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EVALUATION OF ANTIPEROXIDATIVE AND ANTIOXIDANT PROPERTIES OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *PERSEA AMERICANA* MILL. IN RATS FED HIGH LIPID DIET

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ABSTRACT

Hyperlipidemia is an important factor in the pathogenesis of chronic degenerative and inflammatory diseases such as atherosclerosis, diabetes and cancer. *Persea americana* Mill. (Lauraceae) is a plant that has been widely used in folk medicine in the treatment of various ailments including hypertension. This study investigated antiperoxidative and oxidative potentials of aqueous extract of *P. americana* (AEPA) and methanolic extract of *P. americana* (MEPA) respectively in rats. Hyperlipidemia was induced by feeding four-week-old male albino rats with high lipid diet containing cholesterol and cholic acid. Hyperlipidemic rats were administered either AEPA or MEPA orally (10 mg kg^{-1} body weight) for 8 weeks. Control rats received standard chow and water only. The antiperoxidative potential of the extracts was evaluated by measuring the levels of malondialdehyde(MDA), conjugated dienes (CD) and protein carbonyls while the antioxidant effect was assessed by determining the levels of antioxidant enzymes and glutathione (GSH). Administration of AEPA and MEPA lowered oxidative stress as shown by a decrease in protein carbonyl and significant ($p<0.05$) decline in plasma MDA. The extracts elicited an increase in the activities of catalase (CAT) and superoxide dismutase (SOD), and a significant ($p<0.05$) increase in GSH compared to the hyperlipidemic control rats. These findings indicate that the leaf extracts of *P. americana* possess antiperoxidative and antioxidant effects which may be attributed to individual or combined action of the phytoconstituents. This may account for its use in traditional medicine and could be further exploited in the management of diseases associated with hyperlipidemia.

Keywords: *P. americana*, leaf extracts, hyperlipidemia, antioxidant effect, albino rats.

INTRODUCTION

Biological membranes are characterized by the presence of large amounts of polyunsaturated fatty acids (PUFAs) which can undergo oxidation in biological systems by a process known as lipid peroxidation (Porter *et al*., 1995). Peroxidation of membrane lipids may cause impairment of membrane function, decreased fluidity and inactivation of membrane-bound receptors and enzymes (Gutteridge and Halliwell, 1990; Gutteridge, 1995).

Free radical induced oxidative damage has been implicated in the development of various conditions such as diabetes, cancer, cataract, rheumatoid arthritis and atherosclerosis (Parthasarathy *et al*., 1992; Cheeseman and Slater, 1993; Cerutti, 1994). The mechanisms for defense against free radicals include radical scavengers and chain terminators such as vitamins C and E, and antioxidants such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). The various defenses are complementary to one another as they act on different oxidants or in different cellular compartments (Langseth, 1995).

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Persea americana (avocado) is an important edible fruit belonging to the Laurel family, Lauraceae. It is an almost evergreen tree, being shed briefly in dry seasons and grows up to 9 - 20 m in height (Morton, 1987).

The aqueous leaf extracts of *P. americana* have been shown to possess anti-inflammatory activity (Guevarra *et al*.,1998; Adeyemi *et al*., 2002) and antihypertensive/ hypotensive activity (De A Ribeiro *et al*., 1986; Adeboye *et al*., 1999). Also, the aqueous leaf extract of *P. americana* has been reported to possess hypoglycemic/ hypocholesterolemic effects (Antia *et al*., 2005; Brai *et al*., 2007). There are no documented reports on the effects of *P. americana* on lipid peroxidation and antioxidant status. This study investigates the effects of *P. americana* on indices of oxidative stress and antioxidant status in diet-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Preparation of Extracts

Fresh leaves of *P. americana* were collected from a cultivated plant in Lagos. The leaves were authenticated at the Department of Botany, University of Lagos and a voucher specimen (LUH 4199) deposited in the herbarium of the department. The leaves were air-dried

and pulverized. The aqueous and methanolic extracts were prepared by Sohxlet extraction. The extracts were evaporated to dryness in an oven at 40^{o}C and stored in clean sterile vials until used.

