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Research Article

Assessment of Flavonoid Content, Free Radical Scavenging and Hepatoprotective Activities of *Ocimum gratissimum and Spondias mombin* in Rats Treated with Dimethylnitrosamine

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Abstract

Formation of reactive oxygen species (ROS) has been implicated in the metabolism of nitrosamines resulting in oxidative stress. *Ocimum gratissimum* and *Spondias mombin* are valued ethnomedicinally in folkloric medicine. The *in vitro* antioxidant activities and hepatoprotective effects of methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* in rats intoxicated with dimethylnitrosamine were investigated in this study. Results revealed a copious flavonoid content in the extracts of *Ocimum gratissimum* (43.04 ± 4.18 mg/g catechin equivalent [CE]/g extract) and *Spondias mombin* (55.90 ± 6.3 mg/g CE/g extract). The extracts showed high $DPPH^{\bullet}$ radical scavenging activity: *Ocimum gratissimum* (79.4% at 200 µg) and *Spondias mombin* (71.27% at 200 µg), and a significant inhibition of AAPH-induced lipid peroxidation: *Ocimum gratissimum* (84.6% at 25 µg) and *Spondias mombin* (85.29% at 400 µg). The methanol extracts also showed strong reductive potential: *Ocimum gratissimum* (0.806 ± 0.001 at 400 µg) and *Spondias mombin* (0.908 ± 0.022 at 200 µg). Acute oral dimethylnitrosamine (DMN) administration led to hepatotoxicity as evident by elevated levels of ALT and AST. The antioxidant status and oxidative stress were monitored by determining the levels of hepatic reduced glutathione (GSH), hydrogen peroxide (H_2O_2) and glutathione peroxidase (GPx) activity. H_2O_2 generation was significantly enhanced, GSH level was significantly reduced and GPx activity was significantly induced in DMN intoxicated group. However, pretreatments with the extracts, at 100mg/kg and 200mg/kg, ameliorated the levels of ALT, AST and H_2O_2. In addition, the induction of GPx was also decreased by the two extracts at both doses. Moreover, the extracts significantly raised the level of non-enzymic antioxidant, GSH. These findings demonstrated that methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* are potent in reversing the dimethylnitrosamine induced hepatotoxicity and as such will be promising therapeutic agents against free radical mediated diseases.

Keywords: *Ocimum gratissimum, Spondias mombin,* antioxidant, reactive oxygen species, dimethylnitrosamine.

*INTRODUCTION

Dimethylnitrosamine (DMN) belongs to a class of chemicals known as *N*-nitroso compounds, characterized by the *N*-nitroso functional group (–N–N=O), and to the family of nitrosamines (Sax and Lewis, 1987) and has been found in processed meats and industrial products: pesticides, rubber tires, alkylamines, and dyes. It may also form under natural conditions in air, water, and soil as a result of chemical, photochemical, and biological processes, and has been detected in drinking-water and in automobile exhaust (Richardson, 2003; ATSDR, 1989). The formation of reactive oxygen species (ROS) like H_2O_2, superoxide anion ($O_2^{\bullet-}$) and hydroxyl radicals ($OH^{\bullet}$) has been demonstrated during the metabolism of nitrosamines resulting in oxidative stress, which may be one of the key factors in the induction of pathological conditions such as hepatocellular necrosis, carcinogenicity, clastogenicity, neoplastic changes, and tumor formation (Pradeep *et al.,* 2007). There is strong evidence that the toxicological effects of DMN are directly dependent upon the CYP2E1-dependent metabolic conversion of this nitrosamine to highly reactive species coupled to the fact that CYP2E1 in mouse liver stimulated Kupffer cells leading to generation of superoxide and other ROS capable of damaging liver cells (Farombi *et al.,* 2009; Lee *et al.,* 1996).

Plants have been used in traditional medicine for several thousand years (Abu-Rabia, 2005). Plants are potent biochemical factories and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals (Bouayed *et al.,* 2007). The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants. Plants contain many antioxidant compounds that act as major defence against radical-mediated toxicity by preventing or attenuating the damages caused by free radicals (Farombi *et al.,* 1999). The medicinal actions of plants are unique to particular plant species or groups and consistent with this concept is that the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1997).

