

ORIGINAL ARTICLE

Modulation of antioxidant enzymes activities and lipid peroxidation products in diet-induced hypercholesterolemic rats fed ortanique peel polymethoxylated flavones extract

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Summary

The primary aim of this study was to investigate the effects of ortanique peel polymethoxylated flavones extract (PMF^{ort}) on antioxidant enzymes activities and lipid peroxide levels in organs of hypercholesterolemic and normal rats. Thirty Sprague-Dawley rats were fed high cholesterol diets supplemented with 1.5% PMF^{ort} and niacin respectively for 49 days. Hypercholesterolemic rats fed PMF^{ort} had significant reductions in malondialdehyde levels in the liver and brain compared to untreated hypercholesterolemic control rats. This reduction also occurred in the brain of the rats fed niacin. The activities of catalase, glutathione reductase, transferase and peroxidase were significantly reduced in the spleen, brain and liver of hypercholesterolemic rats fed PMF^{ort} compared to control. The activities of these enzymes were only reduced in the brain and liver of rats fed niacin. The results would suggest that PMF^{ort} modulates hypercholesterolemia-associated organ injury and oxidative stress in rat organs. PMF^{ort} could therefore be a suitable candidate of natural origin for prophylactic and therapeutic treatment of hypercholesterolemia-associated oxidative stress and organ injury.

Key words: hypercholesterolemia; ortanique; antioxidant enzymes; polymethoxylated flavones

INTRODUCTION

We have demonstrated in previous publications that Ortanique peel PMF extract (PMF^{ort}) is a very potent hypolipidemic supplement that significantly reduced serum total, LDL, VLDL and triglycerides levels and increased HDL cholesterol levels (Green et al. 2011a). We also derived the mechanism of action

which included reductions in the hepatic activity of the rate-limiting cholesterol synthesizing enzyme 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA HMG-CoA) reductase as well as absorption of cholesterol in the small intestines due to reductions in villi length and goblet cell proliferation. Our histopathological findings also demonstrated that PMF^{ort} ameliorated hypercholesterolemia-associated organ injury (Green et al. 2011b). Therefore another objective that warrants further exploration is to investigate the effects of PMF^{ort} on antioxidant enzymes activity and organ injury. This is important as several studies have shown that hypercholesterolemia usually results in oxidative stress and since flavonoids are potent antioxidants they could be explored as possible supplements for treating hypercholesterolemia-induced oxidative stress.

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Flavonoids are a ubiquitous family of phytochemicals, inclusive of polymethoxylated flavones, that exhibit a broad spectrum of pharmacological properties (Harborne 1993, Middleton et al. 2000, Manthey and Grohmann 2001, Green et al. 2007). They represent a highly diverse class of secondary plant metabolites with about 9000 known structures (Martens and Mithofer 2005). Flavonoids are common in fruits and vegetables and may function as natural antioxidants through their ability to suppress reactive oxygen species (ROS) formation and due to their lower redox potentials (Affany et al. 1987, Halliwell and Gutteridge 1999, Pietta 2000). This is an important property as antioxidants can act as free radical scavengers and may also chelate peroxidant metals, reducing their capacity to produce free radicals. ROS are produced as normal products of aerobic metabolism but can be elevated under certain pathophysiological conditions (Sies 1997, Lee et al. 1998). Reactive oxygen intermediates such as hydroxyl radicals (HO[•]), superoxide anions (O₂^{•-}) and hydrogen peroxide (H₂O₂) at low concentrations may be beneficial or even indispensable in processes such as intracellular messaging and defense against micro-organisms (Schulze-Osthoff et al. 1997, Sun et al. 1998, Vogt et al. 1998). On the other hand high doses and/ inadequate removal of ROS result in oxidative stress, which may cause severe metabolic malfunctions and damage to biological macromolecules (Lledias et al. 1998). A plethora of non-enzymatic and enzymatic antioxidant defenses exists, including superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT).

Pathological conditions that predispose to cardiovascular events, such as hypertension, hypercholesterolemia, and diabetes, are associated with oxidative stress (Mataix et al. 1998, Wassmann et al. 2004). There is considerable evidence that hypercholesterolemia is a major risk factor for progression of coronary atherosclerosis and is associated with an increase in the incidence of myocardial ischemia and cardiac events (Kannel et al. 1971, Smith et al. 1992). One mechanism that may underlie this degradation in coronary vascular function is an alteration in oxidative status. Increased production of several oxidants such as peroxynitrites, as well as peroxidation compounds and oxidative end-products such as PGF_{2α}-isoprostanes as well as a shift in oxidative status with a decrease in nitric oxide (NO) and an increase in oxygen radicals may have a deleterious effect on vascular permeability (Chait et al. 1993, Napoli et al. 1995, Davi et al. 1997). The primary aim of this study was to determine the effect of PMF^{ort} on lipid peroxide levels and the activities of

the endogenous antioxidant enzymes in the major organs of rats to determine whether or not they ameliorate hyperchole-sterolemia-associated organ injury and oxidative stress.

