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Comparison Between Antioxidative and Hypolipemiant Effects of Eicosapentaenoic Acid-docosahexaenoic Acid Rich Sitosterol Ester and A-linolenic Acid Rich Sitosterol Ester in Hypercholesterolemic Subjects

Avery Sengupta and Mahua Ghosh

Hypercholesterolemia is a well established risk factor for cardiovascular disease and oxidative stress. Less is known about the influence of esterified phytosterols on lowering cardiovascular risk and possessing an antioxidative property. In the present study, thirty six rats divided into six groups were investigated to know the role of ω-3 fatty acid rich sterol esters (eicosapentaenoic acid-docosahexaenoic acid rich sterol ester, α-linolenic acid rich sterol ester) on hypercholesterolemia. Hypercholesterolemia was developed in the rats by adding cholesterol in the diet at the level of 1% and they were treated with two therapeutic doses (0.25, 0.5 g kg^{-1} b.wt. day^{-1}) of two sitosterol esters (eicosapentaenoic acid-docosahexaenoic acid rich sterol ester, α-linolenic acid rich sterol ester). Both the sterol esters proved to be hypolipemiant but the effect of eicosapentaenoic acid-docosahexaenoic acid rich sterol ester was more pronounced than α-linolenic acid rich sterol ester. Both the sterol esters proved to be antioxidative by increasing the levels of antioxidant enzymes in the liver of the treated rats but the effect of α-linolenic acid rich sterol ester was about 40% greater than eicosapentaenoic acid-docosahexaenoic acid rich sterol ester. The overall results show that the higher dose (0.5 g kg^{-1} b.wt. day^{-1}) was much effective than the lower dose.

Key words: Antioxidant, hypolipemiant, hypercholesterolemia, EPA-DHA rich sterol ester, ALA rich sterol ester

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INTRODUCTION

Hypercholesterolemia contributes to coronary artery disease (Kannel *et al*., 1997; Castelli, 1988; Castelli *et al*., 1986; Gotto *et al*., 1977). It increases the level of lipid peroxidation product malondialdehyde (MDA) in blood and aortic tissue (Prasad and Kalra, 1989, 1993) and thus makes the tissues more susceptible to increased production of Reactive Oxygen Species (ROS) leading to oxidative stress. The uncontrolled production of ROS leads to the occurrence of cardiovascular disease (Ross, 1993; Moreno and Mitjavila, 2003). ROS promotes oxidation meaning the addition of oxygen to Low-Density Lipoprotein (LDL), contributes to the build up of fatty plaque on artery walls, which can eventually slow or block blood flow to the heart. The susceptibility of an organism to oxidative damage is influenced by the antioxidant defense system's ability to cope with the stress which in turn can be influenced by nutrition intervention with antioxidants (Chow, 1989). Inherent antioxidant defense systems consisting of enzymes, such as Glutathione Peroxidase (GPx), catalase and superoxide dismutase (SOD) and antioxidant nutrients may participate in coping with ROS production (Wills, 1985; Machlin and Bendich, 1987; Leibovitz *et al*., 1990). As antioxidant enzymes have an important role in the protection against free radical damage, a decrease in the activities or expression of these enzymes leads to free radical damage of the tissues (Huang and Fwu, 1993; L'Abbe *et al*., 1991). Thus, an agent is required which can prevent both ROS and atheroma plaque production induced by hypercholesterolemia. Therefore an antioxidative and hypolipemiant substance is essential for the treatment of oxidative stress and coronary artery disease induced by hypercholesterolemia.

Phytosterols are cholesterol-like molecules found in all plant foods, is present in the highest concentrations in vegetable oils. They are present in diet in several forms, but the two most abundant are β-sitosterol and campesterol (Awad and Fink, 2000). Phytosterols have serum cholesterol lowering properties which is believed to be caused by an inhibition of cholesterol absorption from the higher affinity of phytosterols than cholesterol for micelles (Armstrong, 1987). As early as 1951, it was shown that β-sitosterol lowered serum cholesterol in chickens administered with cholesterol (Peterson, 1951). Furthermore, β-sitosterol decreases free oxygen radical and hydrogen peroxide (H_2O_2) levels in blood (Moreno, 2003). Thus β-sitosterol has both hypolipemiant role (by decreasing cholesterol absorption) and antioxidative role. But the disadvantage of phytosterol is that free phytosterol is water and oil insoluble. It is difficult to ingest effective amounts of phytosterol in free form continuously as a food supplement because it has poor solubility in lipids (Jandacek *et al*., 1977).

Omega-3 fatty acids are very important among the polyunsaturated fatty acids. The important metabolically active ω-3 fatty acids are α-Linolenic Acid (ALA), which has plant origin and Eicosapentaenoic Acid (EPA) and docosahexaenoic acid (DHA), which has animal origin. Long chain ω-3 fatty acid consumption may protect the body against the causative factors leading to the cardiovascular disease (Calder, 2004). They also enhance the activities of antioxidant enzymes and thus show antioxidative action.

