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Effect of Dietary Lipids and Drumstick Leaves (*Moringa oleifera*) on Lipid Profile & Antioxidant Parameters in Rats

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ABSTRACT

The study investigated the influence of dietary vitamin E derived from drumstick leaves (DL, Moringa oleifera) on antioxidant status and plasma lipids in male rats, fed diets based on either palm (PO) or peanut oil (PN). Two control (PO & PN) and three experimental diets (PODL, PNDL & POPNDL) differing in dietary fat source supplemented with DL powder were fed (ad libitum, 8 weeks). The vitamin E content of control diets was 30 IU/kg while the experimental diets provided 60 IU/kg of diet. No significant differences in body weight, liver and adipose tissue weights were observed between the 5 groups, despite the difference in the type/source of fat used in the diets. After 8 weeks, the cholesterol and TBARS levels in the livers of rats fed PODL diet were lower than the other groups. In all 5 groups, plasma cholesterol decreased after 8 weeks, however, diets supplemented with DL powder resulted in decreases in both plasma cholesterol and lipid peroxides marked decrease ($p < 0.05$) compared to control group. The increase in plasma vitamin E levels were not significant. The data suggest that the combination of dietary fat and drumstick leaves as a source of antioxidant was beneficial as it reduced plasma cholesterol & lipid peroxidation.

Keywords: Cholesterol; Vitamin E; *Moringa oleifera*; Antioxidant

1. Introduction

It is well documented that diets rich in polyunsaturated fatty acids lead to reduced plasma cholesterol levels and lower atherogenic risk [1-3]. Studies have shown that palm oil is efficacious in lowering plasma cholesterol (TC); it is also associated with significant increases in plasma triglycerides (TG) [4,5]. Furthermore, oxidative damages, including those associated with lipid peroxidation, are generally believed to be a significant factor in many pathological processes. Polyunsaturated oils lead to depletion of plasma α-tocopherol and other antioxidants. Supplementation of diets with α-tocopherol significantly increased plasma α-tocopherol levels and resulted in lesser accumulation of peroxides in plasma [6,7]. Both epidemiological and clinical data have implicated peroxidized plasma lipid in atherogenesis [8,9]. Currently much research is focused on the beneficial effects of bioactive phytochemicals present in micro level in our daily diet. These phytochemicals are abundant in grains, vegetables, fruits, seeds and nuts. They are believed to contribute positively in the prevention of degenerative diseases. The mechanism behind the different beneficial effects of dietary phytochemicals are not fully understood. However, these compounds are known to act as antioxidants, hypocholesterolemic and enzyme modulating agents and phytohormones. Research strongly suggests that protection against degenerative diseases requires the right balance of a multitude of phytoprotectants including essential fatty acids, vitamins, antioxidants and dietary fiber components [10,11].

Thus, with the above concept of interactive mechanism the present study was planned to investigate the effect of dietary lipids and vitamin E supplementation on lipid peroxidation and lipid profile in rats. In order to investigate this point, drumstick leaves (M*oringa olifera*) were incorporated into the diets fed to rats that provided palm oil, peanut oil or blends of palm and peanut oil to test the hypocholesterolemic and antioxidant potential of vitamin E supplementation derived from natural source.

2. Materials and Methods

Forty male Wistar rats, from same colony, of body weights 130 - 140 g were used. They were housed in screen bottom cages with 2 rats in each and were given a standard

casein based diet and water *ad libitum* for 2 weeks to allow acclimatization. They were then weighed, randomly divided into 5 groups of eight each, and fed experimental diets for 8 weeks. Group I receiving standard diet (palm oil based, PO) served as control, group II received peanut oil diet (PN), while groups III (PODL), IV(PNDL) and V(POPNDL) diets were supplemented with vitamin E derived from drumstick leaves. The diet composition was: 62% wheat flour, 18% defatted soya flour, 7% sugar, 9% oil, 1% vitamin mix, 2% mineral mix. Diets were prepared weekly and stored at 4˚C. Samples of diets were analyzed for nutrient composition by AOAC methods [11]. Oils & diets were analyzed for vitamin E content according to the method of Freed [12]. They were weighed initially and once in fortnight thereafter. Blood samples were obtained following an overnight fast after 2 weeks of casein diet and after 8 weeks of experimental diets. Blood was collected using EDTA coated capillary tubes by retro-orbital sinus puncture for measurement of plasma lipids and other biochemical parameters. The diets were formulated as per standard specifications [13].

Plasma lipids were quantified using standard enzymatic kits (Crest Biosystems, Goa, India). Liver was homogenized and the lipid extracted by the method of Folsch *et al.* [14]. Thiobarbituric acid reactive substances (TBARS) [15,16], reduced glutathione [17] and vitamin E were assayed [18] in plasma and liver.

The study was approved by Research & Ethics Committee for Animal experiments, University of Mysore, Mysore.

Statistical Analysis

Data are presented as mean ± SD. Biochemical data was analyzed by ANOVA for comparisons.

3. Results

There was no significant difference in food intake, weight gain, final weight and mean weights of liver, heart and adipose tissue among rats fed different diets (**Table 1**).

