

## Serum lipid profile and retinol in rats fed micronutrient rich edible vegetable oil blend

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**Abstract.** Khan HN, Farooqi H, Ali S, Khan JS. 2010. Serum lipid profile and retinol in rats fed micronutrient-rich edible vegetable oil blend. *Nusantara Bioscience 2: 109-116*. The animal rats were given 10% oil mixed in fat-free diet for one month or six months. In the experiment, the groups of rats were fed with the micronutrient (MN) rich blends mixed previously with 1% cholesterol, and their effects were tested on serum lipid profile. Most significant changes in the High Density Lipoprotein (HDL) cholesterol were observed in one-month study where HDL increased from 24 mg/dl in group to 64 mg/dl in the Mustard palm olein oil blend (MP); in mustard oil (MO) alone fed rats, the HDL was 36 mg/dl. Serum retinol was analyzed as one of the important MN in rats receiving the diet mixed with the blend for various duration of time. The results assume great significance as MO or palm olein oil (PO) alone could not bring the maximum beneficial effects, and the blends appear to have more merit as healthy oils in alleviating adverse health condition such as coronary heart disease (CHD), diabetes, obesity, and hypertension.

**Keywords:** mustard oil, palm olein oil, oil blend, lipid profile, micronutrient, retinol, diabetes, hypertension, coronary heart disease.

**Abstrak.** Khan HN, Farooqi H, Ali S, Khan JS. 2010. Profil serum lipid dan retinol pada tikus yang diberi makanan yang dicampur minyak sayur yang kaya mikronutrien. *Nusantara Bioscience 2: 109-116*. Tikus percobaan diberi minyak 10% dicampur dalam diet bebas lemak selama satu bulan atau enam bulan. Dalam percobaan, kelompok tikus diberi makan yang kaya mikronutrien (MN) dicampur dengan kolesterol 1% sebelumnya, dan efeknya diuji pada profil serum lipid. Sebagian besar perubahan signifikan dalam High Density Lipoprotein (HDL) kolesterol teramati dalam penelitian satu bulan, dimana HDL meningkat dari 24 mg/dl menjadi 64 mg/dl pada campuran minyak mustard-kelapa sawit olein (MP); pada tikus yang hanya diberi minyak mustard (MO) saja, nilai HDL adalah 36 mg/dl. Serum retinol dianalisis sebagai salah satu mikronutrien penting pada tikus yang menerima diet dengan campuran minyak pada berbagai durasi waktu. Hasil menunjuknya secara signifikan bahwa pemberian mustard MO atau kelapa sawit olein (PO) saja tidak membawa efek yang menguntungkan secara maksimum, adapun campuran keduanya tampaknya memberikan manfaat lebih tinggi untuk mengurangi kondisi yang merugikan kesehatan seperti penyakit jantung koroner (PJK), diabetes, obesitas dan hipertensi.

**Kata kunci:** minyak mustard, minyak kelapa sawit olein, campuran minyak, profil lipid, mikronutrien, retinol, diabetes, hipertensi, penyakit jantung koroner.

### INTRODUCTION

Fats and oils are integral to our diets, and are important sources of calorie density in the diet. Besides, they provide essential fatty acids and increase the absorption of fat-soluble vitamins. Oils such as palm olein oil and rice bran oil are considered as good sources of micronutrients (MN) that include  $\beta$ -carotene, tocopherols, and tocotrienols that are known for their beneficial actions in human. Tocotrienols, for example, have been reported for lowering the serum cholesterol level in hypercholesterolemic subjects (Qureshi et al. 1991a) and have been recommended for metabolic disorders such as coronary heart disease (CHD), diabetes, obesity, and hypertension. The MN present in oils are also recognized for a number of other beneficial effects on body, and have been suggested effective in conditions as wide as cancer and kidney stones, to name a few. However, in spite of a high MN content, the

use of unconventional but MN rich oils is limited mainly because of the regional preferences of specific individual oils. In the following section, the effect of three MN rich blends of edible vegetable oils on serum lipid composition and the retinol level is presented. The study is important, as limited data are available on the effects of oil blends on the physiological systems, although extensive research has been conducted on many conventional and unconventional vegetable oils. The oils rich in PUFA, for example, cause a decrease in 'bad' cholesterol and triglycerides. Rice bran oil and palm oil are particularly rich in many MN that have been reported for health benefits (Tomeo et al. 1995). The impact of palm oil on cardiovascular disease and cancer has been investigated (Elson and Qureshi 1995). Tocotrienols from the palm olefin oil inhibit protein oxidation and lipid peroxidation in rat liver microsomes (Kamat et al. 1997). The  $\omega$ -3 fatty acids (linolenic acid) in oils can increase the level of circulating good cholesterol.