Animal Feeding
24 male albino rats were randomly divided into four feeding groups (A-D) of six rats each and hyperlipidemia was induced by feeding the rats with high lipid diet containing 20% groundnut oil, 0.5% cholesterol and 0.25% cholic acid as previously described (Brai *et al.*, 2007). The experimental groups were:

Group A: Normal control
Group B: Hyperlipidemic control (high lipid diet only)
Group C: High lipid diet + 10 mg kg^{-1} b.wt AEPA
Group D: High lipid diet + 10 mg kg^{-1} b.wt MEPA

At the end of the 8 weeks feeding period, blood was withdrawn via cardiac puncture when animals were rendered unconscious under pentobarbital anesthesia (100 mg kg^{-1} b.wt). Blood was collected in heparinized tubes followed by centrifugation at 3,000 rpm for 5 minutes at 4^{o}C to separate the plasma. The plasma was stored in clean tubes at -20^{o}C until analyzed. 0.5 ml aliquot of the whole blood was also collected in heparinized tubes for GPx assay. After sacrificing the rats, the organs were quickly excised and perfused with chilled 1.15 % (w/v) KCl solution, blotted dry, weighed and stored at -80^{o}C. Some portions of the liver were preserved in 10% Formol saline for histopathological analysis.

Biochemical Assays
Standard methods were used to estimate malondialdehyde and conjugated dienes (Buege and Aust, 1978), protein carbonyl (Levine *et al.*, 1990), glutathione (Sedlak and Lindsay, 1968), total protein (Bradford, 1976), catalase (Aebi, 1984), glutathione peroxidase (Paglia and Valentine, 1967) and superoxide dismutase (Misra and Fridovich, 1972).

Histopathological Assessment
Livers of rats from different groups were perfused with 10% neutral formalin solution, dehydrated and embedded in paraffin. Paraffin sections were made and stained using haematoxylin-eosin (H&E). The stained sections were examined under a microscope for histopathological changes in liver architecture, and their photomicrographs were taken.

Statistical analysis
The results were recorded as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Student's t-test. A value of $p<0.05$ was considered significant.

RESULTS

The effects of AEPA and MEPA on plasma MDA level in the rats are as shown in figure 1. Plasma MDA concentration was elevated ($p<0.05$) in the hyperlipidemic control rats compared with the normal control rats. Treatment with AEPA and MEPA reduced plasma MDA concentration ($p<0.05$) suggesting that administration of the extracts inhibited lipid peroxidation in the plasma.

As shown in table 1, MDA concentration was increased ($p<0.05$) in the liver and lungs of rats fed high lipid diet compared to the normal control. Brain MDA concentration was not statistically different ($p>0.05$) in rats treated with the extracts compared with the hyperlipidemic control rats. Also, there were no significant differences ($p>0.05$) in heart and kidney MDA concentrations among the four experimental groups.

The concentrations of conjugated dienes (CD) in the various tissues are as shown in figure 2. CD concentrations were generally higher in rats fed high lipid diet. CD concentration was reduced in the liver and heart of rats treated with AEPA and MEPA. However, there was a significant decline ($p<0.05$) of liver CD level in rats treated with AEPA.

Protein carbonyls were increased ($p < 0.05$) in hyperlipidemic rats compared to the normal control (Fig. 3). Treatment with AEPA helped to lower the concentration of protein carbonyl in the plasma, liver, heart and kidney of the rats. However, rats treated with MEPA showed reduction in protein carbonyl concentration only in the plasma and liver.

Plasma glutathione (GSH) levels in the four groups are shown in figure 4. There was significant ($p<0.05$) depletion in the plasma GSH level in the hyperlipidemic control rats compared with normal control rats. However, treatment with AEPA and MEPA elicited a restoration ($p<0.05$) of GSH level.

As can be seen from table 2, GSH concentration was lower in the liver, kidney, lungs and heart of hyperlipidemic rats. Also, liver and kidney GSH levels were significantly ($p<0.05$) lower in rats treated with AEPA while the brain GSH level was higher ($p<0.05$) compared to normal control rats.