The changes in plasma total cholesterol (TC), triglycerides (TG) in all five groups (before and after 8 wks) are given in **Table 2**. The mean initial plasma TC ranged from 67.4 to 82.5 mg%. After 8 weeks of feeding the respective diets, the TC levels decreased in all 5 groups, however, a significant reduction was observed in the DL supplemented diets. The baseline values of TG ranged from 76.7 to 119.9 mg%. A progressive increase was observed in all 5 groups irrespective of the source/type of fat given. However, diets supplemented with DL resulted in small elevations than control diets. It was interesting to note that DL supplementation to diets based on peanut oil resulted in increase of lesser magnitude.

Table 1. Body weights and organ weights of rats fed on diets containing palm, peanut and mixture of oils with drumstick leaves.

Diets	Body weight (g)		Liver (g)	Heart (g)	Adipose Tissue (g)
	Initial	Final			
PO	152.7 ± 7.29	257.5 ± 13.5	6.7 ± 0.32	0.980 ± 0.81	4.9 ± 0.58
PN	158.2 ± 7.42	263.5 ± 16.4	6.4 ± 0.22	0.980 ± 0.0	4.9 ± 0.99
PODL	157.3 ± 12.03	257.2 ± 12.73	6.9 ± 0.48	0.973 ± 0.53	4.3 ± 0.85
PNDL	151.6 ± 11.05	256.1 ± 18.25	6.6 ± 0.50	0.976 ± 0.59	5.6 ± 0.26
POPNDL	161.7 ± 7.39	256.8 ± 11.24	6.5 ± 0.36	0.970 ± 0.59	4.4 ± 0.76

PO—Palm oil, PN—Peanut oil, PODL—Palm oil + Drumstick leaves, PNDL—Peanut oil + Drumstick leaves, POPNDL—Palm oil + Peanut oil + Drumstick leaves.

Table 2. Plasma lipid profile in rats on diets containing palm, peanut and mixture of oils with drumstick leaves.

Groups	Total Cholesterol (mg/dl)		Total Triglycerides (mg/dl)		HDL (mg/dl)
	Initial	Final	Initial	Final	Final
PO	73.9^{a}	70.6^{b}	76.7^{a}	121.7^{b}	46.3^{b}
PN	67.5^{a}	66.5^{ab}	86.5^{a}	100.9^{a}	41.9^{a}
PODL	72.2^{a}	52.5^{a}	119.8^{c}	153.4^{c}	49.5^{b}
PNDL	82.6^{a}	54.9^{a}	114.6^{b}	122.8^{b}	44.5^{a}
POPNDL	67.6^{a}	58.9^{ab}	106.8^{b}	118.6^{b}	46.2^{a}
SD	±10.53 (25df)	±10.67 (28df)	±11.57 (25df)	±21.07 (28df)	±5.72 (28df)

Values expressed as Mean ± SD for 8 rats; Values carrying superscripts a, b, c in columns differ significantly ($p < 0.05$); Initial—After casein diet, Final—After 8 weeks of experimental diet.

Concentration of lipid peroxides, vitamin E and glutathione in plasma of rats fed different diets are given in **Table 3**. A significant reduction in lipid peroxides was observed in 3 groups *i.e.*, PODL, PNDL and POPNDL after 8 weeks of supplementation with DL. Although DL supplementation resulted in increases in vitamin E levels they were not statistically significant. Perhaps the level and duration of supplementation was inadequate to induce physiological changes. No significant differences between the groups was observed in plasma vitamin E levels post supplementation. There was a significant increase in reduced glutathione in the plasma of DL fed groups. Among the 3 supplemented groups, peanut based diets (IV & V) resulted in higher triglyceride content in the liver (**Table 4**). TBARS levels were the lowest in PODL group indicating decreased lipid peroxidation in hepatocytes.

4. Discussion

This experiment was designed to investigate the effects of dietary lipids and vitamin E supplementation from natural source on plasma cholesterol, vitamin E and lipid peroxides in rats. The diets differed in the source of dietary fat viz., palm and peanut oil. The experimental diets were supplemented with a natural source of vitamin E *i.e.*, drumstick leaves (M*oringa oliefera*), the diets were formulated as per International standards [13]. The diets provided 30 IU of vitamin E per kg diet, which was being derived from palm oil alone in PO diet. Since the vitamin E content of peanut oil was lesser, it was supplemented with synthetic tocopheryl acetate. The level of vitamin E in all 3 experimental diets was equalized by adding necessary amount of DL powder, to a final concentration of 60 IU/kg diet. In the present study, dietary fat alone or together with added vitamin E, was tested as a potential hypocholesterolemic agent and also as an antioxidant.

Table 3. Effects of feeding palm, peanut and mixture of oils with drumstick leaves on plasma lipid peroxide, Vitamin E and Glutathione in rats.

