

***Spondias mombin* L. (Anacardiaceae) enhances detoxification of hepatic and macromolecular oxidants in acetaminophen-intoxicated rats**

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Abstract: Oxidative stress is a common pathological condition associated with drug-induced hepatotoxicity. This study investigated *Spondias mombin* L. aqueous leaf extract on reactive oxygen species and acetaminophen-mediated oxidative onslaught in rats' hepatocytes. Hepatotoxic rats were orally administered with the extract and vitamin C for 4 weeks. The extract dose-dependently scavenged DPPH, hydrogen peroxide and hydroxyl radicals, with IC₅₀ values of 0.13, 0.66, and 0.64 mg/mL, and corresponding % inhibitions of 89, 80, and 90%, respectively at 1.0 mg/mL. Ferric ion was also significantly reduced. The marked ($p < 0.05$) increases in the activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were reduced following treatment with the extract. The extract also significantly ($p < 0.05$) induced the activities of antioxidant enzymes. These inductions reversed the acetaminophen-enhanced reduction in the specific activities of these enzymes as well as attenuated the observed elevated concentrations of auto-oxidized products and rived DNA in the acetaminophen-intoxicated animals. The observed effects competed with those of vitamin C and are suggestive of hepatoprotective and antioxidative attributes of the extract. Overall, the data from the present findings suggest that *S. Mombin* aqueous leaf extract is capable of ameliorating acetaminophen-mediated oxidative hepatic damage via enhancement of antioxidant defense systems.

Keywords: Antioxidants; Carcinogenesis; DNA fragmentation; free radicals; Lipid peroxidation; *Spondias mombin*.

INTRODUCTION

Acetaminophen is a known analgesic, anti-inflammatory and antipyretic agent. It is relatively safe at therapeutic doses but may cause acute centrilobular liver necrosis in overdose events (Meyer, 2001). Although, acetaminophen elimination is primarily through glucuronide and sulphate conjugates in the liver, a small percentage of it is also metabolized by cytochrome P450 to N-acetyl-P-benzoquinone imine (NAPQI) which is a highly reactive species (Jollow *et al.*, 1974; Wong *et al.*, 1981; Savides and Oehne, 1983). Acetaminophen-mediated hepatotoxicity has been linked with NAPQI and is usually detoxified by reduced glutathione (GSH) to mercapturic acid (Moore *et al.*, 1985). When the rate of NAPQI formation overwhelms the detoxification capacity of GSH, auto-oxidation of important tissue macromolecules (which consequently results in hepatic necrosis) occurs.

Since time immemorial, studies have ascribed several metabolic and debilitating disorders including hepatotoxicity to oxidative stress resulting from disequilibrium between generation and neutralization of

pro-oxidants (Sabiu and Wudil, 2011). Accordingly, antioxidants have been advocated and exploited in the management of such disorders due to their ability to prevent undesirable oxidation either by halting ravaging effects of reactive metabolites or simply as reactive oxygen species (ROS) inhibitors. As such, they offer considerable protection against infections and degenerative diseases (Subramaniam *et al.*, 2000). Restriction in the use of synthetic antioxidants is being imposed because of their carcinogenicity, hence the resolve to naturally occurring plant-based antioxidants of relatively low toxicity. Little wonder, a good percentage of the populace in some African countries including Nigeria, relies exclusively on plants as a source of medicine to complement and supplement the increasingly dear orthodox medical services (Fasola and Egunyomi, 2005). One of such plants finding applications in this regard is *Spondias mombin* (SM).

Due to the limited number of orthodox medical facilities in most rural areas of the world, plant extracts have been embraced to treat and manage liver disorders (Sabiu and Wudil, 2011). Though reports indicate that many of these plant formulations exhibit hepatoprotective potentials (Kanchana and Muhammad, 2011; Sabiu *et al.*, 2014),

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their molecular mode of action is still elusive. *Spondias mombin* L. (Anacardiaceae) is known as Iyeye (Yoruba), Ichikara (Ibo), and Tsardarmasar (Hausa) in Nigeria. It's a fructiferous tree and grows to a height of 15-22 m in rain forests and coastal areas (Ayoka *et al.*, 2008). Different parts of the plant have been valued ethnomedically in folkloric medicine due to its potent bioactive principles (Okwu, 2001). Its leaf is rich in antioxidant minerals and vitamins (Njoku and Akumefula, 2007), and has been opined as excellent analgesic and anti-inflammatory remedy against stomach ache and during child birth in women (Villegas *et al.*, 1997; Kramer *et al.*, 2006). Its pharmacological and therapeutic significance as antiulcerogenic, antimalarial, antioxidative, and antimicrobial agent have also been established (Abo *et al.*, 1999; Caraballo *et al.*, 2004; Sabiu *et al.*, 2015).

