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Antioxidant and Modulatory Effect of Ethanolic Extract of Madagascar *Harungana* (*Harungana madagascariensis*) Bark on Cyclophosphamide Induced Neurotoxicity in Rats

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ABSTRACT

The present study focused on the antioxidant and modulatory effects of the ethanolic extract of Madagascar *Harungana* bark on cyclophosphamide induced oxidative stress in rat's brain. Ethanolic extract of Madagascar *Harungana* bark yielded a yellow dye. Subsequently, the phytochemical screening and antioxidant activities, including total phenol content, free radical scavenging ability, reducing power, Fe^{2+} chelating ability and *in vitro* inhibition of lipid peroxidation in rat's brain, were determined. The protective effect of dietary inclusion of these extracts (0.5 and 1.0%) on cyclophosphamide (75 mg/kg body weight) induced oxidative stress in rat's brain was also assessed. The dietary supplementation of Madagascar *Harungana* bark extract caused a significant ($P < 0.05$) decrease in rat's brain MDA content in a dose dependent manner, along with suppression of serum activity of AST, ALT, ALP, and total bilirubin content. The high neuroprotective effect of the extract could be attributed to its high antioxidant properties as typified by its high reducing power, free radical scavenging and Fe (II) chelating abilities. Therefore, dietary supplementation of Madagascar *Harungana* bark as food colourant could modulate neurotoxicity induced by cyclophosphamide administration.

Key words: cyclophosphamide, Madagascar *Harungana* bark, ethanolic extract, antioxidant, neurotoxicity

INTRODUCTION

The brain and nervous system are particularly vulnerable to oxidative stress because of limited antioxidant capacity[(1)]. Oxidative stress is considered to play a prominent role in the causation of many diseases such as inflammation, aging, cancer, strokes, diabetes, heart attacks, arthritis, Alzheimer's dementia and chronic fatigue[(2)].

Cyclophosphamide is an alkylating agent, the most commonly used anticancer and immunosuppressant drug. The mechanism of action of alkylating agents involves the conversion of an active hydrogen atom from the biologically active molecules (DNA, RNA, enzymes or mucopolysaccharides). The alkylation concerns carboxyl groups, amino terminals, phosphate groups. Cyclophosphamide is used in the treatment of chronic and acute leukemia, multiple myeloma, lymphomas and rheumatic arthritis and also in the preparation for bone marrow transplantation[(3,4)]. Although it has tumour selectivity, it also possesses a wide spectrum of toxicities. The crucial factor for the therapeutic and toxic effect of cyclophosphamide is the requirement of the metabolic activation by hepatic microsomal cytochrome P_{450} mixed-function oxidase system[(5)]. This metabolism produces hydroxylated primary active metabolites, acrolein, phosphoramide mustard and nitrogen mustard[(3)]. Cyclophosphamide's antineoplastic effects are associated with the phosphamide mustard while the acrolein is linked with its toxic side effects. Acrolein interferes with the tissue antioxidant defense system, produces highly reactive oxygen free radicals and is mutagenic to mammalian cells[(4)]. It could also potentially inhibit active site cysteine residue of many enzymes. Administration of high doses of cyclophosphamide could cause lethal cardiotoxicity. Reactive oxygen species have been implicated in the development of cardiotoxicity after cyclophosphamide administration[(5)]. Recent findings by Senthilkumar *et al*[(4)] have shown that cyclophosphamide can initiate oxidative stress by inactivating endogenous antioxidant enzymes and also deplete antioxidant metabolites such as glutathione with a resultant disruption of cell membrane, mutation of DNA and loss of structure and function of protein.