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Ocimum gratissimum (Linn) family Labiaceae is an herbaceous plant commonly found in the tropical and sub-tropical region. The plant is used in the treatment of epilepsy, (Osifo, 1992), high fever (Oliver, 1980) and diarrhoea (Sofowora, 1993; Oliver, 1980). Also, decoctions of the leaves are used to treat mental illness and as condiments in cooking. Leaves and flowering tops of the plant yield an essential oil, on steam distillation, which is used to flavour foods, as dental and oral products, in fragrances and aromatherapy, for aromatic baths of fumigations in the treatment of rheumatism and paralysis and in traditional rituals and medicines (Choudary *et al.,* 2001; Pandey and Chounhury, 2001; Yusuf *et al.,* 1998). Phytochemical screening of *Ocimum* extracts reveal that carbohydrates, reducing sugars, lipids, alkaloids, steroids tannins and some other constituents are present. Eugenol is the dominant constituent in the oil of *O. gratissimum*. The eugenol and methyl eugenol are anti-inflammatory, anti-platelet, local anesthetics and antibiotics, but the association with other terpenes has considered the basil leaf primarily as a regulator of gastrointestinal functions: eupeptic, carminative, anti-gastralgic and antispasmodic (Edeoga, 2006; Morales and Simon, 1996). The African basil leaf concoction is known to be used to control illnesses such cholera, diarrhoea, dysentery, typhoid fever, malaria, headache, pains, stomach upset and other domestic and acute illness (Nargarajun *et al.,* 1989).

Spondias mombin Linn. belongs to the family Anacardiaceae commonly called Hog plum (Gbile, 1984; Gill, 1992). All parts of the plants - bark, leaves, root, flowers and fruit juice - have been valued ethnomedically in folkloric medicine (Irvine, 1961; Daniel, 1990). The leaves are a common remedy for various digestive problems including stomach aches, diarrhoea, dyspepsia, gastralgia, colic, and constipation. The leaves are considered to contain antiviral, antibacterial, anticandidal, and antiseptic principles used in numerous microbial problems including colds and flu, cystitis, urethritis, sore throats, herpes, yeast infections, gonorrhea, eye and ear infections, and externally for infected wounds, cuts, burns, and rashes (Taylor, 2004). The juice of the fresh leaves is a remedy for thrust (Bep, 1960). The bark is widely used as contraceptive and abortive agents as well as for tonsilitis, laryngitis, malaria, fever, erysipelas, bladder and kidney stones, snakebite, intestinal ulcers, ovarian and uterine cancer (Taylor, 2004). A decoction of the leaves and bark is employed as a febrifuge as well as for gonorrhoea and leucorrhoea. The fruits decoction is used as a diuretic and febrifuge (Bep, 1960). A tea of the flowers and the leaves is taken to relieve stomach-ache. The gum is employed as an expectorant and to expel tapeworm (USDA-ARS, 2002). Fragrant flowers of *S. mombin* are also used for eye infections and cataracts when prepared as an infusion. *S. mombin* leaves were also reported with smooth muscle relaxant actions, uterine antispasmodic (Uchendu *et al.,* 2005; Akubue *et al.,* 1983), sedative and anticonvulsant actions (Ayoka *et al.,* 2006), anti-anxiety effects mediated by GABAergic transmission (Ayoka *et al.,* 2005), antioxidant actions (Kramer *et al.,* 2002; Calderon *et al.,* 2000), antibacterial actions (Abo *et al.,* 1999; Morton, 1987; Ajao *et al.,* 1985), antiviral actions (Goncalves *et al.,* 2005; Corthout *et al.,* 1992, 1987, 1985), anti-candidal actions (Herforth, 2002), and hemostatic actions (Ramirez *et al.,* 1988) in order to validate some of its traditional uses.

Studies investigating the phytochemical constituents of aqueous and ethanol leaves extracts of *Ocimum gratissimum* and the methanol extract of *Spondias mombin* have reported the presence of tannin, saponin, anthraquinone, alkaloid, glycosides and rich content of flavonoid and phenolics and these were linked to their free radical scavenging and reducing power abilities comparable to that of gallic acid, an established antioxidant (Edeoga *et al.,* 2006; Oboh, 2006; Igwe *et al.,* 2010; Akinmoladun *et al.,* 2007, 2010). Aqueous extractive of *Ocimum gratissimum* had been contrastingly reported to reduce the activities of serum enzymes monitoring liver integrity (Effraim *et al.,* 2000) and histopathologically elicit dose-dependent lesion development in the liver (Effraim *et al.,* 2003) of rabbit used for the toxicity study, even at the minimum dose of 400mg/kg tested. However, recent findings have provided evidences that support its hepatoprotective roles as diet supplementation with *O. gratissimum* as well oral administration of the aqueous leaves extract reduced the serum liver function markers in rats challenged with paracetamol (Aluko *et al.,* 2013) and diesel petroleum (Ujowundu *et al.,* 2011).

Spondias mombin has been identified to inhibit carrageenan induced edema and arthriris in anti-inflammatory studies (Ojie *et al.,* 2013; Abad MJ *et al.,* 1996). Despite the paucity in evidences supporting its hepatoprotective roles, existing studies are conflicting. Although Odunola *et al.,* (2011) reported the amelioration of serum markers of liver damage in rats intoxicated with sodium arsenite, studies of Iweala and Oludare (2013) and Gbolade *et al.,* (2011) maintained that ethanol extract of *Spondias mombin* was unable to protect liver of diabetic rats from the damage inflicted by alloxan despite its hypoglycemic effect.

