Anti-Peroxidative, Protective and Ameliorative Properties of Methanol Extract of All Parts of *Morinda Lucida* Benth in CCl₄-Induced Liver Injury

Adejo GO^{1*}, Atawodi SE², Ameh DA² and Ibrahim S²

¹Department of Biochemistry, Bingham University, Nasarawa State, Nigeria

²Department of Biochemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

Abstract

The methanol extracts of all the various parts of *Morinda lucida* were studied *in vivo* for anti-peroxidative, protective and ameliorative effects against carbon tetrachloride (CCl₄) induced hepatocellular injury. Thiobarbituric acid reactive substances (TBARS) concentration taken as a measure of lipid peroxidation showed pre-treatment with the parts each (100 mg/kg) conferring anti-peroxidative effect while the leaf and bark extracts showed anti-peroxidative effect in the post-treatment test. Markers of hepatic damage viz: AST, ALT, total and conjugated bilirubin also showed significant protection and amelioration against CCl₄-induced liver damage. Lipid profile studies like TG, TC, LDL and HDL showed no statistical difference among the pre-treatment groups and the 'normal control' except in the 'Root+CCl₄' or 'Leaf+CCl₄' groups which also showed no difference statistically from the 'CCl₄ only' group. In the post-treatment (ameliorative) tests also, no statistical difference was found among the groups compared with the 'normal control' except the 'CCl₄ + Root' and 'CCl₄ + Leaf' groups which bore no statistical difference from the 'CCl₄ only' group, as per LDL and HDL respectively. Findings here show the anti-peroxidative and protective properties of *Morinda lucida* comparable to Vitamins C and E, and ameliorative properties comparable to Silymarin against liver injury. These could possibly be reasons supporting its application in folkloric medicine.

Keywords: CCl₄; *Morinda lucida*; Methanol extracts; Liver injury

Introduction

Reactive oxygen species (ROS) are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* which creates oxidative stress resulting in numerous diseases and disorders [1,2] such as cancer [3], cardiovascular disease [4], neural disorders [5], Alzheimer's disease [6] mild cognitive impairment [7], Parkinsons disease [8], alcohol induced liver disease [9], ulcerative colitis [10], ageing [11] and atherosclerosis [12].

The extreme toxicity of oxygen is related to its high capability of generating free radicals which in turn destroys many major biological molecules. They can attack lipids and proteins and destroy membranes. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids [13]. A potent scavenger of these ROS could therefore serve as possible preventive intervention for free radical-mediated diseases [14].

Today, the search for natural compounds and other preparations of plant origin that can be used to prevent or mitigate maladies have been intensified. For instance, some medicinal and food plants such as *Moringa oleifera* [15], *Khaya senegalensis* [16], *Syzygium aromaticum* [17], *Hibiscus esculentus* [18], *Canarium schweinfurthii* [15] have been demonstrated not only to contain phytochemicals with strong antioxidant capacity, but possess diverse powerful medicinal properties.

Morinda lucida Benth, a Rubiaceae, is known commonly as 'brimstone tree' (English), 'Arbre à soufre' (French), and 'Moindo' (Polish). In Nigeria, it is called 'oruwo' or 'erewo' (Yoruba), 'nfia' or 'Ezeogu' (Igbo), Idonzakara (Hausa), 'oge' (Igala), among other many different indigenous tribal names, while in other African countries it is called 'Uhon' (Ivory Coast) and 'hojologbo' (Sierra Leone) [19-21]. It grows in many African countries and it is widely used as a medicine in West Africa. The plant is generally used as ingredients of fever teas, which are usually taken, for the traditional treatment of malaria [22]. The decoction of the stem bark has been reportedly used for the treatment of severe jaundice [23].

This study was thus designed to evaluate the antioxidant effect of the different parts of *Morinda lucida* and to determine the

hepatoprotective/hepatocurative effects of the extract of the different parts of *Morinda lucida* on the integrity of the liver and associate disorders like hyperlipidemia and hyperbilirubinaemia.

Materials and Methods

Chemicals and reagents

All chemicals and reagents (of analytical grade) were obtained from BioVision Inc. (Milpitas, USA), Sigma Aldrich (Germany) or RANDOX Laboratories Randox Ltd. (Ardmore, United Kingdom).

Experimental animals

Male albino rats of Wistar strain weighing between 180-280 g were used. They were obtained from Afkol farms, Jos, Plateau state and housed in Bingham University Animal facility. They were allowed to acclimatize for at least two weeks under the laboratory condition before the experiment was commenced. Except where and when applicable, animal were maintained on standard regular rodent pellet for rats procured from Vital Feeds depot, Gizzard plaza, Mararaba, Nasarawa State. All other protocols for animal management were observed in compliance with the general guidelines for animal experimentation.