MATERIALS AND METHODS

Materials

Chemical reagents were purchased from Sigma (St. Louis, USA). PMFs extract containing tangeretin – TAN (29%), nobiletin – NOB (24%), tetramethylscutellarein – TMS (23%), sinensetin – SIN (10%), hexamethyl-o-quercetagenin – QUE (10%) and heptamethoxyflavone – HMF (4%) was isolated from *Ortanique* peel in reagent grade methanol using a method previously described (Green et al. 2007). The extract was collected and washed extensively with hexane to remove the volatile oil constituents and other hexane soluble contaminants. The hexane-washed residue was dissolved in chloroform, filtered, dried by rotary evaporation, and analyzed for PMF content.

Animals and diets

The animal protocol was approved by the University of the West Indies (Mona) Ethics Committee. Thirty adult Sprague-Dawley rats were obtained from the Animal House of the University of the West Indies, Mona Campus. They were housed in stainless steel cages that were maintained daily. The cages were placed in a room that was kept on a 12/12-hour light/dark cycle and were fed normal rat chow for one week. The rats were weighed (average body weight, 343 g), labeled and placed into five groups of six rats each, as follows: 1. Normal rats fed rat chow, 2. Normal rats fed 1.5% PMF^{ort}, 3. Hypercholesterolemic rats fed a high cholesterol diet (4% cholesterol and 1% cholic acid), 4. Hypercholesterolemic rats fed a high cholesterol diet supplemented with 1.5% PMFs extract and, 5. Hypercholesterolemic rats fed a high cholesterol diet supplemented with the cholesterol reducing drug niacin at a dose of 1.5%. Food and water were provided *ad libitum*. Body weights were monitored weekly, and food consumption was estimated daily for 49 days by giving rats a known amount of food and by re-weighing food each day and calculating the difference. At the end of the feeding period, rats were sacrificed and internal organs were excised, weighed and frozen in liquid nitrogen and stored at –80 °C until required for analysis. Antioxidant enzymes extraction was done according to a modified method of Ibrahim et al. (1997). Weighed frozen tissue

samples were partially thawed in ice-cold 1.55 mol/l KCl in 0.05 mol/l phosphate buffer, pH 7.4 using Teflon Potter-Elvehjem homogenizer. The homogenates were then centrifuged for thirty minutes at 4 °C at 14,000 rpm and supernatant stored -80 °C for determination of antioxidant enzyme activity (glutathione-S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase). The activity of glutathione-S-transferase was determined according to the method of Habig et al. (1974). Glutathione peroxidase activity was determined according to a modified coupled assay procedure of Paglia and Valentine (1967). Glutathione reductase activity was determined by the method of Carlberg and Mannervik (1985). The activity of catalase was measured by monitoring the decomposition of H₂O₂ as described by Stern (1937) and Beers and Siger (1952). The activity of superoxide dismutase (SOD) was measured by using a SOD assay kit obtained from Dojindo Molecular Technologies, Inc. (Rockville, USA) according to instructions within the technical manual, 2006. Lipid peroxidation was determined by the method of Okhawa et al. (1979).

Protein was determined according to the method of Bradford (1976).

Statistical analysis

The results were expressed as mean value \pm the standard error of the mean (SEM). Analysis of the variance (ANOVA) was used to test for the differences among all the groups at the significance level $2\alpha=0.05$. To find out where the significant difference occurred among the groups, the Duncan's multiple range test was used to compare the means. All statistical analyses were done using the statistical program SPSS version 16 (2007).

RESULTS

The effects of PMF^{ort} on lipid peroxidation in organs of hypercholesterolemic rat

Fig. 1 shows the lipid peroxide levels in the organs of rats fed different diets. Rats in the untreated hypercholesterolemic group had significant increases in liver lipid peroxide levels (3382 \pm 480 nmol/MDAg/g wet wt). This increase was however significantly reduced in the groups fed niacin and PMF^{ort}. The reduction in lipid peroxides level in the liver of hypercholesterolemic rats fed niacin (2000 \pm 202.7 nmol/MDAg/g wet wt) was significantly less than that exhibited by the hypercholesterolemic rats fed PMF^{ort} (741 \pm 128 nmol/MDAg/g wet wt). Hypercho-

lesterolemic rats fed PMF^{ort} (809 \pm 293 nmol/MDAg/g wet wt) and niacin (825 \pm 68.76 nmol/MDAg/g wet wt) had significant decreases in the lipid peroxide levels in the brain relative to the untreated hypercholesterolemic group (2873 \pm 367.2 nmol/MDAg/g wet wt). No significant changes were however observed in the lipid peroxide levels in the spleen, heart and kidneys of rats fed niacin or PMF^{ort}.