Catalase (CAT) activities are shown in table 3. Although plasma CAT activity was highest in the hyperlipidemic control, there was no significant difference ($p>0.05$) across the groups. Liver CAT activity was significantly lower ($p<0.05$) in rats treated with AEPA and there were no significant differences ($p>0.05$) in kidney, heart and brain CAT activity among the groups. Lungs CAT activity showed a significant ($p<0.05$) decrease in the

Table 1. Effect of aqueous and methanolic leaf extracts of *P. americana* on tissue malondialdehyde concentration (μM/mg protein) in rats fed high lipid diet.

	Group			
	A	B	C	D
Liver	0.22 ± 0.02	0.32 ± 0.08[b]	0.41 ± 0.06[b]	0.50 ± 0.04[b]
Kidney	0.07 ± 0.01	0.07± 0.01	0.08 ± 0.01	0.07 ± 0.01
Lung	0.05 ± 0.01	0.07 ± 0.04[b]	0.08 ± 0.02[a]	0.09 ± 0.02[a]
Heart	0.29 ± 0.11	0.30 ± 0.04	0.24 ± 0.10	0.32 ± 0.07
Brain	0.40 ± 0.02	0.41 ± 0.03[a]	0.37 ± 0.01[a]	0.32 ± 0.02[b]

Table 2. Effect of aqueous and methanolic leaf extracts of *P. americana* on reduced glutathione levels (μM/mg protein) in rats fed high lipid diet.

	Group			
	A	B	C	D
Liver	27.28 ± 2.44	23.37± 2.83[a]	16.04 ± 1.63[b]	24.98 ± 3.14[a]
Kidney	24.40 ± 2.04	21.48 ± 1.37	17.43 ± 1.62[a]	20.94 ± 1.11
Lung	29.58 ± 3.42	17.85 ± 2.02[b]	19.31 ± 1.68[b]	24.87± 2.44
Heart	27.12 ± 1.89	13.07 ± 1.18[a]	19.60 ± 0.36	18.85 ± 0.60
Brain	1.39 ± 0.10	1.96 ± 0.45	1.54 ± 0.26	0.89 ± 0.21[b]

Table 3. Effect of aqueous and methanolic leaf extracts of *P. americana* on plasma and tissue catalase concentrations (μM /mg protein) in rats fed high lipid diet.

	Group			
	A	B	C	D
Plasma	0.90 ± 0.25	0.71 ± 0.19	0.76 ± 0.17	0.79 ± 0.38
Liver	1.82 ± 0.26	1.34 ± 0.41[a]	0.85 ± 0.24[a]	4.78 ± 1.04[b]
Kidney	1.18 ± 0.24	0.87 ± 0.34	0.65 ± 0.13	0.97 ± 0.20
Lung	3.89 ± 0.88	1.19 ± 0.19[b]	2.43 ± 0.63	1.57 ± 0.38
Heart	1.77 ± 0.28	1.50 ± 0.46	1.86 ± 0.79	1.26 ± 0.40
Brain	1.26 ± 0.53	0.87 ± 0.19	0.94 ± 0.25	1.56 ± 0.33

Table 4. Effect of aqueous and methanolic leaf extracts of *P. americana* on plasma and tissue superoxide dismutase concentrations in rats fed high lipid diet.

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	Group			
	A	B	C	D
Plasma	3.29 ± 0.64	2.30 ± 0.49	2.69 ± 0.34	2.41 ± 0.12
Liver	4.80 ± 0.66	5.48 ± 0.55	4.62 ± 0.84	6.51 ± 1.32
Kidney	4.43 ± 0.38	4.29 ± 0.59	3.65 ± 0.47	3.44 ± 0.63
Lung	4.43 ± 0.89	3.05 ± 0.40	4.12 ± 0.89	4.16 ± 0.59
Heart	6.49 ± 0.98	3.88 ± 0.54[a]	4.50 ± 0.14	4.13 ± 0.22
Brain	2.29 ± 0.40	2.12 ± 0.36	2.58 ± 0.37	2.30 ± 0.31

Values are expressed as means ± SEM for six rats. [a]Significantly different from normal control ($p< 0.05$).
A, fed standard chow; B, fed high lipid diet; C, fed high lipid diet + 10 mg kg^{-1} b.wt AEPA; D, fed high lipid diet + 10 mg kg^{-1} b.wt MEPA daily.

hyperlipidemic control rats and in rats treated with MEPA compared to normal control.