Collection and authentication of plant sample

The different parts of *Morinda lucida* plant were collected from Idah Local Government Area of Kogi State. They were compressed in a plant press and sent to the Herbarium Unit of the Department of Biological

***Corresponding author:** Adejo GO, Department of Biochemistry, Bingham University, Nasarawa State, Nigeria, Tel: 2348068376357; E-mail: dradejogod@gmail.com

Received July 01, 2014; Accepted July 23, 2014; Published July 25, 2014

Citation: Adejo GO, Atawodi SE, Ameh DA, Ibrahim S (2014) Anti-Peroxidative, Protective and Ameliorative Properties of Methanol Extract of All Parts of *Morinda Lucida* Benth in CCl₄-Induced Liver Injury. Nat Prod Chem Res S1:003. doi:10.4172/2329-6836.S1-003

Copyright: © 2014 Adejo GO, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Sciences, Ahmadu Bello University, Zaria for authentication. A specimen with voucher number: 1862 was deposited at the herbarium.

Plant extracts preparation and determination of percentage yield

Fresh plant parts, namely, whole fruit, leaf, bark, and root were collected and shade dried until brittle then pulverized with a mortar and pestle. Powders (25 g each) were then defatted with petroleum ether. Extraction was carried out with three changes of methanol for 5 hours each. The extracts were then pooled together and concentrated with rotary evaporator at 60°C for 3 hrs, then further allowed to dry over water bath at 45°C for 12 hrs. The pasty extracts were then transferred to desiccators containing activated silica gel for between 6-8 weeks until extracts became dry, depicted by constant weights recorded during the weekly weight measurements. Extracts yield were then quantified by method described by Mishra [24].

Animal groupings and treatments

Animals were broadly and randomly divided into various groups of 6 rats each. Groups I-VI served as the normal, CCl₄ (negative control), fruit, leaf, bark or root only groups respectively. The test groups involved the extract pre-treatment (Hepatoprotective effect) and extract post-treatment (Ameliorative effect) groups. To investigate the former, animals received oral administration of 0.20 mg/kg Vitamin E (α -Tocopherol) [25,26] or 0.86 mg/kg Vitamin C (Ascorbic acid) [doses as low as 0.3 mg/kg [27], 30 mg/kg/day [28], and between 50-200 mg/kg have been effectively reported to be used previously] or 100 mg/kg/day of extract (fruit, leaf, bark or root) on days 1 to 7 followed by 0.60 ml/kg CCl₄ injection *i.p.*, 30 min later (on day 7 only), in groups VI -XII respectively. In the latter, animals first received 0.60 ml/kg CCl₄ injection *i.p.* on day 1, followed 30 min later by oral administration of 100 mg/kg of silymarin [29,30] or 100 mg/kg of fruit, leaf, bark or root extract on day 1, in groups XIII-XVII respectively. Afterwards, they received either silymarin or their respective extracts on days 2-7.

Collection of animal samples

Rats were sacrificed after administration of the methanol extracts of the fruit, leaf, bark and root of *M. lucida* for 7 days orally, and single dose of CCl₄ injection (*intra-peritoneally*) on the first or last day for the pre or post treatment groups respectively. On the 7th day, the rats were allowed to fast overnight and then sacrificed under chloroform anesthesia by decapitation. Blood samples for determination of serobiochemical parameters were immediately collected.

Preparation of serum

Sera were prepared by allowing the collected blood to stand for 1 hour to clot. This was thereafter centrifuged at 1,000xg for 15 min to separate the serum which was harvested and stored in a freezer. The serobiochemical parameters studied were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total bilirubin, high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, and triglyceride concentration. At necropsy, all rats were examined to identify gross lesions, and the specimen of the liver were quickly removed and fixed in 10% formal-saline for histopathological studies.

Preparation of tissue homogenates

Liver tissues were removed immediately after sacrifice of rats, and

washed in ice cold normal saline. They were then blotted, weighed and homogenized in 10 vols of ice cold PBS buffer (pH=7.4) followed by centrifugation of homogenate at 11,180xg for 30 min (at 4°C). The supernatant was stored frozen until used for the determination of TBARS [31-33], usually within 24 hrs.

Determination of lipid peroxidation

The assay is based on the reaction of thiobarbituric acid reacting substances (TBARS) with thiobarbituric acid (TBA) to form a pink coloured product. The colour intensity measured at 535 nm is directly proportional to TBARS concentration in the sample. Details of procedure were as previously described by Atawodi [17].

Determination of lipidemic parameters

Total serum triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations were determined as per manufacturer's instructions using assay kits (by Randox).

Determination of liver function

Hepatotoxicity was induced in rats by method described by Gyamfi et al. [32] where single dose of 0.6 ml/kg of tetrachloromethane (CCl₄) was injected *i.p.* AST and ALT activities were determined as per manufacturer's instructions using assay kits (by Randox Laboratories).

Estimation of bilirubin concentrations: Conjugated and total bilirubins were estimated as described by Garber [34] using respective assay kits (by Randox Laboratories).