The effects of PMF^{ort} on catalase activity in organs of hypercholesterolemic rat

Table 1 shows the results of the activity of the antioxidant enzyme catalase in the organs of hypercholesterolemic and normal rats fed their respective diets. Hypercholesterolemic rats whose diets were supplemented with PMF^{ort} and niacin had significant reductions in the activity of catalase in the heart (3.09 \pm 0.09, 18.07 \pm 0.13 μ mol H₂O₂/min/mg protein), liver (1.01 \pm 0.04, 19.94 \pm 0.13 μ mol H₂O₂/min/mg protein), brain (1.13 \pm 0.11, 2.23 \pm 0.13 μ mol H₂O₂/min/mg protein) and kidney (8.48 \pm 0.31, 5.5 \pm 0.69 μ mol H₂O₂/min/mg protein) compared to the untreated hypercholesterolemic group.

The effects of PMF^{ort} on glutathione transferase activity in organs of hypercholesterolemic rat

Table 2 shows the results of the activity of the antioxidant enzyme glutathione transferase in the organs of hypercholesterolemic and normal rats fed their respective diets. Hypercholesterolemic rats whose diets were supplemented with PMF^{ort} had significant reductions in the activity of glutathione transferase in the spleen (0.07 \pm 0.002 nmol/min/mg protein), liver (2.05 \pm 0.12 nmol/min/mg protein), brain (0.26 \pm 0.01 nmol/min/mg protein) and kidney (0.014 \pm 0.02 nmol/min/mg protein) compared to the untreated hypercholesterolemic group. Significant reductions were observed in glutathione transferase activity in the brain (0.28 \pm 0.04 nmol/min/mg protein), liver (2.37 \pm 0.42 nmol/min/mg protein) and kidney (0.02 \pm 0.07 nmol/min/mg protein) but not the spleen (0.16 \pm 0.07 nmol/min/mg protein) of rats fed niacin compared to the untreated hypercholesterolemic rats. There were however, no significant reductions in glutathione transferase activity in the heart in any of the treatment groups.

The effects of PMF^{ort} on superoxide dismutase activity in organs of hypercholesterolemic rat

Fig. 2 shows the effect of the treatment diets on SOD activity in the organs. Hypercholesterolemic rats fed PMF^{ort} and niacin had significant reductions in the activity of SOD in the heart (36.7 \pm 0.33, 36.9 \pm 1% inhibition) and liver (35.01 \pm 1.89, 37.9 \pm 1.05% inhibition) compared to the untreated hyper-

cholesterolemic rats. No significant changes in the SOD activity were observed in the spleen, kidney and brain of the hypercholesterolemic rats whose diets

were supplemented with PMF^{ort} relative to the untreated hypercholesterolemic rats.