These and other such findings encouraged us to use the unconventional MN rich oils for preparing various blends that are being tested in this study on experimental animals such as MP vegetable oil blends.

## MATERIALS AND METHODS

### Materials

Refined vegetable oils such as palm olein oil and expeller-pressed, unrefined mustard oil (MO) were purchased from the local market of Alaknanda market, New Delhi, India; obtained from the manufacturers, and used before the 'best before date'.

### Preparation of oil blends and analysis

A binary blend of the MP represents mustard oil - palm olein oil blend (35:65) in proportions. The blends marked with a superscript 'C' denote the oil blend in which 1% cholesterol was mixed. The blending was based on the fatty acid composition of the oil, which were mixed in such a way that an ideal fatty acid profile was obtained in the blends. Animals were fed the experimental diet and boiled and purified water on an ad libitum basis; the composition of fat-free diet provided to the animals during the study period is provided in Table 1.

**Table-1:** The composition of fat-free diet

Ingredients	Fat free diet (g/100 g diet)
Casein	15.0
Starch	66.3
Salt mixture	04.0
Vitamin mixture	01.0
Cellulose	02.0
Cholesterol	01.0
Cholic acid	00.5
Choline chloride	00.2

Note: **Salt mixture contained:** 4.6% NaCl; 9.3% Na<sub>2</sub>HPO<sub>4</sub>.H<sub>2</sub>O; 25.6% K<sub>2</sub>HPO<sub>4</sub>; 14.5% CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>.H<sub>2</sub>O; 3.3% Fe(C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>).5H<sub>2</sub>O; 34.9% Ca(C<sub>3</sub>H<sub>5</sub>O<sub>3</sub>)<sub>2</sub>.5 H<sub>2</sub>O; 7% MgSO<sub>4</sub>; 0.05% ZnSO<sub>4</sub>.7H<sub>2</sub>O; 0.9% KI; 0.02% Cr(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>3</sub>. **Vitamin mixture contained:** 150 mg riboflavin; 100 mg thiamine; 1000 mg nicotinic acid; 100 mg pyridoxine; 1 mg cyanocobalamin; 500 mg pantothenic acid; 50 mg folic acid; 3750 mg ascorbic acid; 100 mg vitamin K; 100 mg vitamin E; 2,50,000 IU vitamin A; 20,000 IU vitamin D, and starch to make up to 100.

### Chemicals

Authentic standards of tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) and  $\beta$ - carotene were purchased from E. Merck (Germany) and fatty acid methyl esters from Sigma Chemical Company, USA. HPLC grade methanol, acetonitrile, water, n-hexane, alcohol, BF<sub>3</sub>, and isopropanol were procured from Merck (India). Other chemicals and reagents were of analytical grade and were obtained from standard commercial sources in India.

### Measurement of serum lipid profile

Male rats weighing 160-190 g were divided into groups with each group consisting of 6 rats. Three groups were fed 10% oil/blend in diet for one month or six months before analyzing the serum lipid profile. In yet another group, the rats were fed similarly but a high cholesterol diet (HCD). Animals were maintained in individual cages and supplied water and diet ad lib. Daily food intake and weekly body weights were recorded. Following the completion of the tenure of feeding, the animals were sacrificed and 4 mL of blood collected from each animal by cardiac puncture. The liver was dissected, weighed and the liver weight of each animal was calculated as percentage of body weight of each animal calculated. 1 gm of liver from each animal was homogenized in a buffer and total cholesterol (TC), triglycerides (TG), HDL-C LDL + VLDL-C were measured from the serum. The level of triglycerides, cholesterol, and other lipid moieties in rat serum was estimated using the diagnostic kits. Serum triglycerides were hydrolyzed to glycerol and free fatty acids by lipase. The intensity of the color developed was proportional to the triglycerides concentration and was measured spectrophotometrically at 546nm (530 to 570nm) or with Green filter. Normal cholesterol levels are affected by stress, age, hormonal balance, and pregnancy. The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (red quinone), which is measured at 500 nm.