In combination, β-sitosterol and omega-3 fatty acids may offer a more comprehensive strategy for not just optimizing circulating lipid levels, but also for providing antioxidative effects. Sitosterol esters, which is free β-sitosterol esterified with fatty acids, dissolves well in lipids (Jandacek *et al*., 1977). By the process of esterification of phytosterols to long-chain omega-3 fatty acids their hypolipidemic properties is not impaired, yet it enhances their solubility in lipids by 10-fold. Thus the sitosterol esters are more effective in reducing both total cholesterol and triglyceride concentrations in comparison to free sitosterol because the efficacy of cholesterol lowering is often dependent on the dispersion capability in water and oil. Moreover, the β-sitosterol ester can reduce the susceptibility of omega-3 fatty acids towards peroxidation. β-sitosterol can work against lipid peroxidation and omega-3 fatty acids can enhance the activities of antioxidant enzymes, thus both in combination can be a powerful antioxidant. There is no data regarding the effects of sterol esters in therapeutic doses against ROS. Thus, the objective of this study was to compare the antioxidative and hypolipemiant roles of EPA-DHA rich sterol ester and ALA rich sterol ester in rat model.

MATERIALS AND METHODS

Preparation of β-sitosterol esters: A standard β-sitosterol sample was procured from Fluka Chemicals and analyzed at the laboratory by Gas Chromatography (GC). Fish oil (Mega-Shelcal capsules from Elder Pharmaceuticals, India) was used as the source of eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) and the GC analysis of the fish oil showed that the oil contained 32 EPA and 22% DHA. Refined, bleached

linseed oil procured from V.K.V.K. Oil Limited, Kolkata, India, was used as the source of Alpha Linolenic Acid (ALA) and the GC analysis of the oil showed the presence of 54% ALA in the oil. *Thermomyces lanuginosus* lipase (Lipozyme TLIM), used as biocatalyst, was a generous gift from Novozyme India, Ltd., Bangalore, India. Phytosterol esters were formed by enzymatic transesterification reactions in a packed bed reactor and their fatty acid compositions were analyzed by GC (Sengupta *et al*., 2010).

Fatty acid compositional analysis of phytosterol esters: The percent compositions of various sterol esters according to fatty acid compositions were determined by GC. The GC instrument (Agilent, model 6890 N) used was equipped with a FID detector and capillary HP 5 column (30 mL, 0.32 mm I.D, 0.25 μm FT). N_2, H_2 and airflow rates were maintained at 1, 30 and 300 mL min^{-1}, respectively. Inlet and detector temperature was kept at 250 and 275°C, respectively, and the oven temperature was programmed at 65-230-280°C with a 1-min hold at 65°C and an increase rate of 20°C min^{-1} and 1 min hold up to 230 and 8°C min^{-1} with 24 min hold up to 280°C. Sterol esters were fractionated according to the fatty acid composition from which the amount of each fatty acid incorporated in the ester was calculated. The Retention Time (Rt) of each sterol ester had been previously standardized in GC by preparing esters of β-sitosterol with different fatty acids.

Animals and experimental set up: Animal experiments were conducted according to the guidelines of Animal Ethical Committee of Dept. of Chemical Technology, University of Calcutta. Adult male albino rats of Wistar strain were housed and given food and water *ad libitum*. The duration of the experimental period was 32 days. The animals were divided into six groups with six rats in each: Group 1: Vehicle treated control animals, Group 2: Rats were fed with a high cholesterol diet (rat stock diet supplemented with 1% cholesterol) for 32 days, Group 3: Rats received EPA-DHA rich phytosterol ester (0.25 g kg^{-1} b.wt, day^{-1}, oral gavage) for 25 days along with a high cholesterol diet for 32 days, Group 4: Rats received EPA-DHA rich phytosterol ester (0.5 g kg^{-1} b.wt. day^{-1}, oral gavage) for 25 days along with a high cholesterol diet for 32 days, Group 5: rats fed with high cholesterol diet for 32 days and ALA rich phytosterol ester (0.25 g kg^{-1} b.wt. day^{-1}, oral gavage) for the last 25 days, Group 6: Rats fed with high cholesterol diet for 32 days and ALA rich phytosterol ester (0.5 g kg^{-1} b.wt. day^{-1}, oral gavage) for the last 25 days. Peanut oil was used as the vehicle and given to all the groups and its composition is given in Table 1. The rats of Group 2-6 were made hypercholesterolemic by feeding cholesterol through diet for 32 days. After 5 days the rats of Group 3-6 were orally gavaged with the different treatments which continued for the next 25 days along with the additional dietary cholesterol. The oral gavage was given each day in the morning at around 12 o'clock. At the end of the experiment the feeding of rats was stopped and after 12 h fasting, the rats were anesthetized by chloroform and 5 mL of blood was taken from the heart. The plasma was obtained by centrifugation of the blood. The liver was removed, rinsed with ice-cold saline, blotted, weighed and stored at -20°C until analyzed.

