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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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Table of Contents: Volume 8 Number 5 May, 2014

ARTICLES

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Full Length Research Paper

Effects of dietary tyrosine on serum cholesterol fractions in rats

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The present study was undertaken to measure the effects of dietary tyrosine added to rat diet on serum cholesterol levels in the rat. A total of twenty Wistar strain albino rats were fed with different doses of tyrosine enriched diets containing 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g. After 3 weeks of experimental feeding, there was significant increase ($p<0.05$) in total postprandial serum cholesterol of rats fed with graded of tyrosine when compared with the normal control. Same trend was followed in the week 2 of the same feeding pattern. The effects of dietary tyrosine supplementation on cholesterol levels of the high density lipoprotein (HDL) fraction were comparable, but not all significant on the week 3 treatment. However, there was significant decrease ($p<0.05$) in week 2 of rats fed with the different graded doses of the tyrosine meal when compared with the normal control group. In addition, significant increase was also observed in the low density lipoprotein (LDL) as compared to the control after week 2 and 3 of tyrosine meal treatment. These results revealed that tyrosine supplementation in a physiological amount may increase cholesterol levels in the rat when added to diet, with a slow release of tyrosine during digestion.

Key words: Dietary tyrosine, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL).

INTRODUCTION

Food helps humans maintain good health by providing all essential nutrients. Consuming a variety of foods in balanced proportions will prevent deficiency diseases and chronic diet-related disorders. Amino acids have many functions in the body. They are the building blocks for all body protein - structural proteins that build muscle, connective tissues, bones and other structures, and functional proteins in the form of thousands of metabolically active enzymes (Elwes et al., 1989; Fernstrom, 2000). Amino acids provide the body with the nitrogen that is essential for growth and maintenance of all tissues and structures. Proteins and amino acids also serve as a source of energy, providing about 4 calories per gram. Aside from these general

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Table 1. Composition of basal diet (Growers feed, Grand Cereals LTD, Enugu).

Diet composition	Amount (%)
Crude protein	15
Fat	7
Crude fibre	19
Calcium	1.0
Phosphorus	0.35
Metabolisable energy	2550 kcal/kg

functions, individual amino acids also have specific functions in many aspects of human physiology and biochemistry (Salter, 1989).

Tyrosine is a non essential amino acid the body makes from another amino acid called phenylalanine. It is a building block for several important brain chemicals called neurotransmitters, including epinephrine, norepinephrine and dopamine (Moller et al., 1995). Neurotransmitters help nerve cells communicate and influence mood (Thomas et al., 1999). Tyrosine also helps produce melanin, the pigment responsible for hair and skin colour. It helps in the function of organs responsible for making and regulating hormones, including the adrenal, thyroid and pituitary glands (Shurtleff et al., 1994). It is involved in the structure of almost every protein in the body. It is rare to be deficient in tyrosine. Low levels have been associated with low blood pressure, low body temperature and an underactive thyroid (Sole et al., 1985; Deijen and Orlebeke, 1994; Deijen et al., 1999).

Cholesterol is insoluble in the blood; it must be attached to certain protein complexes called lipoproteins in order to be transported through the bloodstream. Low-density lipoproteins (LDLs) transport cholesterol from its site of synthesis in the liver to the various tissues and body cells, where it is separated from the lipoprotein and is used by the cell (Gordon et al., 1989). Cholesterol attached to LDLs is primarily that which builds up in atherosclerotic deposits in the blood vessels hence LDLs are termed ‘bad’ cholesterol (Olson, 1998). High-density lipoproteins (HDLs) may possibly transport excess or unused cholesterol from the tissues back to the liver, where it is broken down to bile acids and is then excreted thereby serving to retard or reduce atherosclerotic buildup, thus, it is termed ‘good’ cholesterol (Lewis and Rader, 2005).

When we take a close look at the diet of depressed people, an interesting observation is that their nutrition is far from adequate. They make poor food choices and select foods that might actually contribute to depression. Salter (1989) has reported that dietary tyrosine aids to reduce stress among troops. Recent evidence by Tumilty et al. (2011) suggests that oral tyrosine supplementation improves exercise capacity in athletes. A lot of research has been carried out on tyrosine. This study is aimed at determining the effects of dietary tyrosine added to rat diet on serum cholesterol levels in rats.

MATERIALS AND METHODS

Reagents

L-tyrosine used in this study was sourced from Sigma Aldrich USA (Lot# SI bb7526V). All other chemicals and reagents used are of analytical grade.

Animals

A total of twenty (20) Wistar strain albino rats weighing between 134 -180 g bred in the animal house of the Department of Zoology, University of Nigeria Nsukka, were used in the experiment. The animals were kept under room temperature and were acclimatized in the new environment for a period of 7 days and fed non purified diet with the following diet composition as shown in Table 1 before the addition of the dietary tyrosine. The use of animal for research studies was ethically approved by the authorized committee of animal ethics, Department of Biochemistry, University of Nigeria, Nsukka.

Experimental design

After the acclimatization period, a total of 20 rats were used for the experiment and was divided into four groups consisting of four rats in each group as follows: Group 1: Control were fed basal diet; Group 2: Rat fed with 0.8 g/100 g of tyrosine diet; Group 3: Rat fed with 1 g/100 g of tyrosine diet; Group 4: Rat fed with 1.2 g/100 g of tyrosine diet.

The treatment lasted for twenty one (21) days in which blood samples of the rats were analysed on day 0, 14, and 21. The animals were anesthetised and blood sample were collected through ocular puncture for biochemical analysis. Blood samples were received into clean dry centrifuge tube and left to clot at room temperature, then centrifuged at 33.5 g for 15 min to obtain the serum. The serum was carefully separated into dry clean Wassermann tubes, using a Pasteur pipette and kept frozen at (-20°C) until estimation of some biochemical parameters.

Cholesterol determination

Total cholesterol was determined according the method of Abell et al. (1952) as outlined in commercially available kits. Determination of the concentration of the serum total HDL and LDL was determined as described by Kameswara et al. (1999).

Cholesterol determination

The method of Abell et al. (1952) was followed.

Principle

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidase.

Test procedure

Three (3) test tubes were set up in a test tube rack and labeled blank, standard and sample, respectively. To the blank, was added (10 µl) distilled H_2O, 10 µl standard specimen was added to the standard test tube and 10 µl sample (serum) was added to the sample test tube. To each of these test tubes was added 1000 µl of the cholesterol reagent. It was thoroughly mixed and incubated for 10 min at room temperature (20-25°C). The absorbance of the sample (A_{sample}) against the blank was taken within 60 min at 500 nm.

Low density lipoprotein (LDL)

Principle

LDL-C can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

Procedure

The serum samples were kept at 2-8°C. The precipitant solution (0.1 ml) was added to 0.2 ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 xg for 15 min. The cholesterol concentration in the supernatant was determined. The concentration of the serum total cholesterol as described by Kameswara et al. (1999) was used.

Calculation

LDL-C (mmol/L) = Total cholesterol (mmol/L) – 1.5 x supernatant cholesterol (mmol/L).

High density lipoprotein (HDL)

Principle

LDL and VLDL are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, HDL present in the supernatant is determined.

Procedure

The precipitant solution, 0.1 ml was added to 0.3 ml of the serum sample and mixed thoroughly and allowed to stand for 15 min. This was centrifuged at 2,000 x*g* for 15 min. The cholesterol concentration in the supernatant was determined. Determination of the concentration of the serum total HDL as described by Kameswara et al. (1999) was used.

Triacylglycerol

Clinical significance

Triacylglycerols measurements are used in the diagnosis and treatment of diseases involving lipid metabolism and various endocrine disorders e.g diabetes mellitus, nephrosis and liver obstruction.