Management and treatment of liver disorders with herbal drugs has a long history, but substantiated scientific data for compliance and efficacy is sparse. Moreover, synthetic drugs may potentiate toxicity and pertinent adverse effects. The local communities in the south-western region of Nigeria have long been using the leaves of SM in the treatment of liver related diseases. The infusion of its leaves extract is administered orally to treat liver diseases (Kadiri *et al.*, 2015). However, the scientific evidence for its pharmacological potential against protein autooxidation, lipid peroxidation, and DNA damage of the hepatocytes is yet to be investigated. It is on this background, thus, that we investigated aqueous leaf extract of SM against oxidative routes associated with acetaminophen-mediated hepatic damage in Wistar rats.

MATERIALS AND METHODS

Chemicals, reagents and assay kits

Acetaminophen was a product of May and Baker Pharmaceuticals Limited, Lagos, Nigeria. Rutin, quercetin, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), FeCl₃, K₃Fe(CN)₆, glutathione disulphide, 1-chloro-2, 4-dinitrobenzene, epinephrine, glucose-6-phosphate, GSH, gallic acid, trichloroacetic acid (TCA), 2,6-dichlorophenol-indophenol, nicotinamide adenine dinucleotide (NADH), and uridine 5'-diphosphoglucuronic acid were procured from Sigma Aldrich Co., St. Louis, Missouri, USA. Assay kits for liver marker enzymes were purchased from Randox Laboratories Ltd., Co. Antrim, United Kingdom. All other reagents used were of analytical grade and obtained in their purest form from reputable companies.

Plant collection and authentication

Fresh leaves of SM were collected in June 2014 from the premises of University of Ilorin, Ilorin, Nigeria. They were authenticated at the University's Herbarium, where a

voucher specimen (no. UIH/14/20567) was prepared and deposited.

Experimental animals

The study was executed following approval (no. HUI/ECULA/014/06/006) from the Ethical Committee on the use of Laboratory Animals of Al-Hikmah University, Ilorin, Nigeria. Albino rats (*Rattus norvegicus*) with a mean weight of 170.00 ± 4.65 g were procured from the Animal House facility of the University and were kept in metabolic cages placed in a well-ventilated room with optimum condition (temperature: 23 ± 1°C, photoperiod: 12 h light/dark cycle, relative humidity: 45-50%). The rats were allowed to acclimatize for 10 days and had *ad libitum* access to food and water.

Preparation of extract

Spondias mombin's leaves were shade-dried to constant weight and subsequently pulverized, weighed and kept airtight prior to extraction. The powdered sample (500 g) was exhaustively extracted in distilled water (5 L) for 48 h with continuous agitation on labcon platform shaker maintained at 150 rpm. The solution obtained was then filtered and the resulting filtrate lyophilized to give 15.5 g (3.1%) residue. The lyophilized sample was reconstituted in distilled water to give the therapeutic doses of the extract used in the study.

Quantification of flavonol content

The amount of flavonols present in the extract was determined by the method of Kumaran and Karunakaran (2007). Briefly, 20 g of AlCl₃ and 50 g of sodium acetate anhydrous powder were dissolved in distilled water and made up to 1 L. Rutin standard curve was prepared by mixing 2 mL of its varying concentrations with 2 mL of AlCl₃ (20 g/L) and 6 mL of sodium acetate (50 g/L). Absorbance was read at 440 nm after 150 min at 20°C. The procedure was repeated with 2 mL of SM extract replacing rutin solution for evaluation and comparison. All determinations were done in triplicates and the flavonols content was evaluated in rutin equivalents (mg/g) using the equation from the standard curve.

In vitro antioxidant assay

Scavenging DPPH radical

The ability of SM leaf extract against DPPH was evaluated adopting the procedure of Turkoglu *et al.* (2007). In brief, equal volumes (2 mL each) of different amounts (0.2-1.0 mgmL⁻¹) of the extract in ethanol and 0.2 mmol/L of DPPH in ethanol were mixed and allowed to stand for 30 min. Following the incubation period at room temperature, the absorbance was read against blank at 517 nm and the inhibition rate on DPPH radical was evaluated by the expression:

Percentage Inhibition (I%) = [(A_{blank} - A_{sample})/A_{blank}] × 100, where A_{blank} and A_{sample} represent the absorbance of the blank and sample respectively. Using standard calibration curve, the concentration of SM extract

resulting in 50% inhibition (IC₅₀) of DPPH radical was estimated.