Table 1: Fatty Acid Profile of Peanut oil (Vehicle)

	Fatty Acid (% w/w)						
Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{22:0}$	$C_{24:0}$
Peanut oil	7.10	4.40	55.60	26.30	2.40	3.10	1.10

Cholesterol absorption: Stools were collected daily, and fecal neutral steroids were isolated by hexane extraction and TLC. Ratios of Cholesterol/Sitosterol (C/S) were determined after feeding the rats with additional cholesterol and sitosterol in the form of ester. Using this method, cholesterol absorption was calculated as follows:

$$\text{Absorption (\%)} = \frac{\text{Faecal C/S}}{\text{Dietary C/S}} \times 100$$

Analysis of plasma lipids: Blood samples for laboratory analyses were taken into EDTA-containing vacuum tubes after 12 h fasting. Plasma was separated by centrifugation and stored at -70°C until analyzed. In the analysis of lipid profile plasma lipids, samples were taken into glass centrifuge tubes and homogenized with different proportions of chloroform/methanol (v/v) and then centrifuged. Redistilled water was added to the mixture and the chloroform layer was separated in a separatory funnel. The chloroform was then evaporated to obtain the plasma lipids. The lipid components such as total cholesterol, HDL cholesterol and triacylglycerol were analyzed using enzyme kits supplied by Merck.

Estimation of hepatic lipid profile: Hepatic lipid was extracted by the method of Folch *et al*. (1951). One gram of tissue was homogenized with 1 mL of 0.74% potassium chloride and 2 mL of different proportions of chloroform and methanol for 2 min and then centrifuged. The mixture

was left overnight and the chloroform layer was filtered through a Whatman filter paper (No. 1). The chloroform layer was dried, the tissue lipid contents were measured and the lipid was used for lipid analysis. The liver and brain lipids were used for the estimation of total cholesterol and triacylglycerol estimation by using standard kits. Phospholipid was estimated using the method of Chen *et al.* (1956).

Estimation of antioxidant enzymes in the hepatic tissue: Measured amounts of liver tissues were taken and homogenized in different concentrations of phosphate buffer. The samples were then centrifuged and the supernatants were used for enzyme assay. The activity of Catalase (CAT) was determined spectrometrically by the method of Aebi (1984). Superoxide Dismutase (SOD) activity was assayed by measuring the auto oxidation of haematoxylin as described by Martin *et al.* (1987). Glutathione Reductase (GSH) was determined by the method of Ellman (1959). Total activity of Glutathione Peroxidase (GPx) (GPx EC.1.11.1.9.) was determined in the tissue homogenates according to Flohe and Gunzler (1984). All the enzyme activities were expressed in terms of enzyme units per mg protein. Protein was determined using the standard method of Lowry *et al.* (1951).

Statistical analysis: All results were expressed as the mean value±SEM. Statistical significance of the difference among values was analyzed by one-way ANOVA. Student-Newman-Keuls (SNK) test was performed after ANOVA. Results were considered significant at $p<0.05$.

RESULTS

Fatty acid composition of phytosterol esters: The transesterification reaction was carried out in the packed bed reactor. In the first set of reaction fish oil was used as a source of EPA and DHA fatty acids and in the second set, linseed oil was used as a source of ALA fatty acid. Analysis of fatty acid composition of sterol-esters by GC showed that almost all the fatty acids present in the different oils (in TAG form) were incorporated in the corresponding esters. Table 2 shows the fatty acid profile of the sterol esters produced in the packed bed reactor which reflects that EPA-DHA rich sterol ester contains 39.20 EPA and 31.98% DHA and ALA rich sterol ester contains 44.52% ALA.

Changes in cholesterol and sitosterol absorption: Figure 1 and 2 show the changes in cholesterol and sitosterol absorption when the rats are fed with two different sterol esters at two different doses. The results showed that cholesterol absorption decreased with the administration of sterol esters. The decrease in absorption was double in the higher dose in case of both the sterol esters. EPA-DHA rich sterol ester decreased the cholesterol absorption almost as much as ALA rich sterol ester. Similar results were observed in case of sitosterol absorption (Fig. 2).

