Int Cardiovasc Res J.2015;**9**(1):28-33.

International Cardiovascular Research Journal

www.ircrj.com

Comparison of the Effects of Edible Oils: Rice Bran, Grape Seed, and Canola on Serum Lipid Profile and Paraoxonase Activity in Hyperlipidemic Rats

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 28 Apr 2014
Revised: 09 Aug 2014
Accepted: 23 Aug 2014

Keywords:
Rice Bran Oil
Canola Oil
Dyslipidemia

ABSTRACT

Background: Dyslipidemia is considered as one of the crucial contributors to cardio-cerebro-vascular diseases.

Objectives: The present study aimed to compare the effects of Rice Barn Oil (RBO), Grape Seed Oil (GSO), and Canola Oil (CO) on dyslipidemia and oxidative stress in experimentally induced hyperlipidemic rats.

Materials and Methods: In the present experimental study, forty hyperlipidemic male Wistar rats were randomly assigned to 4 groups to receive RBO, GSO, or CO or Soy Bean Oil (SBO), as controls, for 4 weeks following a 3-week period of Atherogenic Diet (AD) intake. Blood samples were collected at the beginning of the study, after inducing dyslipidemia, and at the end of the experimental period. Then, the data were entered into the SPSS statistical software (v. 13.0) and analyzed using paired t-test, paired sample Wilcoxon signed rank test, and Kruskal-Wallis test.

Results: AD elevated lipid and/or lipoprotein profile and decreased the paraoxonase activity in the hyperlipidemic rats. The results of paired t-test revealed that RBO led to a significant improvement in serum lipoprotein profile and paraoxonase activity. Besides, a significant difference was found in the GSO group regarding all the measured parameters, except for paraoxonase activity. Moreover, CO diet showed a significant hypolipidemic effect on serum Triglyceride (TG) and Total Cholesterol (TC) and led to a slight improvement in Low Density Lipoprotein-Cholesterol (LDL-C) and High Density Lipoprotein-Cholesterol (HDL-C).

Conclusions: The results of the present study suggested that vegetable oils, including RBO, GSO, and CO, might improve dyslipidemia and oxidative stress in hyperlipidemic rats. Indeed, substituting saturated fatty acids with unsaturated fatty acids in rats' diet had beneficial effects on serum lipid profile and oxidative stress. Comparison of the 3 edible oils showed that GSO had a more profound effect on decreasing hyperlipidemia.

►*Implication for health policy/practice/research/medical education:*

Hyperlipidemia has become a major concern in the pathogenesis of chronic metabolic diseases, especially cardio-vascular diseases. Changes in dietary pattern, especially in dietary oil, are one of the crucial components of controlling the plasma lipid and lipoprotein concentrations. In the present study, the hypolipidemic and antioxidant properties of RBO, GSO, and CO were compared to show which of these three edible oils can better control hyperlipidemia.

1. Background

Hyperlipidemia refers to a group of metabolic disorders characterized by high concentrations of triglyceride and/or cholesterol in blood circulation. Long-term untreated hyperlipidemia is an important contributor to development and progression of micro- and macro-vascular complications, including microangiopathy, cardio-cerebro-vascular diseases, and metabolic abnormalities. The prevalence of hyperlipidemia has dramatically increased worldwide mostly due to the modern lifestyle and increase in consumption of high-fat diets (1).

Dietary fat is a detrimental factor in regulation of plasma

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cholesterol levels. There is increasing evidence to support the hypocholesterolemic effects of vegetable oils rich in Polyunsaturated Fatty Acids (PUFAs), mainly Linoleic Acid (LA) (2).

Rice Bran Oil (RBO) is not a popular oil worldwide, but it is in steady demand particularly in Asian countries as a so-called ''healthy oil'' (2). Recent studies have demonstrated that rice bran and its main lipid components (including unsaturated fatty acids, triterpene alcohols, phytosterols (β-sitosterol), tocotrienols, and tocopherol, may improve the plasma lipid and lipoprotein profile of rodents, rabbits, non-human primates, and humans (3).

