CHANGES IN SERUM LIPID PROFILE AND MALONDIALDEHYDE FOLLOWING CONSUMPTION OF FRESH OR HEATED RED PALM OIL

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SUMMARY: The effect of chronic consumption of fresh and heated red palm oil (RO) on lipid profile and lipid peroxidation was investigated. Thirty male rats were divided into 3 groups, each treated with the following prescribed food: (i) basal diet fortified with 15% weight/weight (w/w) fresh red palm oil (FRO), or (ii) heated once red palm oil (1H-RO) or (iii) heated 5 times red palm oil (5H-RO) for 20 weeks. There was a significant increase ($p<0.05$) in MDA concentration in all 3 groups compared to their respective baseline concentrations and a significant decrease ($p<0.05$) in total cholesterol (TC) concentration. However, the transient changes observed in serum triglyceride and HDL-cholesterol concentration did not attain significant values.

The LDL-cholesterol concentration in 5H-RO group increased significantly ($p<0.05$) compared to pretreatment value, FRO and 1H-RO groups. Ratio of TC/HDL in 5H-RO group initially increased, but dropped to baseline level at the end of the study. There was no significant difference in the ratio of TC/HDL between the groups.

In conclusion, both fresh and heated RO appeared comparable in their effect on serum cholesterol and lipid peroxidation. It appears that long term feeding with fresh and heated RO did not have an adverse effect on serum TG, HDL and TC/HDL ratio. However, it appears that prolonged heating increases LDL-cholesterol level. Further studies are required to ascertain whether the increase in LDL-cholesterol and MDA with heated oil would render it more atherogenic.

Key Words: Heated red palm oil, lipid profiles, lipid peroxidation.

INTRODUCTION

The association among diet, plasma lipid concentrations and atherosclerosis has been well documented and reviewed (1). Atherosclerotic lesions in men and in animals appear to be related to elevated total cholesterol (TC), LDL-cholesterol (LDL-C), decreased HDL-cholesterol (HDL-C) and excess fat consumption. Plasma lipid levels are not only influenced by the amount of fat consumed but by its nature as well. Hegsted *et al.* (2, 3) and Keys *et al.* (4) demonstrated that the saturated fatty acids were twice as cholesterol raising as the polyunsaturated (PUFA).

Palm oil is a major source of the world supply of oils and fats. Palm oil contains an equal proportion of saturated and unsaturated fatty acid, with about 44% palmitic acid, 5% stearic acid (both saturated), 40% oleic acid (monounsaturated), 10% linoleic acid and 0.4% α-linoleic

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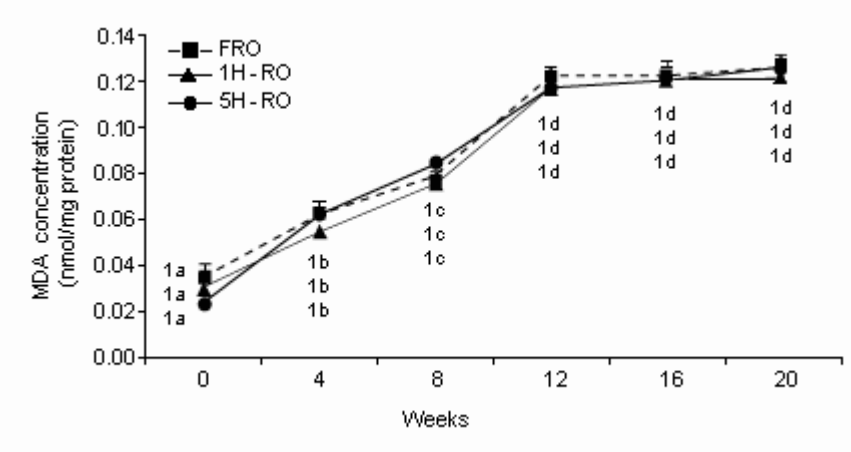
Figure 1 : Effect of fresh and heated red palm oil on serum MDA (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