Plasma and liver GPx activities are shown in figures 5 and 6 respectively. There was a decrease in plasma GPx activity in rats fed high lipid diet compared to normal control rats. Plasma GPx activity was significantly ($p<0.05$) lower in rats treated with AEPA compared to the hyperlipidemic rats. Similarly, GPx activity declined ($p<0.05$) in the liver of rats fed high lipid diets compared to normal control. Table 4 shows plasma and tissue SOD activity. There was no significant ($p>0.05$) difference in

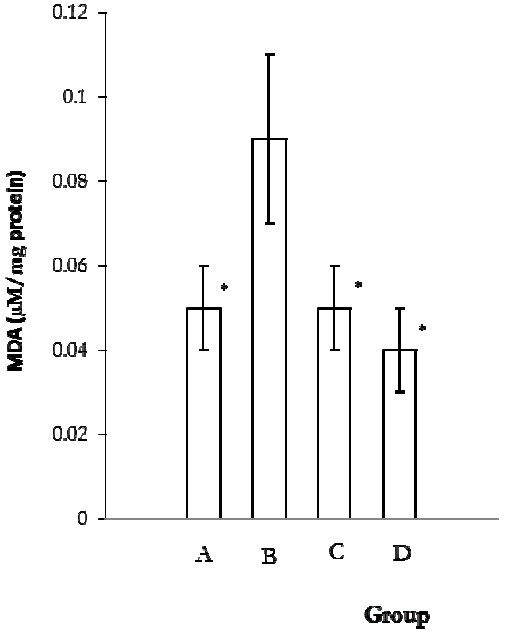

Fig. 1. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on plasma malondialdehyde in rats fed high lipid diet.

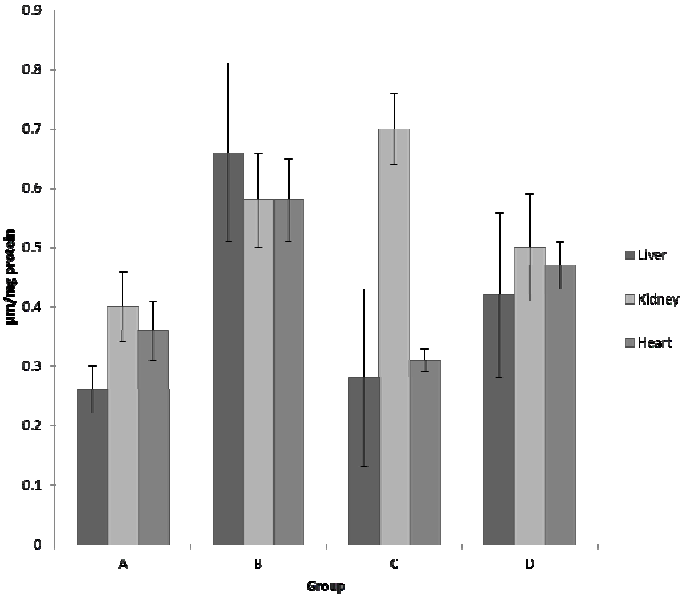

Fig. 2. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on conjugated dienes in rats fed high lipid diet.
Values are means ± SEM (n = 6)
A, standard rat chow; **B**, high lipid diet; **C**, high lipid diet + 10 mg kg^{-1} b.wt AEPA*;* **D**, high lipid diet + 10 mg kg^{-1} b. wt MEPA

the plasma SOD activity across the four experimental groups. However, plasma SOD activity was lowest in the hyperlipidemic control rats. Liver SOD activity was elevated in the hyperlipidemic control and MEPA treated rats in comparison with normal control while kidney SOD activity was lower in the treated rats.