Moreover, grape seed (Vitis vinifera Linn) contains important vitamins, minerals, and polyphenols including flavonoids, proanthocyanidins, and procyanidins (4). Grape Seed Oil (GSO) and its extract (GSE) have been recently shown to have various pharmacological properties, such as chemo- and/or oxidative stress protection (5), as well as anti-inflammatory (6), anti-diabetic, and, through free radical scavenging property, anticancer activities (7).

Canola Oil (CO), which is used widely and is considered as one of the healthiest oils, can not only improve health, but can also reduce the risk of chronic diseases due in part to its special fatty acid composition (i.e., lowest in saturated-, high in mono-unsaturated-, and the best source of omega-3 fatty acids compared to other fat sources). Moreover, studies have indicated that using CO instead of saturated fat reduced the concentration of both Total Cholesterol (TC) and Low Density Lipoprotein Cholesterol (LDL-C), improved insulin sensitivity, and increased the level of tocopherol compared to other dietary sources of fat (8). The fatty acid composition of RBO, GSO, and CO has been listed in Table 1 (8-10).

2. Objectives

The current study aims to compare the effects of RBO, GSO, and CO on blood lipid profile and paraoxonase activity in experimentally induced hyperlipimic rats.

3. Materials and Methods

3.1. Animal Experiment and Diets

In this study, 40 young male Wistar rats with 170 – 200 gr body weight were purchased from Razi Research Center, Shiraz, Iran. The animals were housed in stainless steel cages kept in a temperature-controlled air conditioned room (20 – 22 °C) with 12-h light/dark cycle and 55 ± 5% humidity. The animals spent the adaptation period in this environment one week prior to commencing the experiment.

All the procedures were conducted based on the guide for care and use of laboratory animals. Besides, the study was approved by the Ethics Committee of Animal Experimentation of Shiraz University of Medical Sciences, Shiraz, Iran.

The rats were fed by basal commercial diet (AIN-93M) for 1 week during the adaptation period followed by an Atherogenic Diet (AD) for 3 weeks in order to induce hyperlipidemia. Composition of the AD has been displayed in Table 2. Indeed, the Soy Bean Oil (SBO) content of the Basal Diet (BD) was replaced with coconut oil in AD for 3 weeks. After the AD diet, the hyperlipidemic rats were randomly assigned to 4 groups of RBO, GSO, CO, and SBO each containing 10 animals. The diets of all the four groups were mixed thoroughly with 1% cholesterol and 0.5% cholic acid for 4 weeks.

The rats' food intake was measured daily and their body weight was measured weekly. All the rats were anesthetized with ether following a 16-h fast. Blood samples were drawn from the animals' tails and aortas at the beginning and end of the experiment, respectively. The serum was immediately obtained by centrifugation (at 3000 rpm at 4 °C for 15 min) and was stored at -20 °C until analysis. It should be mentioned that all the parameters were measured

Table 1. Fatty Acid Compositions of RBO, GSO, and CO

	LA	OA	PA	SA	ALA
RBO	34.4%	38.4%	21.5%	2.9%	2.2%
GSO	75%	15%	6%	3%	1%
CO	21%	61%	4%	2%	11%

Abbreviations: RBO, Rice bran oil; GSO, Grape seed oil; CO, Canola oil; LA, Linoleic acid; OA, Oleic acid; PA, Palmitic acid; SA, Stearic acid; ALA, α-linolenic acid

Table 2. Composition of Basal and Atherogenic Diets (g/100 g diet)

Component	(g/100 g BD)	(g/100 g AD)
Casein	14	14
Cornstarch	56.07	56.07
Soybean oil	10	-
Coconut oil	-	10
Cholesterol	-	1
Colic acid	-	0.5
Cellulose	5	5
Mineral mixture	3.5	3.5
Vitamin mixture	1	1
Choline chloride	0.25	0.25
L-cystine	0.18	0.18

Abbreviations: AD, Atherogenic diet; BD, Basal diet

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3.2. Serum Lipid

TC and LDL-C were respectively analyzed based on cholesterol oxidized and enzymatic methods proposed by Allain et al. (11) using commercial kits (Eram Teb, Iran).