acid (both polyunsaturated) (13). Palm oil is a rich source of tocopherols, tocotrienols and carotenoids. Tocopherols and tocotrienols are natural antioxidants. These substances act as scavengers of damaging oxygen free radicals that have been suggested to play an important role in aging, atherosclerosis and cancer (14,15). Carotenoids can function as provitamin to vitamin A of which β-carotene is the most nutritionally active carotene as provitamin A. Provitamin A is converted in the gastrointestinal tract into vitamin A. Like vitamin E, carotenoids also possess antioxidant properties and have been shown to have a protective effect against cancer (16–18). Supplementation of β-carotene has been reported to reduce the occurrence of major cardiovascular events by almost 50% (19). The composition of crude palm oil is different from refined, bleached and deodorized palm oil. Crude palm oil contains a high concentration of carotene; 60–70% of which is β-carotene, 30–35% α-carotene. Refined palm oil (golden oil), which is normally, traded contained high concentrations of vitamin E, but not carotene. In contrast, red palm oil (RPO), which is a non-bleached palm oil, contained approximately 400 ppm of carotene as well as 700 ppm of vitamin E. Malaysian RPO has a high nutritional value and has gained popularity in many Asian countries including Japan.

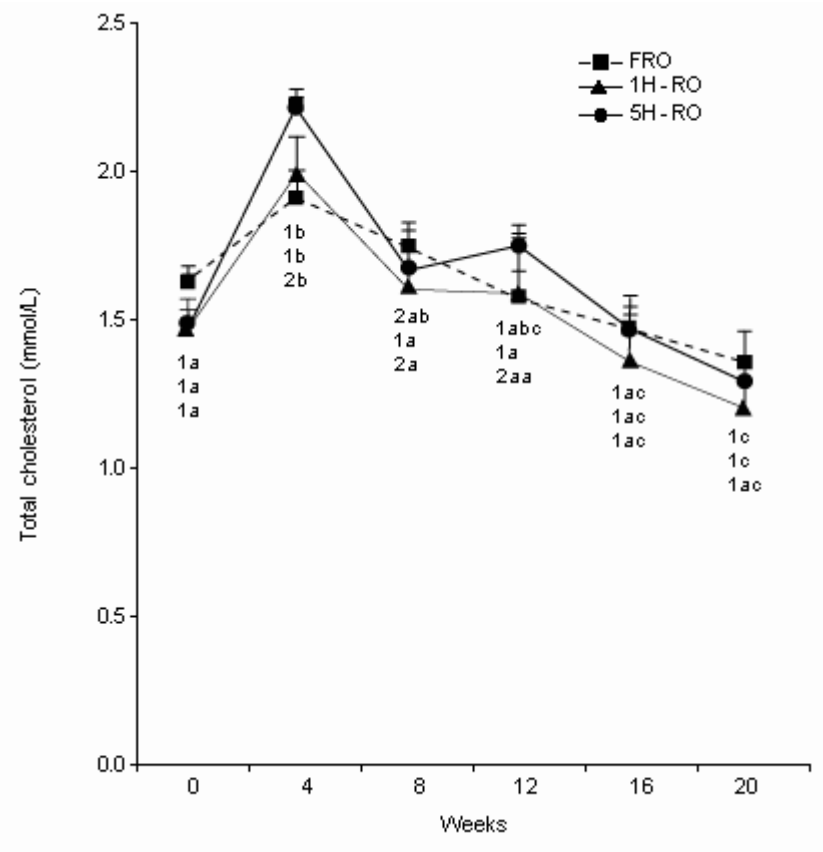
The role played by palm oil and its various fractions in atherosclerosis is not clear. Kritchevsky *et al.* (21) reported that palm oil was atherogenic in rabbits and Rudel *et al.* (22) reported a similar observation. Furthermore, the important physiological role that is played by the minor components present in palm oil is only now becoming apparent. Palm vitamin E and carotenoids have anti-oxidant properties that may help to protect against atherosclerosis.