Principle

The triacylglycerols are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{lipases}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glyserol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4 chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + HCl + 4H_2O$$

Method

A quantity of the sample (0.1 ml) was pipetted into a clean labeled tube and 1.0 ml of trichloroacetic acid (TCA) was added to it, mixed and then centrifuged at 250 rpm for 10 min. The supernatant was decanted and reserved for use.

The mixtures were allowed to stand for 20 min at 25°C and the absorbance of the sample and standards read against the blank was taken at 540 nm.

Calculation

The concentration of triacylglycerol in serum was calculated as follows:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration (mmol/l)} = \text{mmol/l}$$

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 16.0. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation (LSD). The data were expressed as mean ± standard deviation. $P < 0.05$ was considered significant.

RESULTS

The effects of tyrosine supplemented diet on the total cholesterol levels of rats

There was significant increases ($p<0.05$) in total cholesterol of week 2 of rats fed with 0.8 g/100 g of tyrosine as compared to that fed with 1.0 g/100 g and 1.2 g/100 g of the tyrosine supplemented diet. There was also a significant increase ($p<0.05$) of rats fed with 1.0 g/100 g and 1.2 g/100 g of tyrosine meal diet. The same trend was also observed in week 3 (Figure 1). This increases were not dose dependent.

The effects of tyrosine supplemented diet on the HDL levels of rats

The bar shows the result of the HDL of rats fed with tyrosine supplemented diet. There was significant

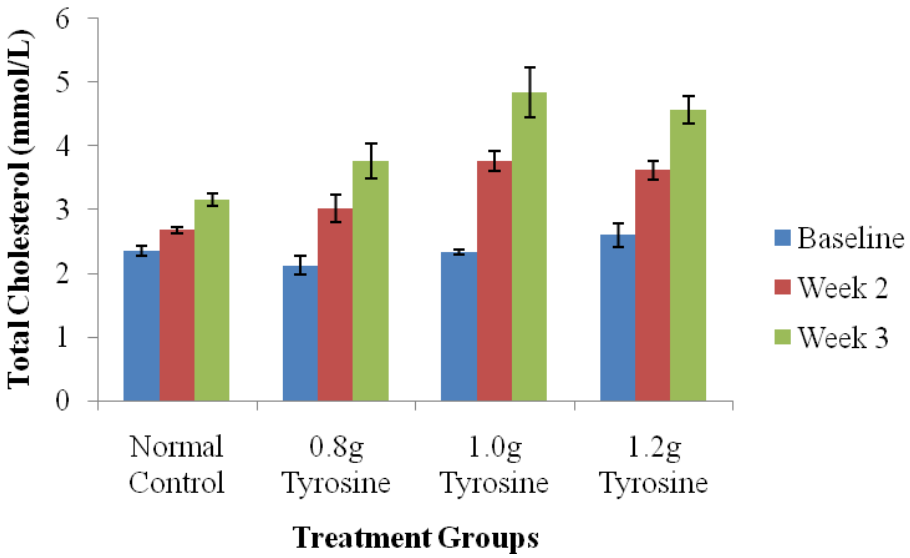

Figure 1. Bar chart representing the effects of tyrosine meal diet on the total cholesterol levels of rats. Values indicadates mean ± SEM (n=4). Significance is at p<0.05.

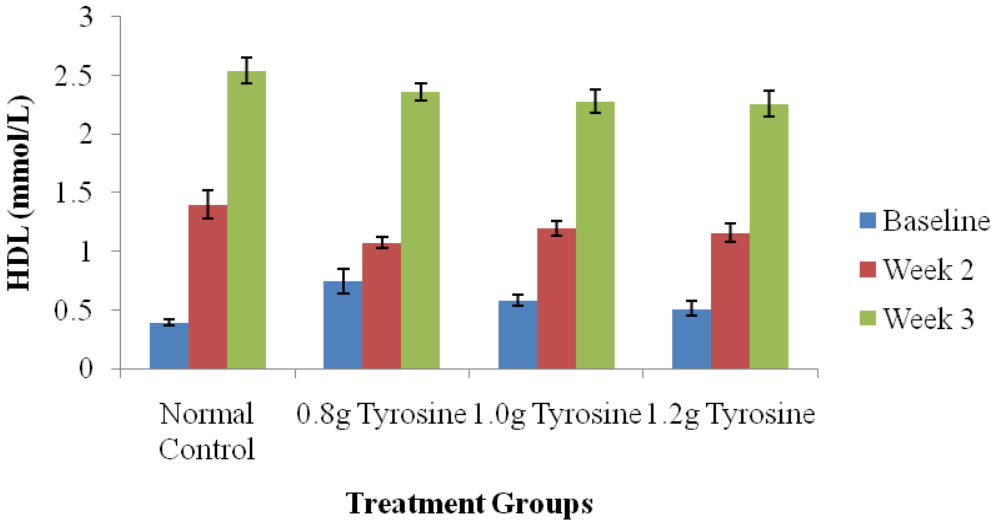

Figure 2. Bar chart representing the effects of tyrosine meal diet on the HDL levels of rats. Values indicadates mean ± SEM (n=4). Significance is at p<0.05.

decrease (p<0.05) in HDL when rats fed with 0.8 g/100 g of tyrosine meal diet was compared to the normal control of week 2. There was no significant difference (p>0.05) in weeks 2 and 3 rats fed with 0.08 g/100 g tyrosine supplemented diet when compared with 1.0 g/100 g and 1.2 g/100 g meal diet (Figure 2).

The effects of tyrosine supplemented diet on the LDL levels of rats

The bar shows the result of the LDL of rats fed with tyrosine supplemented diet. There was significant increases (p<0.05) in week 2 of the LDL of rats fed with 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet as compared to the control. Significant increases (p<0.05) was also observed in of rats fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g tyrosine meal diet at week 3 when compared with the control (Figure 3).

DISCUSSION

The effects of dietary tyrosine on serum lipid profile were

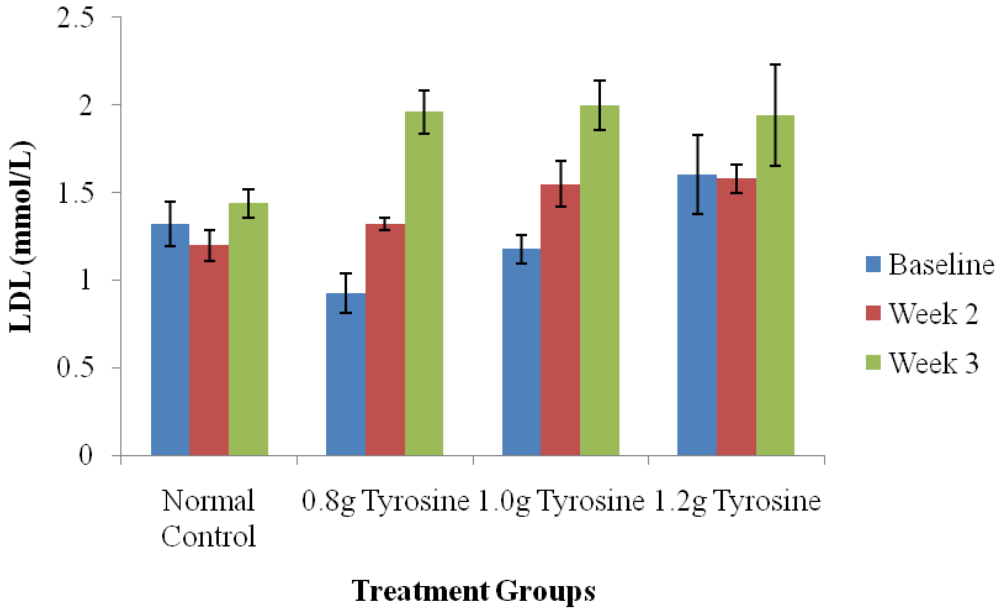

Figure 3. Bar chart representing the effects of tyrosine meal diet on the LDL levels of rats. Values indicadates mean ± SEM (n=4). Significance is at p<0.05.