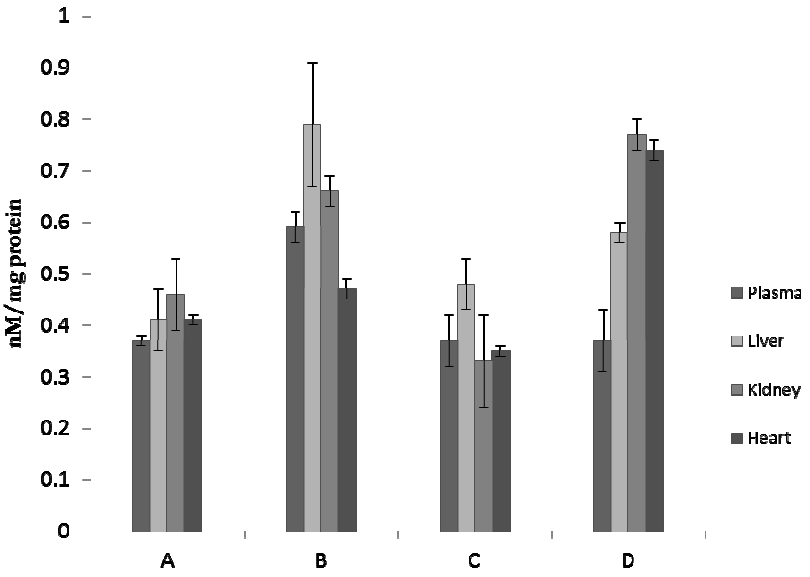

Fig. 3. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on protein carbonyls in rats fed high lipid diet.

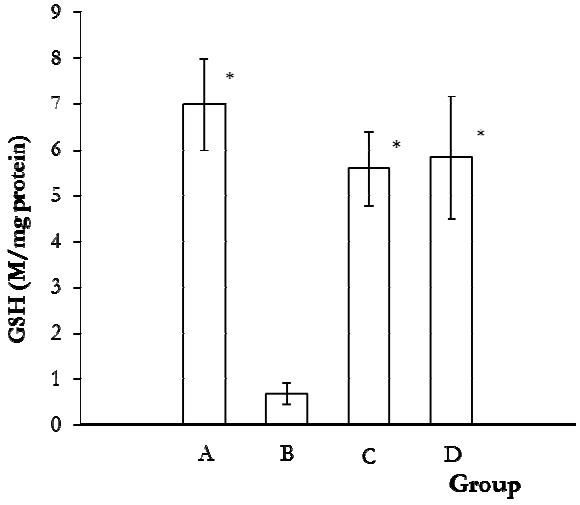

Fig. 4. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on plasma glutathione in rats fed high lipid diet.

Values are means ± SEM (n = 6).*Significantly different from hyperlipidemic control group

A, standard rat chow; **B**, high lipid diet; **C**, high lipid diet + 10 mg kg^{-1} b.wt AEPA*;* **D**, high lipid diet + 10 mg kg^{-1} b. wt MEPA

Also, heart SOD activity was reduced in rats fed high lipid diet compared with normal control rats, with the heart SOD activity in the hyperlipidemic control rats being lower than the treated rats and significantly lower ($p<0.05$) than the normal control. Although lungs SOD activity was slightly lower in the hyperlipidemic control

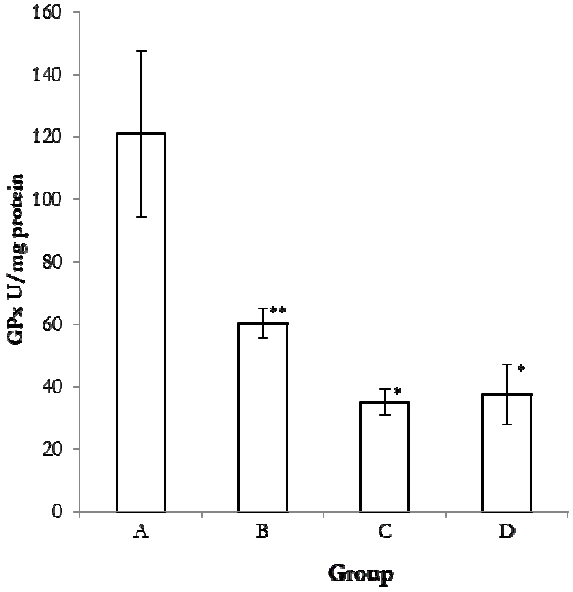

Fig. 5. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on plasma glutathione peroxidase in rats fed high lipid diet.

