

SUNPURE study: A Real World Retrospective Study of Sunflower Oil, Flaxseed Oil, and Rice Bran Oil Combination for Prevention or Cure of Dyslipidemia

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Abstract: *Background:* Unhealthy dietary habits play a crucial role in the development of heart disease. Blending of vegetable oils has been shown to improve lipid profile and inflammatory parameters. *Method:* This was a retrospective, observational, single center real-world study. Anonymized medical records with follow up visits (1 month) for subjects above 18 years were included in the study. This study aimed at determining the effect of a blend of rice bran oil, flaxseed oil and sunflower oil on the glycemc and lipid parameters. *Results:* A total of 73 records were included in the study, of which 56.16% subjects were female. The mean age was 45.67 ± 15.38 years. At one month follow up, significant reductions in total cholesterol (TC) ($p < 0.001$), non-high-density lipoprotein cholesterol ($p < 0.001$), TC/ high density lipoprotein (HDL) ratio ($p = 0.048$), low density lipoprotein (LDL)/HDL ($p = 0.041$), total bilirubin ($p = 0.001$), gamma-glutamyl transferase ($p = 0.021$), alkaline phosphatase ($p = 0.025$), creatinine ($p < 0.001$), calcium ($p = 0.020$), triiodothyronine (T3) ($p < 0.001$), thyroxine (T4) ($p < 0.001$) and thyroid stimulating hormone (TSH) ($p = 0.051$) were observed. *Conclusion:* The blend of rice bran oil, flaxseed oil, and sunflower oil was found to be beneficial in lowering the lipids levels, renal, hepatic, thyroid, and inflammatory markers in Indian population.

Keywords: Rice bran oil, flaxseed oil, sunflower oil, lipid parameters, inflammatory markers

1. Introduction

In developing countries, cardiovascular disease (CVD) continues to be the leading cause of death. India's age-standardized CVD death rate was estimated to be 272 per 100000 population [1]. Obesity, hypertension, family history and dyslipidemia are the common risk factors for the early onset of CVD [2]. Dyslipidemia is the most prevalent and most modifiable risk factor among the common CV risk factors [1]. According to an INTERHEART study, dyslipidemia appears to be the main cause of myocardial infarction in South Asians [3].

Unhealthy lifestyles, particularly dietary habits, play a crucial role in the development of heart disease. Many studies have investigated dietary changes that lower serum lipid levels and reduce CVD [4]. The American Heart Association (AHA) have emphasized on progressive reduction in total body fat, saturated fatty acids, and cholesterol as the primary steps in treating hypercholesterolemia [5]. The lipid profile can be improved through dietary substitution that consists of high saturated

fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) [6].

Recent studies have explored the hypolipidemic effect of vegetable oils which are rich in plant sterols and other unsaponifiable such as tocotrienols and oryzanol. Oryzanol acts as a hypolipidemic agent by preventing cholesterol absorption, increasing fecal excretion, and inhibiting hepatic cholesterol synthesis [7], [8].

Rice bran oil (RBO) has been investigated for its nutritional, chemical, and toxicological properties and was found to be safe for human consumption. The RBO consists of 35% PUFA, 43% MUFA, and 22% SFA. RBO contains oleic acid (38.4%), linoleic acids (34.4%), linolenic acid (2.2%), palmitic acid (21.5%) and stearic acid (2.9%) [9]-[11]. As a result of its high MUFA content, oxidative stability, anti-hypercholesterolemic, antioxidative, and anti-inflammatory properties, RBO is an excellent example of an antiatherogenic functional food [12], [13].

Flaxseed oil is a major source of alpha linolenic acid (ALA) which has a higher bioavailability than the whole seed.

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Flaxseed oil is shown to possess antioxidant, anti-platelet adhesion, and other bioactive properties [14]. It is a rich source of omega-3 and has 55% α -Linolenic with significant amounts of soluble and insoluble fiber with a protective effect on the heart. Flaxseed oil contains 73% PUFA and 18% MUFA [15], [16]. Several studies have shown that flaxseed oil is effective in lowering cholesterol levels and treating chronic inflammatory diseases [17], [18].

Another important edible oil is sunflower oil which contains a high amount of PUFA. It contains approximately 15% saturated, 85% unsaturated fatty acid and consists of 14–43% oleic and 44–75% linoleic acids [19], [20].