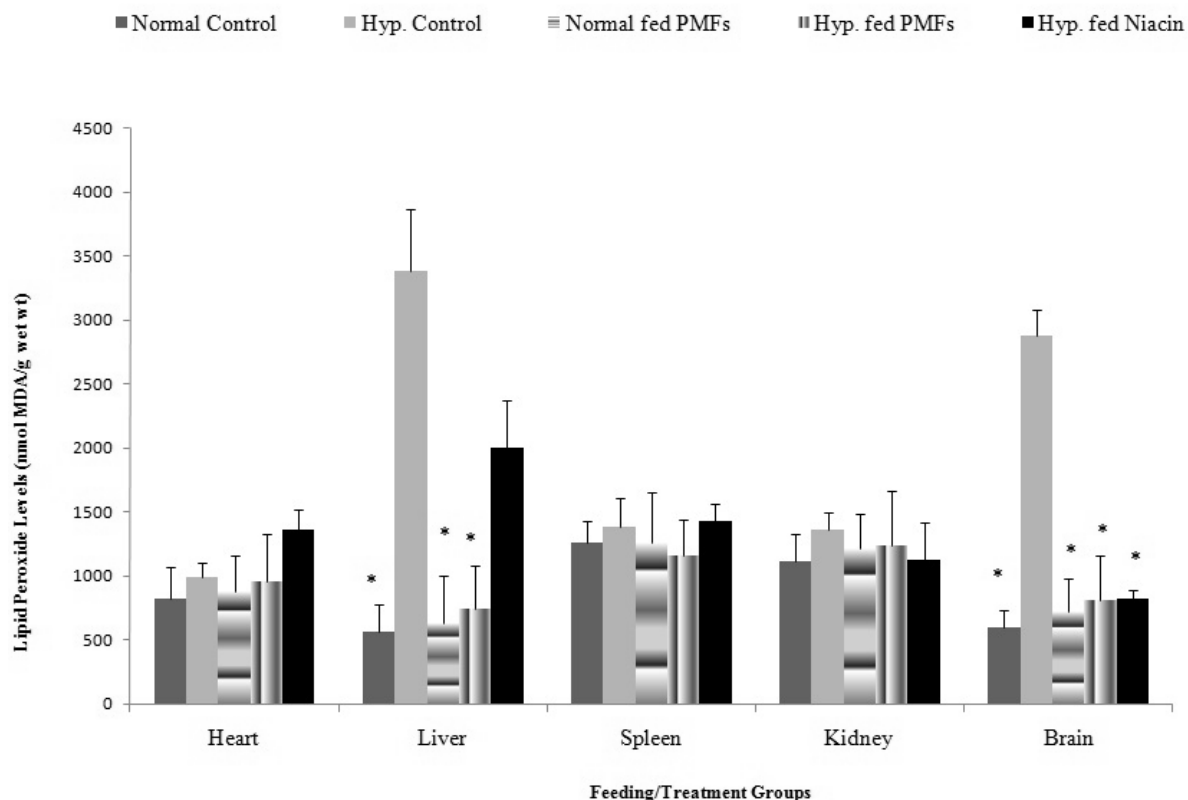


Fig. 1. Effects of ortanique peel PMFs extract on lipid peroxide levels in organ homogenates.

* statistically significant versus Hyp. fed H.C diet, H.C = high cholesterol, Hyp. = hypercholesterolemia

Table 1. Effect of PMF^{ort} on the activity of catalase in rat organs.

Feeding/treatment groups	Catalase activity ($\mu\text{mol H}_2\text{O}_2/\text{min/mg protein}$)				
	Spleen	Brain	Liver	Heart	Kidney
Normal fed normal diet	172 \pm 10.45*	2.95 \pm 0.14*	5.22 \pm 0.22*	10.50 \pm 0.56*	5.82 \pm 0.47*
Normal fed PMFs extract	158.21 \pm 9.62*	3.18 \pm 0.10*	2.37 \pm 0.36*	19.4 \pm 0.10*	4.38 \pm 0.21*
Hyp. fed H.C. diet	223.87 \pm 5.85	21.49 \pm 0.14	114.21 \pm 9.21	216.98 \pm 20.14	15.45 \pm 0.84
Hyp. fed PMFs extract	188.73 \pm 9.77*	1.13 \pm 0.11*	1.01 \pm 0.04*	3.09 \pm 0.09*	8.48 \pm 0.31*
Hyp. fed niacin	229.75 \pm 13.34	2.23 \pm 0.12*	19.94 \pm 0.13*	18.07 \pm 0.13*	5.51 \pm 0.69*

* statistically significant as compared with Hyp. fed H.C diet. H.C = high cholesterol, Hyp. = hypercholesterolemia