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Madagascar *Harungana* (*Harungana madagascariensis)* plant is a genus that is composed of single species belonging to the family of Hypericaceae[6,7]. The family of Hypericaceae is well known for the production of various phenolic compounds such as anthraquinones, xanthones, coumarins, biflavonoids and anthrone derivatives[8-11]. Some of these compounds were reported to exhibit antihypoglycemic[8], antioxidant[12], cytotoxic[13] and platelet aggregation inhibitory[14] activity. Madagascar *Harungana* is a very common small tree reaching 6 m in height and widespread in Tropical Africa. The tree is specifically known for its medicinal values throughout West Africa region and used in treating various ailments[6]. The leaves are used in the treatment of malaria[15]. A mixture of the root and bark is a remedy for dysentery, bleeding and piles[16]. Dalziel[6] reported that the yellow wood (bark) itself yields a yellow dye. The dye can be extracted with hot organic hydroxyl solvents such as ethanol or methanol. Kouam *et al.* [17] reported the isolation of two prenylated anthronoids, harunmadagascarins A and B, from Madagascar *Harungana* stem bark along with six known compounds including two anthronoids: harunganol B and harungin anthrone, one benzophenone: methyl 3-formyl-2,4-dihydroxy-6-methyl benzoate and three pentacyclic triterpenes: friedelin, lupeol and betulinic acid. Harunmadagascarins A and B, harunganol B and harungin anthrone isolated from the Madagascar *Harungana* stem bark were shown to possess high antioxidant activity[17].

In recent years, attention is being focused on medicinal plants for their therapeutic uses due to the twin problems of high prices and side effect of imported drug formulations. There has been particular interest in the antioxidant ability and benefit of phytochemicals in fruits and vegetables[18-21]. Hence the need readily arises to harness both the nutritional and pharmacological potentials of the tropical plants[22]. The use of dyes in food and drinks has been on the increase in recent times. However, there is a dearth of information on the nutritional and therapeutic potential of Madagascar *Harungana* bark as dietary supplements for the management of degenerative diseases. The present study therefore focused on the ability of Madagascar *Harungana* bark to modulate cyclophosphamide induced oxidative stress in rat's brain and the antioxidant properties of the plant.

MATERIALS AND METHODS

I. *Materials*

(I) *Sample Collection*

The extract used for the study was collected from the Chemistry Department, Federal University of Technology, Akure, Nigeria. The extract was prepared from Madagascar *Harungana* bark. All the chemicals used were of analytical grade. Adult Wistar strain albino rats were collected from the animal house of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

(II) *Sample Preparation*

Twenty five grams of the dried defatted (with n-hexane) Madagascar *Harungana* bark was extracted with ethanol in a soxhlet extractor as described by Furniss *et al*[23] for 3 hours at a solute-solvent ratio of 1: 25 (w/v). The solvent was removed from the extract by distillation (atmospheric) and the colourant was transferred to an evaporating dish and dried on a water bath. The extract was later dried in oven at 60-80°C for 30 min-1.5 hour, cooled in a desiccators, weighed and stored.

II. *Phytochemical Screening*

The methods described by Trease and Evans[24] were used for phytochemical screening of Madagascar *Harungana* bark extract for the presence of bioactive compound. The test for tannins was carried out by subjecting 3 g of each plant extract in 6 mL of distilled water, filtered and ferric chloride reagents added to the filtrate. For cardiac glycosides, Killer-Kiliani test was adopted (0.5 g of extract was added to 2 mL of acetic anhydrate plus H_2SO_4). The test for alkaloids was carried out by subjecting 0.5 g of aqueous extract in 5 mL of 1% HCl, boiled, filtered and Mayer's reagent added. The extract was subjected to frothing test for the identification of saponin. The extract was also tested for free glycoside bound anthraquinones. Five grams of extract was added to 10 mL of benzene, filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnings, and potassium hydroxide solution. These qualitative tests were based on the colour change as indication of positive test.

III. *Determination of Total Phenol Content*

The total phenol content of the extract was determined by the method reported by Singleton *et al*.[25]. Appropriate dilutions of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^{o}C and the absorbance was measured at 765 nm with a spectrophotometer. The total phenol content was subsequently calculated and reported in percentage (%). Tannic acid was used as standard phenol.

IV. *DPPH Free Radical Scavenging Ability*

The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al*.[26].

Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

$$\% \text{ DPPH Radical Scavenging Ability} = \text{Abs}_{\text{Ref}} - \text{AbS}_{\text{Sample}}/\text{Abs}_{\text{Ref}}$$

V. *Determination of Reducing Property*

The reducing property of the extract was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu[27]. The aliquot of 2.5 mL was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2.5 mL of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. Five milliliters of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm.