In his long struggle to achieve victory over his problems, man has always turned to plant for his help (Fallah-Hoseini *et al.,* 2006). In recent years there has been increased interest in the application of antioxidants to medical treatments as information is constantly gathered linking the development of human diseases to oxidative stress, and medicinal plants are sources of a wide variety of natural antioxidants. It is on this notion, therefore, that the antioxidative properties and hepatoprotective activities of the methanol leaf extracts of two plant species: *Ocimum gratissimum* and *Spondias mombin* against dimethylnitrosamine (DMN) induced liver injury were investigated in this study.

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MATERIALS AND METHODS

Chemicals and Reagents: Catechin, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH), Ellman Reagent [5′,5′-Dithiobis- (2-nitrobenzoate) DTNB], 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), Hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Company (St. Louis USA). All other reagents and solvents were of analytical grade.

Processing and extraction of plant leaves: The leaves of the plants were collected from within the premises of University of Ibadan Nigeria and authenticated at Botany Department of the same. They were air dried and pulverized.

The methanol extraction was carried out using the soxhlet extraction technique and concentrated using rotary evaporator.

Animals: Male Wister strain rats weighing 150-200g were obtained from the animal house of the Faculty of Veterinary Medicine University of Ibadan and housed in cages in the well-ventilated animal house of the Biochemistry Department, University of Ibadan, where they were provided rat chow produced by Ladokun feeds, Ibadan, Nigeria and water, *ad libitum*, and subjected to natural photoperiod of 12hrs light and 12hrs darkness for the period of the experiment. The animals were acclimatized for 14 days prior the experiment.

Determination of total flavonoid content of the extract: This was determined colorimetrically using the method described by Jia *et al*., (1999). 250 µl of the extract was added to 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$. After 5 minutes, 150 µl of 10% $AlCl_3.H_2O$ was added, followed by 500 µl of 1M NaOH and 275 µl of distilled water after 6 minutes. The solution was properly mixed and the colour intensity of the mixture was read at 510 nm. Catechin was used as the standard.

2, 2 Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity: This was estimated according to the method described by Hatano *et al*., (1988). Extracts (40 – 200 µg) in 4mls of distilled water was added to the methanol solution of DPPH (1 mM, 1 ml). The mixture was shaken and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. DPPH$^{\bullet}$ radical scavenging ability was calculated using this formula:

$$\%RSA = (A_{DPPH} - A_S) / A_{DPPH} \times 100$$

(%RSA = percentage of DPPH discoloration, A_{DPPH} = absorbance of DPPH solution, A_S = absorbance of the solution when the sample was added at a particular level)

Determination of reducing power: This was estimated using the method of Oyaizu (1986). 2.5 ml of 200 mmol/l of phosphate buffer (pH 6.6) and 2.5 ml of 1% K_3FeCN were added to various concentrations of the extract (25- 800 µg/ml). The mixture was incubated for 2 minutes at 50°C and then centrifuged at 1000 g for 8 minutes. 5 ml of the supernatant was then mixed with 5 ml of distilled water and 1 ml of 0.1% $FeCl_3$. The absorbance of the mixture was measured at a wavelength of 700 nm. Catechin was used as the standard.

2, 2' – Azobis (2- aminopropane) hydrochloride (AAPH) – induced lipid peroxidation: This experiment was carried out according to the method described by Neergheen *et al.*, (2005). 200 µl of post-mitochondrial fraction (PMF) of liver homogenate was diluted in 0.1 M potassium phosphate buffer, pH 7.5. Then, 400 µl of extract (100 – 1000 µg) was added followed by 200 µl of AAPH (20 mM) to initiate peroxidation. The mixture was incubated at 37°C for 1 hour and the solution gently shaken at 10 minutes interval. After incubation, 1.6 ml TCA-TBA-HCl stock solution was added. The solution was heated in a boiling water bath for 15 minutes. After cooling, the precipitate was removed by centrifugation and the absorbance of the resulting supernatant was measured at 532 nm. Results were expressed as percentage inhibition of peroxidation with catechin used as standard. Percentage inhibition capacity of the extract was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(A_c - A_{test}) \times 100}{A_c - A_{blank}}$$

A_c = absorbance of control (without extract), A_{test} = absorbance of sample with extract, A_{blank} = absorbance of blank

DMN induced hepatotoxicity study: Animals were divided into eight (8) groups of six (6) animals per group. Rats were pre-treated with the extracts orally for six days before they were challenged with DMN on the seventh day and euthanized on the eighth day. Animals in group 1 served as the control and received normal saline; those in groups 2 and 3 received 100 mg/kg body weight (b.wt) of methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin*, respectively throughout the treatment period while rats in group 4 were given only 20 mg/kg DMN (i.p) dissolved in normal saline, on day 7. *Ocimum gratissimum* at 100 mg/kg b.wt and 200 mg/kg b.wt was respectively administered to the rats in groups 5 and 6 while those in 7 and 8 received 100 mg/kg b.wt and 200 mg/kg b.wt of *Spondias mombin*, respectively for six days prior 20mg/kg DMN (i.p) on the seventh day.