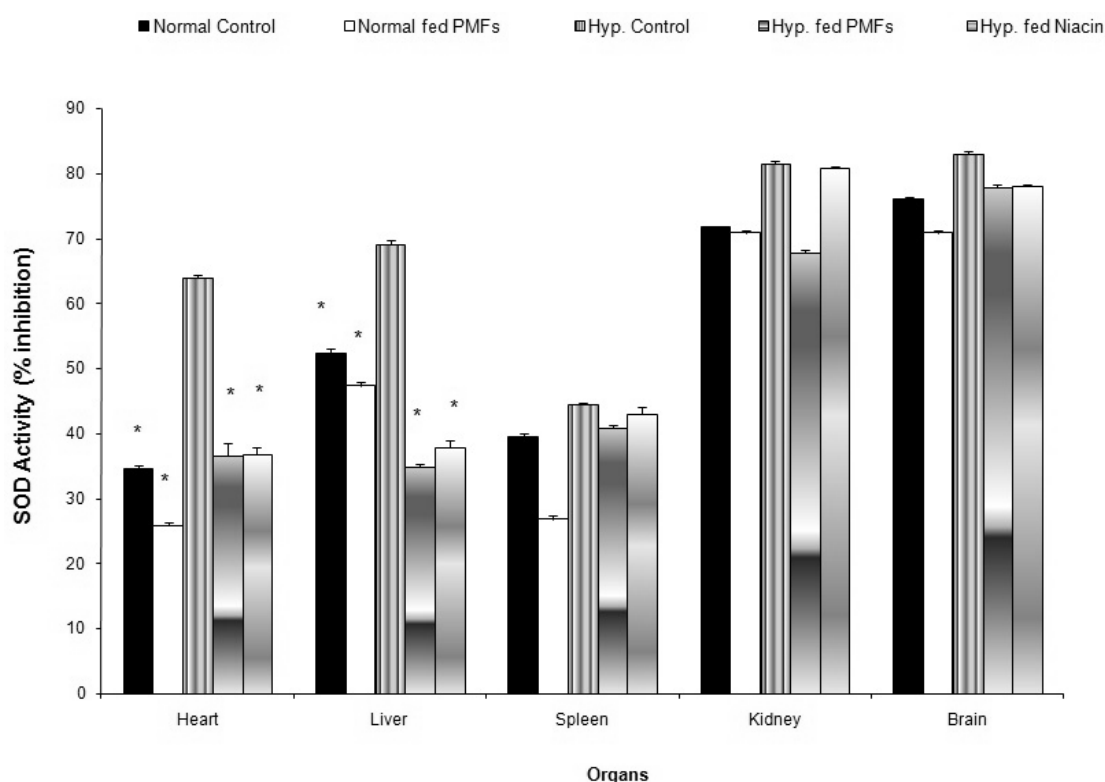


Fig. 2. Effect of PMF^{ort} on the activity of superoxide dismutase in organs of rats.

* symbols as in Fig. 1

Table 2. Effect of PMF^{ort} on the activity of glutathione transferase in rat organs

Glutathione transferase activity (nmol/min/mg protein)					
Feeding/treatment groups	Spleen	Brain	Liver	Heart	Kidney
Normal fed normal diet	0.08±0.002*	0.25±0.03*	1.46±0.55*	0.21±0.013	0.01±0.001*
Normal fed PMFs extract	0.09±0.02*	0.28±0.02*	1.5±0.41*	0.19±0.013	0.016±0.18*
Hyp. fed H.C. diet	0.12±0.02	0.44±0.05	3.54±0.68	0.22±0.038	0.053±0.03
Hyp. fed PMFs extract	0.07±0.002*	0.26±0.01*	2.05±0.17*	0.18±0.12	0.014±0.018*
Hyp. fed niacin	0.16±0.07	0.28±0.04*	2.37±0.42*	0.21±0.057	0.020±0.065*

* symbols as in Table 1

The effects of PMF^{ort} on glutathione reductase activity in organs of hypercholesterolemic rat

Fig. 3 shows the results of the activity of the antioxidant enzyme glutathione reductase in the organs of hypercholesterolemic and normal rats fed

their respective diets. Hypercholesterolemic rats whose diets were supplemented with PMF^{ort} had significant reductions in the activity of glutathione reductase in the liver (18.2 ± 1.15 nmol/min/mg protein), brain (11.45 ± 2.25 nmol/min/mg protein),