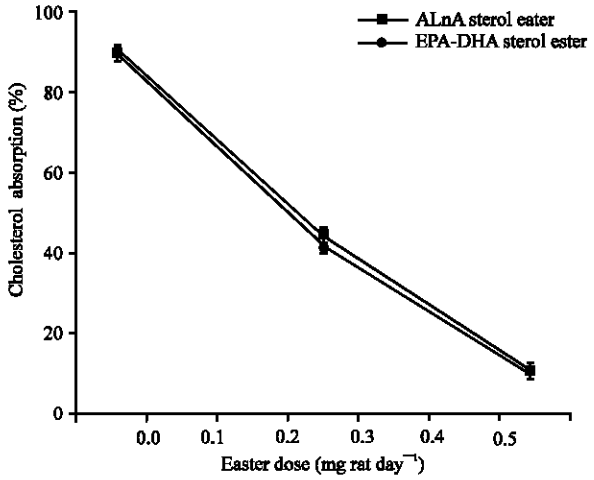

Fig. 1: Comparison between changes in cholesterol absorption by administration of EPA-DHA rich sterol ester and ALA rich sterol ester at two different doses

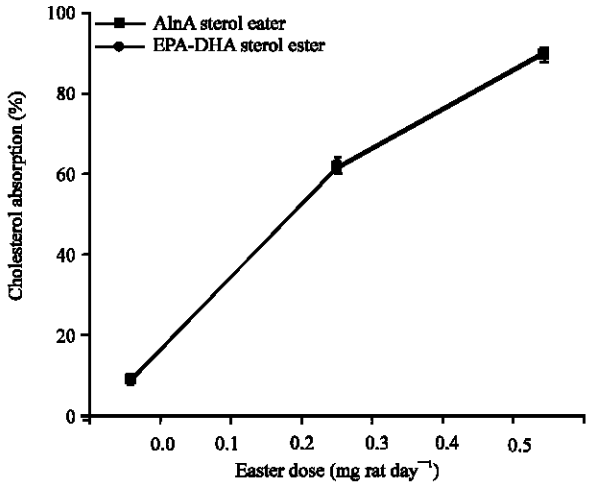

Fig. 2: Comparison between changes in sitosterol absorption by administration of EPA-DHA rich sterol ester and ALA rich sterol ester at two different doses

Table 2: Fatty acid profiles of EPA-DHA rich and ALA rich Sterol esters used as nutraceutical

	Fatty acid (% w/w)						
Sample	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$
Phytosterol-DHA-EPA Ester	0.10±0.00	2.44±0.0	41.23±0.02	1.0±0.15	1.90±0.27	3.05±0.34	2.75±0.64
Phytosterol-ALA Ester	-	12.48±0.60	1.89±0.17	28.28±1.0	912.83±1.01	44.52±1.76	-
	Fatty acid (% w/w)						
Sample	$C_{20:1}$	$C_{20:5}$	$C_{22:0}$	$C_{22:1}$	$C_{24:0}$	$C_{22:6}$	
Phytosterol-DHA-EPA Ester	5.12±0.83	39.20±1.10	5.65±0.37	2.19±0.08	3.84±0.19	31.98±1.63	
Phytosterol-ALA Ester	-	-	-	-	-	-	