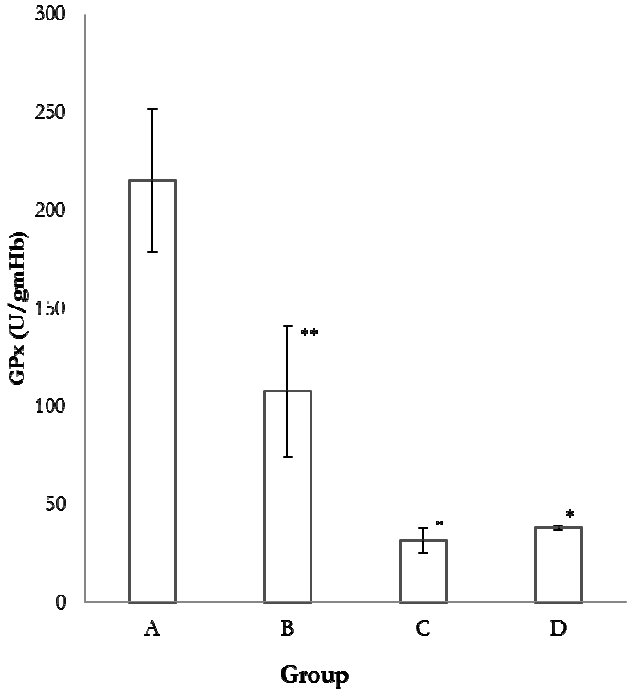

Fig. 6. Effect of aqueous (AEPA) and methanolic (MEPA) leaf extracts of *P. americana* on liver glutathione peroxidase in rats fed high lipid diet.

Values are means ± SEM (n = 6). ** Significantly different from treated rats

A, standard rat chow; **B**, high lipid diet; **C**, high lipid diet + 10 mg kg^{-1} b.wt AEPA*;* **D**, high lipid diet + 10 mg kg^{-1} b. wt MEPA

rats, there was no significant ($p<0.05$) difference in the brain and lungs SOD activity across the experimental groups.

The photomicrographs of the liver sections stained with H & E dye are as shown in figure 7. The liver of rats fed standard chow had preserved lobular architecture while