Analysis of Data: Data were expressed as mean $\pm$ SD. Significance of difference was evaluated by ANOVA and Duncan Multiple Range Test.

Results

Protective influence of different parts of *Morinda lucida* on CCl₄-induced oxidative stress and liver injury: Assay results showed no significant difference ($p < 0.05$) in TBARS concentration between the normal control group, those pre-treated with α -Tocopherol or ascorbic acid or methanol extracts of the different parts of *M. lucida* before CCl₄ injection, nor with the groups administered with the methanol extracts only. However, rats group injected with 'CCl₄ only', was statistically higher than the normal control and all other groups ($p < 0.05$). Similar to TBARS, markers of hepatic damage viz: AST, ALT, total and conjugated bilirubin showed no statistical difference among all the extract pre-treated groups and experimental controls except in the 'CCl₄ only' group where values were significantly higher than all others. Histomicrographs of rats' tissues attests to these (Figures 1 and 2).

Values of lipidemic statuses like triglyceride (TG) concentration, total cholesterol (TC) and low density lipoprotein (LDL), showed 'CCl₄ only' group being significantly higher ($p < 0.05$) than the normal control group and those pre-treated with α -Tocopherol or ascorbic acid or methanol extracts of the different parts of *M. lucida* before CCl₄ injection except in the 'Root+CCl₄' and 'Leaf+CCl₄' where there was no difference found compared to the 'CCl₄ only' group (Tables 1 and 2). HDL concentration showed significantly lower values ($p > 0.05$) in the 'CCl₄ only' group than the normal control and all other groups (Table 1, Figures 1 and 2).

	Treatment group	Triglyceride (mg/dl)	Total Cholesterol (U/l)	HDL (mg/dl)	LDL (mg/dl)
Experimental controls	I (Normal control)	79.60 ± 7.07 ^a	31.12 ± 9.12 ^a	40.44 ± 0.75 ^a	34.95 ± 9.51 ^a
	II (CCl ₄ only)	277.85 ± 18.38 ^b	139.22 ± 8.02 ^b	35.35 ± 1.45 ^b	95.57 ± 5.23 ^b
	III (Fruit only)	78.03 ± 9.56 ^a	26.74 ± 2.33 ^a	42.02 ± 0.72 ^c	10.80 ± 2.64 ^d
	IV (Leaf only)	69.30 ± 5.88 ^a	23.41 ± 3.45 ^a	44.12 ± 0.78 ^{cd}	17.60 ± 1.11 ^d
	V (Bark only)	78.03 ± 9.56 ^a	20.92 ± 2.34 ^a	42.55 ± 0.64 ^c	14.62 ± 1.88 ^d
	VI (Root only)	88.01 ± 09.43 ^a	91.71 ± 11.06 ^c	46.06 ± 0.89 ^d	72.31 ± 5.21 ^e
Hepatoprotective effect	VII (Vit. E+CCl ₄)	90.26 ± 14.35 ^a	24.18 ± 3.19 ^a	41.78 ± 0.89 ^{ac}	24.27 ± 3.13 ^a
	VIII (Vit. C+CCl ₄)	149.56 ± 18.10 ^c	26.68 ± 4.83 ^a	41.24 ± 0.96 ^{ac}	11.42 ± 1.40 ^d
	IX (Fruit+CCl ₄)	77.36 ± 7.25 ^a	30.22 ± 8.17 ^a	40.11 ± 1.71 ^a	26.72 ± 2.11 ^a
	X (Leaf+CCl ₄)	107.35 ± 9.34 ^d	105.36 ± 6.07 ^d	44.22 ± 1.40 ^{cd}	90.93 ± 8.88 ^b
	XI (Bark+CCl ₄)	133.13 ± 18.09 ^c	82.42 ± 11.44 ^c	46.27 ± 0.86 ^d	51.85 ± 9.86 ^c
	XII (Root+CCl ₄)	85.19 ± 9.22 ^a	115.14 ± 9.92 ^d	42.11 ± 0.71 ^c	85.33 ± 9.01 ^b
Ameliorative effect	XIII (CCl ₄ +Silym.)	67.87 ± 4.76 ^a	24.62 ± 2.18 ^a	49.17 ± 0.22 ^d	13.23 ± 4.24 ^d
	XIV (CCl ₄ +fruit)	86.94 ± 6.07 ^a	108.32 ± 8.81 ^{cd}	42.90 ± 0.24 ^c	73.97 ± 9.04 ^e
	XV (CCl ₄ +leaf)	113.66 ± 32.02 ^a	87.04 ± 4.22 ^c	38.75 ± 0.78 ^{bc}	75.27 ± 5.12 ^e
	XVI (CCl ₄ +bark)	83.19 ± 08.16 ^a	23.36 ± 3.49 ^a	46.60 ± 0.59 ^d	25.35 ± 6.47 ^a
	XVII (CCl ₄ +root)	89.30 ± 06.52 ^a	106.91 ± 6.05 ^{cd}	44.17 ± 1.94 ^{cd}	81.20 ± 3.54 ^b

Values are Mean ± SD (n=6). No significant difference between values with same superscripts down the column (p<0.05)

Table 1: Lipid profiles of rat's pre and post treated with methanolic extracts of different parts of *Morinda lucida* upon CCl₄ administration.

