Original Article

Anti-inflammatory properties of blended edible oil with synergistic antioxidants

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ABSTRACT

Background: Blending of oil combines the potency of two edible oils and offers a balance of fatty acids. Various cooking preparations existing across different ethnicities and regions subject oil to different cooking temperatures thereby causing deterioration of the oil due to oxidative stress. In order to prevent the oxidative damage of unsaturated fatty acid, a blend of rice bran oil (RBO) and safflower oil (SO) (70:30) with an antioxidant technology was designed. A controlled trial was carried out to assess the efficacy of the blend on different biomarkers including lipid parameters and some important inflammatory markers that have the potency to lead to various lifestyle diseases. **Study Design:** A prospective, double-blind, randomized, parallel group study (on 80 adult hyperlipidemic patients) was conducted for 3 months. During the study, all the subjects were recommended lifestyle modifications, which included, exercise regime and diet counseling; oil quantity consumed was 1 L/person/month for both the groups. The subjects were divided into two groups; one group, continued with their regularly consumed oil whereas, the other was given the test oil. Biomarkers assessed were lipid profile and seven other inflammatory markers were assessed. **Results:** Low-density lipoprotein cholesterol (LDL-C) the primary marker for cardiovascular diseases showed a decrease of 56.07 ± 04.31 mg/dL and 31.98 ± 03.81 mg/dL ($P < 0.001$ by analysis of variance [ANOVA]) from baseline in test and control group, respectively, during 3 months. Similar reduction trends were observed for total cholesterol where –52.31 ± 13.04 mg/dL and 31.98 ± 04.12 mg/dL ($P < 0.001$ by ANOVA, between the groups) were seen in test and control group, respectively. Oxidized LDL and high sensitivity C-reactive protein showed a reduction of 2.23 ± 1.3 units/dL and 0.87 ± 2.85 mg/L in test group whereas; an increase of 1.04 ± 1.73 units/dL and 0.44 ± 2.37 mg/L was seen in the control group, respectively ($P < 0.05$ by Student's *t*-test, between the groups). **Conclusion:** The study showed that the blend of RBO and SO with antioxidant technology along with other lifestyle changes helps lowering of blood lipids and stated inflammatory biomarkers and thus, in turn may help prevent lifestyle diseases.

Key words: Anti-inflammatory, antioxidants, blended edible oil

Introduction

Correlations between nature and nurture; genetic and environmental factors are known to be the basis for health and disease. Nutrition is one of the major environmental factors that affect health and disease.[1] Dietary fat plays an essential role in our diet. Fats form an important part of our diet. National Institute of Nutrition (NIN), India recommends diet to consist of 15–30% fats.[2]

In India cooking oil forms an integral part of every household and right choice of edible oil is needed for maintaining a healthy life. In most of the western and southern states, there is a strong preference for groundnut

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oil, whereas in the north and east mustard oil is preferred. Palm oil is largely consumed; however, the large quantity of palm oil is utilized for the manufacture of "Vanaspati", which is mostly used in commercial establishments.[3]

However, none of these single edible oils meet the dietary fatty acid recommendation. Hence, the NIN, India in their recent recommendations state that a correct combination or blend of two or more oils should be used to achieve all kinds of fatty acids in the diet.[2]

Furthermore, according to the Food Safety and Standards Authority of India a mixture of two edible oils (1 blend) is allowed in India.[4]

A study carried by Sugano and Tsuji showed that a synergistic blend of safflower oil (SO) and rice bran oil (RBO) when RBO is 20–30% exerted significant reduction in plasma cholesterol and concluded that blending RBO with SO magnify the hypocholesterolemic efficacy compared with the effect of each oil alone.[5] Hence, it was envisaged to have a blend of 70% RBO, and 30% SO, which had proven to show anti-hyperlipidemic effects. Each component of this blend is known to have benefits. Studies with rice bran and RBO have shown to lower exert anti-hyperlipidemic effect and lower blood cholesterol concentrations in both healthy, as well as hyperlipidemic individuals.[6-10] SO rich in polyunsaturated fatty acids (PUFAs) is also known to have low-density lipoprotein cholesterol (LDL-C) lowering potential.[11]

In Indian cooking conditions, oil is subjected to high cooking temperatures and in these conditions the oxidative degradation of oil is accelerated. Even the shelf life stability of vegetable oils in food uses and their applicability depends on its oxidative stability.[12] The oxidative potential of PUFAs is the highest, followed by monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA).[13,14] Hence, it becomes essential to protect the fatty acids from degradation to maximize their benefits in the body. Oxidization of LDL-C (OxLDL-C) *in vivo* is also known to be the first step of atherosclerosis. Antioxidants are known to maintain the integrity of vegetable oils by preventing the oxidation reactions mainly by scavenging free radicals. Different antioxidants work on different levels and hence, no one antioxidant is sufficient to interact at all levels.