VI. *Fe^{2+} Chelation Assay*

The Fe^{2+} chelating ability of the extract was determined by method of Minotti and Aust[28] with slight modification by Puntel *et al.*[29]. Freshly prepared 500 µM $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µL of 0.1 M Tris-HCl (pH 7.4), 218 µL of saline and the extract (25 µL). The reaction mixture was incubated for 5 min, followed by the addition of 13 µL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm with a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated with respect to the reference, which contains all the reagents without the test sample.

$$\% \text{ Fe Chelating Ability} = \text{Abs}_{\text{Ref}} - \text{AbS}_{\text{Sample}}/\text{Abs}_{\text{Ref}}$$

VII. *Lipid Peroxidation Assay*

(I) *Preparation of Tissue Homogenates*

The rats were decapitated under mild diethyl ether anaesthesia and the cerebral tissue (whole brain) was rapidly dissected, placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10, w/v) with about 10 up and down strokes at approximately 1200 rpm in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000 ×g to yield a pellet that was discarded, and a low-speed supernatant (S1) containing mainly water, proteins and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides) was kept for lipid peroxidation assay[30].

(II) *Lipid Peroxidation and Thiobarbibutric Acid Reactions*

The lipid peroxidation assay was carried out by the modified method of Ohkawa *et al.*[31], briefly 100 µL of S1 fraction was mixed with a reaction mixture containing 30 µL of 0.1 M Tris-HCl buffer (pH 7.4), extract (0-100 µL) and 30 µL of the pro-oxidant (250 µM freshly prepared $FeSO_4$). The volume was made up to 300 µL by water before incubation at 37°C for 1 hr. The colour reaction was developed by adding 300 µL of 8.1% SDS (Sodium duodecyl sulphate) to the reaction mixture containing S1, followed by the addition of 600 µL of acetic acid/HCl (pH 3.4) and 600 µL of 0.8% TBA (Thiobarbituric acid). This mixture was incubated at 100°C for 1 hr. The absorbance of TBARS (Thiobarbituric acid reactive species) produced were measured at 532 nm. MDA (Malondialdehyde) produced was expressed as % Control.

VIII. *Bioassay*

The ability of the dietary inclusion of extract from Madagascar *Harungana* bark to prevent cyclophosphamide-induced oxidative stress in rat's brain was evaluated by the method reported by Bhatia *et al.*[32]. The rats were acclimatized for four weeks, during this period, they were maintained *ad libitum* on commercial growers (Guinea feeds). The rats were subsequently divided into four treatment groups. Animals in group 1 and 2 were fed the basal diet [corn flour (67%), groundnut cake (14%), fish meal (5%), groundnut oil (10%), vitamin-mineral premix (4%)], while animals in group 3 were fed basal diet containing 0.5% supplementation of Madagascar *Harungana* bark extract (at the expense of the premix), and those in group 4 were the basal diet containing 1.0% supplementation of Madagascar *Harungana* bark extract (at the expense of the premix). Half to one percent bark extract inclusion gave the desired colouring of the diet. The experiment lasted for 14 days. The rats in groups 2-4 were injected intraperitoneally with cyclophosphamide (75 mg/kg body weight) 24 hours before the termination of the experiment[32,33], while group 1 served as the control. The MDA content of the brain was determined as described above, and serum activities of glutamate oxaloacetate transferase (SGOT), glutamate pyruvate transferase (SGPT), alkaline phosphatase (ALP) and total bilirubin were determined using diagnostic kits.