Recent studies reported that blending vegetable oils (RBO, flaxseed oil, and sunflower oil) can enhance blood lipid profiles, glycemic levels, and inflammatory parameters [21], [23]. The present study was aimed to evaluate the effect of a blend of rice bran oil, flaxseed oil, and sunflower oil on the glycemic and lipid parameters in general population.

2. Methodology

This was a retrospective, observational, longitudinal, single center real-world study, to analyze the data from outpatient and inpatient settings. Anonymized medical records with follow-up visits for subjects above 18 years included in the study. Subjects less than 18 years of age and pregnant women were excluded. The records of 73 subjects were included in the study. During the followup period (1 month), blood samples were collected for assessing the changes in glycemic parameters and lipid profiles.

The study was approved by the Royal Pune Independent Ethics Committee (RPIEC), located in Pune, India (Ethics Approval Number: RPIEC250523; dated 25 May 2023).

The aggregated data extracted from the EMRs was used for statistical analysis. Descriptive analysis was conducted on all variables. Categorical variables, such as dietary habits, were expressed in terms of frequencies and their respective percentages. Continuous variables, including lipid profile (TC, HDL, Non-HDL, LDL, triglycerides, apolipoprotein A, apolipoprotein B, apolipoprotein B/A1 ratio, very low

density lipoprotein, lipoproteinA, hs-CRP, TC/HDL ratio, LDL/HDL ratio), liver function test (total bilirubin, gamma glutamyl transferase (GGT), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase), renal function test (estimated glomerular filtration rate (EGFR), urea, creatinine, calcium, uric acid), thyroid function test (T3, T4, TSH), and glycated hemoglobin (HbA1C), were expressed as mean \pm SD (variables distributed normally) and median (IQR) (variables not distributed normally) based on their statistical distributions. To assess the effect of a blend of rice bran oil, flaxseed oil, and sunflower oil from baseline to one month of use in the aforementioned continuous variables, paired T-test (variables distributed normally) and Wilcoxon Signed Rank test (variables not distributed normally), was used basis on the statistical distribution of the variables. P-value less than 0.05 ($p < 0.05$) was considered as the threshold for establishing statistical significance.

3. Results

A total of 73 records were included in the study, of which 41 (56.16%) subjects were female. The mean age and body mass index (BMI) of the subjects were found to be 45.67 ± 15.38 years and $26.32 \pm 4.98 \text{ Kg/m}^2$, respectively. Among all subjects, 57 (78.08%) subjects were following a non-vegetarian diet. Only 2 subjects (2.74%) did workout and exercise and 1 subject (1.37%) indulged in occasional snacking. All subjects ($n=73$, 100%) had intake of fruits. (Table 1)

Table 1: Dietary habits in study population ($n=73$)

Parameter	n (%)
Diet Pattern	
Non-vegetarian	57 (78.08%)
Vegetarian	16 (21.92%)
Occasional Snacking	
	1 (1.37%)
Fruits Intake	
	73 (100%)
Exercise	
	2 (2.74%)

Dyslipidemia was the most (82.19%) reported comorbid condition with a mean duration of 4.31 ± 2.9 years among all the subjects. (Figure 1)

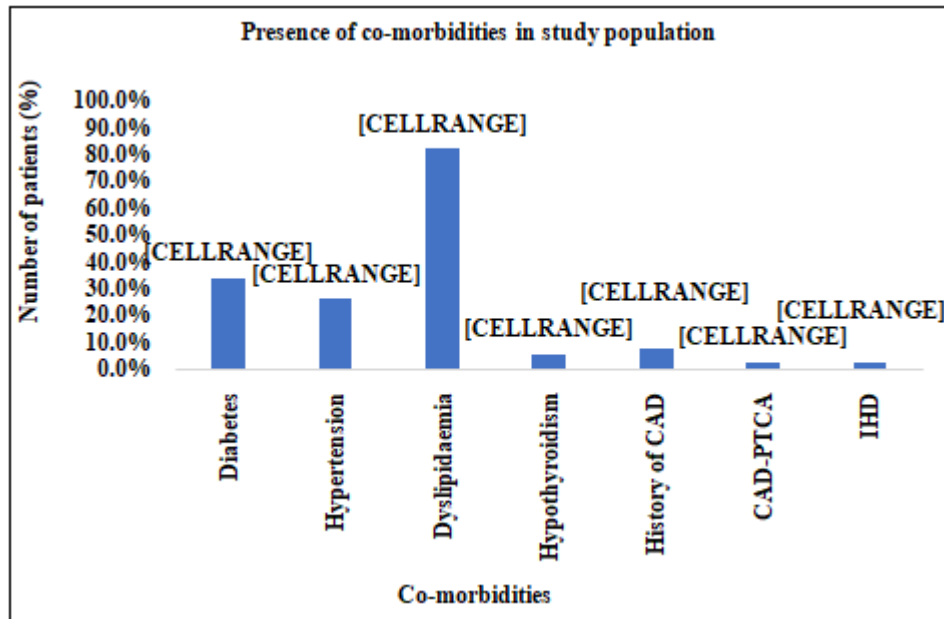


Figure 1: Co-morbidities in study population (n=73)