This antioxidant enriched blend was tested in this prospective, randomized, double-blind, and parallel group study in Indian hyperlipidemic subjects. The technology utilizes the important phytonutrients such as oryzanols, tocopherols, tocotrienols and phytosterols present in the RBO in the right proportion best suited for lowering cardiac risks. A study conducted in the year 2011, stated that SO which is a rich source of essential $n = 6$ PUFA linoleic acid; showed an increase in lean body mass and decrease in fat mass in the trunk region, another observation of the study was the SO improved fasting glucose, hemoglobin A1c, C-reactive protein (CRP), high-density lipoprotein cholesterol (HDL-C) and adiponectin levels.[15] Study conducted on rats showed that gamma-oryzanol along with cycloartenol ferulic acid ester could accelerate the excretion of lipids from the blood. Study conducted on 4 weeks old hamsters were fed on defatted rice bran and/or crude RBO and full-fat rice bran, results showed that a significant decrease in total liver cholesterol levels. Nonhuman primates were fed on semi-purified diets with blended edible oils, which included rice bran as a component of the blend; the observation of the study was that there was a high correlation between the serum cholesterol and LDL-C reduction levels in moneys, who were fed on a standard diet in comparison to the semi-purified diets. Human study revealed that when 300 mg/day gamma-oryzanol was administered for a period of 3 months on hyperlipidemic patients there was a significant decrease in plasma total cholesterol (TC) and LDL-C levels, another observation in the study was that there was an increase in HDL-C levels in the hypercholesterolemic group. Several animal and human studies have already demonstrated their property to improve the plasma lipid pattern of rodents, rabbits, nonhuman primates and humans, reducing total plasma cholesterol and triglyceride (TG) concentration and increasing the HDL-C level.[16]

Subjects and Methods

A prospective, double-blind, randomized and parallel group study was undertaken after approval of the protocol by an Independent Ethics Committee in accordance to Declaration of Helsinki.

Subject population

A total of 86 male and female patients were recruited postproviding their informed consent of which eighty subjects completed the trial and six were lost to follow-up. Adult hyperlipidemic subjects (male/female) of age 25–45 years and having body mass index ≥ 18.5 kg/m^2 and ≤30 kg/m^2 at study entry were included in the study.

The cases were ranging from 21 to 63 years with average age 36.16 years in the reference group which was comparable to 33.63 years among test group, and the difference was not statistically significant. 55.8% cases were male in the reference group, which was comparable to 53.5% among test group, and the difference was insignificant. The inclusion criterion was serum LDL-C of 130–190 mg%.

The patients on lipid lowering or anti-hypertensive drugs such as diuretics, nonselective β blockers within the last 3 months, uncontrolled hypertension, and clinical signs of liver or thyroid disorders, acute attack of coronary heart disease or decomposed congestive heart failure within last 1-month were excluded. Patients are consuming laxatives or is a smoker, has liver disorders were also excluded from the study. Females who were lactating or pregnant were also not included in the study.

Foods

With the help of a computer generated randomization list, the selected patients were randomly assigned to one of the two groups. In one of the groups, the patients were served food prepared in the test oil, that is, blend of 70% RBO and 30 SO with antioxidant technology (Saffola® Total, Marico Ltd., India), and it was designated as "test oil" group. In the other group, the patients were served food prepared the same cooking oil they were using. Double blinding was achieved as both the test oil and the reference oils were coded by the sponsor before providing them to the site. Both test and the reference oils were packed in a similar way making it difficult to identify whether it is a test or any other oil. However, the respective oil codes were printed on the packets for identification, which were never revealed to the study site. Same oil codes were used for developing the randomization code and randomization schedule were provided to the site for oil packet allocation. Control subjects were served with food prepared in same cooking oil that they were previously using such as coconut, sunflower, palm or corn oils. Standard brands of the respective oils available in the market were utilized for this purpose. This group served as the control group. The detailed fatty acid composition of the test oil and control oil is given in [Table 2]. Values were taken as per Nutritive Value of Indian Foods. Gas chromatography technique