Values are expressed as Mean±SEM

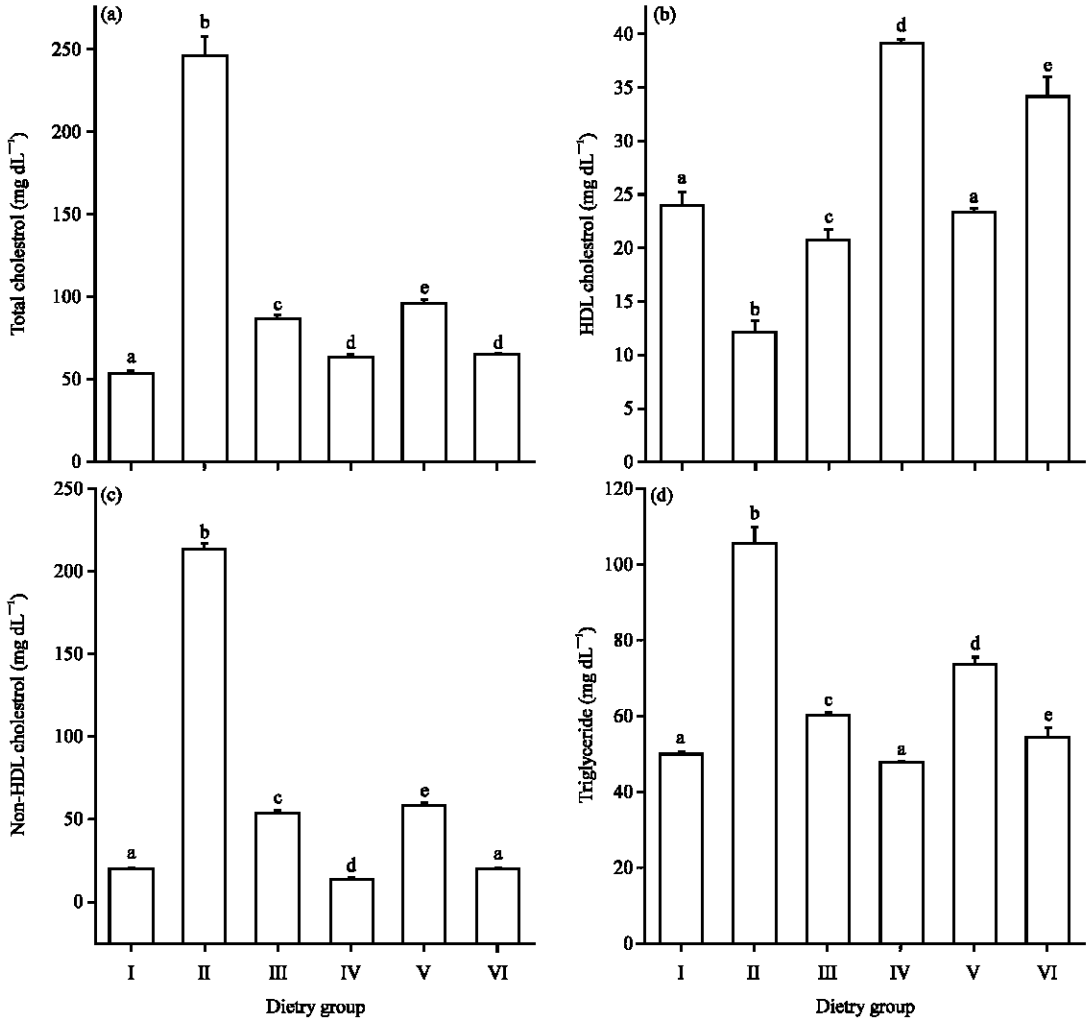

Fig. 3(a-d): Plasma lipid profile of rats of different dietary groups, I: Control; II: Hypercholesterolemic Control; III: EP -DHA rich ester fed in lower dose; IV: EPA-DHA rich ester fed in higher dose; V: ALA rich ester fed in lower dose; VI: ALA rich ester fed in higher dose, Each bar is expressed as overall mean±S.E.M. for six animals, Bars not sharing a common superscript are statistically significant ($p<0.05$)

Changes in plasma lipid profile: The plasma lipid profile of rats of different groups is shown in Fig. 3. The type of fat consumed altered the different lipid concentration in plasma. Rats fed with control peanut oil had plasma total cholesterol of 54.37 mg dL^{-1} which was significantly increased to 246.83 mg dL^{-1} by feeding them high