### Determination of serum retinol by HPLC.

The technique has several advantages over other available techniques for retinol estimation, and is a rapid micro procedure (Bieri et al. 1979). We have used this method for assessing the status of retinol in animals given the oil blend on a Shimadzu (model LC-10ATvp) HPLC equipped with a binary gradient and a multiple wavelength detector (SPD-10Avp) and C-18 column (alpha Bond C18 125A 10 $\mu$ m300 x 4.60mm) protected by a guard column was used. The system was operated using SPINCHROM software. Methanol/HPLC water (95/5) was used as a mobile phase, and the flow rate was set to 1.2 mL/minute. Retention time of retinol was 6.80 minutes at this flow rate in the C-18 column used in this study. In the injector of the machine, 20  $\mu$ l aliquot (from serum) was injected and the results were calculated with the help of an internal standard added to the sample before injection. Detection wavelength was 326 nm.

### Statistical analysis

Data were analyzed by ANOVA to ascertain if the dietary treatments were a source of variance related to various lipid parameters measured. Significance was accepted at the  $p < 0.05$  levels (Snedecor and Cochran 1990).

## RESULTS AND DISCUSSION

The effect of dietary fats on serum lipids and MN profile has long been recognized (Rukmini and Raghuram

1991; Manorama et al. 1996). Public health programs for the prevention of coronary heart diseases (CHD) in developed nations recommend changes in dietary habits, especially in the quality and the quantity of fats. The American Heart Association recommends (AHA Medical/Scientific Statement 1990) that total fat in diet should not exceed 30% of energy calories, out of which the energy percent from saturated fatty acids (SFA) should not exceed 8-10%, PUFA (polyunsaturated fatty acid) 10 energy percent, and monounsaturated fatty acids (MUFA) by difference (that is 10 - 12%). The recommended dietary allowances (RDA) for a balanced diet as per the ICMR Nutrient Requirements and RDA guidelines for Indians (ICMR Expert Group Report: 1989; ICMR 1990) suggest that fat in diet should not exceed 20 energy% with 3 energy% derived from PUFA. In 1985, Lasserre et al reported that linolenic acid (18:3), an essential polyunsaturated fatty acid, should provide 0.5-1% of total calories. These and various other recommendations were made to ensure optimum serum lipid composition in human for nutritional and health benefits. Studies have revealed that the type of fatty acid in diet is critical. Replacement of dietary saturated by unsaturated fatty acids, for example, is a very effective way of lowering serum cholesterol. A desirable serum lipid composition in human is the one, which has low cholesterol (<180 mg/dl), low triglycerides (<100 mg/dl), low LDL-cholesterol and high HDL-cholesterol (Ausman et al. 2005).

Unconventional oils such as rice bran oil, soyabean oil, cottonseed oil, and palm olein oil have been used in suitable proportions for nutritional, health and other benefits as per the consumer's acceptability and market demands. These oils are preferred over other oils for blending for their micronutrient content and economic reasons. Rice bran oil, for example, is well known for its hypocholesterolemic action both in animals and in humans. With the fatty acid composition resembling groundnut oil, rice bran oil has limited PUFA, which provides additional health benefits. Rice bran oil contains oleic acid (38.4%), linoleic acid (34.4%), and linolenic acid (2.2%) as unsaturated fatty acids. Palmitic acid (21.5%) and stearic acid (2.9%) are the saturated fatty acids in rice bran oil. Its unsaponification matter (4.2%) consists of several micronutrients including total tocopherols (81.3mg/g),  $\gamma$ -oryzanol (1.6mg/g) and squalene (320 mg/g). The MN of rice bran oil namely  $\gamma$ -oryzanol and phytosterols help to reduce the triglyceride and elevate the HDL. In studies on the hypocholesterolemic action of rice bran oil (Seetharamaiah and Chandrasekhra 1989), addition of oryzanol at 0.5% level to the diet containing rice bran oil has been observed to significantly decrease the serum total cholesterol.