Table 1: Biomarkers for cardiovascular risk with method reference and detection limit

Markers	Methods	Specificity and sensitivity
Lp (a)	Randox Reagent on the VITROS5, 1 FS Chemistry System	Substances: Bilirubin, hemoglobin, ascorbic acid, TGs, ascorbic acid (L), Apo and intra-lipid were tested for specificity and did not cause interferences, and the sensitivity was found to be 3.4 mg/dL
Apo A1	VITROS Chemistry Products Apo Al Reagent on the VITROS5, 1 FS Chemistry System	Substances: Bilirubin, amoxicillin, carbamazepine, dipyrone, ethamsylate, gentamicin sulfate, intralipid, hemoglobin, ibuprofen, lidocaine, methotrexate, procainamide, propranolol, ranitidine, salicylic acid, simvastatin Bilirubin, carbamazepine, dipyrone, ethamsylate, gentamicin sulfate, intralipid, hemoglobin, ibuprofen, lidocaine, methotrexate, procainamide, propranolol, ranitidine, salicylic acid, simvastatin, theophylline, TG Acetaminophen, N-acetyl-L-cysteine, and valproic acid were tested for specificity on the VITROS Chemistry Products Apo Al Reagent using NCCLS Protocol EP7 and found not to interfere, bias <13 mg/dL (<0.13 g/L) at the standard concentration and the sensitivity was found to be 30–240 mg/dL
Apo B	VITROS Chemistry Products Apo B reagent on the equipment VITRO S5, 1 FS Chemistry System	Substances: Amoxicillin, Ascorbic acid, bilirubin, carbamazepine, dipyrone, ethamsylate, gentamicin sulfate, hemoglobin, ibuprofen, lidocaine, methotrexate, procainamide, propranolol, ranitidine, salicylic acid, simvastatin, theophylline, TG Acetaminophen, N-acetyl-L-cysteine, and valproic acid were tested for specificity on the VITROS Chemistry Products ApoB Reagent using NCCLS Protocol EP7 and found not to interfere, bias <6.4 mg/dL (<0.06 g/L) at the standard concentration and the sensitivity was found to be 35–300 mg/dL
HCY	VITROS Chemistry Products HCY Reagent on the VITROS5, 1 FS Chemistry System	Substances: Acetaminophen, N-acetyl-L-cysteine, adenosine, amoxicillin, ampicillin, ascorbic acid (L), aspirin, atorvastatin, bilirubin, caffeine, clopidogrel hydrogensulphate, creatinine, L-cysteine, doxycyclin, D-penicillamine, enalapril maleate, gemfibrozil, gluthathione, DL-HCY and hemoglobin were tested for specificity on the VITROS Chemistry Products HCY Reagent using NCCLS Protocol EP7 and found not to interfere, bias <13.6% at the standard concentration and the sensitivity was found to be 1.0–50.0 umol/L
CRP	VITROS Chemistry Products hsCRP Reagent on the VITROS5, 1 FS Chemistry System	Substances: Amoxicillin, ascorbic acid, bilirubin, carbamazepine, dipyrone, ethamsylate, gentamicin sulfate, hemoglobin, ibuprofen, lidocaine, methotrexate, procainamide, propranolol, ranitidine, salicylic acid, simvastatin, theophylline, TG Acetaminophen, N-acetyl-L-cysteine, and valproic acid were tested for specificity on the VITROS Chemistry Products hsCRP Reagent using NCCLS Protocol EP7 and found not to interfere, bias <0.28 mg/L at the standard concentration and the sensitivity was found to be 0.10–15.00 mg/L
ELIZA	Capture (sandwich) ELISA 96-well microtiter plate (in duplicate with 2 rows of calibration wells) method based on Holvoet *et al* (Arterioscler Thromb Vasc Biol 21:844–848 2001) with specific murine monoclonal antibody mAb-4E6	The bound conjugate is detected with TMB after acid termination. The colorimetric endpoint was read on an Allere (R) Easy Reader at 450 nm. The sensitivity of the test is based on the OD response on the calibration curve at 0.2 OD (450 nm) corresponding to 5 U/dL. The precision of the test was calculated from 3 samples assayed in 5 replicates with a coefficient of variation of 3.8% in relative arbitrary units against our in-house reference. No significant cross-reactivity or interference between OxLDL and analogs such as LDL, HDL, and VLDL is observed in this ELISA