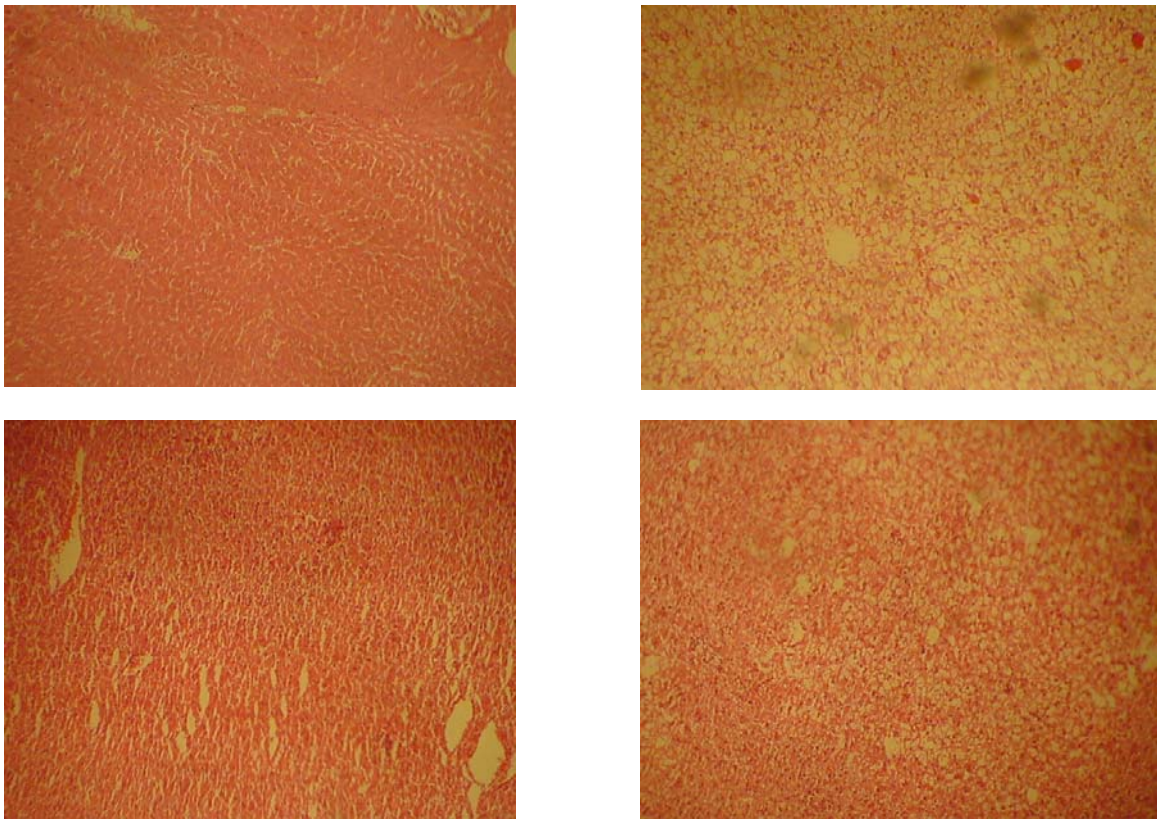

Fig. 7. Photomicrographs of liver sections of experimental groups (H&E x 100).(A) Normal liver architecture; (B) Hyperlipidemic rat with severe fatty change; (C) Hyperlipidemic rat + AEPA (10 mg kg^{-1} b. wt) showing mild fatty change; (D) Hyperlipidemic rat + MEPA (10 mg kg^{-1} b. wt) with moderate fatty change.

severe fatty changes were observed in the liver of hyperlipidemic rats. However, treatment with AEPA and MEPA ameliorated the severe fatty changes caused by the high lipid diet.

DISCUSSION

Increased incorporation of PUFA from vegetable oil dietary sources into plasma lipoprotein has been shown to increase both lipoprotein (Nadine *et al*., 1995) and tissue susceptibility to lipid peroxidation (De Schrijver *et al*., 1992; Skúladóttir *et al*., 1994). Lipid peroxidation products have been shown to rise with increased amount of fatty acids susceptible to peroxidation in the high fat diets (Ima-Nirwana *et al*., 1996). In this study, feeding rats a high lipid diet was found to induce pro-oxidant changes in markers of oxidative stress in the plasma. These changes were manifested as significant depletion of plasma concentration of GSH and non-significant increase in plasma MDA. This finding is similar to an earlier report which showed that feeding rats a high cholesterol diet containing currant oil induced pro-oxidant changes in markers of oxidative stress in the blood (Večeřa Škottová *et al*., 2003). The observed higher levels of MDA, CD and protein carbonyls in the plasma and tissues of hyperlipidemic rats agree with an earlier observation that hypercholesterolemia is associated with increased oxidant stress (Prasad and Kalra, 1993).

Oxidative modification alters the function of proteins and is thought to play an important role in the decline of cellular functions during ageing (Leeuwenburgh *et al*., 1998). Because proteins have many different and unique biological functions, oxidative modifications to proteins can lead to diverse functional consequences such as inhibition of enzyme activities and loss of protein function (Stadtman, 1990). The increase in protein carbonyls content in the hyperlipidemic rats is indicative of oxidative damage as well as chemical modification of proteins in these tissues.