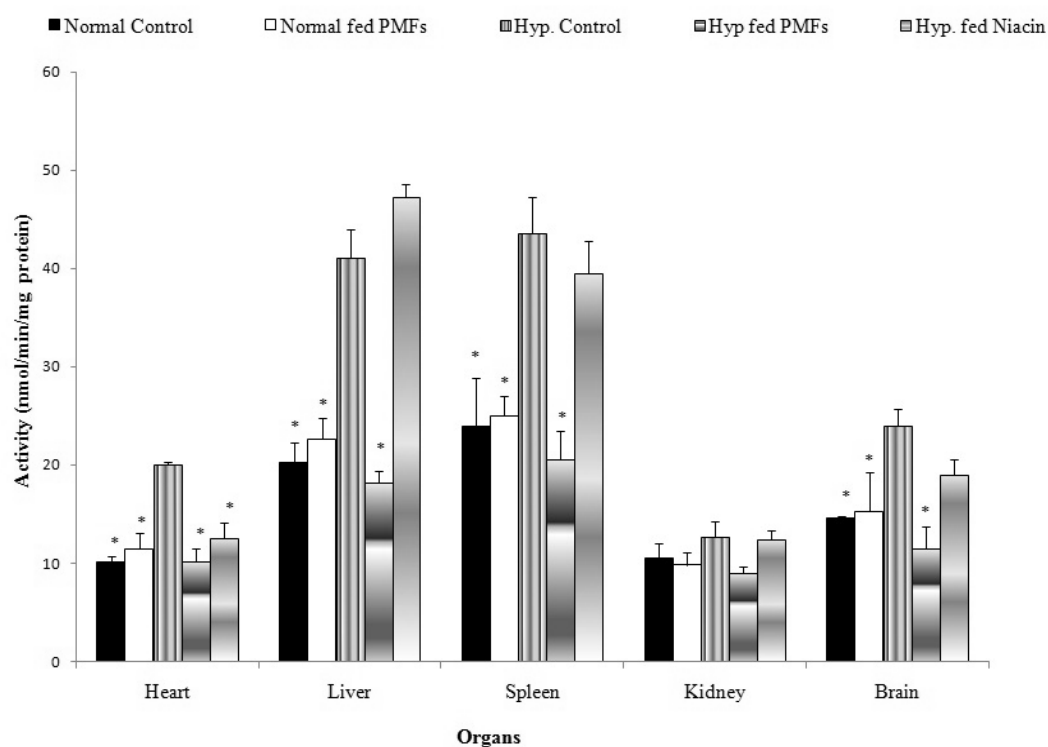


Fig. 3. Effect of PMF^{ort} on the activity of glutathione reductase in organs of rats.

* symbols as in Fig. 1

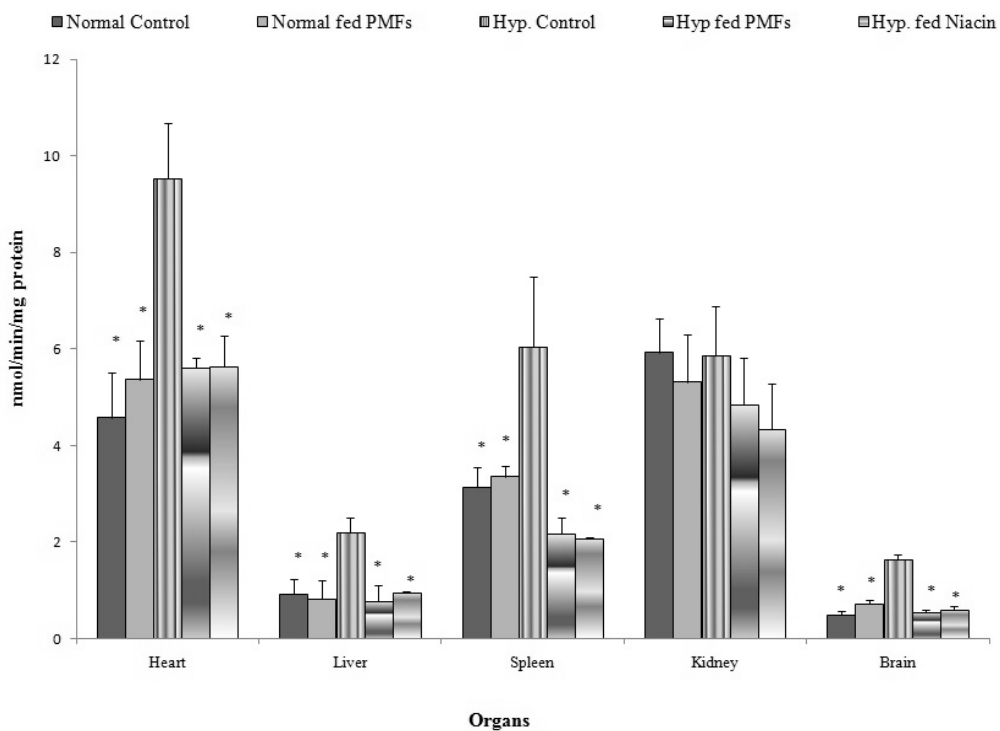


Fig. 4. Effect of PMF^{ort} on the activity of glutathione peroxidase in organs of rats.

* symbols as in Fig. 1

spleen (20.56 ± 2.89 nmol/min/mg protein) and heart (10.24 ± 1.59 nmol/min/mg protein) when compared with the untreated hypercholesterolemic group. There were however, no significant reductions in glutathione reductase activity in the liver (47.23 ± 1.33 nmol/min/mg protein), spleen (39.48 ± 1.66 nmol/min/mg protein) or brain (18.95 ± 3.33 nmol/min/mg protein) except in the heart as a result of niacin supplementation. Also, no significant increase was observed in the activity of glutathione transferase in the kidney in any of the treatment groups.

The effects of PMF^{ort} on glutathione peroxidase activity in organs of hypercholesterolemic rat

Fig. 4 shows the results of the activity of the antioxidant enzyme glutathione peroxidase in the organs of hypercholesterolemic and normal rats fed their respective diets. Hypercholesterolemic rats whose diets were supplemented with PMF^{ort} and niacin had significant reductions in the activity of glutathione peroxidase in the liver (0.78 ± 0.33 , 0.95 ± 0.02 nmol/min/mg protein), brain (0.54 ± 0.07 , 0.59 ± 0.09 nmol/min/mg protein), spleen (2.18 ± 0.33 , 2.07 ± 0.034 nmol/min/mg protein) and heart (5.61 ± 0.22 , 5.64 ± 0.63 nmol/min/mg protein) compared to the untreated hypercholesterolemic group. There were however no significant increase in the activity of glutathione transferase in the kidney in any of the treatment groups.