HCY: Homocysteine, ELIZA: Enzyme-linked immunoassay, CRP: C-reactive protein, LDL: Low density lipoprotein, OxLDL: Oxidized LDL, HDL: High-density lipoprotein, VLDL: Very low density lipoprotein, Apo: Apolipoprotein, Lp (a): Lipoprotein (a), TG: Triglyceride, OD: Optical density

was used to get the fatty acid composition of test oil as per standard AOAC method 969.33 and 996.06. Trans fatty acid was not detected (instrument sensitivity 0.01%). In addition, oil intake was maintained at 1 L oil/person/month in both the groups. Along with this, the subjects were monitored every day for intake of same by trained nutritionist at the study site. The daily menu was planned by the nutritionist and exercise was also recommended to the subjects. Subjects were provided three meals a day, that is, lunch, snacks, and dinner. Each subject collected their meals from the study center daily except for Sundays. Subjects were provided additional oil packets for food preparation at home on Sundays and were also instructed about the method of cooking such as boiling, grilling, roasting, baking, microwaving, poaching, sautéing, steaming. Frying was not allowed. Standard diet charts were used, and individualized diet plans were prepared and followed for each subject based on their daily food intake and energy requirements.

Totally, 15 days run-in period was scheduled for all enrolled subjects during the period subjects were advised to follow restricted diet advised by the study nutritionist. Restricted diet refers to adhering to the individualized diet plan provided to each subject including the quantity of food to be consumed. Outside snacking was completely prohibited. Fried foods were not allowed. Subjects were not allowed to take vitamin or mineral supplements during the study. This was to assess the diet compliance to the advised diet plan. Subjects, which complied with the diet plan were carried forward for the study.

Study protocol

Lifestyle modifications were the first step in the study. Lifestyle modifications included modification in diet as described above. The study fitness trainer advised a meaningful 30–40 min exercise routine at home such as walking, jogging or biking to maintain a healthy weight and establish a more active lifestyle (World Health Organization recommends 75 min of vigorous-intensity aerobic physical activity or 150 min of moderate-intensity aerobic physical activity throughout the week). Adherence to the advised exercise regime was checked during each meeting with the fitness trainer. A good compliance was observed, and adhered to the suggestions. After 2 weeks of stabilization on the low-fat diet, fasting subjects blood samples were collected for estimating lipid profiles and the subjects were allotted to either treatment group randomly. There were five visits for the study during which safety and efficacy assessments were done – screening (day-15), enrollment (day 0), Follow-up visits (1–3 months). Quality of life assessment was done at every follow-up visit. It included questions regarding general well-being, lethargy, fatigability, abdominal discomfort, bowel irregularities and any other specific symptoms expressed by the patients. Opinion of patients regarding taste of the product was also recorded. Adverse events, if any, were recorded. The major outcome measures consisted of lipid profile levels compared to baseline (day 0) as measured on 1, 2 and 3 months. Inflammatory biochemical parameters were also assessed to detect the efficacy of the antioxidant technology of the oil. Markers such as, oxidized lower density lipoprotein (ox LDL), high sensitivity C-reactive protein (hsCRP), Lipoprotein [a] (Lp[a]), tumor necrosis factor alpha (TNF-α). Apolipoproteins A1 (Apo-A1) and B (Apo-B) were also measured [Table 1]. All investigations were done at an NABL accredited laboratory.

Biochemical analysis

Cholesterol oxidase/esterase method was used to assess cholesterol parameters. HDL-C was assessed using non-HDL precipitation method, followed by enzymatic analysis. TG was assessed using enzymatic technique using lipase and glycerol kinase. The amount of very LDL-C (VLDL-C) was estimated using internationally accepted method derivation using the TG value. Immunoturbidimetric techniques were used to assess Lp (a), Apo, homocysteine (HCY) and hsCRP. Competitive enzyme-linked immunosorbent assay was used to measure OxLDL and TNF-α were measured using chemiluminescent immunometric assay.