CAD: coronary artery disease; IHD: Ischemic heart disease, PTCA: percutaneous transluminal coronary angioplasty

After one month of followup, a significant reduction in total cholesterol was observed. ((TC) ($p < 0.001$), non-high-density lipoprotein cholesterol (non-HDL) ($p < 0.001$), TC/HDL ratio ($p = 0.048$), LDL/HDL ($p = 0.041$), total bilirubin ($p = 0.001$), GGT ($p = 0.021$), alkaline phosphatase ($p = 0.025$), creatinine ($p < 0.001$), calcium ($p = 0.020$), T3 ($p < 0.001$), T4 ($p < 0.001$) and thyroid stimulating hormone (TSH) ($p = 0.051$) was observed. (Table 2)

Table 2: Comparison of clinical parameters between baseline and one month follow-up (n=73)

Parameter	Baseline Mean ± SD/Median (IQR)	One month Follow-up Mean ± SD/ Median (IQR)	p-value
Lipid Profile			
TC (mg/dL)	175.11 ± 45.32	160.32 ± 39.68	<0.001*
HDL (mg/dL)	43.63 ± 9.66	42.25 ± 9.9	0.167*
Non-HDL (mg/dL)	130.96 ± 45.16	117.41 ± 37.61	<0.001*
LDL(mg/dL)	98 (41)	96 (30)	0.374
TG(mg/dL)	113 (118)	128 (103)	0.416
Apolipoprotein A (mg/dL)	116 (17)	117 (21)	0.851
Apolipoprotein B (mg/dL)	84 (28.5)	88 (28)	0.177
Apolipoprotein B/A1 Ratio	0.7 (0.3)	0.7 (0.3)	0.534
VLDL (mg/dL)	23 (23.3)	25 (21)	0.525
Lipoprotein(A) (mg/dL)	15.9 (29.45)	17.9 (29.55)	0.080
Hs-CRP	3 (7.85)	3.2 (5.3)	0.549
TC/HDL Ratio	3.9 (1.85)	3.6 (1.55)	0.048
LDL/HDL	2 (1.45)	2.3 (1.05)	0.041
Liver function test			
Total Bilirubin	0.56 (0.38)	0.5 (0.5)	0.001
GGT	20 (14.95)	20.4 (16.1)	0.021
SGOT	21 (8.5)	21 (7.85)	0.721
SGPT	17 (12)	20 (10.6)	0.445
Alkaline Phosphatase	83.31 ± 18.16	77.71 ± 18	0.025
Renal Function test			
EGFR	109 (25.5)	109 (22)	0.079
Urea	18 (8)	19 (7)	0.812
Creatinine	0.68 (0.21)	0.6 (0.2)	<0.001
Calcium	9.2 (0.45)	9.3 (0.6)	0.020
Uric Acid	4.6 (1.88)	4.4 (1.7)	0.651
Thyroid Function test			
T3 (ng/dL)	111.25 ± 22.2	102.81 ± 20.14	<0.001*
T4 (µg/dL)	9.2 (2.95)	8.6 (1.8)	<0.001
TSH (MIU/ML)	3.04 (2.12)	2.4 (1.95)	0.051
Average Sugar Test			
HbA1C	5.6 (1.3)	5.7 (1.55)	0.860

Note: *Paired to test p-values and rest all are Wilcoxon-sign rank test p-values.

EGFR:Estimated glomerular filtration rate; GGT:Gamma-glutamyl transferase; HbA1C:Glycated hemoglobin; HDL: High-density lipoprotein cholesterol; Hs-CRP: High-sensitivity C-reactive protein; LDL:Low-density lipoprotein cholesterol; SGOT:Serum glutamic-oxaloacetic transaminase; SGPT:Serum glutamic pyruvic transaminase; T3:Triiodothyronine; T4:Thyroxine; TC: Total cholesterol; TG: Triglycerides; TSH:Thyroid stimulating hormone;VLDL:Very low-density lipoprotein cholesterol.