IX. *Data Analysis*

The results of the three replicates were pooled and expressed as mean ± standard error (S.E.). Student t-test, one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out[34]. Significance was accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Recently, attention has been focused on the utilization of dyes from plant pigments as food colourant[35,36]. The presence of phytochemicals and phenolic compounds has been shown to contribute immensely to the nutritional quality of food and food products. The ability of dietary supplementation of ethanolic extract of Madagascar *Harungana* bark to modulate cyclophosphamide induced neurotoxicity in rats is highlighted in this study. The phytochemical screening showed the presence of alkaloid, phlobata nin, anthraquinone, saponin, tannin and flavonoids, this finding complements earlier report on the phytochemicals on *Madagascar Harungana* leaf that contains saponin, tannin, phenols, oils, sterols and flavonoids[15]. However, Chu *et al.*[37] reported that many plants are rich sources of phytochemicals and that intake of these plant chemicals has a protective potential against degenerative diseases. This correlates with the findings of Boyer and Liu[38] to the extent that these various phytochemicals have protective and therapeutic effects essential to preventing diseases and maintaining a state of well-being. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure[39,40]. Natural polyphenols are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases[41,42]. The total phenol content of Madagascar *Harungana* bark extract was determined and presented in Table 1. The results revealed that Madagascar *Harungana* bark extract has high total phenolic content (68.9%).

Prevention of the chain initiation step by scavenging various reactive species such as free radicals is considered to be an important antioxidant mode of action[43]; hence the ability of the Madagascar *Harungana* bark extract to scavenge free radicals was assessed in this study. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) is a stable radical, and has been used to estimate the radical scavenging capacities of antioxidants[44]. This is based on a model system whereby antioxidant capacity is measured as the ability to donate a hydrogen atom thus neutralizing DPPH radicals[45]. The DPPH free radical scavenging ability of the Madagascar *Harungana* bark extract (5 µg/mL) is presented in Table 1. It was observed that Madagascar *Harungana* bark had a high free radical scavenging ability (96.7%) as compared to several tropical green leafy vegetables[19,46] and that of mushrooms[47].

Reducing power can be a novel antioxidation defence mechanism; the two mechanisms involved in the reducing power are electron transfer and hydrogen atom transfer[48]. The reducing power as typified by the ability of the Madagascar *Harungana* bark extract to reduce Fe (III) to Fe (II) was determined and the result is presented in Table 1. The Madagascar *Harungana* bark was able to reduce Fe^{3+} to Fe^{2+} (5 µg/mL). The reducing power of the Madagascar *Harungana* bark was higher than what was obtained for some Nigerian pepper fruits *Capsicum spp*[33]. The value was also higher than that of some commonly consumed and under-utilized tropical legumes (cowpea, pigeon pea and African yam bean) as reported by Oboh[49], but lower than that of some green leafy vegetables[20]. The antioxidant activity of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, free radical scavengers, singlet oxygen quenchers and metal chelators[40].

Table 1. Total Phenol, DPPH free radical scavenging ability, reducing power and Fe (II) chelating ability of ethanolic extract of Madagascar *Harungana* bark

Parameter	Contents*
Total phenol (%)	68.9 ± 0.8
DPPH Free radical scavenging ability (%)	96.7 ± 1.1
Reducing property (OD_{700})	0.7 ± 0.2
Fe (II) Chelating ability (%)	86.2 ± 0.5

*Values represents mean of triplicate readings.

Antioxidants can prevent transition metals, by chelation and deactivation, from participating in the initiation of lipid peroxidation and oxidative stress through metal catalyzed reactions; hence the ability of the Madagascar *Harungana* bark extract to chelate transition metals is considered as an antioxidant mechanism[29,43,50]. Iron (Fe), an essential metal needed for normal cellular physiology, is present in biological systems bound to several protein moieties such as hemoglobin, ferritin *etc*. It may also exist in free form in which it is able to participate in Fenton reaction with OH^- and Fe^{3+} as products. The Madagascar *Harungana* bark extract was able to chelate Fe^{2+} (0.3 µg/mL) as shown in Table 1. Chelation of transition metals such as iron is regarded as a preventive antioxidant mechanism; however Madagascar *Harungana* bark extract had high Fe^{2+} chelating ability (68.3%). Furthermore, it is worth noting that there is an agreement among the total phenol content, reducing power, DPPH free radical scavenging ability and the Fe^{2+} chelating ability of Madagascar *Harungana* bark extract. The high antioxidant capacity of Madagascar *Harungana* bark extract could be attributed to its high total phenol content. This finding agrees with earlier reports where correlations were observed between total phenol content and observed antioxidant activities[51,52]. The antioxidant activity of Madagascar *Harungana* bark extract may contribute partially to its therapeutic potentials[15].