Statistical analysis

Statistical analyses were performed with SAS software (version 9.1; SAS institute, Cary, NC, USA). Data are shown as means with their standard errors unless otherwise stated. Analysis of variance was used to test the significance of the difference between the groups for the lipid profile parameters. The inflammatory markers were assessed using Students *t*-test.

Results

Of the 86 patients recruited in the study 80 patients completed the study. Of these data of 39 patients in test group and 41 in the control group were used for the final analysis. The cases were ranging from 21 to 63 years with average age 36.16 years in the reference group which was comparable to 33.63 years among test group, and the difference was not statistically significant. 55.8% cases were male in the reference group, which was comparable to 53.5% among test group, and the difference was insignificant. The baseline lipid profile levels between both the groups were similar.

At the end of 3 months, both the study oil group and control group showed a statistically significant decrease in LDL-C from the baseline ($P < 0.001$). However, the reduction in test group is significantly lower that the control

group. Post 1-month intervention; mean LDL-C showed a significant fall of 16.7% among the control group and 27.0% in test group from baseline. If compared fall was significantly more among test group than the control group. After an interval of 3 months, a reduction of 34.9% was observed in LDL-C levels in the study oil group as against 20.6% for the control group [Figure 1]. TC levels showed a similar trend where the significant reduction of 13.8% and 22.1% was observed in the control and test groups, respectively, in 3 months and the difference between the groups were significant as well ($P < 0.001$) [Figure 1].

High-density lipoprotein cholesterol showed a positive trend and a significant increase of 17.2% was seen in the control and 32.7% in the test groups, respectively. The rise was significantly higher in test group than the control group [Figure 2]. VLDL-C showed a significant fall of 17.1% among the control group and 29% in test group. If compared the fall was more among test group than the control group and the difference was statistically significant. In correlation with VLDL-C, TGs showed similar reductions and the difference was statistically significant [Figure 1]. Table 3 summarizes the reductions observed in both the groups.

Oxidized LDL is another important biomarker as it is a direct assessment of the levels of oxidization in the body. OxLDL showed decrease of 1.8% in the test group and an increase of 0.8% was seen in the control group. The decrease in test group was significant ($P < 0.001$, by Student's *t*-test) when compared with control group thus, reemphasizing the positive effect of the test oil. hsCRP showed a positive trend in test group alone where a decrease of 24.1% was seen in test group and an increase of 15.4% was seen in control. Thus, a positive effect was exerted in the test group alone, and the difference between the groups was statistically significant ($P < 0.05$, by Student's *t*-test).

Table 2: Fatty acid composition of test and control oils

	Test oil#	Palm oil*	Sunflower oil*	Corn oil*
16:0 palmitic acid	15.90	42.00	5.60	10.70
18:0 stearic acid	2.64	4.30	2.20	1.70
18:1 (*n*=9) oleic acid	34.65	43.70	25.10	29.60
18:2 (*n*=6) linoleic acid	43.15	10.00	66.20	57.40
18:3(*n*=3) α-linolenic acid	0.47	-	-	-
22:0 behenic acid	0.37	-	-	-
24:0 lignoceric acid	0.47	-	-	-
SFA	19.00	46.3	9.10	12.70
MUFA	47.00	43.7	25.10	29.60
PUFA	34.00	10.00	66.20	57.40
Total	100	100	100	100

*Values as per nutritive value of Indian foods values of test oil calculated using gas chromatographic technique. #Values of Test Oil calculated using gas chromatographic technique. SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

After treatment HCY showed, insignificant fall of 2.0% among the reference group and a significant fall of 11.2% in test group from baseline. If compared change was more in test group than the control group and the difference was statistically significant ($P < 0.05$, by Student's *t*-test) [Figure 3].

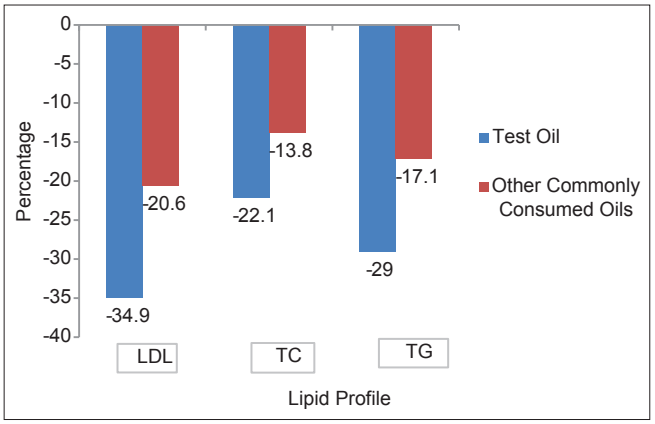

Figure 1: Comparison of changes in mean lipid profile levels (mg/dL) between two groups