Hydrogen peroxide scavenging assay

This assay was evaluated according to the procedure of Ruch *et al.* (1989). Exactly, 3.4 mL of different amounts (0.2-1.0 mg/mL) of SM extract in phosphate buffered saline (pH 7.4) were carefully mixed with about 0.6 mL of 40 mM H₂O₂ and left for 10 min at room temperature. The absorbance was thereafter read at 230 nm and the percentage H₂O₂ inhibitory potential of SM was evaluated from the expression:

% H₂O₂ scavenged = $A_{\text{control}} - (A_{\text{sample}} - A_{\text{extract}}) / A_{\text{control}} \times 100$, A_{control} is the absorbance of the mixture without extract. A_{sample} and A_{extract} represent the absorbance of the mixture with the extract and that of the extract alone, respectively. The IC₅₀ value was thereafter estimated from the calibration curve.

Hydroxyl radical scavenging assay

This was measured as described by Smirnoff and Cumbes (1996). About 2 mL of leaf extract of SM (at concentrations 0.2-1.0 mg mL⁻¹) were mixed with 0.6 mL of ferrous sulfate (8 mM), 0.5 mL of H₂O₂ (20 mM) and 2 mL of salicylic acid (3 mM). Following a 30 min incubation period at 37°C, distilled water (0.9 mL) was added and the resulting mixture centrifuged at 4472 g for 10 min. The absorbance was subsequently read at 510 nm and the IC₅₀ value was evaluated from calibration curve following estimation of percentage hydroxyl radical scavenging capacity of SM as per the expression:

% hydroxyl radical scavenged = $A_{\text{control}} - (A_{\text{sample}} - A_{\text{extract}}) / A_{\text{control}} \times 100$, A_{control} , A_{sample} and A_{extract} represent the absorbance of the mixture without extract, mixture with the extract and that of the extract alone, respectively.

Reducing power

Following the method of Oyaizu (1986), the reducing power of the extract was assayed. About 1 mL of different concentrations of SM leaf extract were mixed with 2.5 mL each of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. After a 20 min incubation period at 50°C, TCA was added to the mixture and the resulting medium centrifuged for 10 min at 3000 rpm. About 2.5 mL of the supernatant obtained was thereafter mixed with equal volume of distilled water and 0.1% FeCl₃ (0.5 mL) prior to absorbance reading at 700 nm.

Induction of liver damage

Hepatotoxicity (liver damage) was achieved following the method of Kanchana and Muhammad (2011). Briefly, the animals were orally administered with 400 mg kg⁻¹ body weight (b.w.) of acetaminophen once daily for 2 weeks.

Animal grouping and treatments

Forty two rats were randomly distributed into six groups of seven animals per group. The rats in group 1 received sterile placebo and served as the control. Group 2

comprised animals induced with liver damage but not treated. Rats in group 3 were placed on 200 mg/kg b.w. of SM leaf extract only, while animals in groups 4, 5 and 6 were hepatotoxic rats treated respectively with the extract at 100 and 200 mg kg⁻¹ b.w. doses and vitamin C (100 mg kg⁻¹ b.w.) for 4 weeks. All administrations were done once daily via oral intubation.

Serum and tissue preparation

Twenty four hours after the last treatments, the animals were sacrificed under halothane anesthesia. Following being unconscious, blood was diligently collected from the jugular veins using sterile scalpel blade. The blood collected was allowed to clot and then centrifuged for 10 min at 300 g for serum preparation. The supernatant obtained was used for the evaluation of serum activities of liver marker enzymes. Excised liver tissues from the animals were cleaned and homogenized in ice-cold sucrose solution (0.25 M, 1:5 w/v). The homogenates were then centrifuged at 10000 g (10 min, 4°C) for post-mitochondrial fractions and the clear supernatant obtained was stored at -20°C before being used for other biochemical assays (Oloyede and Sunmonu, 2009).

Biochemical assay

Assays for glucose-6-phosphate dehydrogenase and liver marker enzymes were performed in accordance with the manufacturer's specifications on the respective kits. Tissue activities of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were analyzed using the procedures of Sinha (1972), Mavis and Stellwagen (1968), Thabrew *et al.* (1987) and Marklund and Marklund (1974), respectively. Ellman (1959) and Hissin and Hilf (1976) protocols were respectively employed in the determination of reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations. Tissue levels of lipid hydroperoxides, conjugated dienes and malondialdehyde were evaluated adopting the methods of Reilly and Aust (2001), while the procedures of Burton (1956), and Levine *et al.* (1990) were used in the quantification of fragmented DNA and protein carbonyls, respectively.