analyzed in this study. The animals fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g of tyrosine supplemented diet showed significant increases (p<0.05) in total cholesterol of week 2 of rats as compared to that fed with 1.0 g/100 g and 1.2 g/100 g of the tyrosine supplemented diet. There was also a significant increase (p<0.05) in rats fed with 1.0 g/100 g and 1.2 g/100 g of tyrosine meal diet. The same trend was also observed in week 3 as reflected in this work. Increased total cholesterol concentration when fed with high tyrosine meal diet allows one to investigate the effects of treatment in dietary protein source (tyrosine), on this fraction. These result followed similar trend in the rise of total cholesterol levels using other sources of proteins (soy protein and casein) as reported by other researchers (Forsythe et al., 1980; Nagata et al., 1982c; Van der Meer, 1983; Van der Meer et al., 1985). Our result showed significant elevation of cholesterol in the serum of experimental rats as compared to the control. Cholesterol is synthesized in the liver, this result demonstrate the ability of tyrosine to influence liver metabolism towards increased synthesis of lipids. The high levels of cholesterol may be due to a number of factors such as the increased availability of fatty acids for esterification (Bopama et al., 1997), reduced catabolism of LDL, inhibition of tissues, activation of acetyl-CoA caboxylase (McCarthy, 2001) and production of triglycerides precursors such as acetyl-Coa and glycerol phosphate (Fatiha et al., 2014). The elevation of cholesterol in the liver might suggest that the dietary tyrosine supplement contain ingredients capable of enhancing the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase (Vega et al., 2003) which are required for cholesterol synthesis.

Atherosclerosis is characterized by liver disease without alcohol which is manifested by the significant lipid deposition in hepatocytes of liver parenchyma as a single macro-vesicular steatohepatitis and can develop fibrosis in cirrhosis which is increasingly recognized as an important cause of mortality (Angulo and Lindor, 2002).

There was significant decrease (p<0.05) in HDL in rats fed with 0.8 g/100 g of tyrosine supplemented diet as compared to the control of week 2. There was no significant (p>0.05) increase in weeks 2 and 3 of rats fed with 0.8 g/100 g tyrosine meal diet when compared with 1.0 g/100 g and 1.2 g/100g supplemented diet. In the present study, in the rats fed higher doses of tyrosine serum HDL continued to have higher amount as in the case of total cholesterol as compared to the normal rats fed supplemented tyrosine diet during the 21 days study. These observations was in constrast with the work of Nagaoka et al. (1990a) who reported that prolong administrations of dietary tyrosine caused hypercholesterolemia in male Wisttar rats. There was significant increases (p<0.05) in week 2 of the LDL of rats fed with 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet when compared with the control. Significant increases (p<0.05) was also observed in week 3 of rat fed with 0.8 g/100 g, 1.0 g/100 g and 1.2 g/100 g tyrosine supplemented diet when compared with the control. Excess dietary tyrosine or certain xenobiotics increases HDL and LDL cholesterol (Kato and Yoshida, 1981; Nagaoka et al., 1985b). In the hypercholesterolemia

seen following feeding of cholesterol, serum LDL cholesterol was relatively increased (Quazi et al., 1983). It seems likely that cholesterol metabolism is quite different in rats fed higher doses of tyrosine from those of rats fed cholesterol-containing diet. Many investigators have already suggested that the hypercholesterolemia seen in feeding dietary tyrosine (Qureshi et al., 1978; Solomon and Geison, 1978) was mediated by enhancement of of cholesterol synthesis in the liver.

Conclusion

The alterations in the serum cholesterol reveals that long term feeding of dietary tyrosine may escalate cholesterol accumulation in the adipose tissues.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Antidenaturation and antioxidative properties of phytochemical components from *Spondias mombin*

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The antidenaturation and antioxidant properties of *Spondias mombin* Linn (Anacardiaceae) methanol leaf extract (SMC) and fractions prepared from it were evaluated in this study. SMC and its fractions: ether (SME), saponin-rich (SMS) and flavonoid-rich (SMF) were phytochemically screened and evaluated for total antioxidant activity (TAA), ability to inhibit deoxyribose degradation (DEO), lipid peroxidation inhibitory activity (LPIA), 2,2 - diphenyl-1-picryl hydrazyl scavenging activity, ability to chelate ferrous ions and protein denaturation inhibitory activity (PRO). The antioxidant and antidenaturation activities were in the order SME > SMF > SMC > SMS. TAA strongly correlated with DEO, LPIA and PRO. The results indicate that *S. mombin* contains a diverse array of phytochemicals with potent antioxidant and bio-preservative properties which can serve as candidates for food preservation and drug development.

Key words: *Spondias mombin*, phytoconstituents, antioxidant activity, protein denaturation.

INTRODUCTION

Recent studies have shown that many diseases are due to oxidative stress resulting from an imbalance between formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA. This leads to damage to these biomolecules for example protein denaturation, DNA degradation and lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular and inflammatory diseases and ageing (Braca et al., 2002; Aukrust et al., 2005). Preservation of the integrity of these molecules is essential for optimum health. Human cells protect themselves against free radical damage by enzymatic antioxidants such as superoxide dismutase (SOD) and catalase, or non-enzymatic ones such as ascorbic acid, tocopherol and glutathione. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements may be vital to combat oxidative damage (Rahman, 2007). *Spondias mombin* Linn belongs to the family Anacardiaceae. The leaves have been reported to possess abortifacient, antidiarrhoeal, antimicrobial,

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antiviral and wound-healing properties and to be rich in vitamin C (Ayoka et al., 2006; Ayoka et al., 2008). The aim of the present study was to evaluate the protective ability of methanol extract and fractions of *S. mombin* leaves against oxidative stress and protein denaturation *in vitro.*

MATERIALS AND METHODS

Chemicals and reagents

DPPH radical (2,2 - diphenyl-1-picryl hydrazyl), thiobarbituric acid (TBA) and 2-deoxyribose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trisodium orthophosphate was obtained from BDH Chemicals Ltd. (Poole, England). Ethylenediaminetetraacetic acid was obtained from Fisher Scientific Ltd (UK). Other chemicals and reagents used were of analytical grade.

Sample extraction and fractionation

The leaves of *S. mombin* were obtained from Ore town, Ondo State, Nigeria and were authenticated at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria (voucher number IFE-16747). Air dried leaves were powdered and 923.2 g macerated in 3.5 L of 80% methanol for 48 h. The mixture was filtered and the filtrate obtained was concentrated using a rotary evaporator and then lyophilized to obtain the methanol crude extract (SMC) (4.93% yield). The extract (15 g) was defatted with n-hexane (3 × 50 ml) and then partitioned between ether and water (1:1, 3 × 100 ml). The ether portion was concentrated to give the ether fraction (46.67% yield). The aqueous portion was extracted with n-butanol (2 × 50 ml) and the butanol extract partitioned with KOH (2 × 50 ml). The butanol portion was collected and concentrated to give the saponin-rich fraction (2.67% yield). The potassium hydroxide portion was partitioned between HCl and butanol (1:1, 2 × 50 ml). The butanol portion was collected and concentrated to yield the flavonoid-rich fraction (1.33% yield).

Animal handling and care

Male Wistar rats (200 - 250 g) were used. The animals were handled in accordance with the international guide for the care and use of laboratory animals (Committee for update of the guide for the care and use of laboratory animals, 2011).

Phytochemical screening

Phytochemical tests were carried out on samples as previously described (Edeoga et al., 2005; Sofowora, 2006).