Lipid peroxidation is thought to proceed via radical indicated abstraction of hydrogen atoms from methylene

carbons in polyunsaturated fatty acids[53]. Malondialdehyde (MDA) is one of the end products of lipid peroxidation and the extent of lipid peroxidation is estimated by measuring MDA levels. MDA is a reactive aldehyde and is one of the many reactive electrophilic species that cause toxic stress in cells and form advanced glycation end products (AGEs). The production of MDA is used as a biomarker to measure the level of oxidative stress in an organism[54]. The ability of Madagascar *Harungana* bark extract to inhibit lipid peroxidation was determined on rat's brain tissue *in vitro* (Figure 1). The results revealed that Madagascar *Harungana* bark extract caused a dose-dependent inhibition in MDA production in the brain tissue. This clearly indicates that Madagascar *Harungana* bark extract exerted an antioxidant effect. This inhibitory effect of the extract on MDA production in isolated brain may be attributed to the total phenol content of Madagascar *Harungana* bark extract[21].

Furthermore, the protective ability of Madagascar *Harungana* bark extract was assessed *in vivo*. Wistar albino rats were placed on basal diet with varying levels of Madagascar *Harungana* bark extract inclusion (0.5% and 1.0%); however, there was no significant ($P > 0.05$) difference in the daily feed intake (Table 2). The intraperitoneal administration of a single dose of cyclophosphamide (75 mg/kg body weight) prior to the termination of the experiment caused a significant ($P < 0.05$) increase in the MDA content of the rat brain (Figure 2). This increase in the brain MDA content following intraperitoneal administration of cyclophosphamide agreed with earlier report by Bhatia *et al.*[32]. The administration of intermittent massive dosage of cyclophosphamide has been found advantageous in chemotherapy[5]. The cellular mechanism of cyclophosphamide toxicity is mediated by an increase in the free radicals through intracellular phosphamide mustard and acrolein, the principal alkylating metabolism of cyclophosphamide[4]. Acrolein interferes with the tissue antioxidant defense system and produces highly reactive oxygen free radicals, which is mutagenic to mammalian cells[4].

Free radical induced lipid peroxidation has been suggested to alter the membrane structure and function, thus causing cellular abnormalities such as mutations and cell death[4]. Hence, an increase in free radical production mediated by cyclophosphamide metabolites which in turn stimulated lipid peroxidation, shall lead to an increase in MDA production in the brain cells of rats.

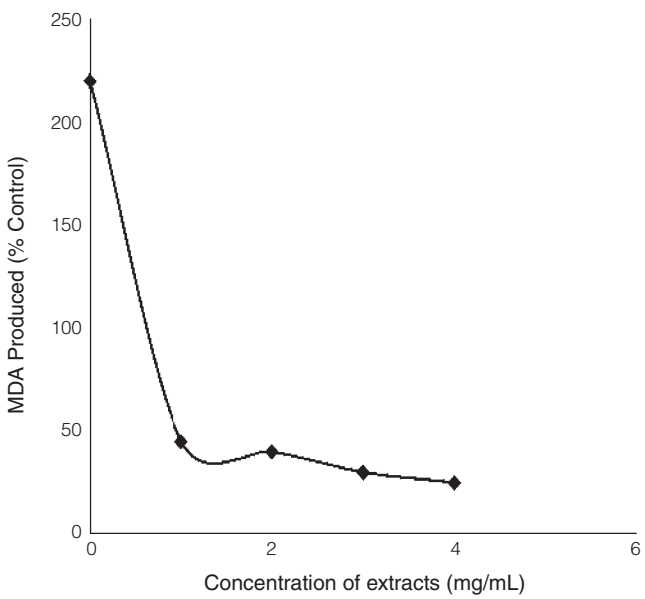

Figure 1. Inhibition of lipid peroxidation in rat's brain by Madagascar *Harungana* bark extract (*in vitro*).