Similarly, unconventional oil in India, the palm olein oil, is a rich source of  $\beta$ -carotene and tocotrienols. Dietary tocotrienols reduce the concentrations of plasma cholesterol, apolipoprotein B, thromboxane B<sub>2</sub>, and platelet factor 4 in pigs with inherited hyperlipidemias (Qureshi et al. 1991b). These and other facts have prompted researchers to use unconventional oil for health, economic and industrial benefits as the presence of a large amount of MN and antioxidants in selected unconventional

oils confer nutritional benefits and oxidative stability to the blends prepared from these oils. In 1984, Suzuki et al. have demonstrated that rice bran oil (RO) in combination with safflower oil (SFO) that has high PUFA, can significantly bring down the serum cholesterol to normal values within 8 days. A study in human volunteers feeding a blend of RO: SFO could effectively bring down the cholesterol below 120 mg/dl in a week. Safflower oil is rich in PUFA (75%) and there are several reports available on the adverse effects of PUFA (Grundy 1975). SFO, when fed to animals or human, reduces serum total cholesterol but at the same time, it lowers the HDL levels. On the other hand, rice bran oil does not only significantly reduce total cholesterol, triglycerides, LDL, and VLDL levels but also significantly increases the HDL, which is an important benefit of blending SFO and RO.

Hypothesizing the health benefits of the constituents (MN plus fatty acids) present in rice bran oil and palm olein oil, and the traditional acceptability of mustard oil, we prepared the MN rich binary edible vegetable oil blends that were expected to have nutritional and health benefits particularly with regard to the serum lipid and MN composition. The oil blend was fed to rats in diet strictly in accordance with the procedure described in the methods section earlier, and the serum lipid profile was studied following a six-month or a one-month treatment period.

### Long-term feeding

#### *Serum cholesterol and triglyceride*

The results of the six-month study of feeding the oil/blend mixed in diet to rats have been summarized in Table 2. As shown in Table 2, total cholesterol in control animals receiving normal diet was 54 mg/dl. The level of cholesterol in rats receiving the MP group it was 52 mg/dl. Incidentally, this blend was the richest as far as the MN level of all the blends used in this study is concerned, and had a fatty acid composition close to the recommended international standards. As described earlier, presence of unsaturated fatty acids in diet is a very effective way of lowering serum cholesterol. An intriguing hypothesis to explain this decrease takes into account the kinks in unsaturated fatty acids; the lipid esters containing PUFA require more space in lipoproteins and thereby sterically exclude cholesterol. Because a PUFA occupies a greater spatial area, the LDL can carry fewer lipids and their lipid content is thereby lowered. PUFA would cause a drop in the lipoprotein concentration and the VLDL synthesis that would be responsible for the reduced LDL synthesis (Lewis et al. 1961). Further, the unsaturated fats cause plasma cholesterol to be transferred to the tissue pools, and the cholesterol transferred into the liver could cause a temporary depression of lipoproteins (Spritz and Mishkell 1965).

Lipid-lowering action of rice bran oil has been extensively studied (Rukmini and Raghuram 1991). In fact, edible grade crude palm oil (*Elaeis guineensis*) is one of the richest natural sources of  $\beta$ -carotene (7.5 mmol/L). In the present study, we have used refined palm olein oil that retains substantial quantity of  $\beta$ -carotene.

The mustard oil based blends particularly the MP blend could cause a reduction in the level of cholesterol particularly in the group of rats with cholesterol overload (1% cholesterol mixed in the blend). In a six-month study on MP fed blends, the cholesterol level in 46 in MP<sup>C</sup>. These values were well below the levels seen in the normal control rats (54 mg/dl) and also lower than the values observed in mustard oil alone treated animals (47 mg/dl). The values in MP groups alone were 52 mg/dl, respectively. The MP blend (without cholesterol supplementation) appears to be not effective in bringing down the cholesterol level. However, in cholesterol-supplemented group, there was some lowering of cholesterol in MP<sup>C</sup> fed rats in the six-month study. A possible explanation might be a feedback effect due to a long-term feeding of cholesterol on cholesterol biosynthesis involving control via HMG Co-A reductase (Yung et al. 2010)

#### Lipoprotein

LDL, VLDL, and HDL play important role in CHD. Accumulation of oxidized low-density lipoproteins in macrophages and smooth muscle cells causes foam cell formation - an initial step in atherosclerosis (Ozer et al. 1995). The results of the analysis of serum for LDL, VLDL, and HDL are presented in Table 2. In the six-month study, the LDL values in all the groups were higher than the rats fed normal diet. This was expected as the gravimetric analysis for determining the lipid content of the normal diet showed that the amount of oil in normal diet (as extracted by hexane) was less when compared to the groups of rats supplied with the single oil or the blend. To the blend fed as well as the single oil fed rats, 10% oil mixed with fat-free diet was supplied. In the normal control rats (with usual diet), the LDL level was 12 mg/dl. In palm olein oil and mustard oil fed rats, the values were - respectively - 15, and 19 mg/dl. Significantly, the mustard oil based blends performed much better, and more interestingly, in the palm olein oil containing blend of mustard oil (MP blend), the value was lowest (9 mg/dl). In MP<sup>C</sup> fed blends, the values were 36 mg/dl, respectively. This increase of LDL in cholesterol-supplemented rats needs some explanation. In this group, cholesterol-supplementation resulted in lowering of the LDL value (Table 2). The hypolipidemic action of rice bran oil seems to be an obvious reason.