Analysis of data

Reactive species inhibitory activities were presented in percentages. Other data were presented as mean ± standard deviation (SD) of seven replicates. One way analysis of variance (ANOVA) using SPSS software package for windows (Version 16) for differences between means was used to detect differences between the treatment groups and were considered statistically significant at p < 0.05.

RESULTS

Flavonol estimation and in vitro ROS scavenging effects of *S. mombin*

Aqueous leaf extract of SM was found to contain 49.59 ± 0.03 mg rutin g⁻¹ of flavonol. fig. 1-4 revealed the *in vitro*

antioxidant potentials of aqueous leaf extract of *S. mombin*. The extract dose-dependently inhibited and scavenged the generated radicals in all the assays investigated. The reducing power of the extract against ferric ion was also commendable. The capability of the extract to scavenge DPPH radical revealed a remarkable effect judging by the IC_{50} value (0.13 mg/mL) when compared with vitamin C (0.26 mg/mL) (table 1). For the hydrogen peroxide and hydroxyl radicals, SM exhibited good antioxidant effect (with IC_{50} values of 0.66 and 0.64 mgmL⁻¹, respectively) and competed well with vitamin C (0.56 and 0.53 mgmL⁻¹) (table 1). Similarly, the reducing power ability of SM revealed a significant potential against ferric ion, with the highest dose (1.0 mg/mL) exhibiting the most potent effect (fig. 4). The respective coefficient of determination (R^2) values and regression equations for the assays are also presented in table 1.

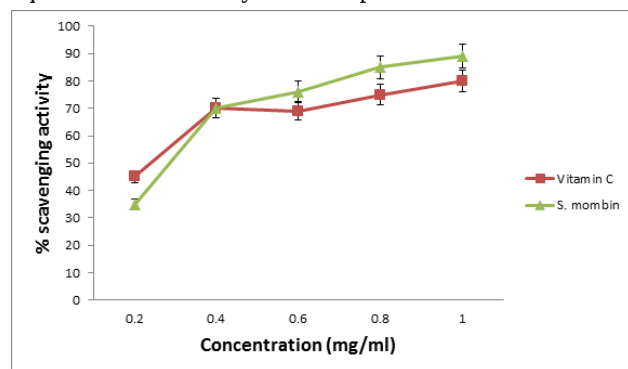


Fig. 1: DPPH scavenging effect of *S. mombin* leaf extract.

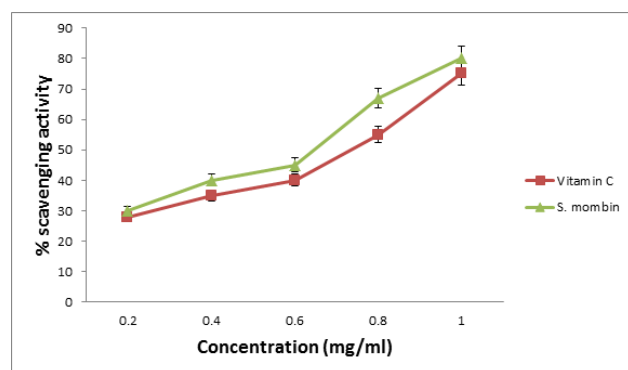


Fig. 2: Hydrogen peroxide scavenging effect of *S. mombin* leaf extract.

Liver function enzymes

The effect of aqueous leaf extract of SM on the specific serum activities of ALP, ALT and AST in the experimental animals is shown in table 2. Administration of 400 mgkg⁻¹ b.w. of acetaminophen for 2 weeks brought about a significant ($p < 0.05$) increase in the activities of these enzymes when compared with the normal control and vitamin C treated animals. Following treatment with the extract, the elevated activities of the assayed enzymes induced by acetaminophen were markedly ($p < 0.05$) attenuated in a dose-dependent manner.

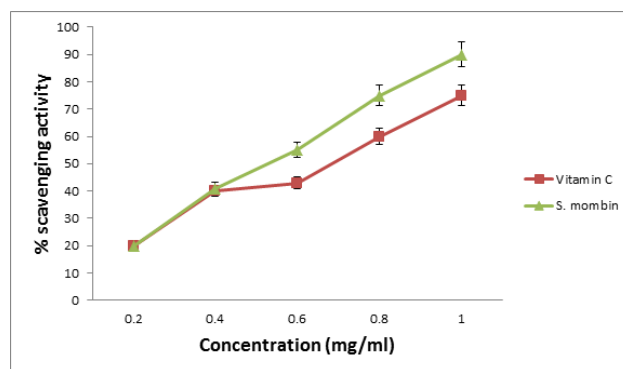


Fig. 3: Hydroxyl ion radical scavenging effect of *S. mombin* leaf extract.