Table 2. Average feed intake of Madagascar *Harungana* bark extract

Group	Average feed intake (g/rat/day)*
Control 1	12.1 ± 2.3^a
Control 2	11.8 ± 2.1^a
Madagascar *Harungana* bark extract (0.5%)	13.8 ± 1.3^a
Madagascar *Harungana* bark extract (1.0 %)	12.9 ± 1.7^a

*Values represents mean of fourteen readings; Values with the same alphabet are not significantly different ($P > 0.05$).

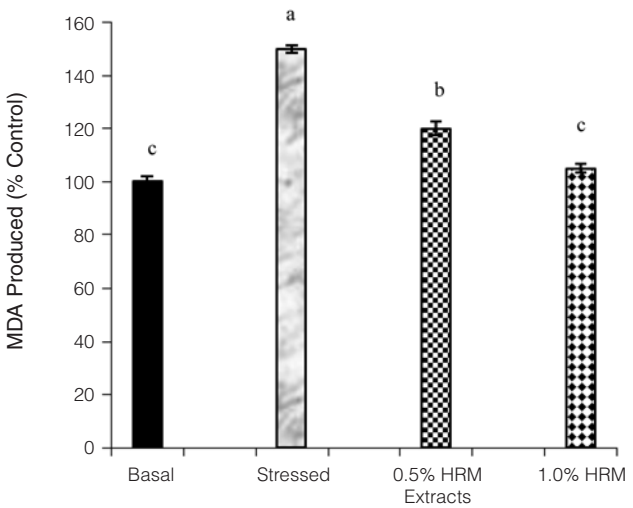

Figure 2. Inhibition of cyclophosphamide induced lipid peroxidation in rat's brain Madagascar *Harungana* bark extracts (*in vivo*).
Bars with the same alphabet are not significantly different ($P > 0.05$).
Basal: Rats placed on diet excluded from Madagascar *Harungana* bark extract.
Stressed: Rats placed on diet excluded from Madagascar *Harungana* bark extract but injected with cyclophosphamide.
0.5% HRM: Rats placed on 0.5% dietary inclusion of Madagascar *Harungana* bark extract and injected with cyclophosphamide.
1.0% HRM: Rats placed on 1.0% dietary inclusion of Madagascar *Harungana* bark extract and injected with cyclophosphamide.

Coupled with the fact that, the brain has limited access to the bulk of antioxidants produced by the body, neurons are first cells to be affected by the shortage of antioxidants and are most susceptible to oxidative stress[21,33].

However, dietary inclusion of Madagascar *Harungana* bark extract (0.5 and 1.0%) caused a significant ($P < 0.05$) decrease in the brain MDA content in a dose dependent manner (Figure 2). This inhibition in MDA production may be due to that Madagascar *Harungana* bark extract is rich in biologically active phytochemicals with antioxidant activities such as phenols. The high antioxidant activities of the Madagascar *Harungana* bark (*in vitro* and *in vivo*) could be attributed to its high phenolic content (Table 1). These findings agree with many earlier reports where a correlation was established between phenolic content and antioxidant properties of some plant foods[19,37,46].

Cyclophosphamide administration was also observed to significantly ($P < 0.05$) increase aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin levels in the serum. Increased activities of these enzymes in the serum are well-known diagnostic indicators of liver injury. The results of the changes in AST, ALT and ALP are shown in Figures 3, 4, and 5 respectively. Rats fed continuously with basal diet followed by cyclophosphamide administration (stressed) had significant ($P < 0.05$) increase in the serum AST, ALT and ALP compared to rats fed only basal diet. This indicated that cyclophosphamide induced oxidative stress on the liver which consequently resulted in the significant rise in AST, ALT and ALP in the serum.

