, all the animals were fasted over-night, sacrificed the next

day and blood obtained via cardiac puncture.

Liver function indices
The blood was centrifuged at 3000 rpm for 10 minute to obtain sera. The liver function assay for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP) were performed using standard method (CESSCCCP, 1974; Singh, 2007).

Haematological parameter
The heamoglobin level of the rats was monitored by the cyanomethhaemoglobin method. A 4-ml aliquot of Darpkin solution was placed in a test tube containing 20 µml of blood homogenized vigorously and allowed to stand for 5 minute. The absorbance of the medium was obtained at 540 nm in an ultraviolet/visible light spectrophotometer, which was previously calibrated at same wavelength with a reagent blank. The Hb level was then derived from the standard curve of haemoglobin.

Anti-nutrients and heavy metals
The levels of tannins, phytate, oxalate, cyanide, resin, iron, cadmium, mercury, lead and aluminium in *S. mombin* leaf, were determined by the methods described in Pearson (1976).

Phytochemical analysis
The classes of phytocompounds present in the leaf extract were determined using standard methods (Trease and Evans, 1983; Harborne, 1998).

Statistical analysis
All data were expressed as mean ± S.E.M. Means were compared for statistically significant difference using one-way analysis of variance (ANOVA). Effects were considered

RESULTS

The phytochemical composition of the extract, as shown in the appendix A, indicated limited amount of triterpenes and free reducing sugars, moderate amount of saponins, glycosides, resins and tannins, and abundance of phytates. This was confirmed by the levels of its anti-nutrient presented in Table 1.

TABLE 1: Levels of some anti-nutrients in the pulverised leaves of *Spondias mombin*

Anti-nutrient	*Percent level*
Phytate	**7.85 ± 1.02**
Oxalate	**2.57 ± 0.96**
Tannins	**2.51 ± 0.79**
Resins	**0.40 ± 0.06**
Cyanide	**0.04 ± 0.01**

Among the anti-nutrients (Table 1), phytates had the highest concentration per 100 g of sample on dry basis. Low limits of resins and cyanide were recorded. The levels of some heavy metals in the leaf were shown in Table 2.

Table 2: Levels of some critical heavy metals in the pulverised leaves of *Spondias mombin*.

Heavy metal	***Limit detected (mg/100 g)***
Iron	7.86 ± 1.34
Mercury	0.09 ± 0.01
Cadmium	0.56 ± 0.17
Aluminium	6.80 ± 2.06
Lead	0.48 ± 0.19

Iron and aluminium were 7.86 ± 0.24 and 6.80 ± 0.19 mg/100 g, respectively, while low limits of mercury and cadmium were noted.

Analysis of some haematological indices revealed that sub-chronic administration of oral doses of *S. mombin* induced a non-dose dependent increase in haemoglobin (Table 3).

TABLE 3: Levels of haemoglobin in the treated and normal animals

Treatment Group	*Haemoglobin level (g/100 ml)*
Normal animals	13.60 ± 0.46
250 mg/kg	14.23 ± 0.77
1,000 mg/kg	12.60 ± 2.60

From Table 4, the levels of AST, ALT and ALP at 250 mg/kg oral dose of the leaf extract were generally lower than the corresponding values for the control. This was confirmed by the levels of the serum transaminases at 1,000 mg/kg oral dose, which was significantly lower than the normal values ($p < 0.05$). However, ALP level at the later dose was insignificantly higher than the corresponding normal value ($p < 0.05$). These observations may indicate that the leaf extract of *yellow mombin* possess hepato-protective effect.

TABLE 4: Serum level of liver function indices in the treated and normal animals

Liver function Indices	*Treatment Group*		
	Normal Animals	*250 mg/kg*	*1,000 mg/kg*
AST			
(µg/L)	118.00 ± 16.64	116.33 ± 29.74	*100.00 ± 3.46
ALT			
(µg/L)	26.67 ± 6.11	22.67 ± 2.31	*21.33 ± 3.06
ALP			
(µg/L)	50.00 ± 39.20	*45.27 ± 40.14	*61.05 ± 2.41

Significant at $p < 0.05$ with respect to the normal.

DISCUSSION

It is of great concern that most herbal medicines used by Nigerian traditional medicine practitioners are often used in their crude forms, which may contain several deleterious constituents like heavy metals and anti-nutrients. The toxic effects of these herbal preparations on target organs of animals and humans have been of medical concern (Chan, 1997; Pak *et al.*, 2004).

In this investigation, an attempt was made to evaluate the toxicological profiles of the aqueous-ethanol extract of *S. mombin* in the Wistar albino rat model following sub-chronic administration. Its effect on Hb level showed that *S. mombin* had no deleterious effect on haematological parameters; but may be considered as a haemoglobin booster. Since, the Hb level in treated animals was higher than those of the untreated (normal) animals.

From Table 4, the results obtained in extract treated rats at 250 mg/kg were significantly lower than those of the control ($p < 0.05$). However, at a higher dose of 1,000 mg/kg, the extract insignificantly increased the plasma level of ALP as compared to the control ($p < 0.05$), while those of AST and ALT were significantly lower than the corresponding values for the normal control ($p < 0.05$). The observations indicated that the leaf extract of *S. mombin* was not toxic to hepatocytes at oral doses of 250 and 1,000 mg/kg for the period of administration. Rather, the drug candidate exerted a protective effect on the rat hepatocytes.

Increase in serum enzymes such as AST, ALT and ALP, are commonly used in the diagnosis of liver injury, especially, by chemicals (Pitter and Ernest, 2003). Since the findings of the current investigation are the converse. Thus the leaf extract of *S. mombin* is not toxic to rat hepatocytes.

CONCLUSION

The findings indicated that the aqueous-ethanol extract of *S. mombin* leaf is not hepato-toxic in rats up to the oral dose of 1,000 mg/kg over a period of three weeks. Thus its safety on sub-chronic administration in ethno medicine is hereby confirmed.

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