Groups	Lipid Peroxide (n mol/ml)		Vitamin E (mg%)		Glutathione (m mol/ml)	
	Initial	Final	Initial	Final	Initial	Final
PO	4.0^{a}	2.9^{b}	2.0^{a}	3.6^{a}	0.76^{b}	0.78^{a}
PN	3.9^{a}	2.4^{a}	2.0^{a}	3.7^{a}	0.41^{a}	0.63^{a}
PODL	3.9^{a}	2.1^{a}	1.9^{a}	3.8^{a}	0.78^{b}	1.02^{b}
PNDL	3.8^{a}	1.8^{a}	2.3^{a}	3.7^{a}	0.64^{b}	0.85^{ab}
POPNDL	4.5^{a}	1.8^{a}	2.1^{a}	3.9^{a}	0.58^{ab}	1.04^{b}
SD	±0.76 (22)	±0.84 (32)	±0.51 (21)	±0.52 (33)	±0.15 (18)	±0.18 (27)

Values expressed as Mean ± SD for 8 rats; Values carrying superscripts a, b, c in columns differ significantly ($p < 0.05$).

Table 4. Liver lipid, TBARS in the liver of the rats fed on diets containing palm, peanut and mixture of oils with drumstick leaves.

Groups	Total Cholesterol*	Total Triglycerides*	TBARS (µmole/g)
PO	8.4 ± 1.25	8.9 ± 0.15	6.1 ± 0.55
PN	8.6 ± 0.28	8.2 ± 2.15	6.4 ± 1.1
PODL	7.8 ± 0.11	8.7 ± 1.7	4.8 ± 1.1
PNDL	9.3 ± 1.15	9.6 ± 2.8	6.4 ± 1.6
POPNDL	8.4 ± 0.86	8.8 ± 2.8	5.6 ± 0.86

*Expressed as mg/g liver weight. Values expressed as Mean ± SD for 8 rats.

All diets were well accepted by the animals. The organ weights were comparable in all 5 groups. These results are in good agreement with reported studies carried out with rats fed dietary phenolic compounds [9,19].

Supplementation of DL powder resulted in a significant decrease in plasma cholesterol compared to the 2 control diets. Palm oil is known to exhibit hypocholesterolemic effect [4], although such a trend was observed in the PO diet, it was not significantly lower. Supplementing both PO and PN diets with higher levels of vitamin E proved to be beneficial in lowering plasma cholesterol. It is reported that oils rich in PUFA's such as sunflower, safflower and peanut may decrease the absorption of vitamin E. PUFA's may also enhance the *in vivo* oxidation of vitamin E, thus limiting its availability and clinical benefit [20]. Thus, PUFA based diets require additional vitamin E for lowering plasma cholesterol.

α-tocopherol, biologically and chemically the most active form of vitamin E is the major lipid-soluble antioxidant known to break the chain of free radical mediated lipid peroxidation of PUFA's [21]. It functions as a potent inhibitor of lipid peroxidation in biological cells, cell membranes and plasma [21,22]. Animal tissues respond to dietary *α*-tocopherol and supplementation has shown to significantly decrease lipid peroxides in plasma with a concomitant increase in *α*-tocopherol content in plasma [6] and in muscle [22]. In the present study, supplementing vitamin E in the form of DL powder did result in increases in plasma levels after 8 weeks, however, the mean increases between the groups were not statistically different. DL supplemented groups also had the lowest levels of TBARS after 8 weeks. This finding may be associated with the PUFA and vitamin E content of the diet. Increased unsaturation of dietary lipid, increases susceptibility to lipid oxidation *ex vivo* thus increases in plasma lipid peroxides are expected with increasing dietary lipid unsaturation. Contrary to this association, greater decreases in plasma lipid peroxides and increase in glutathione were observed in diets III, IV and V, which con-

tained drumstick powder. Supplementation with vitamin E might have resulted in this beneficial effect. This reiterates the fact that requirements of vitamin E are associated with PUFA content [23]. These data suggest that dietary vitamin E supplementation may be more efficient antioxidant in rats fed peanut oil based diets. The overall lipid oxidation process may not depend solely on the fatty acid composition of the oil, but also on the content of antioxidants, such as vitamin E and carotenoids, found naturally in drumstick leaves. Dietary supplementation of vitamin E did not result in significant differences in plasma levels of vitamin between the groups maybe attributed to the fact that the supplementation was not at supra-physiological level. In this study, vitamin E was added at twice the level in the experimental diets compared to the control diet and was derived from a natural source. Studies reported in the literature have used pure isolated forms of antioxidants for supplementation [6, 9,22]. This study differs from others as it explores the supplemental utility of a natural source of vitamin E (drumstick leaves) on plasma lipid profile and antioxidant status in rats.

5. Conclusion

In conclusion, supplementation with dietary vitamin E as beneficial as it reduced plasma cholesterol and lipid peroxidation and raised vitamin E levels. Recent reports have suggested that vitamin E is not the only antioxidant responsible for inhibiting lipid oxidation [23,24]. This indicates that other natural antioxidants derived from dietary intake could also be involved in the prevention of lipid oxidation. In this context, drumstick leaves appear to possess "a package of natural antioxidant" compounds such as vitamin E, C, carotenoids and polyphenols, deserves further evaluation as potential antioxidant agent. Consumption of foods containing a variety of compounds with antioxidant activities has greater nutritional significance in the management of hyperlipidemia.

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