DISCUSSION

In hypercholesterolemia, one of the mechanisms that might be activated and might hinder coronary vascular function is a shift in scavenging activity and redox status, a state known as increased oxidative stress (Montilla et al. 2004). Numerous studies show that a close relationship exists between high blood cholesterol and atherosclerosis, it has also been suggested that this relationship may be dependent on enhanced oxidative stress (Domagala et al. 1989, Ohara et al. 1993, Prasad et al. 1997). Studies have also shown that cholesterol rich diet increases the formation of peroxynitrite, a toxic reaction product of superoxide and nitric oxide in the rat myocardium (Onody et al. 2003). Work done by Eddy et al. (1998) showed that rats that develop hypercholesterolemia consuming a high fat diet had elevations in renal lipid peroxidation products compared to rats that were fed normal rat chow.

Parmar and Kar (2007) reported that citrus peel extract reduced lipid peroxidation levels in the liver and kidneys of animal model of diet-induced atherosclerosis and thyroid dysfunctions. To our

knowledge, this study is the first report on the effect of PMF^{ort} on lipid peroxide levels in hypercholesterolemic rats. The results showed that supplementation of the diet of hypercholesterolemic rats with PMF^{ort} resulted in significant reductions in the lipid peroxide levels in the liver and brain compared to the untreated hypercholesterolemic group. Supplementation of the diet of hypercholesterolemic rats with niacin however significantly increased lipid peroxide levels in the liver relative to the hypercholesterolemic group fed PMF^{ort}. No significant changes were however observed in the lipid peroxide levels in the kidney, spleen and heart in any of the groups studied.

Studies have shown that antioxidant therapy attenuates elevations in lipid peroxidation products (Eddy et al. 1998). The PMFs extract may have reduced lipid peroxidation by acting as antioxidants and hence aiding the endogenous antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase and catalase) involved in the scavenging/inactivation of the reactive oxygen species or redox metal ions before lipid peroxidation takes place (Sung and Park 1999).

Several studies show that in nearly all regions of the world heart failure is both common and on the rise (McMurray and Stewart 2000, Mendez and Cowie 2001). Cardiovascular diseases such as hypertension, diabetes and hypercholesterolemia are risk factors for heart failure and hence studies aimed at reducing or preventing the occurrence of these disorders have increased in recent years (Sanderson and Tse 2003). Several studies show that feeding animals a high cholesterol diet results in intracellular lipid accumulation in cardiomyocyte as well as alterations in the structural and functional properties of the myocardium (Melax and Leeson 1975, Hexeberg et al. 1993, Onody et al. 2003). Work done by Prasad et al. (1997) showed that diet-induced hypercholesterolemia produces oxidative stress in the myocardium. Hypercholesterolemia also resulted in increases in the levels of lipid peroxidation product malondialdehyde (MDA) and chemiluminescence (M-CL), a measure of antioxidant reserve, and activities of antioxidant enzymes (CAT, and GSH-P_x) but a decrease in the activity of SOD in cardiac muscles of rabbits (Prasad et al. 1997).

Prior to this study the effect of PMFs on antioxidant enzymes activities in the heart of hypercholesterolemic rats was never assessed. The results in this study showed that supplementation of the diets of hypercholesterolemic rats with PMF^{ort} and niacin resulted in significant reductions in the activities of catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase in

the heart compared to the untreated hypercholesterolemic group. No significant differences were however observed in the activity of glutathione transferase in the heart in any of the groups studied.

Several studies have shown that feeding rats a high cholesterol diet results in hepatic steatosis and elevations in antioxidant enzymes activities in the liver (Mahfouz and Kummerow 2000, Perlemuter et al. 2005). Reports on the hepatic activity of antioxidant enzymes in hypercholesterolemic rats tend to indicate elevated activities as a result of citrus consumption. Kim et al. (2004) reported that superoxide dismutase, catalase, and glutathione reductase activities were all significantly increased by supplementation of the citrus flavonoid naringin in hypercholesterolemic mice compared to control. Deyhim et al. (2006) also reported elevations in antioxidant enzymes activities in orchidectomized rats as a result of citrus juice consumption. In this study, however, supplementation of the diets of hypercholesterolemic rats with PMF^{ort} resulted in significant reductions in the hepatic activities of catalase, glutathione peroxidase, glutathione transferase and glutathione reductase compared to the untreated hypercholesterolemic group. Supplementation of the diets of hypercholesterolemic rats with niacin also resulted in significant reductions in the hepatic activities of catalase and glutathione peroxidase but did not significantly alter the activity of glutathione reductase compared to the untreated hypercholesterolemic group.