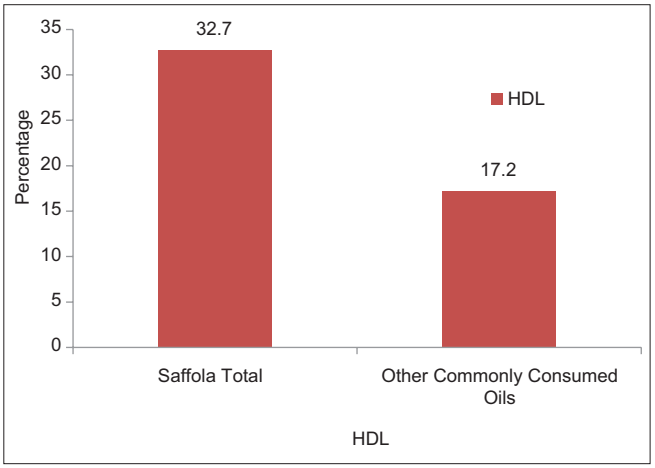

Figure 2: Comparison of changes in mean high-density lipoprotein levels (mg/dL) between two groups

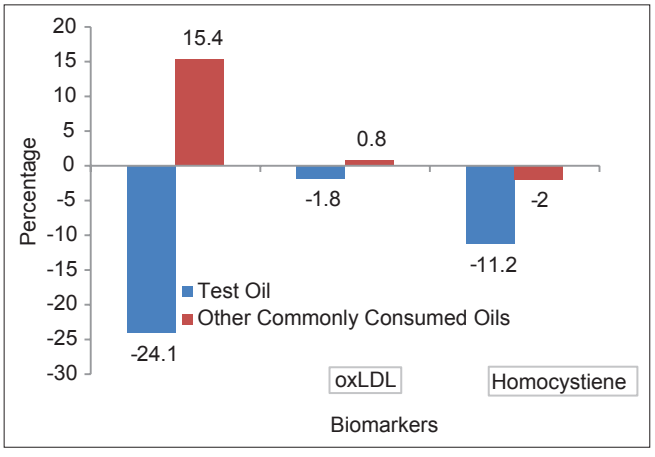

Figure 3: Comparison of changes in mean inflammatory parameters between two groups

When compared the benefit seen in terms of mean difference levels among test group had 2 times more reductions as compared to the control group for OxLDL, hsCRP and HCY levels. Table 4 summarizes the reductions for the above mentioned three biomarkers.

Apolipoprotein-B exhibited a significant decrease in both the groups. Apo-A1 levels showed no significant change. Lp[a] levels showed a decrease in test group and a slight increase in the control group. Differences in TNF-α level were comparable in both the groups post 3 months. Similar trend was seen for Apo-B/Apo-A1 levels. As trends were comparable, the data for these markers are not shown.

Another interesting observation was the significant decrease observed in mean fat and fat mass in the test group. The test group showed a 2.6% and 0.61% decrease in fat mass and body fat levels, respectively, among test group and an insignificant decrease of 1.1% and 0.31% in fat mass and body fat levels, respectively, in control group.

Quality of life and other biochemical parameters were comparable in both the groups post-analysis.