With regard to the values for VLDL, palm olein oil treated rats showed lowest VLDL levels (approximately, 15 mg/dl in each group). In single vegetable oil fed rats, VLDL was highest in mustard oil group (33 mg/dl). Among blends, the MP blend also showed higher VLDL value (33 mg/dl). On the contrary, the value in MP<sup>C</sup> blend was 20 mg/dl. As shown above, palm olein oil group had the lowest LDL as well as VLDL (15 mg/dl). Thus, the MN present in palm olein oil might have contributed towards the low value of VLDL in the MP<sup>C</sup> group. HDL-cholesterol is an important scavenger of surplus cholesterol transporting it from the cell membrane to the liver where it is metabolized or converted into the bile acids. In mustard oil and palm olein oil alone treated rats, the values were 32 and 33 mg/dl respectively. In all groups in this study (except for the cholesterol loaded groups), the HDL levels were higher than the value reported from the group fed control diet (29 mg/dl). As expected, in the group of rats supplied with extra cholesterol (1% mixed in the blend), the HDL values were lower in comparison to the increased values observed following the non-cholesterol blend feeding. Respectively, the values were 26 mg/dl for MP<sup>C</sup>. The obvious reason for this is the increased cholesterol load.

Briefly, six-month study results suggest that in comparison to the control values. On the other hand, the MP blend resulted in a decrease in HDL and did not affect the cholesterol level. MP blends also increased triglycerides. In cholesterol-supplemented groups, the three blends could lower the total cholesterol, but had no beneficial effects on HDL. Triglycerides decreased in MP<sup>C</sup> rats.

#### Body weight

The effect of feeding fat on body weight is well established. Results presented in Table 2 depict the effect of a six-month feeding study of various oils (mixed with diet) on the animal body weight. The increase in the body weight of rats fed palm olein oil and mustard oil alone was 80 and 59 mg/dl, respectively, after six months. In MP it was 79 mg/dl. In <sup>C</sup> supplemented groups viz. MP<sup>C</sup>, the increase in body weight was also least. In MP<sup>C</sup> it was 66 mg/dl. Presence of mustard oil in the blend appears to have a beneficial effect on health with regard to the body weight increase. This has implication in patients with lifestyle diseases - diabetes and cardiovascular disease.

**Table 2.** The effect of long-term feeding of the MN rich blend on serum cholesterol and triglyceride levels in rats and the animal body weight.

Oil	Serum(mg/dl)		Lipoprotein (mg/kg)			Body weight(g)		
	Cholesterol	Triglyceride	LDL	VLDL	HDL	IBW	FBW	Weight gain
Control	54.17 ± 19.00	124.6 ± 21.3	12.0 ± 4.50	24.9 ± 4.27	29.0 ± 10.1	n.a	n.a	n.a
MO	48.68 ± 06.70	166.4 ± 14.1	19.2 ± 10.7	33.3 ± 10.2	32.0 ± 06.0	265.0	420.0	155.0 (59%)
PO	42.25 ± 03.20	75.7 ± 30.23	14.8 ± 9.70	15.2 ± 5.2	33.6 ± 9.12	275.8	495.2	219.4 (80%)
MP	52.50 ± 10.80	166.6 ± 51.30	8.71 ± 5.00	33.4 ± 5.2	23.5 ± 4.35	278.3	498.2	219.9 (79%)
MP <sup>C</sup>	46.40 ± 13.50	98.2 ± 38.20	22.2 ± 5.30	19.7 ± 5.3	25.78 ± 9.6	305.8	510	204.2 (66%)

Note: 1.5 mL of oil was administered to each rat, which corresponds to 10% oil in diet. <sup>C</sup> represents the blend containing 1% cholesterol. Male rats were used in this study for six months. The values in bracket indicate percent increase. IBW: Initial body weight, FBW: Final body weight.