In addition, serum High Density Lipoprotein Cholesterol (HDL-C) was assessed utilizing heparin manganese precipitation procedure and a commercial kit (Zist Shimmy, Iran) (12). Besides, Triglyceride (TG) concentration was determined enzymatically by a commercial kit (Man, Iran) based on modification of the lipase-glycerol phosphate oxidized method (13).

3.3. Paroxonase Activity

The enzyme activity was assayed following purification of paraoxon (Sigma, USA) according to the procedure proposed by Rodrigo et al. (14).

3.4. Statistical Analyses

All data analyses were performed using the SPSS statistical software, version 13. The results have been presented as mean ± Standard Deviation (SD). Lipid profile and paraoxonase activity were compared by paired t-test, paired sample Wilcoxon signed rank test, and Kruskal-Wallis test. Additionally, $P < 0.05$ was considered as statistically significant.

4. Results

At the beginning of the experiment, no significant differences were found among the four groups regarding body weight and TG, TC, LDL-C, and HDL-C levels. Also, the four groups were similar with respect to paraoxanase activity at the beginning of the study (data not shown).

Feeding by AD for 3 weeks resulted in development of hyperlipidemia and reduction of serum paraoxonase activity in all the experimental rats. Indeed, a significant increase was observed in the rats' serum TG, TC, LDL-C, and HDL-C levels after being fed with AD for 3 weeks ($P < 0.001$) (Table 3).

Feeding with RBO resulted in a statistically significant reduction in serum levels of TG, TC, and LDL. Feeding with RBO also led to a significant increase in HDL-C level and paroxonase activity ($P < 0.05$, Table 4). Similarly, a significant decrease was observed in TG, TC, and LDL levels in the group fed by GSO for four weeks ($P < 0.05$, Table 4). In this group, HDL-C level increased significantly, while paraoxonase activity did not change noticeably (Table 4).

CO also resulted in lower levels of TG, TC, and LDL-C and higher HDL-C level and paraoxonase activity. However, no significant differences were found between the mean levels of LDL-C and HDL-C (Table 4).

Comparison of the mean values of the measured parameters in the four study groups using one-way ANOVA only indicated a significant difference regarding TG concentration (Table 5). In addition, the results of Tukey's multiple comparison test demonstrated a significant

Table 3. The Effect of AD on Lipid Profile and Paraoxonase Activity in the Rats Fed for 3 Weeks

	BD (n = 40)	AD (n = 40)	P value
TG (mg/dL)	66.57 ± 17.54	127.30 ± 29.30	< 0.001
TC (mg/dL)	53.10 ± 11.69	125.47 ± 30.90	< 0.001
LDL (mg/dL)	20.90 ± 10.29	33.57 ± 9.31	< 0.001
HDL (mg/dL)	17.40 ± 5.14	22.40 ± 5.81	< 0.001
PON1 (IU/mL)	31.38 ± 8.81	24.61 ± 9.43	< 0.001

Abbreviations: AD, Atherogenic diet; BD, Basal diet (AIN-93M); PON1, paraoxonase activity
*paired t-test

Table 4. The Effect of RBO, GSO and CO on Lipid Profile and Paraoxonase Activity in the Rats Fed for 4 Weeks

		Before	After	P value
RBO	TG (mg/dL)	125.50 ± 18.42	98.90 ± 21.02	< 0.001
	TC (mg/dL)	68.60 ± 8.07	119.70 ± 17.33	< 0.001
	LDL (mg/dL)	26.30 ± 4.60	32.32 ± 4.20	0.006
	HDL (mg/dL)	27.10 ± 6.26	22.50 ± 4.94	< 0.001
	PON1 (IU/mL)	28.60 ± 11.73	20.64 ± 4.58	0.034
GSO	TG (mg/dL)	71.40 ± 19.13	132.20 ± 25.03	< 0.001
	TC (mg/dL)	62.40 ± 10.14	125.90 ± 37.63	< 0.001
	LDL (mg/dL)	28.10 ± 7.53	32.31 ± 8.33	0.015
	HDL (mg/dL)	24.20 ± 6.42	20.90 ± 4.55	0.012
	PON1 (IU/mL)	30.04 ± 6.76	26.23 ± 2.77	0.096
CO	TG (mg/dL)	96.60 ± 27.47	144.30 ± 38.03	< 0.001
	TC (mg/dL)	67.40 ± 22.02	131.60 ± 28.75	0.003
	LDL (mg/dL)	30.70 ± 8.05	34.20 ± 7.89	0.065
	HDL (mg/dL)	26.20 ± 7.08	21.60 ± 8.26	0.354
	PON1 (IU/mL)	24.23 ± 8.41	20.47 ± 8.53	0.002