The increased levels of these enzymes and metabolites in the serum could be attributed to the activity of acrolein; a product of cyclophosphamide metabolism. Acrolein interferes with the body antioxidant system; a breach in antioxidant defense system would result in proliferative production of reactive oxygen species (ROS) which would in turn attack hepatocyte membrane by disrupting its structure and function, the result of which is a leak of these enzymes into the circulation. Similar studies by Mythili *et al.*[5], Senthilkumar *et al.*[4] and Sulkowska *et al.*[3] showed that intraperitoneal injection of cyclophosphamide resulted in decrease in the activity of antioxidant enzymes and an increase in serum marker enzymes and metabolites for liver function. ALT and AST enzymes located in the liver cells, shall leak into the general circulation when liver cells are injured[55]. ALT is regarded as a more specific indicator of liver inflammation, while AST may be elevated in diseases of other organs such as heart and muscle[55].

In acute liver injury, such as acute viral hepatitis, ALT and AST may be greatly elevated, sometimes over 1,000 u/L[55]. Mild or moderate elevation of ALT or AST are non-specific and may be caused by a wide range of liver diseases[55]. This correlates with the report of Oboh[49], in that an increase in serum levels of both ALT and AST indicates possible damage to the liver. However,

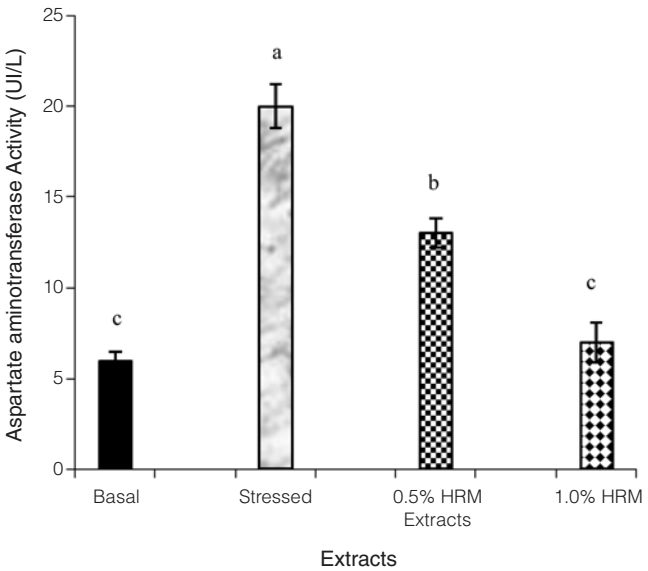

Figure 3. Inhibition of cyclophosphamide induced elevation of rat's serum aspartate aminotransferase activity by Madagascar *Harungana* bark extract (*in vivo*).
Bars with the same alphabet are not significantly different ($P > 0.05$).

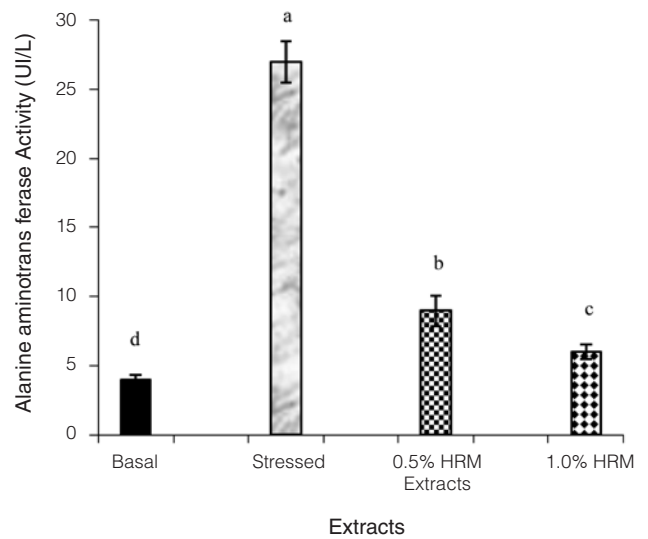

Figure 4. Inhibition of cyclophosphamide induced elevation of rat's serum alanine aminotransferase activity by Madagascar *Harungana* bark extract (*in vivo*).
Bars with different alphabets are significantly different ($P \leq 0.05$).

there was a significant ($P < 0.05$) decrease in the serum ALT, AST and ALP of those rats fed on diet containing Madagascar *Harungana* bark extract. This decrease was also dose-dependent, indicating that the extract was able to protect the hepatocytes from damage. The mechanism by which the extract exerted this activity is not clearly understood. However, it may be due to antioxidant phytochemicals which facilitate hepatocyte healing and protect the liver cells from free radical damage[56].