### Short-term feeding

#### *Serum cholesterol and triglyceride*

The results of one-month study are presented below. These data do not truly reflect the picture produced by the six-month study protocol; although in this study also the level of serum cholesterol was reduced following palm olein oil feeding, the blend fed rats reported values that do not show much variation. Serum cholesterol could be reduced to a significantly low level (Table 3). In fact, a study in normocholesterolemic human volunteers (Sanders and Reddy 1992) has shown that consumption of realistic amounts of rice bran oil does not contribute to a reduction in plasma cholesterol concentration in men consuming the oil for three weeks.

Feeding mustard oil for one month could slightly decrease serum total cholesterol (79 mg/dl versus 74 mg/dl). In MP rats, the value was 77 mg/dl that increased to 88 mg/dl in MP<sup>C</sup> rats (Table 3). The possible reason for the discrepancy could be the short duration of treatment that did not allow the physiological system to adjust to the new dietary habit that ensured that 10% oil blend must be supplied in diet.

The triglyceride levels measured in rats fed blend for one month were found to be lowest in palm olein oil rats. In MO rats the value was comparable to the control, which was not the case in the six-month study. In six-month study, lowest triglyceride values were observed in the MP<sup>C</sup> blends (Table 3). In one-month study, the values in these groups were also less than the value in mustard oil group (214 mg/dl) but only marginally. In case of triglyceride levels, the cholesterol-supplemented rats reported lesser values when compared to the animals fed with the blend without cholesterol (Table 3). For MP<sup>C</sup> group, the values for triglycerides were 209 mg/dl. In MP groups, triglyceride values were 284 mg/dl, respectively. The results suggest that one-month feeding of blends could bring down the level of triglycerides in animals supplemented with cholesterol, which has nutritional and health implications as described above.

#### *Lipoprotein*

With respect to the level of VLDL in one-month study, the highest level was observed for the MP blend (Table 3). For palm olein oil it was 22 mg/dl; the control value for VLDL was 48 mg/dl. Among all the blends, the VLDL value was lowest in MP<sup>C</sup> blend. In MP<sup>C</sup> rats, VLDL levels were 42 mg/dl, respectively. In MP rats, these values were higher and were reported to be 45 mg/dl (respectively). VLDL synthesis has been associated with LDL. A depressed VLDL synthesis is concluded to be responsible for the reduction of LDL synthesis (Lewis et al. 1961). The data were consistent with others. In the blend fed rats (except for the MP<sup>C</sup> rats), the LDL was around 41 mg/dl. This is interesting because, in cholesterol supplemented rats, VLDL values were more in comparison to the MP groups. In case of LDL, the values in different groups were quite similar (with the exception of MP<sup>C</sup>). In MP<sup>C</sup>, depressed VLDL could easily be associated with decreased LDL.

In mustard oil and palm olein oil alone rats, the values were almost similar (35 mg/dl). Among the blends, mustard based blend showed significant rise in HDL in one-month study; here, the values were 64 mg/dl for MP, respectively. In rats fed blend mixed with 1% cholesterol for one month, HDL values were 32 mg/dl, respectively, for MP<sup>C</sup>.

Comparison of one-month and six-month study data on serum lipid profile showed that while in one-month study HDL decreased in palm olein oil alone and mustard oil alone rats, in six-month study, HDL increased in all groups (palm olein and mustard oil fed groups). Cholesterol did not change in mustard oil fed rats; it decreased in rats given palm olein oil for one-month. Triglyceride was low in all groups (mustard oil and palm olein oil) after one-month treatment, and remained low in palm olein oil rats following six-month treatment; it increased in mustard oil-fed rats. In six-month study, cholesterol, however, decreased in all groups receiving single oil. However, after six-month MP caused a slight decline in HDL. MP groups after one month, but after six months, the level was unaffected in MP. In six-month study, triglyceride increased in MP. Triglyceride also in one-month feeding was unchanged in MP. However, in six-month feeding study, cholesterol decreased in this group; triglyceride remained unchanged. In MP<sup>C</sup> group of rats, HDL decreased after one-month period and cholesterol increased. After six-month, HDL in this group was normal (with respect to control) and cholesterol reduced. Triglyceride decreased in MP<sup>C</sup> following six-month feeding. However, in rats receiving cholesterol-supplemented blends in diet for one month, triglyceride was low in all. All values discussed here are in comparison to the control rats fed normal diet in which no oil was mixed. The results suggest superiority of the blends over single edible vegetable oils. An important point to be noted while comparing the values of similar parameters in one-month and six-month study experiment is the difference in actual values for the same parameter. These differences were due to the kits that were supplied by different manufacturers. The two studies were performed at different times.