Discussion

Dietary fat plays an important and essential role. However,

Table 3: Impact of test oil on lipid profile

Mean lipid levels (mg/dL) x̄±SD	Duration (months)	Reference (*n*=41)	Test (*n*=39)	*P*
LDL-C	Baseline	155.17±13.04	160.53±17.45	0.125 (NS)
	3 months	123.19±13.23	104.46±18.17	
	Mean difference (baseline-day 90)	−31.98±03.81*	−56.07±04.31*	0.001*
	P	0.001	0.001	
TC	Baseline	232.47±13.34	236.92±10.55	0.101 (NS)
	3 months	200.49±13.03	184.61±17.82	±
	Mean difference (baseline-day 90)	−31.98±04.12*	−52.31±13.04*	0.001*
	P	0.001	0.001	
HDL-C	Baseline	38.47±06.88	39.76±06.53	0.392 (NS)
	3 months	45.07±07.21	52.75±06.28	
	Mean difference (baseline-day 90)	06.60±01.40*	12.99±01.00*	0.001*
	P	0.001	0.001	
VLDL-C	Baseline	38.88±10.53	38.61±08.61	0.900 (NS)
	3 months	32.23±10.80	27.40±08.47	
	Mean difference (baseline-day 90)	−06.65±01.19*	−11.21±00.69*	0.001*
	P	0.001	0.001	
TG	Baseline	194.40±52.65	193.05±43.07	0.900 (NS)
	3 months	161.14±53.98	137.02±42.37	
	Mean difference (baseline-day 90)	−33.26±05.93*	−56.03±03.43*	0.001*
	P	0.001	0.001	

By ANOVA. NS: Not significant, *Significant. SD: Standard deviation, LDL-C: Low density lipoprotein cholesterol, TG: Triglycerides, TC: Total cholesterol, HDL-C: High density lipoprotein cholesterol, VLDL-C: Very low density lipoprotein cholesterol, ANOVA: Analysis of variance

Table 4: Comparison of changes in mean inflammatory parameters between two groups

Mean levels (x̄±SD)	Duration (days)	References	Test	*P*
hsCRP (mg/L)	*n*	40	34	
	Baseline	2.85±3.04	3.61±3.83	0.383 (NS)
	3 months	3.29±3.90	2.74±2.38	
	Mean difference (baseline-day 90)	0.44±2.37	−0.87±2.85	
	(*P* value) one-tailed	(0.124) NS	(0.957) NS	0.018*
	(*P* value) two-tailed	(0.247) NS	(0.084) NS@	0.037*
HCY (umol/L)	*n*	40	34	
	Baseline	20.35±11.29	20.38±09.94	0.990 (NS)
	3 months	19.94±10.57	18.09±09.49	
	Mean difference (baseline-day 90)	−0.41±3.76	−2.29±3.97	
	(*P* value) one-tailed			
	(*P* value) two-tailed	(0.494) NS	(0.002)*	0.041*
OxLDL (units/dL)	*n*	41	39	
	Baseline	122.49±32.58	123.91±29.94	0.839 (NS)
	3 months	123.53±32.71	121.68±29.78	
	Mean difference (baseline-day 90)	1.04±1.73*	−2.23±1.30*	
	(*P* value) one-tailed	(0.001)	(0.001)	0.001*
	(*P* value) two-tailed	(0.001)	(0.001)	0.001*

By Student's *t*-test. *Significant, @Significant at 10%. NS: Not significant. Subjects that exhibited lab values beyond the normal analytical range were considered as outliers. hsCRP: High sensitivity C-reactive protein, OxLDL: Oxidized low-density lipoprotein, SD: Standard deviation, HCY: Homocysteine

no single oil can provide the recommended dietary fat ratio. Every oil is composed of three fatty acids, that is, SFA, MUFA, and PUFA

World Health Organization guidelines state that the PUFA: SFA ratio should be 0.8–1. The American Heart Association also recommends a balance of fatty acids in the ratio of 1:1:1 (SFA: MUFA: PUFA). Bhattacharyya *et al*., (2013), reported that no single oil showed the required amount of micronutrients for the stability of oil with balanced SAFA/MUFA/PUFA to provide standard nutritional quality.[17] Blended oils are known to have a greater thermal, as well as oxidative stability along with their nutritional benefits.[18] No single oil provides the recommended dietary fatty acid ratio and hence, it is essential to blend oils. However, blending needs to be achieved basis research. In this study, the base of a Japanese study was applied to arrive at the 70:30 RBO: SO combination.

Patented Losorb Technology was also employed in the blend. Losorb technology is the selection of a group of additives with changes in the way oil is processed that make our oils different from other oils. This technology brings forth a synergistic effect of the additives and results in lower uptake of oil by foods.

According to the National Cholesterol Education Program's Adult Treatment Panel III (ATP III) report, lowering LDL-C should be the primary target of any lipid-lowering therapy.[19] According to ATP III classification, LDL-C levels between 130 and 159 mg% are considered as borderline high and those between 160 and 189 mg% as high. For borderline high category, lifestyle changes including dietary modifications have been recommended. On the other hand, though therapeutic lifestyle changes are first-line management for patients in the second category, they ultimately require LDL-lowering drugs to reduce the risk.[20] Hence, one needs to concentrate on lifestyle related modifications to control LDL-C levels. Hence, the fact that in the control group with dietary and exercise modifications alone a reduction of 20.6% in LDL-C levels is promising however, the fact that a reduction of 34.9% was observed in LDL-C levels in the test group emphasizes the importance of choosing the right kind of oil in the diet.