Red palm oil is a refined, deacidified, deodorized palm oil with up to 85% of its carotene and tocopherol content retained. Manorama *et al.* (29) reported that palm oil decreases TC, LDL-C, TG and increases HDL-C. Much of the fat consumed in our diet has been exposed to heat during cooking. In deep fat frying, often the fat is kept hot for a long period of time at 180°C and moisture and air are mixed into the hot oil. The fried food absorbs this heated oil.

The oxidation of oil produces oxygen-derived free radicals and hydroxylated products that are harmful to tissues of the body (30) producing adverse effect like hemolytic anemia, increased blood clotting time and hepatomegaly (31). Reproductive toxicity, elevation of TC and free fatty acid levels of various tissues, thrombocytopenia and enhanced platelet aggregation levels have also been documented (32,33).

The harmful effect of the thermally oxidized palm oil on lipid profiles and its relation with the development of atherosclerosis have not been much explored. This aspect is also important, as studies have reported that lipoprotein oxidation is a necessary step in the development of fatty streaks into atherosclerotic plaques (34,35).

Figure 2 : Effect of fresh and heated red palm oil on serum total cholesterol (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



In practice, there is a tendency for the thermally heated oil to be re-used repeatedly in frying and cooking. In view of the potential hazardous effect of the heated oil on health, we undertake this study to see whether thermally oxidized red palm oils can alter serum lipid profile and MDA.

MATERIALS AND METHODS

Thirty male rats of Sprague-Dawley species (200-250 g) were randomly divided equally into 3 groups. The first group was fed on fresh palm oil diet while the second and the third groups were fed on the thermally oxidized red palm oil diet (heated once and heated 5 times). The animals were housed in stainless steel cages at room temperature of 27 $\pm$ 2°C and were quarantined for a two-week period prior to introduction of the test diets. All the test and control animals had free access to food and tap water for 20 weeks. The fasting serum lipid and MDA were taken at baseline and at intervals of 4 weeks for 20 weeks.

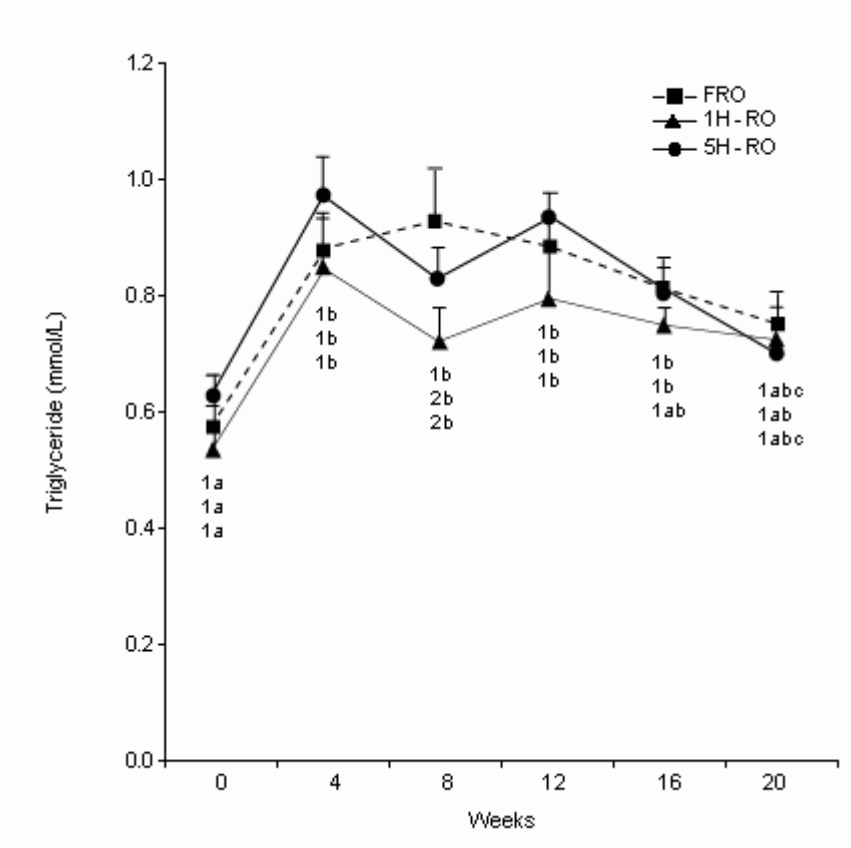
Source and preparation of palm oil diets

