AHC -08

Methanol Extract of *Spondias mombin* Leaf Protects the Liver against Acetaminophen-Induced Hepatotoxicity in Albino Rats

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Abstract
This study investigated the protective effect of *Spondias mombin* against acetaminophen-induced hepatotoxicity. Thirty female albino rats assigned to six groups of five rats per group were used to study the acute toxicity effect of the plant. Rats in groups 2-6 received 500, 1000, 2000, 4000 and 5000 mg/kg body weight of the extract while rats in group one received distilled water to serve as the normal control. The rats were observed for 48 h for signs of toxicity. Another thirty rats assigned to six groups (A-F) of five rats per group were used for biochemical studies. Rats in groups D-F were pre-treated with 100, 200 and 400 mg/kg of the extract while group C rats received 100mg/kg silymarin two times daily for five days before intoxication with 2000 mg/kg acetaminophen. Group A rats received distilled water alone while rats in group B were pre-treated with distilled water and intoxicated with acetaminophen to serve as normal and negative controls respectively. Sera for biochemical [Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total protein, albumin and total bilirubin] analyses were collected 18 h post acetaminophen intoxication. Results indicate that there were no signs of toxicity even at the highest dose of 5000mg/kg. The activities of ALT, AST and serum levels of total bilirubin of the extract and silymarin-pretreated rats were significantly ($p<0.05$) reduced when compared with that of the negative control group. Total protein and albumin values were not affected. It was concluded that the methanol extract of *Spondias mombin* leaf possesses hepatoprotective properties.

Keywords: Acetaminophen, hepatotoxicity, rats, *Spondias mombin*

Introduction

Spondias mombin otherwise known as *Spondias purpurea* or yellow mombin belongs to the family of Anacardiaceae. It is a native of America and can be found in Africa. It is a small deciduous tree used in traditional medicine as diuretic, febrifuge, astringent and in treatment of urethritis, cystitis and other inflammatory disorders (Ayoka *et al.,* 2008).

Liver disorders constitute major problems worldwide. The majority of injury to the liver may be as a result of chronic alcohol consumption, poor nutrition, infections, hepatotoxicants or drug abuse (Francesca, 2012). Acetaminophen or paracetamol® is a common over-the-counter analgesic and antipyretic drug that is abused daily (Keffe and Friedman, 2004). Acetaminophen-induced hepatic injury is attributed to the production of N-acetyl-p-benzoquinone, a highly reactive metabolite of acetaminophen (Vermeulen *et al.,* 1992).Injury to the liver can be assessed by evaluating the activities of certain enzymes that are localized in the hepatocytes or hepatobilliary system such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase etc. These enzymes appear in the serum only when there is injury to the liverand or some tissues such muscles, bones and thus can be used to assess the status of the liver (Dobbs *et al.,* 2003). Other parameters that can be used to assess the status of the liver include total protein, total bilirubin, albumin etc (Aba *et al*., 2014a).

Herbs have been in use since time immemorial in management of hepatic disorders. The aim of this study is to evaluate the possible hepatoprotective properties of *S. mombin* in acetaminophen-induced hepatotoxicity in albino rats.

Materials and Methods

Cold maceration method was used (Aba *et al.,* 2014b). Five hundred gram (500 g) of air-dried pulverized *Spondias mombin* leaves were soaked in 1, 500ml of 80% methanol with intermittent shaking after every 2 h interval for 48 h. Thereafter, it was filtered with No1 Whatmann filter paper and concentrated *in vaccuo*.

Female albino Wistar rats weighing between 120 and 150 g and obtained from animal house of Veterinary Medicine Faculty, University of Nigeria, Nsukka were used for this study. Randox assay kits for estimation of serum AST, ALT, Bilirubin, total protein and albumin were used. The chemicals and reagents were of analytical grades. Acetaminophen was from M&B while Legatex 70® was the brand name of silymarin used.

Acute toxicity study was carried out followed by the study to investigate relevant biochemical parameters. Acute toxicity of methanol leaf extract of *S. mombin* was evaluated using 30 female rats that were randomly assigned into six groups of five rats per group. Groups 2-6 rats received the extract at increasing doses of 500, 1000, 2000, 4000 and 5000 mg/kg respectively while rats in group 1 received 10ml/kg distilled water to serve as normal control. Signs of toxicity such as dullness, excitement, death were looked out for during the 48 h study.

A total of 30 female rats assigned to 6 groups of 5 rats per group were used for the study. Rats in group A were administered with 10ml/kg distilled water (DW) alone and served as normal control. Group B rats received 10ml/kg DW before administration of acetaminophen while rats in group C were pretreated with silymarin before intoxication with acetaminophen. Rats in groups D-F were pretreated with 100, 200 and 400 mg/kg body weight of the methanol leaf extract of *Spondias mombin* prior intoxication with acetaminophen.
All pretreatment with distilled water, silymarin or the extract were done two times daily for five days per Os using gastric tube. Acetaminophen was administered on the 5th day at the dose of 2000 mg/kg body weight. Eighteen hours (18 h) post acetaminophen intoxication, blood samples (sera) were obtained via the retrobulbar plexus of the eye for assay of some biochemical parameters (ALT, AST, total bilirubin, total protein and albumin).