Biochemical Assay: After 24 hour of DMN intoxication, the animals were sacrificed by cervical dislocation and blood samples were collected by orbital venous plexus bleeding for biochemical analyses and liver tissues were excised. Excised livers were quickly rinsed in ice-cold 1.15% KCl, dried, weighed and then homogenized in ice- cold isotonic phosphate buffer of pH 7.4, using a dilution factor of 1.4. The liver homogenate was further centrifuged at 10,000 g for 15 minutes in a cold centrifuge in order to obtain the post-mitochondrial fraction. Protein concentration was determined by the method of Lowry *et al*. (1951). Reduced glutathione (GSH) was determined at 412 nm using the method described by Jollow *et al*. (1974); hydrogen peroxide (H_2O_2) generation by Wolff's method (1994); GPx activity by Rotruck *et al.*, (1973) and biochemical analyses of the serum enzymes for aspartate amino-transferase (AST) and alanine aminotransferase (ALT) were by the method described by Reitman and Frankel (1957).

Histological Study: A portion of liver tissues from each group was collected and preserved in 10% neutral buffered formalin for histopathological studies. These tissues were processed and embedded in paraffin wax after which thin sections of 5-6 µm thickness of liver tissue were cut and stained with hematoxylin and eosin. The thin sections of liver were made into permanent slides and examined photomicroscopically.

Statistical analysis: The results obtained were expressed as mean standard error (S.D.). Statistical comparison was performed between the groups by Analysis of Variance (ANNOVA) at $p < 0.05$.

RESULTS

The findings from the study revealed that the methanol extracts of *Ocimum gratissimum* and *Spondias mombin* possessed copious amount of flavonoid with that of *Spondias mombin relatively higher than that of Ocimum gratissimum* (Figure 1).

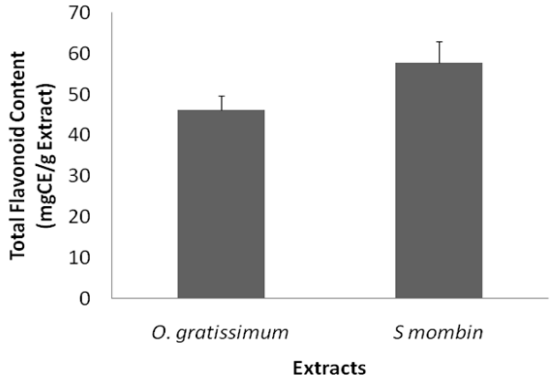

Fig. 1
Total flavonoid contents of methanolic extracts of *gratissimum* and *Spondias mombin*

The two methanol extracts exhibited DPPH• radical scavenging activities with increasing concentrations with 20 µg showing percentage radical scavenging activity of 63.1% for *O. gratissimum* and 2.83% for *Spondias mombin*, and that of 200 µg showing 79.4% and 71.57% for *O. gratissimum* and *S. mombin*, respectively (Table 1).

Methanol leaves extracts of *Ocimum gratissimum* and *Spondias mombin* were able to prevent the generation of AAPH-induced peroxyl radicals via two different patterns: the *Spondias mombin* extract, at 50 µg/ml and 400µg/ml showed significant ($p<0.05$) dose dependent pattern of inhibition, 46.08% and 85.29% respectively, comparable with a known antioxidant, catechin: 51.96% at 50µg/ml (Table 2). However, the methanol extract of *Ocimum gratissimum* inhibited the radical generation significantly ($p<0.05$) at lower concentration, 71.8% at 50 µg/ml, than at higher concentration, 51.3% at 200 µg/ml (Table 2).

The extracts also displayed excellent reducing ability by significantly ($p<0.05$) terminating radical chain reactions in an increasing degree as their concentrations increased from 50 – 800 µg: 0.26 – 1.13 and 0.46 – 1.94 for *Ocimum gratissimum* and *Spondias mombin*, respectively (Table 2). The reductive potential of *Spondias mombin* is equivalent to that of catechin at 100 µg/ml.

Table 1:
DPPH• Radical scavenging ability of the Methanol extracts of *Ocimum gratissimum* and *Spondias mombin.*

Plant species	Parameter	Extract Dose (µg/ml)							
		Control	**20**	**40**	**80**	**100**	**200**	**Catechin (50)**	**Catechin (60)**
Ocimum gratissimum	**ABS***	0.78± 0.00	0.29± 0.001	0.28± 0.002	0.24± 0.001	0.22± 0.001	0.16± 0.001	0.38± 0.001	
	%RSA		63.1	64.4	68.9	71.0	79.4	50.5	
Spondias mombin	**ABS***	0.76 ± 0.002	0.73 ± 0.004	0.71± 0.002	0.54± 0.004	0.41± 0.002	0.21± 0.002		0.68± 0.003
	%RSA		2.83	5.43	28.21	45.21	71.57		9.40

* The values are mean ± SD (n = 3)