For diabetes patients, after one month followup, a significant reduction in total bilirubin ($p=0.003$), GGT($p=0.018$), alkaline phosphatase ($p=0.006$), calcium ($p=0.055$),uric acid ($p=0.054$), T4 ($p=0.025$) was observed.(Table 3)

Table 3: Comparison of clinical parameters between baseline and one month follow-up for diabetes patients (n=25)

Parameter	Baseline Mean ± SD/ Median (IQR)	One month Follow-up Mean ± SD/ Median (IQR)	p-value
Lipid Profile			
TC (mg/dL)	176.52 ± 52.27	167.04 ± 44.21	0.150*
HDL (mg/dL)	46 (16)	43 (15.5)	0.532
Non-HDL (mg/dL)	131.6 ± 50.48	123.24 ± 41.16	0.177*
LDL (mg/dL)	94.12 ± 38.4	98.12 ± 34.91	0.440*
TG (mg/dL)	131 (119.5)	160 (83.5)	0.819
Apolipoprotein A (mg/dL)	119.4 ± 18.06	122.76 ± 17.08	0.159*
Apolipoprotein B (mg/dL)	88.8 ± 24.83	95.36 ± 23.97	0.106*
Apolipoprotein B/A1 Ratio	0.75 ± 0.2	0.77 ± 0.19	0.569*
VLDL (mg/dL)	26 (24)	31 (16.5)	0.972
Lipoprotein(A) (mg/dL)	17.4 (36.35)	23 (28.5)	0.076
Hs-CRP	3.3 (10.2)	2.9 (5.1)	0.079
TC/HDL Ratio	4 ± 1.24	3.91 ± 1.1	0.568*
LDL/HDL	2.03 ± 0.9	2.26 ± 0.82	0.090*
Liver function test			
Total Bilirubin	0.56 (0.34)	0.4 (0.4)	0.003
GGT	24 (28.55)	22 (17.9)	0.018
SGOT	20 (11.5)	18.6 (7.15)	0.241
SGPT	17 (11.5)	18 (10.5)	0.853
Alkaline Phosphatase	84 (26)	76 (16.5)	0.006
Renal Function test			
EGFR	97.88 ± 18.58	96.52 ± 23.73	0.650*
Urea	23.2 ± 7.8	22.69 ± 6.33	0.758*
Creatinine	0.66 (0.34)	0.7 (0.3)	0.248
Calcium	9.2 (0.4)	9.4 (0.55)	0.055
Uric Acid	4.6 (2.5)	4 (2)	0.054
Thyroid Function test			
T3 (ng/dL)	103.84 ± 23.03	95.88 ± 20.78	0.062*
T4 (µg/dL)	9.4 (3.3)	8.5 (1.7)	0.025
TSH (MIU/ML)	3.34 ± 1.81	3.65 ± 2.1	0.239*
Average Sugar Test			
HbA1C	6.9 (2.6)	7 (2.55)	0.925

Note:*Paired to test p-values and rest all are Wilcoxon-sign rank test p-values.

EGFR:Estimated glomerular filtration rate; GGT:Gamma-glutamyl transferase; HbA1C:Glycated hemoglobin; HDL: High-density lipoprotein cholesterol; Hs-CRP: High-sensitivity C-reactive protein; LDL:Low-density lipoprotein cholesterol; SGOT:Serum glutamic-oxaloacetic transaminase; SGPT:Serum glutamic pyruvic transaminase; T3:Triiodothyronine; T4: Thyroxine; TC: Total cholesterol; TG: Triglycerides; TSH:Thyroid stimulating hormone;VLDL:Very low-density lipoprotein cholesterol.

For dyslipidemia patients, after one month followup, a significant reduction in high-sensitivity C-reactive protein (Hs-CRP) ($p=0.033$), alkaline phosphatase ($p=0.041$) wasobserved. (Table 4)

Table 4: Comparison of clinical parameters betweenbaseline and one month follow-up fordyslipidemia patients(n=13)