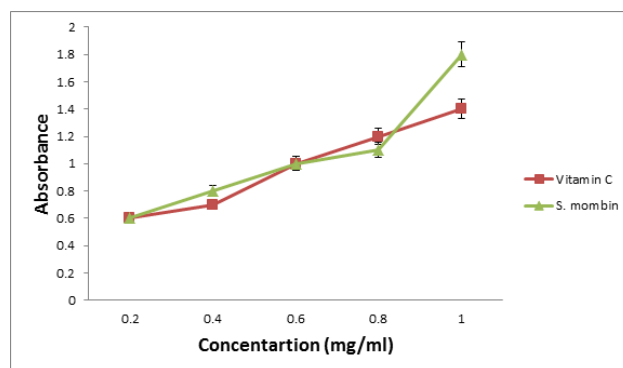


Fig. 4: Reducing power potential of *S. mombin* leaf extract.

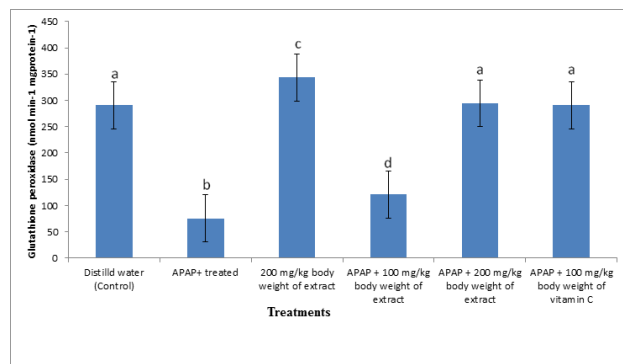


Fig. 5: Specific activity of glutathione peroxidase following administration of aqueous extract of *S. mombin* leaf in acetaminophen-treated rats. Values are mean $\pm$ standard deviation (SD) of seven determinations. ^{abcd} Bars with different superscripts for the parameter are significantly different ($p < 0.05$). APAP = Acetaminophen.

Biomarkers of oxidative stress

Antioxidant enzymes

The activities of enzymes including GPx, CAT, SOD, glucose-6-phosphate dehydrogenase and GR were remarkably ($p < 0.05$) induced by SM leaf extract. These inductions significantly ($p < 0.05$) reverted the observed reduction in their activities and the effect compared favourably with that of vitamin C (figs. 5, 6 and 7).

Table 1: IC₅₀ (mg/mL) of DPPH, hydrogen peroxide, and hydroxyl radicals scavenging capabilities of *Spondias mombin* aqueous extract.

Assay	Parameters	Vitamin C	SMAE
DPPH	IC ₅₀	0.26	0.13
	R ²	0.821	0.774
	Regression equation	y=61.50x+34.10	y=37.50x+45.30
H ₂ O ₂	IC ₅₀	0.56	0.66
	R ²	0.957	0.928
	Regression equation	y=63.50x+14.30	y=57.00x+12.40
OH [•]	IC ₅₀	0.53	0.64
	R ²	0.996	0.968
	Regression equation	y=87.00x+4.00	y=65.00x+8.60

SMAE= *Spondias mombin* aqueous extract.

Table 2: Effect of aqueous leaf extract of *Spondias mombin* on specific activities of some marker enzymes of the hepatocytes of acetaminophen-treated rats.

Treatments	Alkaline phosphatase (U/L)	Alanine amino transferase (U/L)	Aspartate amino transferase (U/L)
Sterile placebo (Control)	65.23 ± 0.02 ^a	41.15 ± 0.30 ^a	80.36 ± 0.16 ^a
APAP treated	116.21 ± 0.03 ^b	152.82 ± 0.20 ^b	139.18 ± 1.05 ^b
200 mg/kg body weight of extract	59.65 ± 0.02 ^a	53.61 ± 0.40 ^a	78.05 ± 0.81 ^a
APAP + 100 mg/kg body weight of extract	80.23 ± 0.01 ^c	55.56 ± 0.35 ^a	105.17 ± 1.15 ^c
APAP + 200 mg/kg body weight of extract	59.25 ± 0.01 ^a	52.70 ± 0.22 ^a	93.36 ± 0.11 ^a
APAP + 100 mg/kg body weight of Vitamin C	63.61 ± 0.02 ^a	48.39 ± 0.18 ^a	84.04 ± 0.12 ^a

Table 3: Effect of aqueous leaf extract of *Spondias mombin* on the levels of non-enzymic antioxidant system of acetaminophen-treated rats.