#### *Body weight*

The results depicting the change in the body weight of rats fed the oil and the blends are presented in Table 3. When compared to the increase in body weight in rats fed mustard oil alone mixed in fat-free diet (50 mg/dl). Among the groups exhibiting highest increase in body weights following oil administration in diet for one month were MP<sup>C</sup> group (67 mg/dl) and the palm olein oil group (61 mg/dl). In other blend fed rats the values were comparable among themselves. Overall, the results displayed for the six-month study.

As described earlier in this text, the blend of palm olein oil and mustard oil, palm olein oil have been found effective in influencing the serum lipid profile of rats fed the oil mixed in diet. The blend also appeared quite effective in bringing down the serum lipid profile values when the rats were loaded with cholesterol (1% in the blend) in a one-month or a six-month long feeding study. It not only consistently bring down the cholesterol,

**Table 3.** The effect of short-term feeding of the MN rich blend on serum cholesterol and triglyceride levels in rats and the animal body weight.

Oil	Serum(mg/dl)		Lipoprotein (mg/kg)			Body weight (g)		
	Cholesterol	Triglyceride	LDL	VLDL	HDL	IBW	FBW	Weight gain
Control	78.93 ± 17.24	238.1 ± 40.00	23.87 ± 8.3	47.63 ± 7.9	38.54 ± 15.9	n.a	n.a	n.a
MO	74.00 ± 11.60	214.0 ± 37.50	22.14 ± 3.7	42.80 ± 7.5	35.75 ± 11.9	236.2	354.0	117.8 (50%)
PO	60.89 ± 19.22	29.0 ± 33.36	22.37 ± 4.6	45.80 ± 2.3	34.62 ± 07.0	216.3	348.7	132.4 (61%)
MP	77.01 ± 13.37	225.9 ± 44.56	42.50 ± 9.2	45.18 ± 2.4	64.37 ± 15.83	268.0	395.0	115.0 (47%)
MP <sup>C</sup>	88.21 ± 06.18	209.2 ± 43.80	23.87 ± 8.3	41.90 ± 4.1	32.08 ± 06.32	305.8	510.0	204.2 (67%)

Note: 1.5 mL of oil was administered to each rat, which corresponds to 10% oil in diet. <sup>C</sup> represents the blend containing 1% cholesterol. Male rats were used in this study for one month. The values in bracket indicate percent increase. IBW: Initial body weight, FBW: Final body weight.

triglycerides and other biochemical changes, but also found to be the best edible oil blend with respect to the increase in body weight in both long-term as well as short term studies.

The blends capable of lowering cholesterol and triglycerides have health benefits. Diet influences the risk factors for CHD, which include the elevated levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), and serum triglycerides and reduced levels of high-density lipoprotein cholesterol (HDL-C). The fat content in diet readily modulates these risk factors. A high intake of saturated fatty acids and cholesterol in diet may lead to hypercholesterolemia, largely through an increase in LDL-C. On the other hand, polyunsaturated fatty acids have a hypocholesterolemic effect in human. A large number of studies contributing to the polyunsaturated hypothesis have used diets that provide dietary fat calories derived almost entirely from the polyunsaturated fatty acids. Contribution of energy from saturated fatty acids was hence almost negligible. This created an artificial dietary environment that is seldom applicable in daily life. Although the hypocholesterolemic effect of the polyunsaturated can often be observed in a high-fat diet, its effects are less clear in a low-fat diet. The mono-unsaturated, long regarded as neutral in its effect on serum and lipoprotein cholesterol, can also lower cholesterol. Besides this, as described elsewhere, MN such as oryzanol, tocotrienols, etc. exert direct effects on cholesterol synthesis. Presence of these micronutrients in blends can account for their beneficial effects on serum lipid profile (Amos 2007).