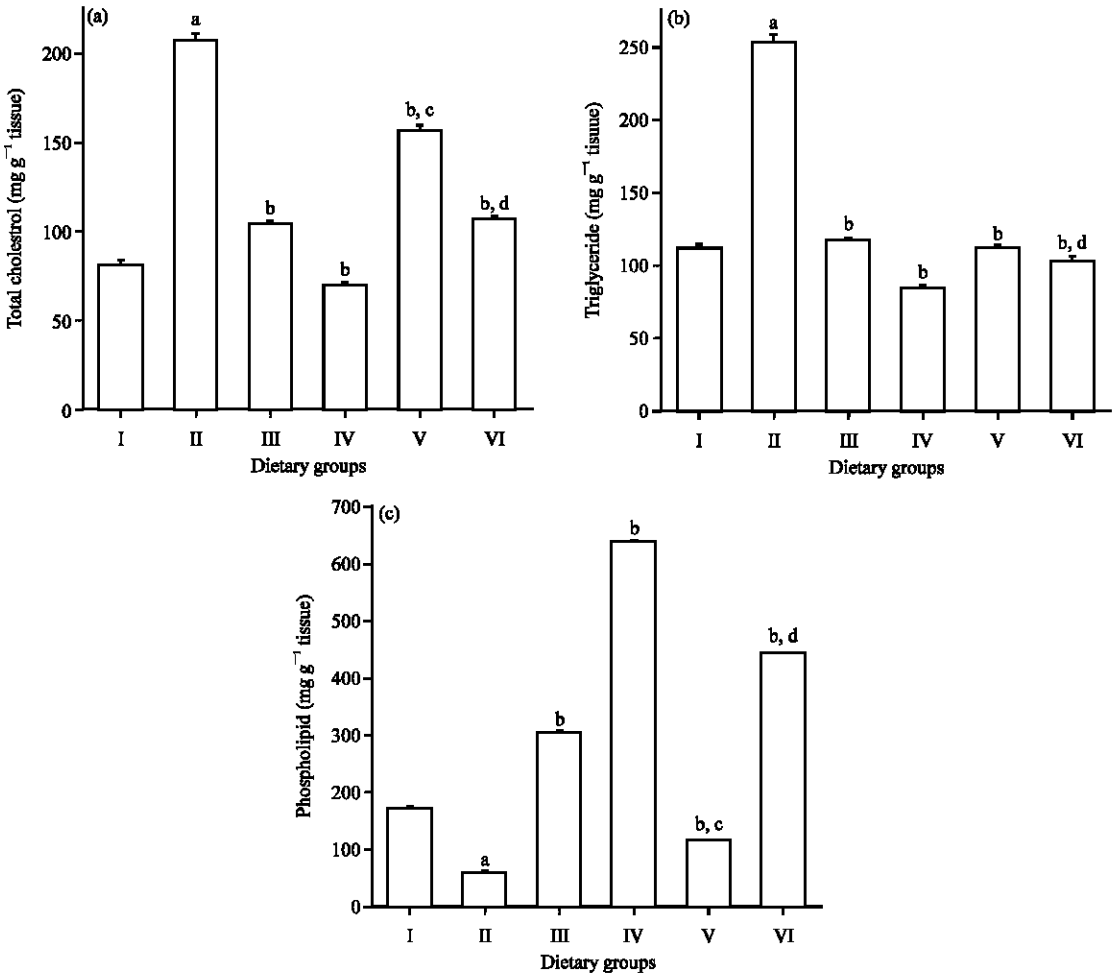

Fig. 4(a-c): Liver lipid profile (mg g^{-1} tissue) of rats of different dietary groups, I: Control; II: Hypercholesterolemic Control; III: EPA-DHA rich ester fed in lower dose; IV: EPA-DHA rich ester fed in higher dose; V: ALA rich ester fed in lower dose; VI: ALA rich ester fed in higher dose, Each bar is expressed as overall mean±S.E.M. for six animals, The superscript letters represent statistical significance at $p<0.05$, [a]Comparisons are made between Groups I and II, comparisons are made between Groups II and III-VI, [c]Comparisons are made between Groups III and V, [d]Comparisons are made between Groups IV and VI

cholesterol diet. Both the doses of phytosterol ester brought about a decrease in total cholesterol which was much more in case of EPA-DHA rich sterol ester. The higher doses produced better hypocholesterolemic effect than the lower dose. Similar results were found in case of estimation of non-HDL cholesterol levels and triacylglycerol levels. Thus the phytosterol esters lowered the levels of non-HDL cholesterol and triacylglycerol significantly ($p<0.05$). The level of HDL cholesterol, which was known as good cholesterol, increased significantly ($p<0.05$) by treating the rats with phytosterol esters. The increase in the HDL level was much more in case of the rats treated with EPA-DHA rich sterol ester.

Changes in hepatic lipid profile: The liver lipid profile of rats of different groups is shown in Fig. 4.The type of fat consumed altered the different lipid concentration in plasma. Rats fed with control Peanut oil had total cholesterol of 81.71 mg g^{-1} tissue which was significantly increased to 207.93 mg dL^{-1} by feeding them high cholesterol diet. Both the doses of phytosterol ester brought about a decrease in total cholesterol which was much more in case of EPA-DHA rich sterol ester. The higher doses produced better hypocholesterolemic effect than the lower dose. Thus the phytosterol esters lowered the levels of triacylglycerol significantly ($p<0.05$). Phospholipid levels in the liver decreased in hypercholesterolemia, but administration of phytosterol esters increased its level.