Treatments	Reduced glutathione (nmol mg protein ⁻¹)	Peroxidized glutathione (nmol mg protein ⁻¹)	GSH: GSSG ratio
Sterile placebo (Control)	64.25 ± 0.20 ^a	11.45 ± 0.11 ^a	5.61 ± 0.18 ^a
APAP treated	30.88 ± 0.43 ^b	27.24 ± 1.10 ^b	1.13 ± 0.18 ^b
200 mg/kg body weight of extract	93.36 ± 1.18 ^c	11.51 ± 0.11 ^a	8.11 ± 0.11 ^c
APAP + 100 mg/kg body weight of extract	58.68 ± 2.26 ^a	15.86 ± 0.32 ^c	5.20 ± 0.15 ^a
APAP + 200 mg/kg body weight of extract	62.87 ± 2.15 ^a	11.65 ± 0.57 ^a	5.40 ± 0.09 ^a
APAP + 100 mg/kg body weight of Vitamin C	66.44 ± 1.18 ^a	11.62 ± 0.30 ^a	5.72 ± 0.12 ^a

Table 4: Effect of aqueous leaf extract of *Spondias mombin* on lipid peroxidised products of the hepatocytes of acetaminophen-treated rats.

Treatments	Conjugated dienes (nmol mg protein ⁻¹)	Lipid hydroperoxide (nmol mg protein ⁻¹)	Malondialdehyde (nmol mg protein ⁻¹)
Sterile placebo (Control)	20.49 ± 0.20 ^a	14.62 ± 0.21 ^a	5.24 ± 0.23 ^a
APAP treated	72.32 ± 0.35 ^b	100.11 ± 0.27 ^b	17.33 ± 0.62 ^b
200 mg/kg body weight of extract	16.32 ± 0.73 ^a	8.88 ± 0.18 ^a	5.21 ± 0.17 ^a
APAP + 100 mg/kg body weight of extract	46.71 ± 0.45 ^c	47.65 ± 0.75 ^c	8.07 ± 0.36 ^c
APAP + 200 mg/kg body weight of extract	31.32 ± 0.36 ^d	28.18 ± 0.12 ^a	5.53 ± 0.36 ^a
APAP + 100 mg/kg body weight of Vitamin C	34.55 ± 0.08 ^d	31.45 ± 0.15 ^d	5.32 ± 0.46 ^a

Data are mean ± standard deviation (SD) of seven determination. ^{abcd} (in Tables 2-4) Values with different superscripts for each parameter are significantly different (P<0.05). APAP=Acetaminophen

Non-enzymic antioxidant

Acetaminophen administration caused a significant ($p < 0.05$) reduction in the level of reduced glutathione and a corresponding significant ($p < 0.05$) increase in the level of oxidised glutathione. These trends were significantly everted in a dose-dependent manner in animals treated with aqueous leaf extract of SM. It does competed well with vitamin C (table 3).

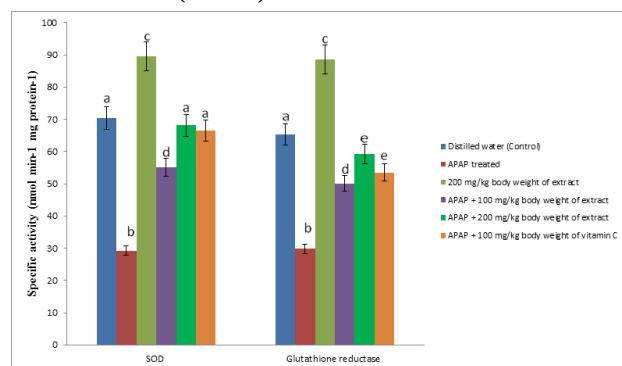


Fig. 6: Specific activities of SOD and Glutathione reductase following administration of aqueous extract of *S. mombin* leaf in acetaminophen-treated rats. Values are mean $\pm$ standard deviation (SD) of seven determinations. ^{abcde}Bars with different superscripts for each parameter are significantly different ($p < 0.05$). APAP = Acetaminophen.