RPO was obtained from Malaysian Palm Oil Promotion Council of Malaysia. The heated form was thermally oxidized as described by Owu *et al.* (33). Fresh palm oil was heated at 150°C in a stainless steel pot for 20 mins intermittently for 5 rounds, with a cooling interval of 5 hours. The two heated diets were formulated by mixing 15% (w/w) of each heated oil with commercial rat chow obtained from Gold Coin.

Determination of serum lipids and lipoprotein

Blood was extracted from the orbital vein after 12 hours of fasting. Serum total cholesterol (TC), HDL-cholesterol and triacylglycerols were analyzed by the enzymatic method using kits (Boehringer Mannheim). The addition of phosphotungstic acid and magnesium ion to the sample led to the precipitation of various lipoproteins on centrifugation. The HDL contained in the supernatant portion can then be separated and measured using enzymatic methods. The LDL-cholesterol was calculated by using the Friedwald formula (36).

Figure 3 : Effect of fresh and heated red palm oil on serum triglyceride cholesterol (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



Determination of plasma malondialdehyde (MDA)

The MDA content in the serum was determined using a method described by Ledwozyw *et al.* (37).

Statistics

The data is presented as the mean±SEM. The result obtained was analyzed using the Kruskal-Wallis test. The Mann-Whitney and Wilcoxon Signed Rank test were used where appropriate. A value of $p<0.05$ was considered significantly.

RESULTS

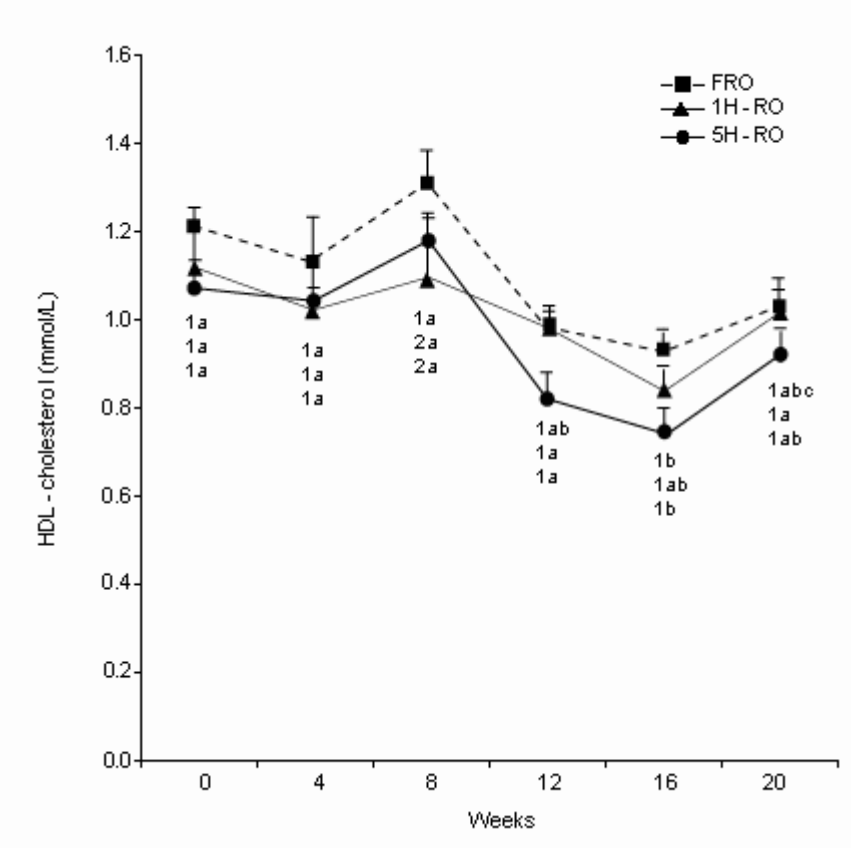
There was a significant increase in serum MDA in the groups fed with fresh (FRO), heated once (1H-RO) and heated 5 times (5H-RO) red palm oil from 4 weeks onwards compared to their respective baseline values and more or less reaching a plateau beginning from 12 weeks onwards. However, there was no difference in MDA concentrations-between groups throughout the study period (Figure 1).