Table 2:
Reducing power and inhibition capabilities of AAPH-induced lipid peroxidation of the Methanol extracts of *Ocimum gratissimum* and *Spondias mombin.*

Plant species	Grouping	Conc. (µg/ml)	Parameters		
			AAPH-induced LPO		**Reducing Power**
			ABS*	**(% Inhibition)**	**ABS***
Ocimum gratissimum	**Control**	**-**	0.117 ± 0.001	-	0.260 ± 0.001
	1	**25**	0.018 ± 0.001	84.6	
	2	**50**	0.033 ± 0.001	71.8	0.420 ± 0.010
	3	**100**	0.046 ± 0.002	60.7	0.450 ± 0.010
	4	**200**	0.057 ± 0.001	51.3	0.540 ± 0.002
	5	**400**	0.062 ± 0.001	47.0	0.810 ± 0.001
	6	**800**	0.083 ± 0.001	29.1	1.130 ± 0.001
	Catechin	**100**	0.023 ± 0.001	80.3	0.530 ± 0.001
	Catechin	**200**	0.150 ± 0.001	87.2	
Spondias Mombin	**Control**	**-**	0.102 ± 0.010	-	0.340 ± 0.010
	1	**25**	0.081 ± 0.010	20.69	
	2	**50**	0.055± 0.02	46.08	0.460 ± 0.030
	3	**100**	0.040± 0.01	60.78	0.620 ± 0.040
	4	**200**	0.026±0.02	74.51	0.910 ± 0.020
	5	**400**	0.015± 0.01	85.29	1.380 ± 0.060
	6	**800**	0.012 ± 0.01	88.24	1.940 ± 0.020
	Catechin	**50**	0.049 ± 0.01	51.96	
	Catechin	**100**			0.650 ± 0.020

* The values are mean ± SD (n = 3)

Table 3:
The effect of the Methanol extracts of *Ocimum gratissimum* and *Spondias mombin* on hepatotoxic and oxidative stress parameters in rats with *DMN induced* liver damage.

Plant species	Parameters	TREATMENT GROUPS				
		Control (Normal Saline)	**Extract Only (100mg/kg)**	**DMN Only (20mg/kg)**	**Extract (100mg/kg) + DMN (20mg/kg)**	**Extract (200mg/kg) + DMN (20mg/kg)**
Ocimum gratissimum	**ALT (U/L)**	54.0 ± 3.66	46.22 ± 4.62*	76.70 ± 8.55*	58.40 ± 6.97**	55.44 ± 3.23**
	AST (U/L)	170.74 ± 10.53	173.70 ± 17.06	223.36 ± 17.10**	167.20 ± 1.39**	170.62 ± 13.24**
	GSH (mg/ml)	5.44 ± 0.83	5.50 ± 0.45*	3.42 ± 0.38*	4.50 ± 1.13**	6.08 ± 1.06**
	GPx (unit/mg protein	43.47 ± 2.44	58.87 ± 7.34	57.87 ±7.34*	39.92 ± 1.16**	32.10 ± 1.65**
	H_2O_2 (µmole/mg protein)	30.40 ± 1.71	27.90 ± 1.27*	35.50 ± 3.80*	28.58 ± 3.22**	31.92 ± 3.28**
Spondias mombin	**ALT (U/L)**	54.27 ± 3.66	37.36 ± 3.70*	76.70 ± 8.55*	52.98 ± 6.34**	56.35 ± 4.04**
	AST (U/L)	170.74 ± 10.53	157.43 ± 7.74*	223.36 ± 17.10*	176.02 ± 5.35**	171.18 ± 10.84**
	GSH (mg/ml)	5.44 ± 0.83	4.65 ± 0.49	3.42 ± 0.38*	6.08 ± 0.86**	7.25 ± 0.89**
	GPx (unit/mg protein)	43.47 ± 2.44	47.45 ± 5.62	57.38 ± 10.30*	39.40 ± 2.11**	34.94 ± 5.12**
	H_2O_2 (µmole/mg protein)	30.40 ± 1.71	27.54 ± 2.49*	35.50 ± 3.80*	30.15 ± 1.40**	28.13 ± 2.84**

The results are shown as Mean ± SD (n = 6), DMN = Dimethylnitrosamine
* Significantly different from the control ($p<0.05$); ** Significantly different from the DMN ($p<0.05$)