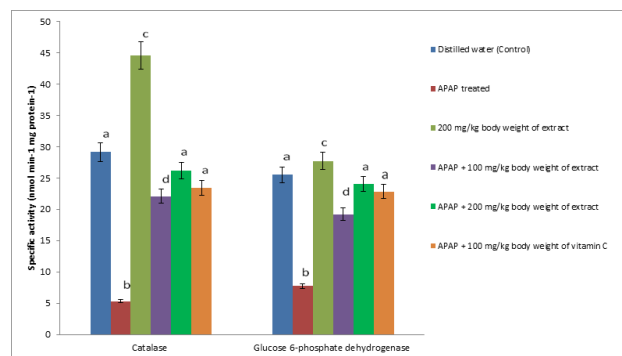


Fig. 7: Specific activities of catalase and glucose-6-phosphate dehydrogenase following administration of aqueous extract of *S. mombin* leaf in acetaminophen-treated rats. Values are mean $\pm$ standard deviation (SD) of seven determinations. ^{abcde}Bars with different superscripts for each parameter are significantly different ($p < 0.05$). APAP = Acetaminophen.

Macromolecular auto oxidation

Acetaminophen-mediated elevation in the levels of protein carbonyl, malondialdehyde, lipid hydroperoxides, conjugated dienes and percentage DNA fragmentation were significantly ($P < 0.05$) attenuated by aqueous leaf extract of SM (tables 4 and 5).

DISCUSSION

Oxidative damage resulting from deleterious influence of free radicals has been linked to acetaminophen-mediated

hepatotoxicity and the overall consequential effects are usually disconcerting (Balamurugan, 2007). When the body's antioxidant defense system is overwhelmed by catastrophic free radicals events, timely intervention with exogenous antioxidants augments the cellular defense system thereby preventing cell death.

Inhibitory effects on DPPH radical and ROS are models for testing antiradical potentials of plant formulations (Sabiou *et al.*, 2016a). Therefore, the results of absorbance and % inhibition/scavenging against radical propagation showed reduced levels of these species due to the scavenging ability of aqueous extract of SM. This compared favorably well with the effects elicited by vitamin C (a reference standard) and indicative of the extract's capability to halt cascade of reactions involving free radicals by sacrificing either electron or hydrogen atom there by making them relatively stable and preventing likelihood of cellular damage. The tending of R^2 values of the assays towards 1.0 is another predictive and supporting fact for the excellent antioxidative potential of SM.

The extent of drug-induced hepatotoxicity can be assessed by the release of enzymes like AST, ALT and ALP via the hepatocyte membrane into circulation (Jaeschke *et al.*, 2003). In this study, the elevated activities of AST, ALT and ALP in acetaminophen treated animals may be indicative of liver damage and cell necrosis resulting from formation of NAPQI in excess of GSH detoxification capacity. This is in agreement with previous studies (Balamurugan, 2007; Kanchana and Mohamed, 2011) where overdose of acetaminophen proved toxic to hepatocytes. Consequently, the significant reduction in the specific activities of these enzymes in rats treated with aqueous extract of SM indicates that it was able to ameliorate the deleterious influence of acetaminophen. This observation suggests hepatoprotective potential of the extract at the tested regimen.

The decreased specific activities of the assayed antioxidant enzymes could be due to their excessive mobilization towards detoxification of NAPQI and ROS during acetaminophen hepatotoxicity. This might have led to unguided oxidative attack on cellular macromolecules that consequently results in necrosis (Sabiou *et al.*, 2014). This finding is consistent with the reports of Chinnasamy *et al.* (2011) and Sabiou *et al.* (2016b) where similar reductions in activities of ROS detoxifying enzymes were associated with inordinate formation of NAPQI and ROS in acetaminophen-mediated hepatotoxicity in rats. Thus, excellent reversion of acetaminophen-induced reduction in the activities of ROS detoxifying enzymes by SM leaf extract indicates antioxidative activity. This could be ascribed to the tendency of the extract to scavenge NAPQI and ROS or enhance ROS detoxifying enzymes.

The observed attenuation in the level of GSH could be due to depletion of GPx and GR, as well as formation of

Table 5: Effect of aqueous leaf extract of *Spondias mombin* on protein carbonyl and fragmented DNA of acetaminophen-treated rats.

Treatments	Protein carbonyl (nmol mgprotein ⁻¹)	Fragmented DNA (%)
Sterile placebo (Control)	8.45 ± 0.02 ^a	10.63 ± 0.30 ^a
APAP treated	15.63 ± 0.03 ^b	98.75 ± 0.30 ^b
200 mg/kg body weight of extract	8.32 ± 0.03 ^a	11.57 ± 0.20 ^a
APAP +100 mg/kg body weight of extract	12.27 ± 0.02 ^c	40.32 ± 0.30 ^c
APAP + 200 mg/kg body weight of extract	9.88 ± 0.05 ^a	18.91 ± 0.20 ^a
APAP + 100 mg/kg body weight of Vitamin C	9.85 ± 0.05 ^a	18.11 ± 0.20 ^a