#### Effect of blend feeding on serum retinol

Retinol is an important lipid-soluble micronutrient in animals that can be measured in serum. A molecule is a form of vitamin A that is formed in the body using  $\beta$ -carotene as a precursor. Its level in the blood is dependent on the type of food consumed. Crude palmolein oil is one of the richest natural sources of  $\beta$ -carotene and is cheaper than the conventional edible vegetable oils in India, although most of the carotenoids are lost during refining. Nutritional evaluation of crude palm oil has been done in rats (Manorama and Rukmini 1991), and no adverse effects could be observed as judged by the growth rate, feed—efficiency ratio, protein—efficiency ratio, net protein utilization, digestibility, fat absorption, nitrogen balance,

phosphorous and calcium retention, serum enzymes, and hematology. These parameters were comparable to the control values. Palm oil feeding could cause an increase in serum retinol that has been well documented. In this study, we have tested serum retinol in rats that were fed the three binary blends prepared using mustard, palm or rice bran oil in specific proportions. Table 4 depicts the results of a study on serum retinol estimated in rats receiving the oil at a dose level of 1.56 g per day. Measurement of serum retinol after one-month feeding revealed higher levels in all groups in comparison to the control rats (30  $\mu$ g/dl). While in palm olein oil, it was 45  $\mu$ g/dl. Lower value in palm olein oil rats could be due to the use of refined palm oil; losses of  $\beta$ -carotene due to refining process have been reported. This statement is supported from the  $\beta$ -carotene level in palm olein that has been discussed in earlier reports on the MN level in various oils. Unpredictably the values in palm oil rats were similar to the mustard oil fed rats (44.46 and 45.50  $\mu$ g/dl) following one-month feeding. When the blends were compared, MP reported the highest increase (46  $\mu$ g/dl); in MP<sup>C</sup> also, the value was 46  $\mu$ g/dl. Overall, one-month study results suggest that MP or MP<sup>C</sup> rats had the highest retinol level in all blends, and the blend was found to be effective in both normo- and hypercholesterolemic rats. Cholesterol seems to have no effect on serum retinol level in blend fed animals.

**Table 4:** Serum retinol level in rats receiving oil blend in diet for various duration of time.

Group	30 days	180 days	240 days
Control	30.30 ± 5.62	37.00 ± 5.68	37.19 ± 6.63
MO	44.46 ± 4.88	42.73 ± 6.16	48.28 ± 8.82
PO	45.50 ± 10.4	37.17 ± 6.70	37.17 ± 6.60
MP	45.89 ± 16.9	43.81 ± 2.07	43.81 ± 2.07
MP <sup>C</sup>	46.10 ± 8.05	47.98 ± 9.72	47.98 ± 9.72

Note: Values are expressed as mg/dl (Mean ± S.D.). The oil was fed to rats at a dose level of 1.56 g (1.75 mL) per animal per day that is equivalent to 10% daily oil requirement. The group denoted by superscript <sup>C</sup> has 1% cholesterol dissolved in oil.

Following six-month feeding study, retinol level increased in control rats from 30  $\mu$ g/dl to 37  $\mu$ g/dl. This value sustained when measured following 8 months feeding study. In palm olein oil group, retinol level was

brought down to 37 µg/dl from 45 µg/dl reported in one-month study. Of particular interest is the fact that after six-months, retinol in control and palm olein fed rats was exactly similar. A simple but valid explanation can be the loss of β-carotene in the palm olein rats. It should be noted that the same batch of palm olein was used throughout this study. The higher retinol level observed in 30 days study could be due to higher levels of β-carotene in fresh oil; β-carotene deteriorated on subsequent storage. Interestingly, in mustard fed animals the level reported in one-month study were maintained for six-months, and following 240 days (8 months) feeding, slight increase in retinol could be found. In MP, the value decreased slightly. In cholesterol-supplemented rats, initial values were maintained until the end of the study, and cholesterol does not seem to affect the serum retinol level in blend fed rats.

### CONCLUSION

The present study demonstrated the substantial hypolipidemic action of PO blends which may be due to their minor components. These minor components appear to play a major role in reducing the level of circulating lipids in rats. They appear to lower TC, LDLC, TG levels. The cholesterol-lowering effect of PO has been attributed to tocotrienols and other components of the unsaponifiable matter. Tocotrienols inhibit endogenous synthesis of cholesterol through the HMGCOA reductase. It can be suggested that PO blends should be considered for edible purposes in spite of the high unsaponifiable matter, which may confer some benefit. Comparison of two blends among themselves revealed a maximum increase in MP rats; in MP<sup>C</sup> also, the value was similar. Overall, one-month study results suggest that MP or MP<sup>C</sup> rats had the highest retinol level in all blends, and the blend was effective in both normo- and hypercholesterolemic rats. The effect of feeding the individual blend in diet to rats and to measure serum lipid profile and serum micronutrient retinol. The blends of PO with these oils not only bring about a favorable circulating lipid profile but may also result in economic advantage of lower prices as PO is cheaper oil than SFO and SNO in the current retail market in India.

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