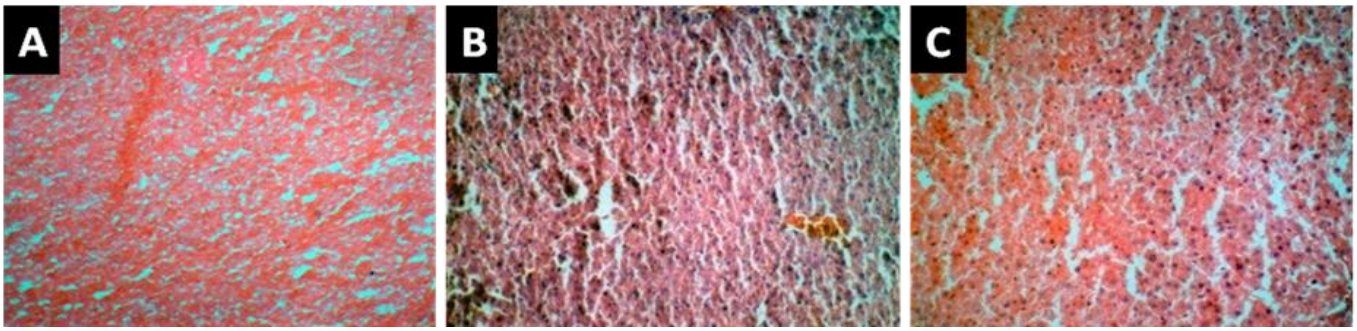

Plate 1
Photomicrographs of liver from normal saline (A), 100mg/kg *Ocimum gratissimum* (B) and 100 mg/kg *Spondias mombin* (C) treated rats

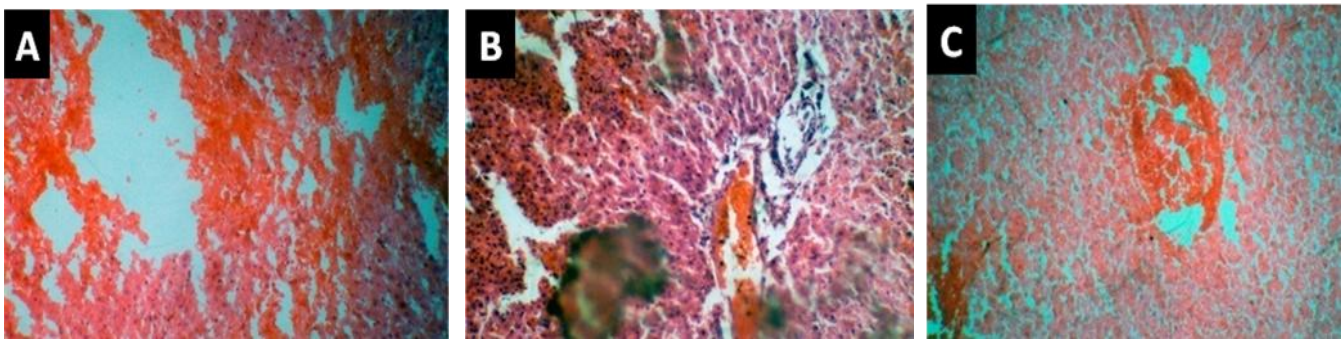

Plate 2
DMN-induced histological features in rat livers. A. Photomicrographs showing massive vacuolar degeneration and hemorrhages in the parenchyma. B. Photomicrographs showing severe portal congestion with mild periportal cellular infiltration. C. Photomicrographs showing marked central venous portal congestion.

Protective effects of methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* on DMN-induced liver injury were evaluated by determining the levels of ALT, AST, GSH, GPx and H_2O_2, and the results are presented in Table 3.

This study showed that 20mg/kg DMN administration led to liver damage as observed by the increased activity of serum ALT and AST (markers of early acute liver damage). However, pretreatment with the extracts of *Ocimum gratissimum* and *Spondias mombin* at both concentrations (100 and 200 mg/kg b.wt) significantly ($p<0.05$) reduced the activity of ALT and AST (Table 3). In addition to the build-up of hepatotoxicity marker enzymes observed in the 20 mg/kg intoxicated group, it was also noticed that the level of H_2O_2 generated and activity of GPx were significantly higher. The *Ocimum gratissimum* and *Spondias mombin* extracts display excellent ameliorative effect by significantly lowering the levels of H_2O_2 generated and activities of GPx in rats pre-treated with both 100mg/kg and 200mg/kg of the two extracts (Table 3). DMN intoxication decreased significantly the level of the non-enzymic antioxidant, reduced glutathione (GSH) when compared with the control. To potentiate the protective effects of the *Ocimum gratissimum* and *Spondias mombin* extracts, pre-treatments at 100mg/kg and 200mg/kg significantly ($p<0.05$) increased the level of GSH.