Aspartate aminotransferase and alanine aminotransferase activities were determined following the method of Reitman-Frankel colourimetric method (Reitman and Frankel, 1957) while total protein was by direct Biuret method (Lubran, 1978) and total bilirubin by Jendrassik-Grof method (Doumas *et al.,* 1973.). Albumin was determined by bromocresol blue method (Doumas *et al.,* 1971).

One-way Analysis of Variance was used to compare the means of parameters across the group. Variant means were separated with Duncan's Multiple Range Test and probability levels less than 0.05 was considered significant (Aba *et al.,* 2014b).

Results and Discussion

The activities of ALT and AST were significantly ($p<0.05$) higher in group B rats (Negative control) compared to all other rat groups eighteen hours (18 h) post acetaminophen administration (Table1). This could be due to hepatic injury associated with acetaminophen administration (Prescott, 1980). Activities of serum enzymes such as ALT and AST are elevated in cases of liver injury (Savide and Oehme, 1983). Groups treated with silymarin and 400 mg/kg body weight of *S. mombin* methanol leaf extract recorded statistically similar ALT activity compared to that of the normal control. The AST activity of the rats treated with silymarin was similar ($p>0.05$) to that of the normal control rats but were significantly ($p<0.05$) lower than that of the extract-treated rats. The reductions in the activities of ALT and AST of the extract or silymarin-pretreated groups in comparison to the negative control groups is an indication that the silymarin and extract protected hepatocytes against injury induced by acetaminophen.

Table 1: Effect of methanol extract of *Spondias mombin* leaf on some biochemical parameters of acetaminophen-induced hepatotoxicity in rats

Group/Parameters	ALT (IU/L)	AST(IU/L)	Bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)
Group A	30.00±3.11^{a}	53.47±9.97^{a}	0.24±0.00^{a}	6.52±0.24^{a}	3.51±0.99^{a}
Group B	150.01±5.22^{c}	299.80±8.04^{c}	1.16±0.01^{c}	6.19±0.15^{a}	3.41±0.00^{a}
Group C	30.05±0.97^{a}	58.25±11.23^{a}	0.29±0.00^{a}	6.35±0.45^{a}	3.40±1.11^{a}
Group D	50.12±15.24^{b}	61.52±10.33^{b}	0.42±0.04^{b}	6.43±0.12^{a}	3.52±0.21^{a}
Group E	42.00±7.42^{b}	64.31±5.99^{b}	0.41±0.04^{b}	6.44±0.05^{a}	3.50±1.10^{a}
Group F	31.44±1.16^{a}	61.29±16.40^{b}	0.40±0.05^{b}	6.23±0.09^{a}	3.49±1.00^{a}

Different superscripts along the same column (across the groups) indicate significant difference at $p<0.05$
A –Distilled Water (DW) pretreatment + 2000 mg/kg Acetaminophen B - 100mg/kg Silymarin pretreatment + 2000 mg/kg Acetaminophen, C- 100mg/kg Silymarin pretreatment + 2000 mg/kg Acetaminophen, D- 100 mg/kg *S. mombin* extract pretreatment +2000 mg/kg Acetaminophen E- (200 mg/kg *S. mombin* extract pretreatment +2000 mg/kg Acetaminophen F- (400 mg/kg *S. mombin* extract pretreatment +2000 mg/kg Acetaminophen

There was also significant ($p<0.05$) decreases in total bilirubin levels of the extract-treated groups when compared to the negative control. Total bilirubin value of the silymarin-treated was significantly ($p<0.05$) lower than that of the extract-treated but statistically similar ($p>0.05$) with that of the normal control rats. Serum bilirubin (probably unconjugated bilirubin) may have accumulated in the acetaminophen intoxicated rats due to

impairment of hepatic function. Lower serum levels of bilirubin in the extract and silymarin-treated groups indicate improvement of hepatic function due to possible protection offered by the extract and silymarin. Hepatocytes are involved in bilirubin conjugation. This function is impaired in hepatic injury (Aba *et al.,* 2014a).

The total protein and albumin values did not vary significantly across all the groups through the duration of the experiment. This may be due to the fact that the experiment did not last for a longer time as to impair secretory function of the liver and induce significant changes in total protein and albumin values of the rats. The half life of serum proteins is about 2-3 weeks thus it takes liver injury beyond this period to elicit significant changes in values of the proteins (Andersen *et al.,* 2014).

Conclusion

We conclude that the methanol leaf extract of S*pondias mombin* is safe and posseses hepatoprotective effect against acetaminophen-induced liver injury.

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