	Group	TBARS (nmol/mg protein)	AST (U/l)	ALT (U/l)	Conjugated Bilirubin (mg/dl)	Total Bilirubin (mg/dl)
Experimental controls	I (Normal control)	94.3 ± 7.5 ^{a,c}	23.57 ± 3.07 ^{ab}	49.11 ± 6.6 ^a	0.12 ± 0.06 ^a	0.24 ± 0.03 ^a
	II (CCl ₄ only)	177.2 ± 15.0 ^b	90.73 ± 6.22 ^c	89.63 ± 10.49 ^c	0.51 ± 0.03 ^b	0.62 ± 0.02 ^b
	III (Fruit only)	93.4 ± 6.3 ^{a,c}	17.15 ± 2.85 ^a	28.14 ± 1.43 ^d	0.30 ± 0.02 ^c	0.37 ± 0.07 ^c
	IV (Leaf only)	63.8 ± 4.1 ^a	26.47 ± 6.01 ^b	28.32 ± 5.81 ^d	0.19 ± 0.08 ^a	0.30 ± 0.05 ^a
	V (Bark only)	88.3 ± 10.0 ^{a,c}	11.25 ± 3.82 ^a	20.38 ± 6.42 ^d	0.13 ± 0.05 ^a	0.15 ± 0.02
	VI (Root only)	91.6 ± 14.2 ^{a,c}	12.66 ± 1.58 ^a	26.44 ± 8.20 ^d	0.28 ± 0.08 ^c	0.38 ± 0.06 ^c
Hepatoprotective effect	VII (Vit. E+CCl ₄)	92.6 ± 7.1 ^{a,c}	64.31 ± 8.03 ^d	52.37 ± 2.48 ^a	0.30 ± 0.03 ^c	0.42 ± 0.05 ^c
	VIII (Vit. C+CCl ₄)	37.1 ± 3.2 ^a	62.87 ± 4.05 ^d	44.54 ± 1.49 ^{ab}	0.26 ± 0.03 ^c	0.40 ± 0.05 ^c
	IX (Fruit+CCl ₄)	76.7 ± 2.8 ^{a,c}	10.95 ± 4.04 ^a	48.83 ± 7.04 ^a	0.16 ± 0.02 ^a	0.26 ± 0.05 ^a
	X (Leaf+CCl ₄)	58.9 ± 22.0 ^a	33.08 ± 8.44 ^b	53.09 ± 4.12 ^a	0.25 ± 0.06 ^c	0.39 ± 0.08 ^c
	XI (Bark+CCl ₄)	109.3 ± 8.6 ^c	21.26 ± 3.56 ^{ab}	29.15 ± 4.12 ^d	0.25 ± 0.04 ^c	0.30 ± 0.02 ^a
	XII (Root+CCl ₄)	77.3 ± 9.1 ^{a,c}	30.67 ± 2.49 ^b	32.26 ± 3.16 ^{bd}	0.36 ± 0.06 ^d	0.46 ± 0.04 ^c
Ameliorative effect	XIII (CCl ₄ +Silym.)	96.4 ± 6.8 ^c	10.38 ± 1.66 ^a	22.42 ± 3.41 ^d	0.10 ± 0.03 ^a	0.24 ± 0.05 ^a
	XIV (CCl ₄ +fruit)	148.5 ± 10.6 ^b	35.38 ± 7.18 ^b	30.83 ± 7.35 ^d	0.19 ± 0.04 ^a	0.30 ± 0.08 ^a
	XV (CCl ₄ +leaf)	117.1 ± 22.0 ^c	45.74 ± 9.79 ^{bd}	28.53 ± 3.52 ^d	0.25 ± 0.04 ^c	0.31 ± 0.06 ^a
	XVI (CCl ₄ +bark)	121.7 ± 8.3 ^c	28.97 ± 3.48 ^b	21.48 ± 3.04 ^d	0.32 ± 0.02 ^c	0.36 ± 0.04 ^c
	XVII (CCl ₄ +root)	138.0 ± 19.3 ^b	28.17 ± 6.52 ^b	18.35 ± 2.26 ^d	0.33 ± 0.01 ^c	0.37 ± 0.03 ^c

Values are Mean ± SD (n=6). No significant difference between values with same superscripts down the column (p<0.05)

Table 2: TBARS concentration and liver function parameters of rats pre and post treated with methanolic extracts of different parts of *Morinda lucida* upon CCl₄ administration.