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Bilirubin is formed during the breakdown of red blood cells and is also a good indicator of liver function. The elevation in serum bilirubin levels indicates possible erythrocyte haemolysis and inability of the liver to metabolize the bilirubin, which could have be occasioned by high dose of free radicals or their oxidation products. Cyclophosphamide administration to rats fed with basal diet was observed to show significant ($P < 0.05$) increase in serum bilirubin level (Figure 6) when compared with rats fed with basal diet without cyclosphosphamide. However, there was a significant decrease ($P < 0.05$) in the serum bilirubin level of those rats fed on diet containing Madagascar *Harungana* bark extract. This decrease was also dose-dependent. Hence, this study also revealed the hepatoprotective potentials of the ethanolic extract of Madagascar *Harungana* bark extract.

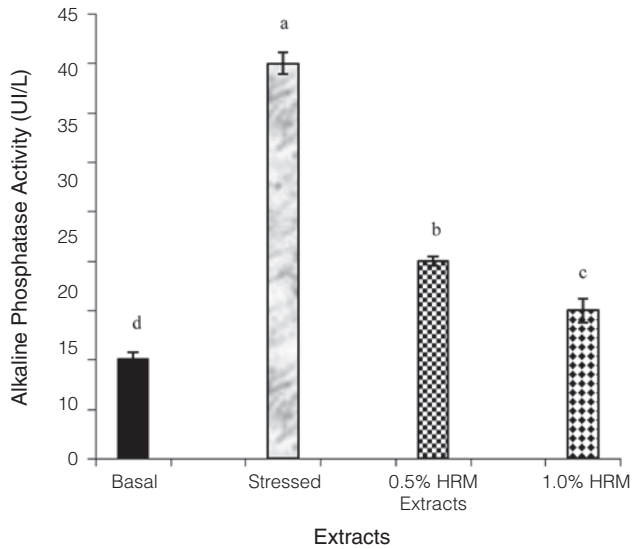

Figure 5. Inhibition of cyclophosphamide induced elevation of rat's serum alkaline phosphatase Activity by Madagascar *Harungana* bark extract (*in vivo*).
Bars with different alphabets are significantly different ($P \leq 0.05$).

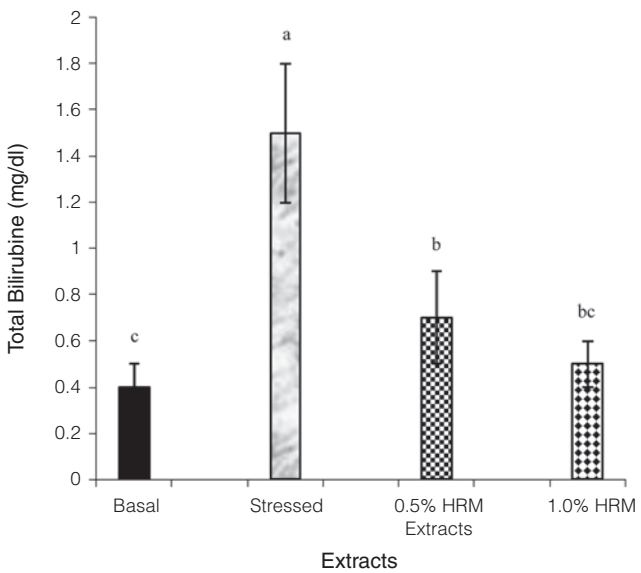

Figure 6. Inhibition of cyclophosphamide induced elevation of rat's serum total bilirubin by Madagascar *Harungana* bark extracts (*in vivo*).
Bars with the same alphabet are not significantly different ($P > 0.05$).

CONCLUSIONS

Ethanolic extract of Madagascar *Harungana* bark exhibited high inhibitory effect of cyclophosphamide induced neurotoxicity and hepatotoxicity. These modulatory properties could be attributed to their high antioxidant properties as typified by the high reducing power, free radical scavenging ability, Fe (II) chelating ability, and total phenol content.

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