Parameter	Baseline Mean ± SD/ Median (IQR)	One month Follow-up Mean ± SD/ Median (IQR)	p-value
Lipid Profile			
TC (mg/dL)	163.23 ± 44.55	152.54 ± 37.18	0.319
HDL (mg/dL)	42.77 ± 9.9	42.69 ± 10.86	0.976*
Non-HDL (mg/dL)	120.31 ± 41.77	109.31 ± 33.76	0.272*
LDL (mg/dL)	81.08 ± 29.9	85.54 ± 30.6	0.595*
TG (mg/dL)	154 (96.5)	150 (70.5)	0.346
Apolipoprotein A (mg/dL)	116.38 ± 15.82	121.77 ± 13.95	0.174*
Apolipoprotein B (mg/dL)	84.15 ± 20.96	86.38 ± 20.43	0.714*
Apolipoprotein B/A1 Ratio	0.7 (0.25)	0.7 (0.3)	0.833
VLDL (mg/dL)	30 (19.5)	29 (14)	0.507
Lipoprotein(A) (mg/dL)	16 (45.3)	20 (33.45)	0.583
Hs-CRP	3.3 (13.7)	1.7 (4.45)	0.033

Parameter	Baseline Mean \pm SD/ Median (IQR)	One month Follow-up Mean \pm SD/ Median (IQR)	p-value
TC/HDL Ratio	3.9 (1.75)	3.8 (1.8)	0.220
LDL/HDL	1.8 (1.15)	2.3 (1.2)	0.291
Liver function test			
Total Bilirubin	0.71 (0.45)	0.7 (0.6)	0.152
GGT	20 (12)	22 (17.95)	0.363
SGOT	18 (9.5)	21 (9.6)	0.916
SGPT	15 (10.9)	18 (12)	0.421
Alkaline Phosphatase	92.1 \pm 21.93	74.38 \pm 23.99	0.041*
Renal Function test			
EGFR	94 (20)	91 (26)	0.476
Urea	27 (13)	22 (7.5)	0.151
Creatinine	0.69 (0.26)	0.7 (0.25)	0.964
Calcium	9.2 (0.55)	9.5 (0.25)	0.077
Uric Acid	4.7 (2.97)	5.4 (2.65)	0.506
Thyroid Function test			
T3 (ng/dL)	103.77 \pm 32.41	95.62 \pm 23.5	0.293*
T4 (μ g/dL)	9.2 (3.05)	8.1 (2.7)	0.255
TSH (MIU/ML)	4.11 (3.04)	3.2 (3.65)	0.169
Average Sugar Test			
HbA1C	6.9 (2.8)	7 (2.65)	1.000

Note:*Paired to test p-values and rest all are Wilcoxon-sign rank test p-values.

EGFR:Estimated glomerular filtration rate; GGT:Gamma-glutamyl transferase; HbA1C:Glycated hemoglobin; HDL: High-density lipoprotein cholesterol; Hs-CRP: High-sensitivity C-reactive protein; LDL:Low-density lipoprotein cholesterol; SGOT:Serum glutamic-oxaloacetic transaminase; SGPT:Serum glutamic pyruvic transaminase; T3:Triiodothyronine; T4:Thyroxine; TC: Total cholesterol; TG: Triglycerides; TSH: Thyroid stimulating hormone; VLDL: Very low-density lipoprotein cholesterol.

4. Discussion

The present study aimed to evaluate the effect of a blend of RBO, flaxseed oil, and sunflower oil in the general population and showed a beneficial effect on lowering the lipids, renal, hepatic, thyroid, and inflammatory markers in Indian population.

According to the Indian Dietetic Association, a BMI of 23 and above is considered as overweight and a BMI of 25 and above as obesity [24]. Previous studies have reported obesity and dyslipidemia as the crucial factors in inducing oxidative stress and the release of proinflammatory cytokines, which are the risk factors for CVD [25]. Similar findings were observed in the current study where the mean BMI of the subjects was found to be $26.32 \pm 4.98 \text{Kg/m}^2$, and dyslipidemia was the most (82.19%) reported comorbid condition among all the subjects.

Several studies have revealed that specific oil blends can improve blood lipid profiles and other cardiometabolic parameters, when using blended vegetable oils that are widely consumed in Asia [26]-[27]. In the current study, after one month of follow up, a significant reduction in TC ($p < 0.001$), non-HDL ($p < 0.001$), TC/HDL ratio ($p = 0.048$), and LDL/HDL ($p = 0.041$), was observed. In concordance with the present study findings, Sumanto et al., reported the blend of refined RBO, flaxseed, and sesame oils, significantly reduced TC ($p < 0.0001$), LDL ($p < 0.0001$), TG ($p < 0.0001$), apoB ($p < 0.0001$), and HDL ($p < 0.0001$) [26]. A single blind crossover Indian study by Kennedy et al., demonstrated RBO and sunflower oil blend significantly reduced TC and LDL in hyperlipidemic (8% and 7%) and normolipidemic (4% and 5%) patients respectively [8]. The possible mechanism of action by which gamma oryzanol reduces cholesterol may be by inhibition of β -Hydroxy β -methylglutaryl-CoA (HMG-CoA) reductase, a key enzyme

involved in cholesterol synthesis in the liver. Moreover, sterols in the gamma-oryzanol molecule may also decrease cholesterol absorption [8], [28], [29].