Ameliorative influence of different parts of *Morinda lucida* on CCl₄-induced oxidative stress and liver injury: No significant difference (p<0.05) in TBARS concentration among the normal control group and those post-treated with either silymarin or methanol extracts of the leaf or bark of *M. lucida* after CCl₄ administration, but significantly lower than the fruit or root post-treated and the 'CCl₄ only' groups (p>0.05) (Table 1). Markers of hepatic damage viz: AST, ALT, total and conjugated bilirubin showed no statistical difference between the normal, all the extract post-treated groups after CCl₄ administration and experimental controls except in the 'CCl₄ only' group where values were significantly higher (p<0.05) than all the others groups. These were also indicated in the histomicrographs (Figures 1 and 2).

Lipidemic statuses viz: triglyceride (TG) concentration, total cholesterol (TC) and low density lipoprotein (LDL), showed 'CCl₄ only' group being significantly higher (p<0.05) than the normal control group, the silymarin group, and those post-treated with methanol extracts of the different parts of *M. lucida* after CCl₄ administration

except in the 'CCl₄+Root' group, with respect to LDL concentration (Table 2). In HDL concentration, there was significantly lower values (p>0.05) in the 'CCl₄ only' group than the normal control and all other groups except in the 'CCl₄+Leaf' group (Table 1, Figures 1 and 2).

Discussion

Treatments with plant extracts in hepatotoxicity studies for seven days have shown to be enough time to express hepatoprotective or ameliorative effect on induced liver damage [35-37]. In the hepatoprotective experiment, the C (water soluble) and E (lipid soluble) vitamins were used as the standard compounds due to their known roles in conferring protection against lipid peroxidation [38,39]. Since they are basically vitamins which are supplied to the body through diets, they offer protective effects against metabolic insults even before an ailment is fully established. Silymarin was employed as standard drug owing to its role as drug widely employed in the treatment of hepatic function impairment [40]. Silymarin, has been extensively

Figure 1: Photomicrographs of liver sections of CCl_4 -induced hepatotoxicity in rats pre-and-post treated with 100 mg/kg of *Morinda lucida* extracts (H & E stain x250), showing a=normal control; b, e(i), f(i)= CCl_4 only silymarin only and fruit only, respectively; c, d, e(ii), f(ii)=pre-treatment for 7 days with vit.C, E, Silymarin or extract of fruit respectively, before CCl_4 administration; and e(iii), f(iii)=post-treatment for 7 days with Silymarin or fruit extract respectively, after CCl_4 administration.

Figure 2: Photomicrographs of liver sections of CCl_4 -induced hepatotoxicity in rats pre-and-post treated with 100 mg/kg of *Morinda lucida* extracts (H & E stain x250), showing g(i), h(i), i(i)=leaf, bark or root only; g(ii), h(ii), i(ii)=pre-treatment for 7 days with extract of leaf, bark or root respectively, before CCl_4 administration; and g(iii), h(iii), i(iii)=post-treatment for 7 days with extract of leaf, bark or root respectively, after CCl_4 administration.

studied and has shown to possess anticancer properties. It also has antioxidant properties, anti-metastatic activity and tumor suppressive effect on hepatocarcinogenesis [30,41].

From the acute toxicity study in rats using all the different parts of *Morinda lucida*, it was observed that the methanol extracts of parts of the plant were non-toxic, as indicated by the absence of any observed adverse effect even at the highest dose of 5000 mg/kg. According to Lorke [42], such compounds are considered non-toxic. Thus, the minimal 100 mg/kg/day dose of the methanol extracts of the fruit, leaf, bark and root of *Morinda lucida* was selected primarily, to ascertain potency, and also based on previous studies [43].

In this study also, the concentration of reactive oxygen species (ROS) measured as thiobarbituric acid (TBARS) concentrations, which is an index of lipid peroxidation [33] was increased in 'CCl₄ only' rats group but significantly decreased in all the rats pre-treated with the different parts of *M. lucida* extracts. Also, among the rats treated with the different parts of extracts of *M. lucida* after administration of CCl₄, there was a significant decrease in the TBARS concentration among the groups that received leaf or bark extracts. While free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation [39] which results in destruction of cell structures, lipids, proteins and nucleic acids [44], there was a clear evidence of the intervention of the extracts against the generation of free radicals.

Considering hepatoprotection/amelioration, there is replete of literature that serum enzymes particularly, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) present in hepatic and biliary cells [45] are usually released from the hepatocytes and leak into circulation causing increase in their levels in the serum under hepatocellular injury or inflammation of biliary tract cells [45-47]. Hence, the elevated levels of ALT and AST are generally attributed to injury caused to the hepatocytes by carbon tetrachloride which affected the normal functions of the liver, since these enzymes are indisputably, markers of liver injury.