Another important factor that was considered while devising the test oil was the properties of vegetable oils which predispose them to auto oxidation reactions. In Indian cooking conditions get oil usually gets exposed to high temperatures in the presence of oxygen and moisture. A number of chemical reactions occur due to these conditions, including oxidation, hydrolysis, and polymerization of unsaturated fatty acids causing a change in the composition of the frying medium as well as results into a production of volatile and nonvolatile oxidized products. The oxidative potential varies for the fatty acids in the order of PUFA > MUFA > SFA. PUFA has always been known to have cardio protective effect. Hence, there is a need to protect PUFA and the oil from deterioration and the same can be achieved using an antioxidant technology.[21] All this leads to health effects as seen in animal studies.[22-26]

Thus, it is essential to protect the oils and prevent formation of these compounds to prevent further adverse health effects. The antioxidant technology employed thus helps protect the oil from degradation and prevents the formation of these compounds. This was tested using certain physical and chemical parameters to establish efficacy of the system over other single oils consisting of naturally present antioxidants. This helps the oil to be stable in Indian cooking conditions.

Additional inflammatory parameters were assessed to prove the efficacy of the oil blend with antioxidants in the body. Recent advances in science have highlighted the fundamental role of inflammation in mediating all stages of atherosclerosis. Studies have showcased that the inflammation predicts outcomes of patients with cardiovascular issues. Elevation of inflammatory markers prospectively defines the risk of atherosclerotic complication thus adding to prognostic information provided by conventional risk factors like LDL-C. Hence, it was of prime importance to also study the effect of the test oil on these markers and the positive trend observed was encouraging.[27]

Oxidized LDL has been studied since 25 years and is gaining popularity as it is the underlying cause for atherosclerosis.[28] OxLDL have been reported to play an important role in the pathogenesis of atherosclerosis. When subjected to oxidative stress, the cells of the arterial wall, that is, endothelial cells, smooth muscle cells, and macrophages, can OxLDL *in vitro*. There is now evidence that suggests that oxidative modification of LDL is of first step that leads to macrophage uptake of LDL *in vivo* thus, making its assessment of prime importance.[29] In this study, the significant reductions in OxLDL levels were seen and hence, strengthen the theory of the efficacy of the antioxidant technology employed. Reductions were also, seen in hsCRP levels, which is considered as an independent risk marker for heart disease.[19] hsCRP provides a novel method for detecting individuals at a high risk of plaque rupture. Many prospective studies have proved the efficacy of hsCRP as a strong independent predictor of future diseases. Thus, it plays an important role as an adjunct for global risk assessment in primary prevention of cardiovascular

disease (CVD).[30] Data from a number of laboratories also state that elevated HCY levels are a risk factor for vascular diseases.[31] The multitude of relationships between elevated plasma HCY and CVDs make it another important marker to study, and reductions seen in the study was considered as a positive impact of the oil tested.

When compared the benefit seen in terms of mean difference levels among test group had 2 times more reductions as compared to the control group for OxLDL, hsCRP and HCY levels.

Furthermore, it is important to note that dietary interventions alone showed a slight nonsignificant increase in hsCRP and OxLDL levels in the control group whereas, a reduction was seen in test group. Other markers assessed were comparable. It was very encouraging to see the significant reduction from baseline levels in the test group for body fat and fat mass levels whereas, no significant change was seen in the control group with only dietary modifications. This could be used to establish a link between choosing the right dietary fat in the prevention of obesity. However, further multi-centric studies and studies with larger sample size may be required to help understand and establish the efficacy of this oil composition for the body fast and anthropometric parameters.

Thus, it can be concluded that lifestyle modifications along with substitution of cooking oil by the blend of RBO: SO (70:30) with antioxidant technology results in significant lowering of LDL-C levels and has positive benefits on the inflammatory markers thus having a positive impact in overall health especially cardiac health in hyperlipidemic subjects.

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Conflict of interest

There are no conflicts of interest.

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