The potential link between dyslipidemia and renal damage was first postulated by Moorhead et al (1982). Since then numerous dietary studies showed that hypercholesterolemia causes oxidative stress in the kidneys of experimental animal models (Stulak et al. 2001, Chade et al. 2002, 2007, Montilla et al. 2004). Supplementation of the diets of hypercholesterolemic rats with PMF^{ort} and niacin resulted in significant reductions in the activities of catalase and glutathione transferase in the kidney but had no significant effect on the activities of glutathione reductase, glutathione peroxidase or superoxide dismutase compared to the untreated hypercholesterolemic group.

There is a very high possibility for oxidative damage to brain tissue because of its high rate of oxidative metabolic activity, intensive production of reactive oxygen metabolites, relatively low antioxidant capacity, low repair mechanism activity, non-replicating nature of its neuronal cells, and the high membrane surface to cytoplasm ratio (Evans 1993, Reiter 1995). Brain tissue contains various types of antioxidants, some of them unique to the brain (Shohami et al. 1997). The two major types of

antioxidant systems in the brain are enzymes and low-molecular weight antioxidants (LMWA). Superoxide dismutase, catalase and peroxidase, as well as some supporting enzymes are present. The activities of SOD and glutathione peroxidase, are quite high, whereas the activity of catalase is relatively low (Sinet et al. 1980, Reiter 1995).

In this study supplementation of the diets of hypercholesterolemic rats with PMF^{ort} resulted in significant reductions in the activities of catalase, glutathione transferase, glutathione peroxidase and glutathione reductase in the brain compared to the untreated hypercholesterolemic rats. Supplementation of the diets of hypercholesterolemic rats with niacin however, only resulted in significant reductions in the activities of catalase and glutathione transferase in the brain compared to the untreated hypercholesterolemic rats.

Several studies show that oxidative stress is implicated in brain injury after ischemia and reperfusion. Lipid peroxidation, protein oxidation, and DNA damage are induced by the ROS produced during cerebral ischemia (Traystman et al. 1991, Chan 2001). PMF^{ort} could therefore be used prophylactically and therapeutically in treating injuries to the brain caused by hypercholesterolemia.

To our knowledge, there are no published reports on the effects of PMFs on antioxidant activities in the spleen of hypercholesterolemic rats. Supplementation of the diet of hypercholesterolemic rats with PMF^{ort} resulted in significant reductions in the activities of glutathione peroxidase, glutathione reductase, glutathione transferase and catalase but resulted in no significant changes in the activities of superoxide dismutase compared to the untreated hypercholesterolemic group. Supplementation of the diet with niacin significantly reduced the activity of glutathione peroxidase but resulted in no significant changes in the activities of catalase, glutathione transferase, glutathione reductase or superoxide dismutase compared to untreated hypercholesterolemic group. PMF^{ort} could therefore be beneficial in the prevention or amelioration of splenic injury by aiding the endogenous antioxidant system in the spleen.

Oxidative stress in hypercholesterolemia may occur by virtue of augmented generation of ROS and oxidation of LDLs (Lerman and Textor 2001, Chade et al. 2002). PMF^{ort} may be decreasing the activities of the endogenous antioxidant enzymes by acting as antioxidants and are being preferentially oxidised by free radicals. This property of PMF^{ort} is important as both animal and human studies show a positive role for antioxidants in the prevention of coronary heart disease – CHD (Kritharides and Stocker 2002).

On evaluation of integrity of organs following consumption of PMF^{ort}, the results suggest that the integrity of the organs – kidney, spleen, brain, liver and heart – was preserved in each, as there were no significant increases in lipid peroxides levels or antioxidant enzymes activities in the organs of normal or hypercholesterolemic rats fed PMF^{ort}. In the hypercholesterolemic state, the levels and activities of lipid peroxidation products and antioxidant enzymes in rat organs were increased. In fact PMF^{ort} ameliorated oxidative stress caused by hypercholesterolemia, as shown in the significant reductions in lipid peroxide levels as well as reductions in antioxidant enzyme activities in the liver, heart, kidney, spleen and brain. PMF^{ort} could therefore be a suitable candidate for further investigations that involve prophylactic or therapeutic treatment of hypercholesterolemia.

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DISCLOSURE

No conflict of interest.

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