The determination of serum bilirubin is also an important marker for the diagnosis of several diseases, particularly high levels of bilirubin are strongly associated with hemolysis, blockage of the biliary tract, and liver disease [48]. Serum bilirubin (conjugated and total) levels were significantly elevated by CCl₄ injection (Table 1). However, these were remarkably reduced by treatment with the methanolic extracts of various parts of *M. lucida* before and after CCl₄ injection, suggesting the capabilities of the extracts to confer both hepatoprotection and amelioration, comparable to silymarin (Table 1, Figures 1 and 2). These findings have shown that the methanol extracts of *M. lucida* protected the rat livers against CCl₄-induced damage and lowered the serum bilirubin *in vivo*.

With respect to lipid profile, evidence of significant decrease in triglyceride, total cholesterol and LDL concentrations among the hepatoprotective and ameliorative rat groups indicated a strong triglyceride and cholesterol lowering effect of the extracts except the leaf and root extracts in the hepatoprotective and ameliorative groups respectively (Table 2, Figures 1 and 2). Triglyceride, total cholesterol and LDL concentration among the 'CCl₄ only' group was significantly very high, indicating the establishment of the hypercholesterolemic effect of the hepatotoxin. The observed increase in HDL among the rat groups among the hepatoprotective and ameliorative groups indicated the hyperlipidemia-lowering effect of the different methanol extracts of *M. Lucida* except in the 'CCl₄+leaf' (ameliorative) group where values were similar to the 'CCl₄ only' group as shown by the significantly low

HDL concentrations due to the effect of CCl₄ (Table 2). Like other plants constituents [49] the methanol extracts of parts of *M. lucida* (particularly the bark and leaf) reduced TG level. It could thus, be suggested that these extracts increased lipase activity which hydrolyzed TG. Among the lipids, increased blood level of TC, LDL and VLDL as well as lowered level of HDL have been identified as contributors in the development of hyperlipidemia [50] which is as a result of pathological conditions like liver disease and diabetes mellitus [51-53]. *M. lucida* may have acted as inhibitor for enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which is the key enzyme in *de novo* cholesterol biosynthesis [54,55]. This reduction could be beneficial in improving lipid metabolism and complications arising from same [56-62]. The present investigation has shown that extracts of parts of *M. lucida* improved liver synthetic activity.

Conclusion

There were reduced levels of thiobarbituric acid reactive substances (TBARS), ALT and AST, and concentrations of conjugated and total bilirubin, triglycerides, LDL and total cholesterol but higher HDL in the sera of animals that received CCl₄ either before or after 7 days of administration of the various extracts particularly the bark and leaf. All these indicated the anti-peroxidative, hepatoprotective/ameliorative effects of these extracts on the integrity of the liver and associated disorders like hyperlipidemia and hyperbilirubinaemia. The findings in this work suggest that *Morinda lucida*, with particular reference to the leaves and bark could be utilized as important sources of natural antioxidants with health benefiting properties, and could be useful as additives in food or in nutraceuticals and functional foods and in biopharmaceutical industries.

References

- Halliwel B (1994) Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 344: 721-724.
- Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L (2007) Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *J Inflamm (Lond)* 4: 15.
- Kinnula VL, Crapo JD (2004) Superoxide dismutases in malignant cells and human tumors. *Free Radic Biol Med* 36: 718-744.
- Singh U, Jialal I (2006) Oxidative stress and atherosclerosis. *Pathophysiology* 13: 129-142.
- Sas K, Robotka H, Toldi J, Vecsei L (2007) Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. *J Neurol Sci* 257: 221-239.
- Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G (2000) Oxidative stress in Alzheimer's disease. *Biochim Biophys Acta* 1502: 139-144.
- Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, et al. (2006) Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* 27: 262-269.
- Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. *Chem Res Toxicol* 13: 135-160.
- Arteel GE (2003) Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* 124: 778-790.
- Ramakrishna BS, Varghese R, Jayakumar S, Mathan M, Balasubramanian KA (1997) Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. *J Gastroenterol Hepatol* 12: 490-494.
- Hyun DH, Hernandez JO, Mattson MP, de Cabo R (2006) The plasma membrane redox system in aging. *Ageing Res Rev* 5: 209-220.
- Upston JM, Kritharides L, Stocker R (2003) The role of vitamin E in atherosclerosis. *Prog Lipid Res* 42: 405-422.
- Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160: 1-40.
- Nanasombat S, Teckchuen N (2009) Antimicrobial, antioxidant and anticancer