Data are mean ± standard deviation (SD) of seven determination. ^{abc}Values with different superscripts for each parameter are significantly different (P<0.05). APAP=Acetaminophen

NAPQI in excess of GSH detoxification capacity (Gini and Muraleedhara, 2010). Conversely, acetaminophen-mediated elevation in the concentration of oxidized glutathione may be ascribed to either GSH auto-oxidation or its mobilization towards formation of GPx. The reduction in the ratio of GSH to GSSG caused by acetaminophen intoxication reveals possible oxidative onslaught on the hepatocytes. Thus, the maintenance of GSH level coupled with corresponding high GSH: GSSG ratio and low GSSG levels in the liver of acetaminophen-intoxicated animals following treatment with SM leaf extract suggests inherent anti oxidative potential and further supports that it offered considerable level of hepatoprotection at the tested regimen. This submission is in conformity with the report of Gini and Muraleedhara (2010) that administration of plant extracts resulted in improved non-enzymic status in drug-intoxicated rats.

Oxidative insult to a cell induces per oxidation of membrane-bound lipids whose reactive metabolites elicit ravaging influence on macromolecules. Acetaminophen has been linked with lipid peroxidation and may aid elevated level of per oxidized products (conjugated dienes, lipid hydro peroxides and malondialdehydes) in hepatotoxicity (Muriel *et al.*, 1992). Therefore, the significantly increased levels of these products may depict haphazard oxidative onslaughts of acetaminophen on membrane-bound lipids. This might have disrupted membrane fluidity, orientation/organization as well as modified and inflicted functional loss of proteins and DNA base pairs (Niki, 2009). Das (1994) has also linked mutation with increased levels of conjugated dienes. The attenuation of acetaminophen-mediated increases in these per oxidative products by the aqueous extract of SM is suggestive of considerable level of protection on the membrane lipids. This could be adduced to ability of the extract to enhance detoxification of reactive metabolites, which might have aided per oxidation of polyunsaturated lipids of the hepatocyte membrane.

Protein carbonyl formation is a useful indicator commonly used to ascertain the level of protein auto-oxidation in cells. The significant increase in its concentration in the acetaminophen-intoxicated rat might be attributable to covalent binding of NAPQI to mitochondrial proteins (Jaeschke and Bajt, 2006). This

resultantly induced formation of nitrate ion that incapacitates the Ca²⁺ pump of the hepatocyte membrane. By effect, hypercalcemia that further hinders mitochondrial function and ATP production might have occasioned the observed elevated level of carbonyl product. The reversion of this trend however, by aqueous extract of SM further substantiates its possible potential to incapacitate reactive metabolites through induction of antioxidant defense systems of the hepatocyte.

Hydroxyl radical mediated oxidative damage and calcium ion accumulation are important events in the pathogenesis of DNA fragmentation. These events either promote tissue necrosis or carcinogenesis, which subsequently results in cell death (Cooke *et al.*, 2006). Thus, the significantly increased level of fragmented DNA in the liver of acetaminophen-treated rat is informative of genotoxicity. It also suggests probable initiation of carcinogenesis. Jaeschke and Bajt (2006) reported similar increase in damaged DNA due to acetaminophen intoxication. The decrease in the level of fragmented DNA in the liver of acetaminophen-treated rats by aqueous extract of SM leaf also buttressed its possible anti oxidative and antigenotoxic activities. The extract might have mimicked an antigenotoxic agent, thereby augmenting DNA repair or synthesis system (Brahmi *et al.*, 2011).

Generally, the attributes elicited by aqueous leaf extract of SM may be ascribed to its secondary metabolites with known antioxidative activity. This property is pertinent to their redox effects in neutralizing free radicals and annihilating ravaging chain reactions of ROS (Zheng and Wang, 2001). Their inhibitory effects on carcinogenesis as well as biological and radical scavenging activities have also been documented (Agrawal, 1989; Tung *et al.*, 2007). Impressively too, the flavonoid and phenolic contents of SM have been quantified (Sabiou *et al.*, 2015), and similar anti oxidative attributes as revealed by these class of compounds have also been established for flavonols (Miliauskas *et al.*, 2004).

CONCLUSION

Overall, it is evident from the present study that aqueous leaf extract of *Spondias mombin* enhanced detoxification

of acetaminophen-mediated liver injury by facilitating the specific activities of ROS detoxifying enzymes, thereby forestalling auto oxidation of cellular macromolecules and hepatotoxicity. Hence, SM leaf extract elicits auspicious attributes suggestive of a formidable agent in the management of drug-induced oxidative hepatic disorders.

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