Total antioxidant activity

Total antioxidant activity (TAA) was determined as previously described (Umamaheswari and Chatterjee, 2008). A reagent solution containing 0.6 M H_2SO_4, 4 mM ammonium molybdate and 28 mM sodium phosphate was prepared. Mixtures of the samples and reagent solution in tubes were incubated at 95°C for 90 min and absorbance was read at 695 nm against a blank containing distilled water in place of the samples. Results were expressed in µg/ml ascorbic acid equivalent (AAE).

Ability to inhibit deoxyribose degradation

The ability of extracts and fractions to inhibit deoxyribose degradation (DEO) was assessed using the method of Kumar et al. (2009). Briefly, different concentration of samples were added to a reaction mixture containing deoxyribose (20 mM), phosphate buffer (500 mM, pH 7.4), hydrogen peroxide (20 mM) and $FeSO_4.7H_2O$ (0.5 mM). The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by the successive addition of 2.8% TCA and 0.6% TBA solution. The tubes were subsequently incubated in boiling water to develop the pink coloured MDA-$(TBA)_2$ adduct. The absorbance was measured at 532 nm in a spectrophotometer.

Lipid peroxidation inhibitory activity

Evaluation of Fe^{2+}- induced lipid peroxidation inhibitory activity (LPIA) of extracts and fractions in the brain (LPB), liver (LPL) and testes (LPT) of rats was performed following the method described by Sabir and Rocha (2008). Rats were sacrificed by administration of an overdose of anaesthetic. The tissues (brain, liver, testes) were quickly removed and placed on ice. A 10% homogenate of each tissue was prepared in 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged for 10 min at 3000 rpm to yield a pellet that was discarded and a low speed supernatant which was used for thiobarbituric acid reaction.

DPPH radical scavenging activity

DPPH antiradical activity (DRS) of extracts and fractions was evaluated using a spectrophotometric method (Mensor et al., 2001). DPPH (1 ml, 0.3 mM) was added to 1 ml of extract or standard and allowed to react at room temperature. The absorbance was read after 30 min at 517 nm and converted into percentage antioxidant activity.

Iron chelating ability

The ability of extract and fractions to chelate ferrous ions (ICA) was evaluated as previously described (Puntel et al., 2005). EDTA was used as the reference compound.

Evaluation of protein denaturation inhibitory activity

The antidenaturation activity (PRO) was evaluated using a modified method of William et al. (2002) with egg albumen as protein source. Egg albumen (0.5 ml) in 0.2 M phosphate buffer (pH 7.4) and 0.5 ml of extract or fractions were incubated at 27°C for 15 min. Thereafter, the mixture was heated at 60°C for 10 min. The absorbance was read at 660 nm after cooling. Results were expressed as percentage inhibition of protein denaturation.

Statistical analysis

Data are presented as mean ± SEM (n = 3) and were analysed statistically by one-way ANOVA, followed by Duncan's multiple range test. The bivariate correlation analysis was performed, quoting the Pearson correlation coefficients and test of significance. Significance was accepted at $P < 0.05$.

RESULTS

Table 1 presents the phytochemical groups detected in

Table 1. Phytochemical constituents of extract and fractions of *Spondias mombin.*

Test	Sample			
	SMC	SME	SMF	SMS
Saponins	++	-	-	+++
Flavonoids	++	-	+++	-
Steroids	+++	-	++	-
Terpenoids	++	-	-	+++
Tannins	+++	++	-	-
Alkaloids	+	-	-	-
Cardiac glycosides	+++	++	-	+

- indicates absence and + indicates presence.

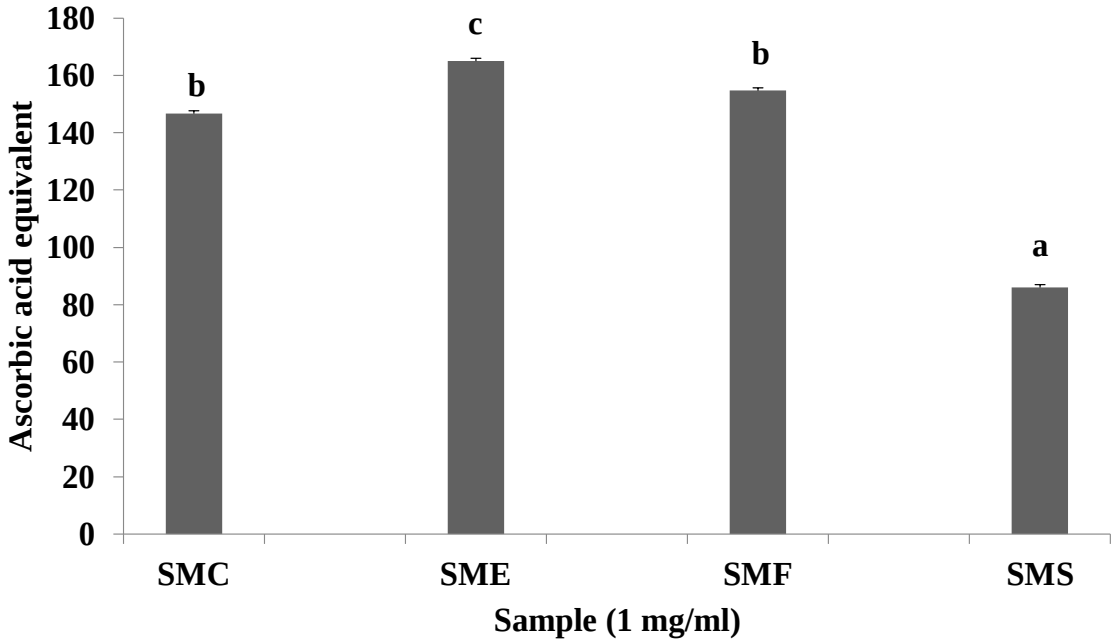

Figure 1. Total antioxidant activity of extract and fractions. Values are given as mean ± SEM (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

the samples. Fractionation resulted in the localization of phytochemicals in the fractions based on solvent properties. SMF and SMS were enriched in flavonoids and saponins respectively. The seeming contaminants may be due to hybrid phytochemicals. The ether fraction was enriched in tannins and cardiac glycosides.

SME had the highest TAA and SMS the least (Figure 1). However, SMC demonstrated the highest DEO followed by SMF while SMS had the least with IC_{50} values of 48.01 ± 0.21, 103.81 ± 0.58 and 270.59 ± 13.89 µg/ml respectively (Figure 2).

Samples, in general, had comparable LPB. SMF and SME showed good LPL while SME also demonstrated remarkable LPT - with respect to the reference standards employed (Figure 3a to c). DRS was highest in SME (Figure 4) while ICA was comparable in the fractions at 50 - 250 µg/ml (Figure 5). PRO followed the same trend as TAA (Figure 6) with SME demonstrating the best antidenaturation activity.

Table 2 presents the relationship between total antioxidant activity of extract and fractions and their inhibitory potentials. The correlation of TAA with PRO, DEO and LPIA are shown in Figures 7a to c. The total antioxidant activity (TAA) correlated strongly with PRO (r^2 = 0.966), DEO (r^2 = 0.801) and inhibition of lipid peroxidation in rat organs (r^2 = 0.901).