The serum TC in all groups fed with 5H-RO transiently increased in 4th week of feeding. However, serum TC then started to fall and was significantly lower in groups FRO and 5H-RO at the end of study period compared to respective baseline level ($p<0.05$). A similar tendency observed in group 1H-RO. There was no difference in TC among the 3 groups throughout the study period except for the transient increase in serum TC whereby the increase was significantly lower for group FRO ($p<0.05$) (Figure 2).

There was a significant increase in serum TG in all groups from the 4th to the 16th week of study compared to their respective baseline values ($p<0.05$). However, the serum TG finally came down to baseline levels at the 20th week. There were differences between groups throughout the study period except the 8th week when group FRO attained a higher increase in TG (Figure 3).

There was a delayed and transient significant decrease in HDL-C for groups FRO and 5H-RO. However, this reverted back to baseline values at 20th week. Again there was no difference between groups in HDL-C throughout the study period (Figure 4).

Figure 4 : Effect of fresh and heated red palm oil on serum HDL-cholesterol (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



There was a significant increase in LDL-cholesterol in all groups at 4th week of feeding. However, the concentration of LDL-cholesterol in this group came down almost to baseline value after 16 weeks of feeding. A similar pattern was also seen in the group fed with 1H-RO. There was a significant increase in LDL-C throughout the study period in the group fed with 5H-RO compared to the baseline. Again, there was no difference in LDL-C between groups. However, at the end of the study period the LDL-cholesterol in 5H-RO group was significantly higher compared to FRO and 1H-RO (Figure 5).