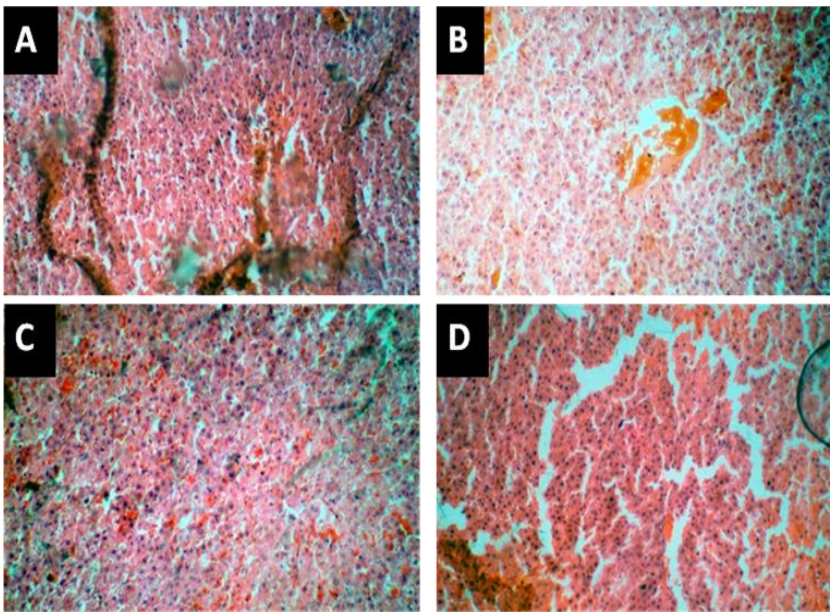

Plate 3
Effects of methanol extracts of *Ocimum gratissimum and Spondias mombin* on DMN-induced histological changes in rat livers. **A**. Rat liver treated with DMN + *Ocimun gratissimum* (100mg/kg) showing no visible lesion. **B**. Rat liver treated with DMN+ *Ocimun gratissimum* (200mg/kg) showing moderate central venous portal congestion. **C**. Rat liver treated with DMN + *Spondias mombin* (100mg/kg) showing mild portal and sinusoidal congestion. **D**. Rat liver treated with DMN+ *Spondias mombin* (200mg/kg) showing mild portal congestion.

Histological examination of liver samples also substantiated the hepatoprotective properties of the methanol extracts of *Ocimun gratissimum* and *Spondias mombin.* Livers of the rats challenged with DMN revealed massive vacuolar degeneration, severe haemorrhages in the parenchyma tissue and marked central venous and portal congestion. (Plate 2), when compared with the control liver (Plate 1), which has similar histological features with the hepatocytes of the rats fed with the methanol extracts only. (Plate 1). This could be due to the formation of highly reactive free radicals because of oxidative stress caused by DMN. Overall, the *Ocimum gratissimum* and *Spondias mombin* – pretreated rats restored the integrity of the hepatocytes relatively with mild portal and sinusoidal congestion and diffuse vacuolar degeneration (Plate 3).

DISCUSSION

The medicinal effects of plants are often attributed to the antioxidant activity of the phytochemical constituents, mainly the phenolics (Thabrew, 1998; Akinmoladun *et al.*, 2007). The antioxidant activity of the phenolics is due to their redox properties which are free radical quenchers (Rice-Evans *et al.,* 1996). Flavonoids are polyphenolic compounds found in small quantities in numerous plant foods, including fruit and vegetables, tea, wine, nuts, seeds, herbs and spices (Hertog *et al*., 1995; Graf *et al*., 2005). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health - they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (O'Byrne *et al*., 2002).

In this study, methanol leaves extracts of two different plant species; *Ocimum gratissimum* and *Spondias mombin* were assessed for antioxidant activities and hepatoprotective properties against dimethylnitrosamine (DMN) induced hepatotoxicity.

Figure 1 shows the flavonoid content present in the methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* evaluated as mg Catechin equivalent per gram of each extract. Effraim *et al.,* 2000 and Igwe et al., 2010 reported the presence of flavonoids and saponins in the leaves of *O. gratissimum* and *Spondias mombin*, respectively. Flavonoids are reported to exhibit antioxidant activity (Robak and Grygleaski, 1998). The biological function of flavonoids includes protection against allergies, inflammation, platelets aggregation microbes, ulcer and tumors (Okwu and Okwu, 2004; Farquar, 1996). Flavonoids represent the most common and widely distributed groups of plant polyphenols. Flavonoids are free radical scavengers, super antioxidants which prevent oxidative cell damage and have strong anticancer activity (Salah *et al.,* 1995). As antioxidants, flavonoids provide anti-inflammatory actions (Okwu, 2001a, b), this may be the reason behind the folkloric use of *Spondias mombin* in the treatment of intestinal troubles (Okwu and Okwu, 2004). The bioactivity of the polyphenols may be related to their ability to chelate metals, inhibit the lipooxygenase pathway and scavenge free radicals (Decker, 1997). In food systems, flavonoids can act as free radical scavengers and terminate the radical chain reaction that occurs during the oxidation of triglycerides (Hendrich *et al.,* 1999; Bravo, 1998).

The DPPH radical has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors. Promising natural antioxidant must be able to scavenge $DPPH^{\bullet}$ radical.(Soares *et al.,* 1997). Methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* exhibited this with increasing concentration (Table 1). The abilities of these extracts to scavenge $DPPH^{\bullet}$ radical portray their expected ability to scavenge both reactive oxygen and nitrogen species, *in vivo*.