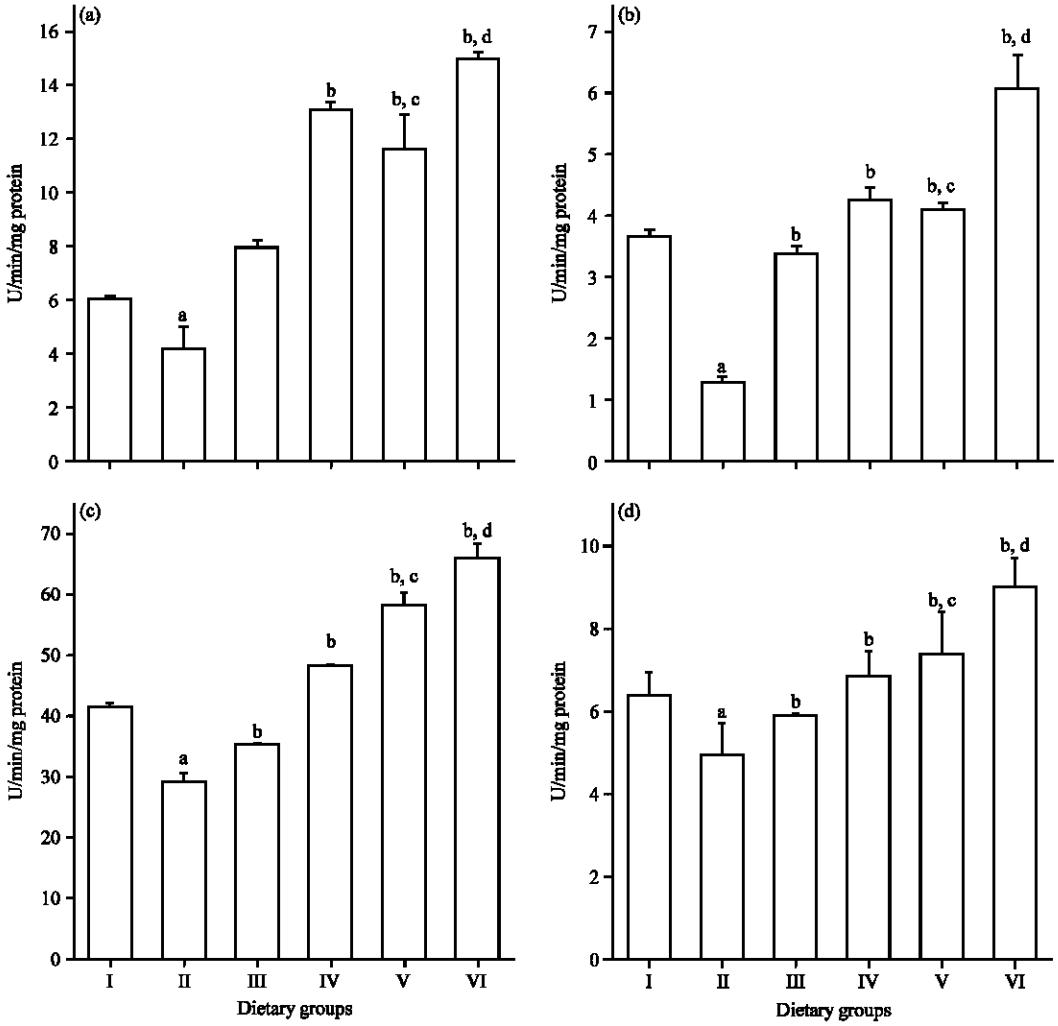

Fig. 5(a-d): Effect of phytosterol esters on hepatic antioxidant enzyme status on HCD fed groups, compared with the control animals, I: Control; II: Hypercholesterolemic Control; III: EPA-DHA rich ester fed in lower dose; IV: EPA-DHA rich ester fed in higher dose; V: ALA rich ester fed in lower dose; VI: ALA rich ester fed in higher dose, a-CAT, b-SOD, c-GSH, d-GPx, Each bar is expressed as overall mean±S.E.M. for six animals, The superscript letters represent statistical significance at $p<0.05$, [a]Comparisons are made between Groups I and II, comparisons are made between Groups II and III-VI, [c]Comparisons are made between Groups III and V, [d]Comparisons are made between Groups IV and VI

Changes in antioxidant enzyme levels: Figure 5 reveals the effect of two different doses (25 and 50 mg rat day^{-1}) of two different phytosterol esters (EPA-DHA rich sterol ester and ALA rich sterol ester) on hepatic antioxidant enzymes on hypercholesterolemic groups (HCD). It was seen from the result that hypercholesterolemia decreased the levels of antioxidant enzymes in Group II significantly. Treatment with the two sterol esters increased the antioxidant enzyme levels. The effect of ALA rich sterol ester was more antioxidative compared to EPA-DHA rich sterol ester and the higher dose produced better effect than the lower one.

DISCUSSION

The study revealed that cholesterol absorption decreased and sitosterol absorption increased with the administration of both the sterol esters and at both the therapeutic dosages. However the effect of the higher dosage was much more in comparison to the lower one. But the effect of both the sterol esters was almost similar. From this we can conclude that decrease associated with cholesterol absorption was due to the presence of β-sitosterol. It is well established that phytosterols, structural analogues of cholesterol, have been shown to

substantially reduce total and LDL-cholesterol concentration. The basic mechanism of action of phytosterol is that, in appropriate conditions, they can become efficiently incorporated into the micelles in the intestinal lumen, displace the cholesterol, and lead to its precipitation with other, non-solubilized phytosterols. Cholesterol absorption, both from the diet and from the enterohepatic circulation, is strongly reduced in the presence of phytosterols and the unabsorbed cholesterol is excreted in the faeces, increasing its concentration at this level. It can also be seen from the results that phytosterol absorption increases with the increase in ester dosage, which proved that sterol ester increased the absorptibity and acceptability of phytosterol than free sterol administration. This is in agreement with the study of Jandacek *et al.* (1977) which stated that the esterification of phytosterols to long-chain omega-3 fatty acids doesn't impair their hypolipidemic properties, yet enhances their solubility in oil by 10-fold.