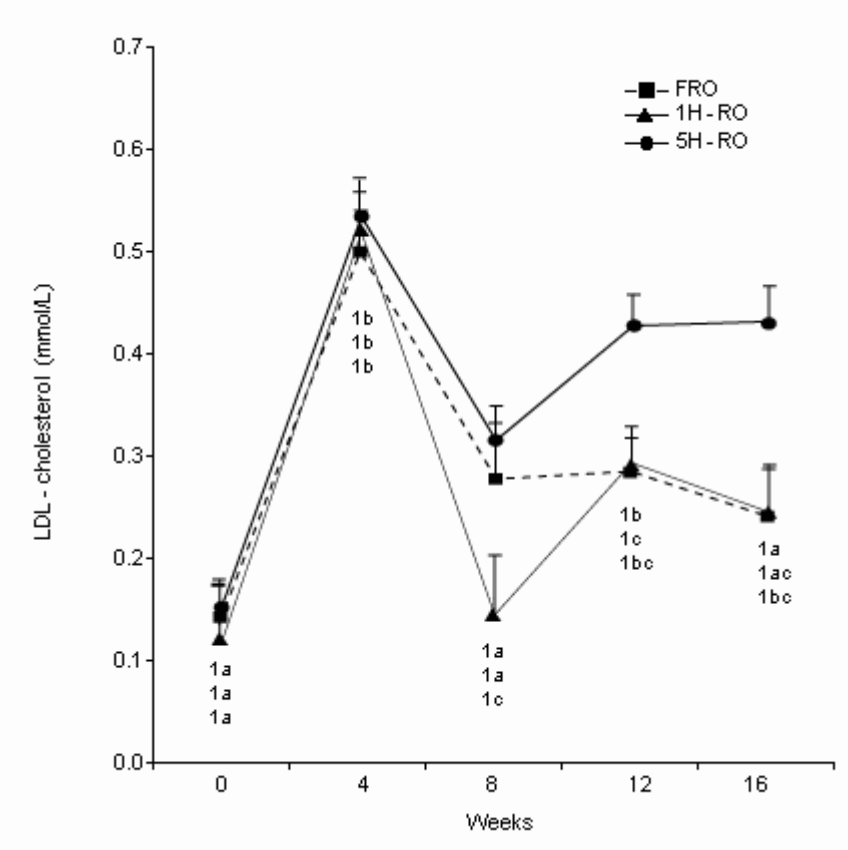
There was a significant increase in TC/HDL-C ratio in all 3 groups after 4, 12 and 16 weeks of feeding compared ($p<0.05$) to their respective baseline. However, the TC/HDL-C ratio dropped to baseline level at the end of the study period. After 4 weeks of feeding the TC/HDL-C ratio was significantly higher in 1H-RO and 5H-RO groups compared to fresh palm oil. A similar pattern was also seen at 12th and 16th weeks of study. However, during this period, TC/HDL-C ratio was significantly higher in the group fed with 5H-RO compared to FRO and 1H-RO (Figure 6).

There was no significant difference in TC/HDL-C ratio between the groups at the end of the study period.

DISCUSSION

The addition of 15% red palm oil caused a temporary increase in total-cholesterol (TC). The highest increase in total-cholesterol occurred after one month of study. The raised TC was higher in the group fed with 5H-RO compared to FRO and 1H-RO. However, after prolonged feeding, the TC was significantly reduced in the three groups. The reduction in TC occurred earlier in the fresh red palm oil group. The changes were observed much later in heated oil group which occurred after 3 and 4 months of feeding in 1H-RO and 5H-RO groups respectively. This finding suggests that prolonged feeding with

Figure 5 : Effect of fresh and heated red palm oil on LDL-cholesterol (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



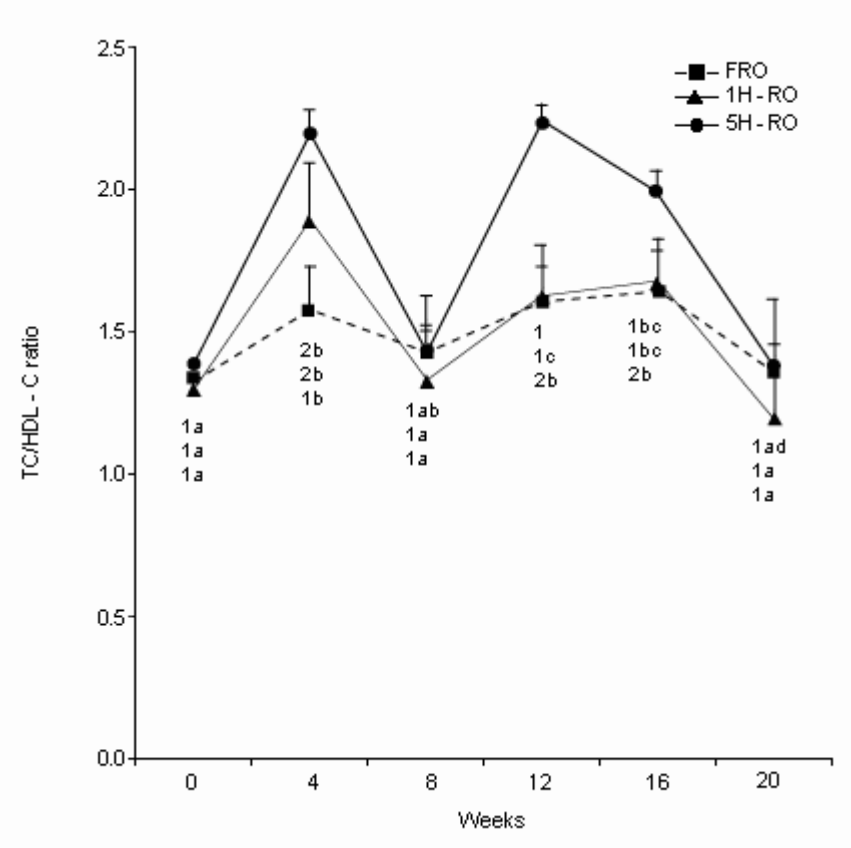
RO reduced serum total cholesterol. Heated RO did not appear to be more hypercholesterolemic than fresh RO. The effect of fresh RO in this study was in agreement with Manorama *et al.* (29) who also reported that RO reduced serum total cholesterol. The cholesterol lowering effect of RO could be attributed to its high content of vitamin E and β-carotene.