- activities of Thai local Vegetables Journal of Medicinal Plants Research 3: 443-449.
15. Atawodi SE (2010) Polyphenol Composition and *in vitro* Antioxidant Potential of Nigerian *Canarium schweinfurthii* Engl. Oil. Advances in Biological Research 4: 314-322.
 16. Atawodi SE, Atawodi JC, Pala YI, Dakwo P (2009a) Assessment of the Polyphenol Profile and Antioxidant Properties of Leaves, Stem and Root Barks of *Khaya senegalensis* (Desv.). Electronic Journal of Biology 5: 80-84.
 17. Atawodi SE, Atawodi JC, Pfundstein B, Spiegelhalter B, Bartsch H, et al. (2011) Assessment of the Polyphenol Components and *In Vitro* Antioxidant Properties of *Syzygium aromaticum* (L.) Merr. & Perry. Electronic Journal of Environmental, Agricultural and Food Chemistry 10:1970-1978.
 18. Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, et al. (2009b) Polyphenol composition and antioxidant potential of *Hibiscus esculentus* L. fruit cultivated in Nigeria. J Med Food 12: 1316-1320.
 19. Adomi PO, Umukoro GE (2010) Antibacterial activity of aqueous and ethanol crude extracts of the root barks of *Alstonia boonei* and preliminary phytochemical test of *Morinda lucida*. Journal of Medicinal Plants Research 4: 644-648.
 20. Dike IP, Obembe OO, Adebisi FE (2012) Ethnobotanical survey for potential anti-malarial plants in south-western Nigeria. J Ethnopharmacol 144: 618-626.
 21. Nweze NE (2012) In vitro anti-trypanosomal activity of *Morinda lucida* leaves. African Journal of Biotechnology 11: 1812-1817.
 22. Awe SO, Makinde JM (1998) Evaluation of sensitivity of *Plasmodium falciparum* to *Morinda lucida* leaf extract sample using rabbit in vitro microtest techniques. Indian Journal of Pharmacology 30: 51-53.
 23. Oliver-Bever B (1986) Medicinal Plants in tropical West Africa. Cambridge University Press, USA, 89-90.
 24. Mishra BB, Gautam S, Sharma A (2006) Microbial decontamination of tea (*Camellia sinensis*) by gamma radiation. Journal of Food Science 71: M151-M156.
 25. Al Shamsi MS, Amin A, Adeghate E (2004) Beneficial effect of vitamin E on the metabolic parameters of diabetic rats. Mol Cell Biochem 261: 35-42.
 26. Adikwu E, Deo O (2013) Hepatoprotective Effect of Vitamin C (Ascorbic Acid). Pharmacology & Pharmacy 4: 84-92.
 27. Jurima-Romet M, Abbott FS, Tang W, Huang HS, Whitehouse LW (1996) Cytotoxicity of unsaturated metabolites of valproic acid and protection by vitamins C and E in glutathione-depleted rat hepatocytes. Toxicology 112: 69-85.
 28. Oliveira CP, Gayotto LC, Tatai C, Della Nina BI, Lima ES, et al. (2003) Vitamin C and vitamin E in prevention of Nonalcoholic Fatty Liver Disease (NAFLD) in choline deficient diet fed rats. Nutr J 2: 9.
 29. Achliya GS, Kotagale NR, Wadodkar SG, Dorle AK (2003) Hepatoprotective activity of panchagavya ghrita against Carbontetrachloride induced hepatotoxicity in rats. Indian Journal of Pharmacology 35: 308-311.
 30. El Mesallamy HO, Metwally NS, Soliman MS, Ahmed KA, Abdel Moaty MM (2011) The chemopreventive effect of *Ginkgo biloba* and *Silybum marianum* extracts on hepatocarcinogenesis in rats. Cancer Cell Int 11: 38.
 31. Raja S, Ahamed KF, Kumar V, Mukherjee K, Bandyopadhyay A, et al. (2007) Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats. J Ethnopharmacol 109: 41-47.
 32. Gyamfi MA, Yonamine M, Aniya Y (1999) Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. Gen Pharmacol 32: 661-667.
 33. Usuh IF, Akpan EJ, Etim EO, Farombi EO (2005) Antioxidant Actions of Dried Flower Extracts of *Hibiscus sabdariffa* L. On Sodium Arsenite - Induced Oxidative Stress in Rats. Pakistan Journal of Nutrition 4: 135-141.
 34. Garber CC (1981) Jendrassik-Grof analysis for total and direct bilirubin in serum with a centrifugal analyzer. Clin Chem 27: 1410-1416.
 35. Prabhat KD, Prasanna P, Somya RP, Ranjan S (2009a) Comparison between ethanolic extracts of the plant *Moringa pterygosperma*, against carbon tetrachloride induced hepatopathy. The Pharma Research 2: 138-145.
 36. Das PK, Panda P, Pani SR, Sethi R (2009b) Hepatoprotective activity of plant *Argemone mexicana* (Linn.) against carbon tetrachloride (CCl₄) induced hepatotoxicity in rats. International Journal of Pharmaceutical Research and Development-Online 8: 1-20.
 37. Dawada S, Varsha Z, Dabhadkar D, Shital P (2012) Hepatoprotective Activity of *Cassia fistula* root against Carbon tetrachloride-Induced Hepatic Injury in rats (Wistar). International Journal of Pharma Sciences and Research (IJPSR), 3: 368-378.