Abbreviations: RBO, Rice bran oil; GSO, Grape seed oil; CO, Canola oil; PON1, paraoxonas activity.
*paired t-test; Values are presented as means ± SD

Table 5. Comparison of the Effects of SBO, RBO, GSO, and CO on Lipid Profile and Paraoxinase Activity in the Hypercholestrolemic Rats Fed for 4 Weeks

	SBO	RBO	GSO	CO	P value
TG (mg/dL)	58.60 ± 10.94	98.90 ± 21.03	71.40 ± 19.13	96.60 ± 27.47	< 0.001
TC (mg/dL)	61.60 ± 16.49	68.60 ± 8.07	62.40 ± 10.14	67.40 ± 22.02	0.662
LDL (mg/dL)	31.60 ± 14.04	26.30 ± 4.66	28.10 ± 7.53	30.70 ± 8.05	0.564
HDL (mg/dL)	26.20 ± 7.08	27.10 ± 6.26	24.20 ± 6.42	26.20 ± 7.08	0.648
PON1 (IU/mL)	39.84 ± 24.5	28.59 ± 11.7	30.04 ± 6.76	24.23 ± 8.41	0.151

Abbreviations: SBO, Soy bean oil; RBO, Rice bran oil; GSO, Grape seed oil; CO, Canola oil; PON1, paraoxonase activity.
* Kruskal-Wallis, one-way ANOVA; Values are presented as means ± SD

difference between the hyperlipidemic rats receiving SBO and those receiving RBO (P = 0.001), CO (P = 0.001), and GSO (P < 0.05) regarding TG level.

5. Discussion

Hyperlipidemia has become a major concern in pathogenesis of chronic metabolic diseases, especially cardiovascular diseases. Changes in dietary pattern, especially in dietary oil, are among the crucial components of controlling the plasma lipid and lipoprotein levels (1). In the present study, the hypolipidemic and antioxidant properties of RBO, GSO, and CO were compared to show which one of these three edible oils can better control hyperlipidemia. Our results demonstrated that feeding AD for 3 weeks could induce hyperlipidemia and decrease paraoxonase activity in male Wistar rats. This was in an agreement with the results of the previous studies (15, 16).

A number of animal and/or human studies have demonstrated that RBO is as effective as other vegetable oils in lowering plasma cholesterol levels (17), needless to say that in some cases, RBO lowered plasma cholesterol level more effectively than other commonly used vegetable oils rich in LA (18). The results of the present study indicated that RBO-containing diet showed hypolipidemic effects on plasma TC, TG, and LDL-C levels. It also led to an increase in HDL-C levels. This effect can be attributed to existence of specific components in RBO, such as γ-oryzanol (and its constituents, triterpene alcohols) and tocotrienols (2).

Oryzanol is an antioxidant compound associated with decrease in plasma cholesterol, cholesterol absorption, and platelet aggregation (19). The most notable property of γ-oryzanol is the ability to lower the plasma cholesterol level (20). Human studies, although sparse, have successfully used 300 mg/d of γ-oryzanol to lower LDL levels (21).

Sterols are also a component of γ-oryzanol, but most of the sterols in RBO are free phytosterols. These compounds act in the gastrointestinal (GI) tract to inhibit the absorption of cholesterol. Vissers et al. showed that consuming margarine containing 2.1 g/d of plant sterols from RBO for 3 weeks caused a 9% reduction in LDL level in normolipidemic subjects (22). Rice bran contains a variety of phenolic compounds with antioxidant activity (18). These compounds may be responsible for increasing the paraoxonase activity, as shown in the present study.