A good correlation between antioxidant activity and reducing power in some plant extracts has been established. Therefore, reducing power may be used as an indicator of potential activity (Yen *et al.,* 2001). The presence of reductants (antioxidants) in the herbal extracts causes the reduction of Fe^{3+}/ Ferric cyanide complex to ferrous form. The reducing power increased with an increase in extracts concentration. The data showed that all the samples increased in their reductive potential when the concentrations of the extracts were increased (Table 2). The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds (Rice-Evans *et al.*, 1995). This feat is typical of chain-breaking endogenous primary cytoprotective enzyme (Glutathione Peroxidase (Lindenau *et al.,* 1998) and antioxidant (α-Tocopherol (Papas, 1999c).

Thermal decomposition of AAPH, a peroxyl radical initiator, produces peroxyradical ($R^{\bullet}$) that can attack the polyunsaturated lipids initiating peroxidation. This simulates what happens in the living systems where free radicals most

importantly $OH^{\bullet}$, generated as part of the body's normal metabolic process or produced, especially beyond the antioxidant capacity, primarily or secondarily in several pathological conditions, initiate peroxidation of lipids and hence damage to other biomolecules via the production of carbonyl derivatives e.g. Malondialdehydes (MDA) (Saha *et al.,* 2008; Valko *et al.,* 2004; Prior, 2003; Lee *et al.,* 2003; Tshibangu *et al.,* 2002; Polterait, 1997). Methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* significantly inhibited lipid peroxidation induced by AAPH. Though the pattern of inhibition experienced with *Ocimum gratissimum* was invertedly dose dependent (Table 2), this depicted that *Ocimum gratissimum* may be more suitable as lipid peroxidation inhibitor at lower concentration thus, might be able to block lipid peroxidation at both the initiation and propagation steps, in vivo, at lower concentration.

The liver is the primary target for DMN toxicity (Farombi *et al.,* 2009; Lee *et al.,* 1996). In this study, it was evident that acute dimethylnitrosamine administration led to liver injury as observed by the ample increase in the levels of aminotransferases (ALT and AST). These enzymes, which are normally located in the cytoplasm, are released into the circulation after the cellular damage. This is consistent with the studies of Farombi *et al.,* (2009) and Adaramoye *et al.,* (2009).

DMN has been suggested to cause the generation of reactive oxygen species (ROS) resulting in oxidative stress, alteration of the antioxidant defence system in the tissues and cellular injury (Ismael *et al*., 2007; Farombi *et al.,* 2009). In this study DMN intoxication elevated the level of hydrogen peroxide (H_2O_2) and reduced significantly the level of the non-enzymic antioxidant, reduced glutathione (GSH). These were followed by a concurrent increased activity of glutathione peroxidase (GPx) (Table 3). GSH is one of the most important cellular antioxidants since it supplies the electrons for the reduction of peroxides by the action of GPx (Hwang *et al.,* 1992). Thus, the observed decline in GSH content in acute DMN challenged hepatocytes may be due to its utilization by Glutathione peroxidase (GPx) to challenge the prevailing oxidative stress under the influence of reactive oxygen species, H_2O_2. This observation therefore, suggests that acute exposure to DMN alters the expression and activities of GPx due to toxic metabolites generated during its biotransformation. This observation is in line with the works of Ismael and Abd El Rahiem (2007); Lindenau *et al.,* (1998) and Łukaszewicz-Hussain *et al.,* (2004). However, Tanguchi *et al.,* (1994), observed a decreased activity of liver GPx after administering 30 mg/kg b.wt DMN in a single dose to rats. This may be as a result of the high concentration of the toxicant, which in a way might be injurious to GPx itself.

However, pre-treatments of methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin*, at 100 mg/kg b.wt and 200 mg/kg b.wt, respectively, prior to *DMN induced* liver injury significantly ($p<0.05$\) ameliorated the damage as observed in the decrease in both ALT and AST activities. This keeps up with the various findings of Ulicna *et al*., (2003); Reinke *et al*., (1998) and Obi *et al*., (1998).

The ability of cells to up-regulate their levels of intracellular glutathione (GSH) synthesis in times of oxidative stress will be an important factor in the ability of the cell to protect itself from toxicity (Hwang *et al.,* 1992). To further potentiate the protective effects of the extracts in pre-treated groups of animals, the oxidative stress was subdued and the antioxidant status was drastically boosted as revealed by the significant ($p<0.05$) increases in the levels of GSH; the lowered levels of the generated H_2O_2, comparable with the normal, and the restored levels of GPx activities.

Antioxidant based drugs or formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (Hennebelle *et al.,* 2006, Augustin *et al.,* 2005, Prior, 2003, Trease and Evans, 1989). In this study it was confirmed that the Methanol leaf extracts of *Ocimum gratissimum* and *Spondias mombin* prevented DMN induced hepatotoxicity by scavenging free radicals, inhibiting the peroxidation of lipids, augmenting cellular redox potential and promoting the dissipation of build-up of free radicals thus, the extracts exhibited potent antioxidant and radical scavenging activities that confer a promising therapeutic potential for free radical mediated diseases.

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