 38. Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, et al. (2002) Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 76: 549-555.
 39. Chanda S, Dave R (2009) *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research 3: 981-996.
 40. Kucharz EJ, Kott T (1994) Hepatoprotective activity of silymarin in rats treated with high doses of acetylsalicylic acid or naproxen. Medical Journal of the Islamic Republic of Iran 8: 183-185.
 41. Tripathi YB, Tripathi P, Arjmandi BH (2005) Nutraceuticals and cancer management. Front Biosci 10: 1607-1618.
 42. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54: 275-287.
 43. Adeneye AA, Agbaje EO (2008) Pharmacological evaluation of oral hypoglycemic and anti-diabetic effects of fresh leaves ethanol extract of *Morinda lucida* benth. in normal and alloxan-induced diabetic rats. African Journal of Biomedical Research 11: 65-71.
 44. Saikat S, Chakraborty R, Sridhar C, Reddy YSR, Biplab D (2010) Free radicals, antioxidants, diseases and phytomedicines: Current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research 3: 91-100.
 45. Jensen JE, Stainberg SE, Freese P, Marino E (2004) Liver function tests. Journal of Digestive Disorders 6: 1-3.
 46. Mourelle M, Amezcua JL, Hong E (1987) Effect of rioprostil and colchicine on CCl₄-acute liver damage in rats. Relationship with plasma membrane lipids. Prostaglandins 33: 869-877.
 47. Romero G, Lasheras B, Sainz Suberviola L, Cenarruzabeitia E (1994) Protective effects of calcium channel blockers in carbon tetrachloride-induced liver toxicity. Life Sci 55: 981-990.
 48. BIOO Scientific Corporation (2009) BIOO Research Products: Total Bilirubin Assay Kit Manual. Max Discovery Total Bilirubin Assay Manual – 3460-10.
 49. Lee KT, Sohn IC, Kim DH, Choi JW, Kwon SH, et al. (2000) Hypoglycemic and hypolipidemic effects of tectorigenin and kaikasaponin III in the streptozotocin-induced diabetic rat and their antioxidant activity *in vitro*. Arch Pharm Res 23: 461-466.
 50. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340: 115-126.
 51. Sharma MK, Khare AK, Feroz H (1983) Effect of neem oil on blood glucose levels of normal, hyperglycemic and diabetic animals. Indian Medical Gazette 117: 380-383.
 52. Pushparaj P, Tan CH, Tan BK (2000) Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 72: 69-76.
 53. Pepato MT, Baviera AM, Vendramini RC, Perez Mda P, Kettelhut Ido C, et al. (2003) *Cissus sicyoides* (princess vine) in the long-term treatment of streptozotocin-diabetic rats. Biotechnol Appl Biochem 37: 15-20.
 54. Gebhardt R, Beck H (1996) Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids 31: 1269-1276.
 55. Eidi A, Eidi M, Esmaeili E (2006) Antidiabetic effect of garlic (*Allium sativum* L.) in normal and streptozotocin-induced diabetic rats. Phytomedicine 13: 624-629.
 56. Cho SY, Park JY, Park EM, Choi MS, Lee MY, et al. (2002) Alteration of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. Clin Chim Acta 317: 109-117.
 57. Prohp TP, Onoagbe IO (2012) Acute toxicity and dose response studies of aqueous and ethanol extracts of *Triplochiton scleroxylon* K. schum. (sterculiaceae). International Journal of Applied Biology and Pharmaceutical Technology 3: 400-409.
 58. Tanko Y, Yaro AH, Isa AI, Yerima M, Saleh MIA, et al. (2007) Toxicological and hypoglycaemic studies on the leaves of *Cissampelos mucronata* (Menispermaceae) on blood glucose levels of streptozotocin-induced diabetic wistar rats. Journal of Medicinal Plants Research 1: 113 -116.

-
59. Santhrani T, Maheswari E, Saraswathy GR (2013) Carbamazepine provoked hepatotoxicity: attenuation by vitamin C. *Oxid Antioxid Med Sci* 2: 37-43.
60. Puntel RL, Roos DH, Grotto D, Garcia SC, Nogueira CW, et al. (2007) Antioxidant properties of Krebs cycle intermediates against malonate pro-oxidant activity in vitro: a comparative study using the colorimetric method and HPLC analysis to determine malondialdehyde in rat brain homogenates. *Life Sci* 81: 51-62.
61. Unekwojo EG, James O, Olubunmi AR (2011) Suppressive, Curative and Prophylactic Potentials of *Morinda lucida* (Benth) against Erythrocytic Stage of Mice Infective Chloroquine Sensitive *Plasmodium berghei* NK-65. *British Journal of Applied Science & Technology* 1: 131-140.
62. Ozbek H, Ozturk M, Ozturk A, Ceylan E, Yener Z (2004) Determination of Lethal Doses of Volatile and Fixed Oils of Several Plants. *Eastern Journal of Medicine* 9: 4-6.