Compared to the control group in the hypercholesterolemic (HCD) group there was a sharp increase in the Total Cholesterol (TC), triglyceride (TG) and Low-Density Lipoprotein Cholesterol (LDL-C) levels. When the HCD group was fed with the phytosterol esters, there was significant lowering ($p<0.05$) in plasma TC concentration, TG and LDL-C levels. The nutraceutical effect of the higher dose (0.5 g kg^{-1} b.wt. day^{-1}) in case of both the esters was more beneficial than the lower dose (0.25 g kg^{-1} b.wt. day^{-1}). In comparison to ALA rich sterol ester, EPA-DHA rich sterol ester produced much more hypocholesterolemic effect. Administration as esters signifies that they must first be hydrolyzed by the pancreatic lipase in order to be effective and to be included in the micelles. Therefore, the phytosterol ester is first hydrolyzed to phytosterol and fatty acids inside the living system. Phytosterols have been shown to inhibit the uptake of both dietary and endogenously produced (biliary) cholesterol from intestinal cells. The potential mechanisms by which phytosterol inhibits cholesterol absorption include inhibition of bile salt cholesterol micellar formation and competition with the brush border for cholesterol uptake (Ikeda *et al.*, 1988), changing micellar solubilization, intracellular esterification and/or incorporation into chylomicron (Child and Kuksis, 1983). At present, reduced cholesterol solubilization in bile salt micelles is proposed as a major factor in inhibited absorption of cholesterol by phytosterols (Ikeda and Sugano, 1983). Such inhibition results in a decrease in serum total and LDL-cholesterol (LDL-C) levels. The second hydrolytic product is omega-3 or n-3 fatty acids. They are pleiotropic molecules with a broad variety of biological actions including hypotriglyceridaemic, anti-aggregatory, anti-inflammatory and antiarrhythmic responses (Garg *et al.*, 2006). There is considerable evidence to suggest that omega-3 fatty acid supplementation is involved in improved vascular function and lipoprotein profile, lower arterial pressure, diminished thrombogenicity and modification of atherogenic processes, all of which are important cardiovascular preventative actions. However the effect of EPA-DHA towards hypolipemiant actions is much greater in comparison with ALA. Thus the combination of EPA-DHA rich sterol ester produced better nutraceutical effects on plasma lipid profile.

High plasma cholesterol represents a major risk factor for atherogenesis. Higher the free radical level, higher the production of cholesterol internally from liver. Liver being the major organ responsible for cholesterol transport, metabolism and excretion, it is reasonable to study hepatic lipemic-oxidative disturbances in hypercholesterolemic diet induced atherogenesis. A study conducted by Lu *et al.* (2007) showed that a high-cholesterol intake increased the tissue content of TBARS and depleted glutathione in the liver, indicating an overall prooxidative state. Hypercholesterolemia disturbs the prooxidant-antioxidant balance in favor of pro-oxidation in liver tissues and thus the antioxidant enzyme level decreases.

The activities of antioxidant enzymes like CAT, SOD, GSH and GPx form the first line of defense against ROS and decrease in their activities contribute to the increased oxidative stress. Increased hepatic oxidative damage to the major biomolecules was accompanied by deteriorating antioxidant enzyme status in the HCD fed groups of our study, signifying heightened oxidative injury. Endogeneous antioxidant enzymes, such as SOD, CAT, GPx, as well as antioxidant nutrients, can help to protect cells against free radical damage. Since antioxidant enzymes play an important role in controlling lipid peroxidation (Huang and Fwu, 1993), an increase in the activities of these enzymes could delay the progression of hypercholesterolemia. In the present study there was a decrease in the activity of the four antioxidant enzymes namely SOD, Catalase, GSH and GPx of the liver of hypercholesterolemic rats. But administration of phytosterol esters caused an increase in the activity of the enzymes. Increase in the activity of the enzyme can attribute to decrease in the oxidative stress as well as increase in the oxidative stress. In our study the increase in the activity of the antioxidant enzymes by administration of sitosterol esters decrease oxidative stress. This is because one of the hydrolytic product of sterol ester, β-sitosterol is an antioxidant able to reduce DNA damage, reduce the level of free radical in our cells

and to increase the level of typical antioxidant enzymes (Paniagua-Perez *et al*., 2008). The decrease in the level of antioxidant enzymes in the liver residing within the vasculature finally lead to the elimination of free radicals by the generation of water and oxygen (Strehlow *et al*., 2003). Thus oxidative stress produced by hypercholesterolemia was decreased by increasing the level of antioxidant enzymes in the liver by administration of sterol esters. The effect of ALA rich sterol ester was much better than EPA-DHA rich sterol ester. This is because the amount of polyunsaturated fatty acids is more in EPA-DHA rich sterol ester in comparison to ALA rich sterol ester and more the amount of polyunsaturated fatty acids more is the chances of lipid peroxidation.

CONCLUSION

The aforesaid discussion concludes that EPA-DHA rich sterol ester provides greater hypolipemiant effects in comparison with ALA rich sterol ester, while ALA rich sterol ester provides more antioxidative effect in comparison to EPA-DHA rich sterol ester. The optimum dose of esters should be given in the dosage of 0.5 g kg^{-1} b.wt./day for the treatment of Cardiovascular Disease and other metabolic disorders.

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