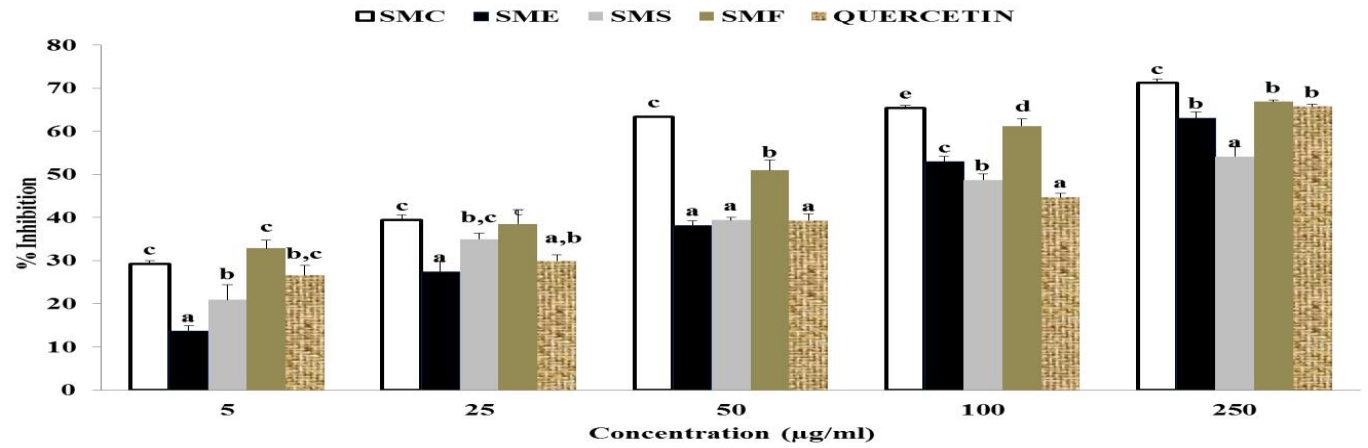

Figure 2. Ability of extract and fractions to inhibit the degradation of 2-deoxyribose. Values are given as mean ± SEM (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

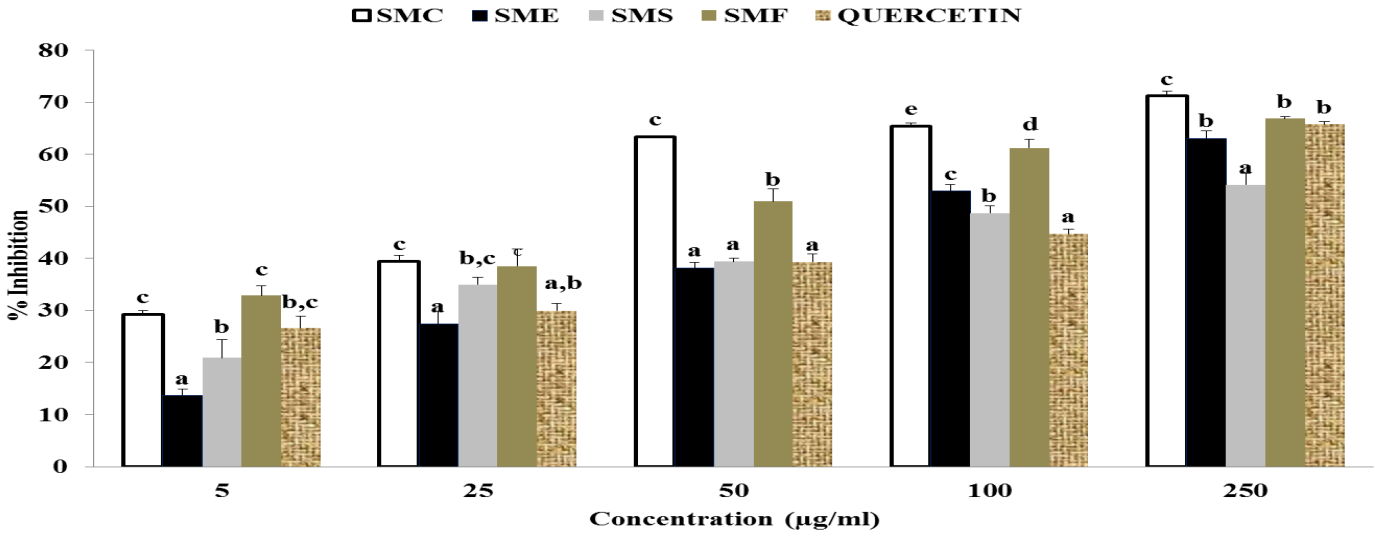

Figure 2. Ability of extract and fractions to inhibit deoxyribose degradation. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

DISCUSSION

Herbal medicine is becoming increasingly popular worldwide and scientific evidence of efficacy of these herbs is continually emerging from controlled preclinical and clinical trials. The medicinal value of herbs lies in their unique content of phytochemicals that elicit definite physiological actions in the mammalian body. Flavonoids and polyphenols are potent water-soluble antioxidants and free radical scavengers which can prevent oxidative cell damage and can lower risk of various pathologies (Urquiaga and Leighton, 2000). The mechanism of action of flavonoids is through scavenging or chelation of free radicals. The hydroxyl groups of flavonoids and other phenolic compounds confer scavenging ability (Cook and Samman, 1996; Yildrim et al., 2000). Flavonoids and other phenolic derivatives with antioxidant and anti-aging properties have been identified in *S. mombin* leaves (Corthout et al., 1992). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity (Gwotmut and Uwafor, 2001) and the presence of these phytochemicals may be associated with the reported oxytocic and abortifacient activities of the plant's leaf extract (Offiah and Anyawu, 1989). These phytochemicals may also account for the plant's reported anti-microbial, anti-bacterial, molluscicidal (Corthout et

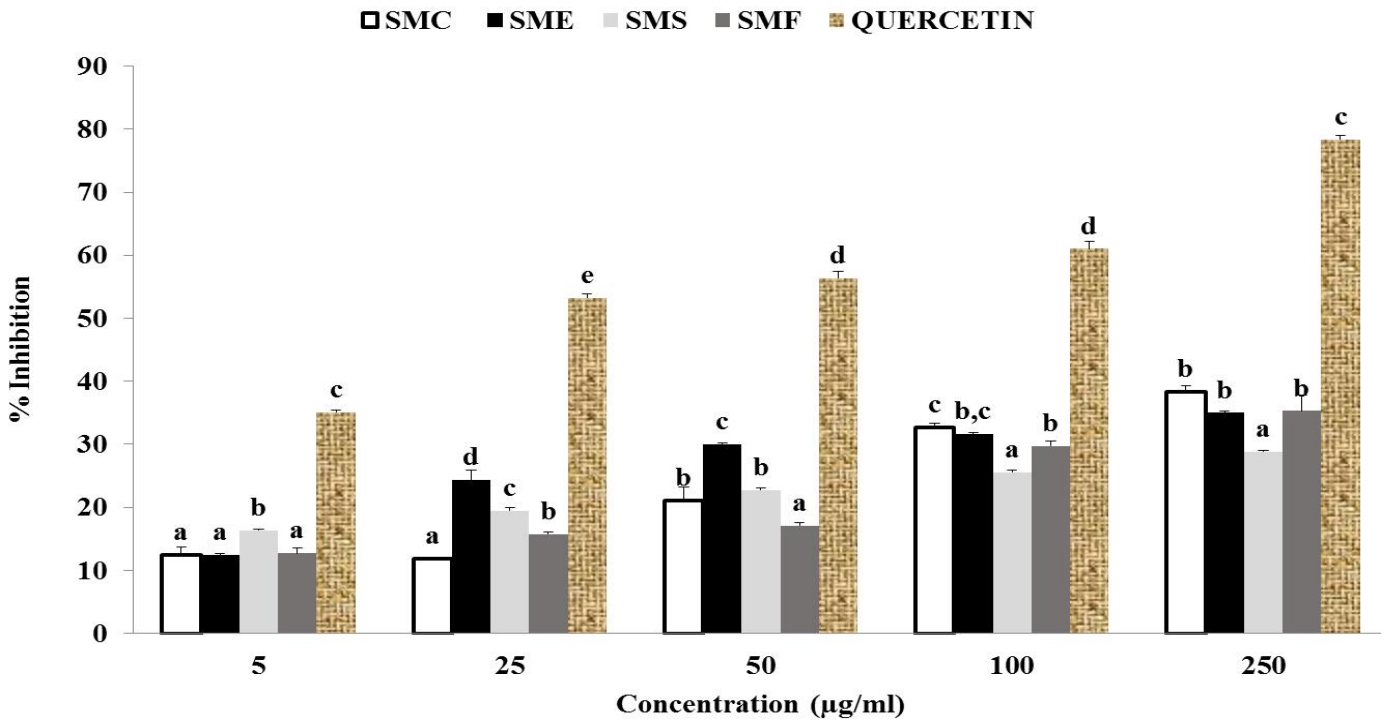

Figure 3a. Lipid peroxidation inhibitory activity of extract and fractions in rat brain. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