The administration of AEPA and MEPA helped to lower oxidative stress in the treated rats as shown in the decline of indices of oxidative stress in the treated rats. The leaves of *P. americana* are known to be rich in flavonoids (King and Knight, 1992; Arora *et al*., 2000), which are antioxidants and free radical scavengers. They have the ability to alter peroxidation kinetics by modifying the lipid packing order and decreasing fluidity of the membrane (Cooper and Kristal, 2000). These changes

could sterically hinder diffusion of free radicals and restrict peroxidative reactions. Hence it may be possible that flavonoids are responsible for the antioxidant effect of AEPA and MEPA. These findings suggest that the leaf extract of *P. americana* would inhibit oxidation of lipoproteins during oxidative stress.

Glutathione levels are maintained by the activities of glutathione reductase and GSH synthases. GSH level in the blood is a sensitive indicator of antioxidant status in circulation and plays a pivotal defensive role against oxidative insults as an endogenous scavenger of free radicals (Spolarics and Meyenhofer, 2000; Piemonte *et al*., 2001). The 10-fold decline in plasma GSH concentrations in the hyperlipidemic rats was restored to almost normal level by treatment with AEPA and MEPA. This suggests that AEPA and MEPA could improve antioxidant status in circulation by causing an increase in the concentration of plasma GSH thus protecting against oxidative damage. However, there was no significant decrease in hepatic GSH concentration in the untreated hyperlipidemic rats. Maintenance of liver GSH under conditions of increased lipoperoxidation has been suggested as a supportive and a compensatory mechanism (Piemonte *et al*., 2001; Valencia *et al*., 2001) reflecting higher capacity of liver to maintain GSH concentration compared to erythrocytes (Soltys *et al*., 2001). The results of this study indicate lower concentration of liver GSH after high lipid feeding. This is similar to an earlier report that liver GSH concentration was decreased after feeding rats with a high cholesterol diet containing lard fat (Večeřa Škottová *et al*., 2003). A decrease in liver GSH is often related to hepatic fatty infiltration in different experimental models (Raza *et al*., 2000; Vendemiale *et al*., 2001). The pathophysiological consequences of GSH depletion have been extensively studied. GSH, GPx, and GST depletion promote generation of reactive oxygen species and oxidative stress with the subsequent cascade of effects affecting the functional and structural integrity of cell and organelle membranes Murray *et al*., 1993). Treatment with AEPA and MEPA caused repletion of GSH in the hyperlipidemic rats thus increasing the antioxidant status in circulation to fight against oxidative insults.

SOD functions as an antioxidant to convert $O_2^{\cdot -}$ to the less toxic H_2O_2 and therefore has a protective role against the possible deleterious effects of $O_2^{\cdot -}$ (Harris, 1992). This could account for the higher levels of plasma SOD activity in the hyperlipidemic rats. The elevation of SOD and CAT activities in the hyperlipidemic rats is probably a response to increased production of lipid peroxides. Enzymes are known to fit into a genetic scheme of regulation in that their concentrations in the cell are rapidly elevated in response to transcriptional regulators that sense sudden changes in oxidant levels (Kakkar *et al*., 1997). The increase in CAT activity, a specific H_2O_2 scavenger may be due to an increase in H_2O_2 formation in the tissues. The increase in CAT activity indicates enhancement of the antioxidant enzyme.

The decline in GPx activity in rats treated with AEPA and MEPA could be attributed to the involvement of GPx in free radical scavenging in the rats.

The opposing responses of CAT and GPx, both of which breakdown H_2O_2, are in agreement with earlier reports (Kakkar *et al*., 1997; Bhor *et al*., 2004). It has been suggested that there is the existence of compensatory mechanisms in response to increased oxidative stress such that tissues lacking one of the enzymes may be critically dependent upon another (Bhor *et al*., 2004).

CONCLUSION

The study has shown that the administration of extracts of *P. americana* helped to reduce oxidative damage in hyperlipidemic rats as revealed by the decline of indices of oxidative stress. Also, *P. americana* leaf extracts improved the antioxidant status in circulation in the rat by causing an increase in the concentration of plasma GSH, an endogenous antioxidant that plays a pivotal role in the defense against oxidative insults.

However, these results show that the AEPA appears more beneficial and could further be exploited as a potential botanical in the management of diseases associated with hyperlipidemia.

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