Fresh and heated red palm oil was found to induce transient, but more prolonged hypertriglyceridemia beginning from the 4th to the 16th weeks. However, it dropped to almost baseline value after 20 weeks of feeding. Furthermore heated RO did not appear to be more hypertriglyceridemic compared to fresh RO throughout the study period. The effect of RO on serum triacylglycerols was comparable to our earlier work (38) which reported that feeding with RO for 12 weeks did not increase serum TG in rabbits.

There was also a transient fall in HDL-cholesterol concentration with fresh and heated RO feeding at 16th week of study. However, the HDL-concentration reverted back to baseline values at the end of the study period, which indicated that RO did not interfere with HDL-concentration. The effect of RO on HDL was not affected by heating since there was no significant difference in HDL-concentration among the groups throughout the study period. The effect of RO on HDL-cholesterol in this study was not in agreement with Manorama *et al.* (29) who found that RO increased HDL-cholesterol in human. Manorama's study differed from this study in their short duration of feeding which was only for 15 days compared to 5 months in this study. Manorama *et al.* (29) used human volunteers instead of rats.

There was also a transient increase in LDL-cholesterol in rats fed with fresh and 1H-RO at 4th week of study. However, as with triacylglycerol and HDL-cholesterol, the values came down to almost baseline level at the end of 16 weeks of study. In contrast, the increase in LDL-cholesterol in rats fed with 5H-RO was sustained throughout

Figure 6 : Effect of fresh and heated red palm oil on TC/HDL-C ratio (Different numbers or alphabets denote significant differences ($p<0.05$) between and within groups respectively).



the study period. The findings suggest that the RO heated 5 times appears to cause a prolonged increase in LDL-cholesterol in rats.

The TC/HDL-C ratio did not differ among 3 groups at the end of the study period although there was a temporary increase in all groups at 4th, 12th and 16th weeks of study.

An important observation was the sustained increase in serum MDA in all the groups throughout the study period. This may indicate that the presence of high vitamin E and β-carotene in RO did not prevent lipid peroxidation. It also appears that heating did not have an effect on serum MDA. This finding is in contrast with our earlier work (38) which reported that RO reduced serum MDA in rabbits fed with RO for 12 weeks. Again, the duration of study and species difference may attribute to the different finding.

In conclusion, both fresh and heated RO appeared comparable in their effect on serum cholesterol and lipid peroxidation. It appears that long term feeding with RO may not have any effect on serum TG, HDL and TC/HDL-C ratio that would be adverse to human health. However, it appears that consumption of RO heated for a prolonged period may result in a sustained increase in LDL-cholesterol level. Further studies are required to ascertain whether the increase in LDL-cholesterol and MDA with heated oil would render it more atherogenic.

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