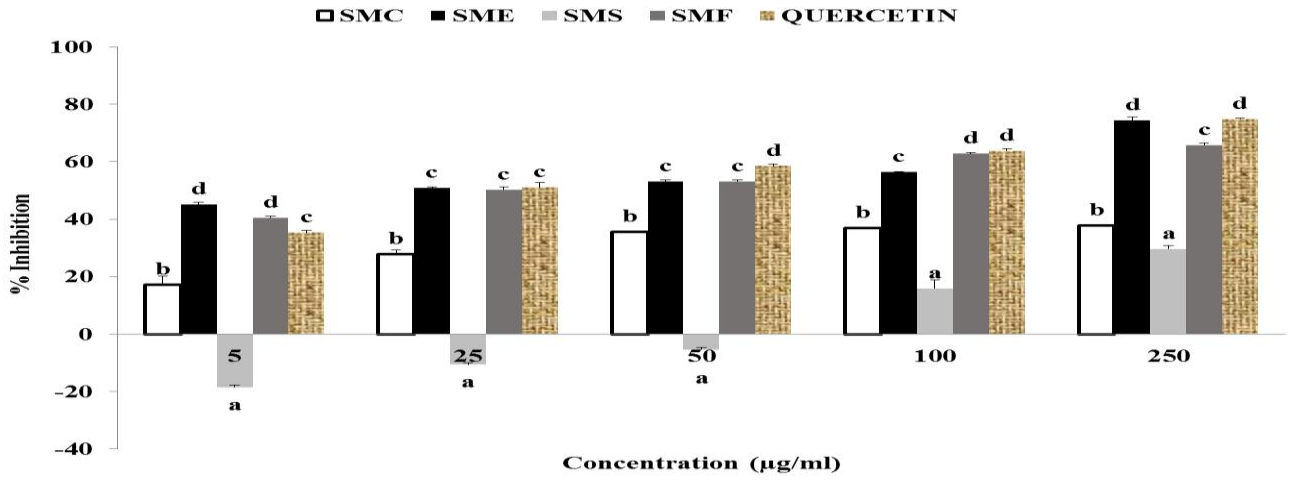

Figure 3b. Lipid peroxidation inhibitory effect of extract and fractions in rat liver. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

al., 1994), anti-viral (Corthout et al., 1992), anti-malarial (Caraballo et al., 2004) and anti-helminthic (Ademola et al., 2005) properties.

The total antioxidant activity shown by *S. mombin* extract and fractions (Figure 1) reflects free radical scavenging ability, reductive potential and the ability of the extracts to deactivate by chelation, the transition metals involved in initiation of free radical induced macromolecular damage. It is a reflection of the aggregate overall activity shown in multiple antioxidant assays (Figures 2 to 5).

The extract and fractions showed ability to inhibit heat-induced protein denaturation (Figure 6). When a protein is denatured, the secondary and tertiary structures are

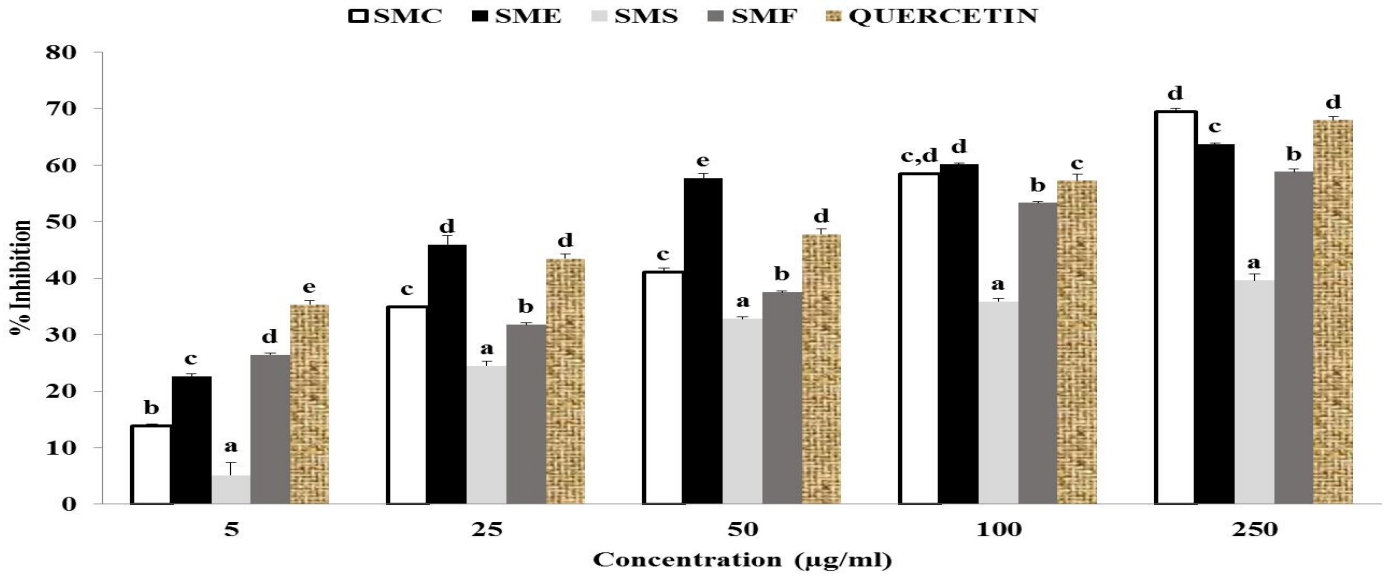

Figure 3c. Lipid peroxidation inhibitory effect of extract and fractions in rat testes. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

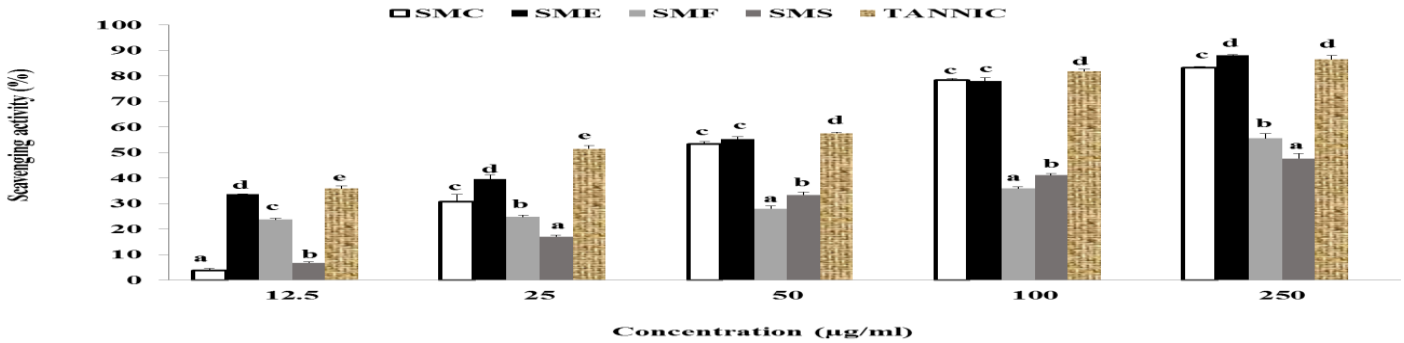

Figure 4. DPPH scavenging activity of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

altered but the peptide bonds of the primary structure between the amino acids are left intact. Since all the structural levels of the protein determine its function, the protein can no longer perform its function once it has been denatured. *S. mombin* extract and fractions may inhibit protein denaturation by preventing the disruption of hydrogen bonds and non-polar hydrophobic interactions responsible for the secondary structure and tertiary structure of proteins. They may also compete with water molecules in the interaction with amide nitrogen and carbonyl oxygen thus helping to stabilize the helical structure of the protein.