After one month of follow up, the current study showed a significant reduction in total bilirubin ($p = 0.001$), GGT ($p = 0.021$), alkaline phosphatase ($p = 0.025$), creatinine ($p < 0.001$), and calcium ($p = 0.020$). In concordance, Stuglin et al., reported that use of flaxseed oil for 4 weeks significantly decreased serum levels of creatinine [30]. Rana et al., in albino rats, reported a decrease in plasma alkaline phosphatase activity in RBO fed group (490.00 ± 57.12) as compared to the groundnut oil group (583.30 ± 33.00) [31]. Few studies have reported modulation of risk factors by RBO in renal disease [32], [33]. Probably, the blends of these edible oils reduce the calcium absorption, which help in reducing formation of renal calculi and aid in prevention of renal disease [34], [35].

RBO, sunflower oil and flaxseed oil have shown a beneficial effect in diabetes patients. Data from different studies recommend the consumption of a blend of edible oils in diabetes patients [22]. In the present study, for diabetes patients after one month follow up, a significant reduction in total bilirubin ($p = 0.003$), GGT ($p = 0.018$), alkaline phosphatase ($p = 0.006$), calcium ($p = 0.055$), uric acid ($p = 0.054$), T4 ($p = 0.025$) was observed.

Dyslipidemia is a consequence of the interplay between genetic faults and environmental factors (such as nutrition, exercise, and pharmacological effect), with the type of fatty acids in the diet having an influence on the blood lipid profile [36]. The current study reported a significant reduction in Hs-CRP ($p = 0.033$), alkaline phosphatase ($p = 0.041$) after one month follow up in patients with dyslipidemia. Moreover, RBO has been shown to protect against oxidative stress and inflammation [37]. Many

epidemiological reports have revealed the anti-inflammatory characteristics of gamma oryzanol, cycloartenylferulate and ferulic acid in RBO [38], [39]. Rao et al. showed that RBO can reduce serum concentrations of CRP, an indicator of acute inflammation, and interleukins [40]. Marjaan et al., reported a decrease in the pro-inflammatory and pro-oxidative mediators (for example, CRP, interleukin (IL)-1b) with consumption of RBO and/or its main components [23].

After one month of follow up, the present study showed a significant reduction in T3 ($p < 0.001$), T4 ($p < 0.001$) and TSH ($p = 0.051$). Yohnosuke et al., reported that gamma oryzanol extracted from RBO produced a significant reduction in the elevated serum TSH levels in hypothyroid patients [41]. Further, Saeed et al., reported that flaxseed oil has a beneficial effect on thyroid function by enhancing oxidative status in male rats [42]. The lack of change in serum thyroid hormone levels after gamma oryzanol consumption suggests that the blend of these edible oils inhibit TSH secretion by a direct action on the hypothalamus or pituitary gland [43].

Different edible oils have been recognized to regulate blood lipid levels for almost a century. According to several studies, a blend of edible oils reduces the lipid parameters, glycemic levels, and inflammatory markers [21]-[23]. RBO, flaxseed oil and sunflower oil blend, has been recognized as a healthy blend of oils for cardiovascular health because of its lipid lowering properties. The major phytochemical found in RBO is gamma-oryzanol, and phytosterols in flaxseed oil and sunflower oil has been shown to improve levels of blood lipids and reduce oxidative stress, thereby possibly reducing CVD risk [29], [44].

5. Conclusion

The blend of rice bran oil, flaxseed oil, and sunflower oil significantly lowered total cholesterol, non-high-density lipoprotein cholesterol, TC/HDL ratio, LDL/HDL, total bilirubin, gamma-glutamyl transferase, alkaline phosphatase, creatinine, calcium, uric acid, T3, T4, thyroid stimulating hormone, high-sensitivity C-reactive protein, and alkaline phosphatase. Hence, the blend of these edible oils could be considered beneficial for lowering the lipids, renal, hepatic, thyroid, and inflammatory markers in the Indian population.

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