S. mombin extract and fractions also demonstrated the ability to inhibit the degradation of deoxyribose (Figure 2). Hydroxyl radicals, produced through iron-catalyzed decomposition of deoxyribose molecule can cause severe deleterious effect on biological macromolecules (Graf et al., 1984).

The extract and fractions inhibited lipid peroxidation in the brain, liver and testes of rats *in vitro* as shown in Figures 3a to c. The overproduction of ROS can result in a direct attack on the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Iron can stimulate lipid peroxidation by the Fenton reaction and can also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can perpetuate the chain reaction (Halliwell et al., 1991). Free radical induced formation of lipid peroxides is thought to play an important role in the etiology and pathogenesis of a number of oxidative stress related diseases. As a result of lipid peroxidation, a number of unstable intermediary metabolites (mainly aldehydes

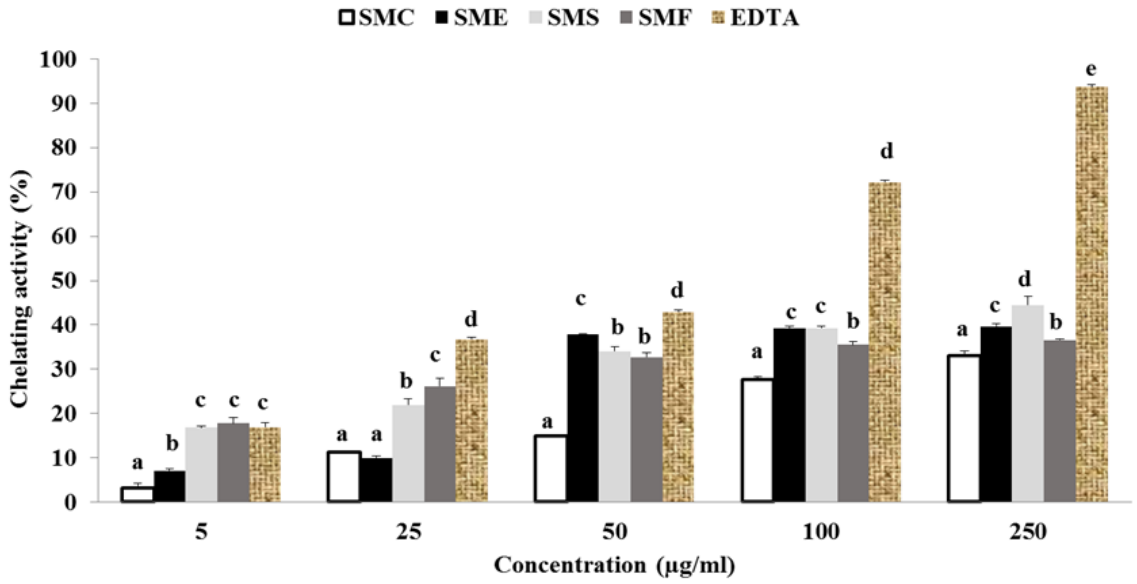

Figure 5. Chelating ability of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

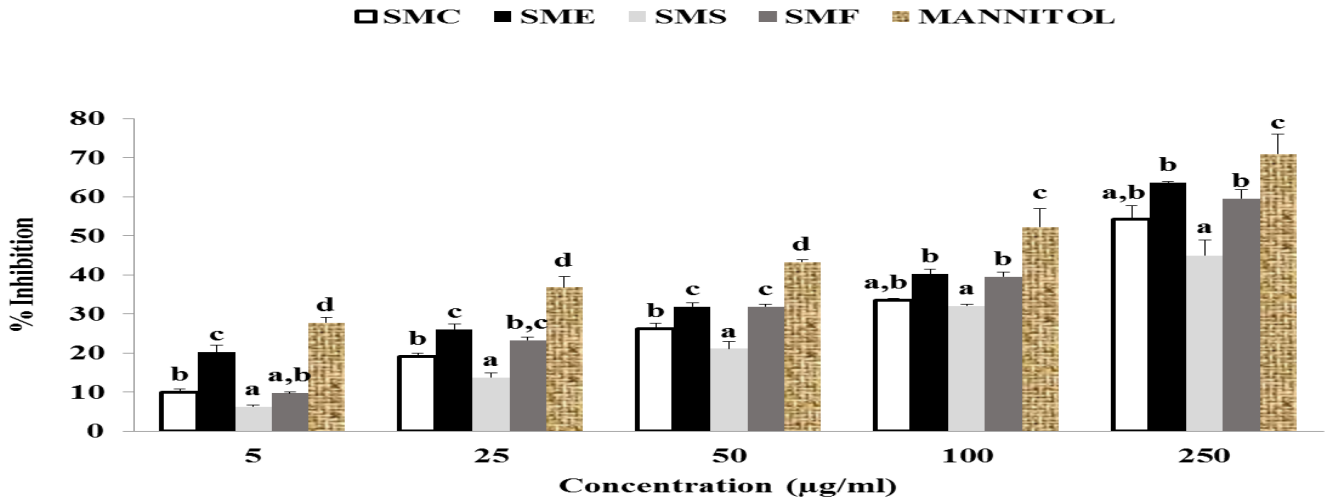

Figure 6. Protein denaturation inhibitory effect of extract and fractions. Values are given as mean ± SD (n = 3). Values with the same alphabet for each sample concentration are not significantly different.

such as MDA and 4-HNE) are produced from cellular molecules (Halliwell, 1993; Erba et al., 2005).

As observed in Table 2 and Figures 7a to c, the total antioxidant activity of *S. mombin* extract and fractions correlated with DEO, LPIA and PRO. This suggests that the antioxidant property is a major underlying factor of the protective properties shown by the plant.

In general, SME had the highest activity followed by SMF, SMC and SMS. The high activity shown by SME may be due to the presence of high amounts of tannins. Tannins are metal ion chelators (Karamać, 2009), and have shown potential antiviral (Lü et al., 2004), antibacterial (Akiyama et al., 2001), and antiparasitic effects (Kolodziej and Kiderlen, 2005). Souza et al. (2006) reported that tannins have anti-inflammatory and antiulcer activity in rodents. The activity exhibited by SMF was close to that of SME and this may be as a result of the presence of flavonoids and phenolic compounds.

Free radicals are constantly generated in the living system and they can cause extensive damage to tissues and biomolecules leading to various diseases, especially degenerative diseases. Agents with antioxidant properties have been found to be useful in treating these disorders. Therefore, a huge body of scientific research is

Table 2. Relationship between total antioxidant activity and inhibitory potentials of extract and fractions.

Test	Sample			
	SMC	SME	SMS	SMF
PRO	0.972**	0.971**	0.969**	0.976**
DEO	0.734	0.944**	0.881*	0.887*
LPB	.908*	0.939**	0.952**	0.971**
LPL	0.853*	0.971**	0.967**	0.904*
LPT	0.833*	0.839*	0.785	0.940**

*Correlation is significant ($P < 0.05$); **Correlation is significant ($P < 0.01$). SMC, Crude extract; SME, Ether fraction; SMS, Saponin-rich fraction; SMF, Flavonoid-rich fraction; PRO, protein degradation inhibitory activity; HRS, hydroxyl radical scavenging activity; LPB, lipid peroxidation inhibitory activity in rat brain; LPL, lipid peroxidation inhibitory activity in rat liver; LPT, lipid peroxidation inhibitory activity in rat testes.

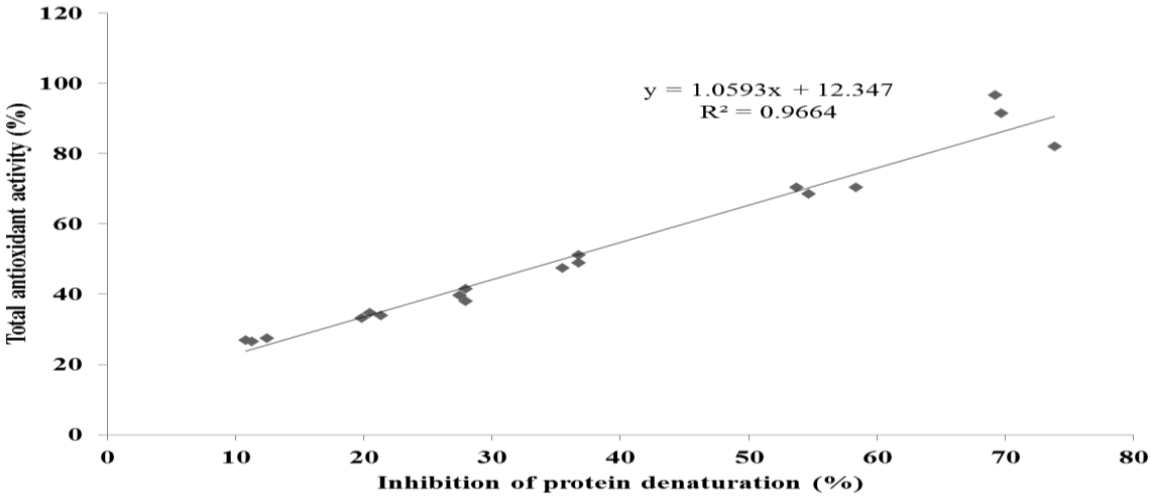

Figure 7a. Relationship between total antioxidant activity and protein denaturation inhibitory potential. Values are given as mean ± SD (n = 18).

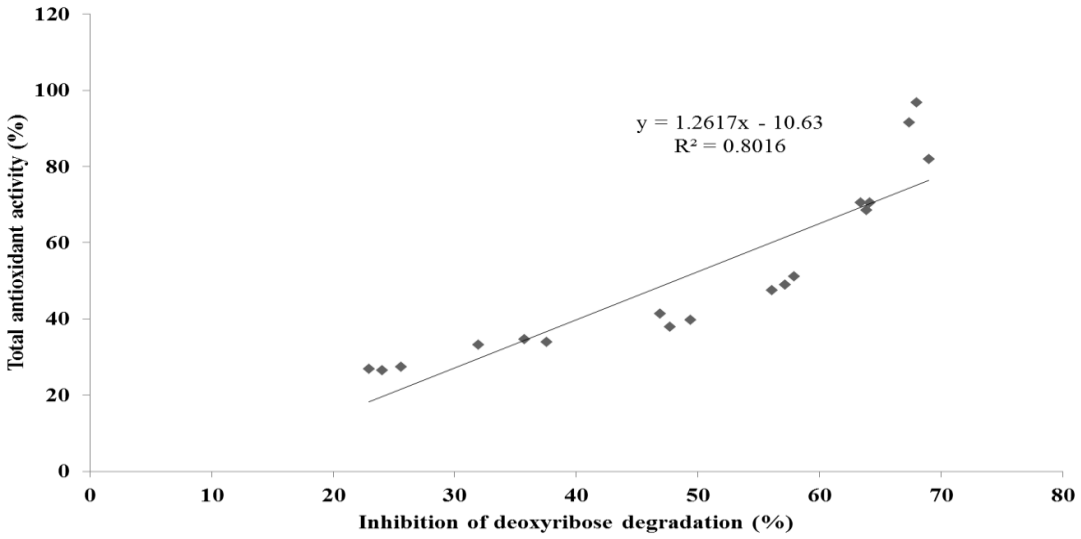

Figure 7b. Relationship between total antioxidant activity and Hydroxyl radical scavenging activity of extract and fractions. Values are given as mean ± SD (n= 18).

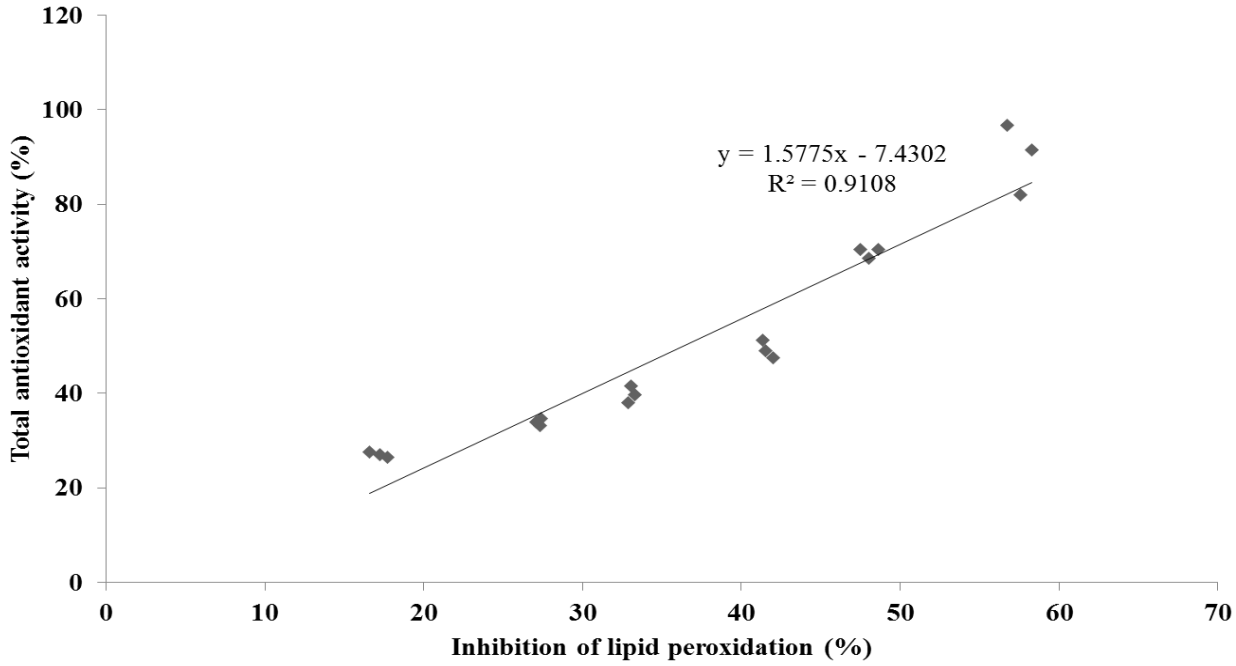

Figure 7c. Relationship between total antioxidant activity and lipid peroxidation inhibitory potential. Values are given as mean ± SD (n= 18).

focused on exploring for safe and effective antioxidants and to encourage the consumption of natural antioxidants from food supplements and traditional medicines (Souza et al., 2006; Yazdanparast and Ardestani, 2007) and, many natural antioxidants have been isolated from different parts of plants (Yazdanparast et al., 2008). The results obtained in this study indicate that extract and fractions from *S. mombin* are significant source of antioxidants, which might be helpful in combating oxidative stress and assaults on biomolecules from various sources.

Conflict of Interest

The authors declare that there are no conflicts of interests.

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