Rice bran is also a rich source of tocotrienols (23) which are unsaturated forms of tocopherols. Tocotrienols possess excellent antioxidant activity in vitro and are considered to be superior to tocopherols in suppressing Reactive Oxygen Species (ROS). Additionally, tocotrienols reduce HMG-CoA reductase, thus limiting cholesterol synthesis. Moreover, tocotrienols are bioavailable; plasma tocotrienol concentrations increased by 16 folds following consumption of 50 mg rice bran daily for 4 weeks (24). However, clinical studies have not generally shown improvement in lipid profiles of hypercholesterolemic subjects supplemented with 160 to 200 mg tocotrienols per day (25, 26).

The results of this study showed a significant decrease in TG level in the RBO group. Lower plasma TG level has been reported to be associated with RBO consumption in both humans (27) and rats (28). However, the precise mechanism has not been thoroughly elucidated yet (29).

GSO contains high concentrations of Vitamin E, LA, flavonoids, resveratrol, and Oligomeric Proanthocyanidins (OPCs). OPCs can also be found in grape skin, although in lower concentrations, and have been proved to encourage brain, heart, eyes, and skin health as an efficient antioxidant (30). GSO showed a significant hypolipidemic effect in the present study. However, despite the high amounts of antioxidant activity, no significant increase was observed in paraoxonase activity in the GSO group. This must be due in part to the fact that antioxidant activity of grape seed may decrease while processing the seed for oil extraction.

Our findings also revealed that GSO markedly suppressed the elevation of serum TG and cholesterol levels in the hyperlipidemic rats. These results confirmed the in vitro activity of GSE, indicating that acute antihyperlipidemic activities of GSE may be mediated through the inhibition of both lipid digestion and absorption (6). The researchers of the same study also reported that long-term supplementation of GSE reduced plasma lipid profiles and prevented high-fat diet-induced obesity and the related metabolic pathways in hamsters by improving either adipokine secretion or oxidative stress (6). Moreover, Adisakwatana suggested that long-term supplementation with GSE might reduce plasma lipid profile mediated through inhibition of pancreatic lipase, cholesterol esterase, cholesterol micellization, and bile acid binding (1).

Binding to bile acids and increasing their fecal excretion have been hypothesized as another possible mechanism of GSO in lowering plasma cholesterol levels. Ngmukote indicated that gallic acid, catechin, and epicatechin exhibited a primary bile acid-binding capacity (glycodeoxycholic acid and taurocholic acid). They also showed in their previous study that taurocholic acid, glycodeoxycholic acid, and taurodeoxycholic acid were bound by GSE by 30%, 70%, and 25%, respectively (31). They suggested that the ability of GSE to bind to bile acids may be linked to its gallic acid, catechin, and epicatechin content (31). Indeed, their findings obviously indicated that the 3 major

polyphenols in grape seed; i.e., gallic acid, catechin, and epicatechin, inhibited pancreatic cholesterol esterase. These also bind to bile acids and reduce the solubility of cholesterol in micelles.

CO contains low Saturated Fatty Acids (SFAs), is almost rich in Mono-Unsaturated Fatty Acids (MUFAs), and has some PUFAs. It has a sustainable balance between ω-6 and ω-3 fatty acids, as well (8). Our results demonstrated that diet containing an oil rich in MUFAs, including CO, could significantly improve TG, TC, LDL, and HDL levels. Similar results were also found in the previous studies using Oleic Acid (OA) and LA-rich diets (32), the effect of which, at least partially, can be attributed to the special fatty acid composition of CO (32). These two unsaturated fatty acids make up 61% and 21% of the total fatty acids in CO, respectively (8). Hypocholesterolemic effect of both OA and LA has been well documented in the previous studies (32). These observations suggested that cholesterol improving components of CO might be due in part to either reducing synthesis or increasing removal of lipoprotein particles rather than alteration in the cholesterol content of the LDL fraction. Spady and Dietschy reported that SFA, unlike OA and LA, increased the suppressive impact of dietary cholesterol on hepatic LDL- receptor activity in hamsters. This observation coincides with the observation that high OA or LA intakes led to a significant increase in the fractional catabolic rate of LDL particles in humans (32).

Although fish oil rich in Eicosapentaenoic Acid (EPA) has been shown to decrease serum TG level, Alpha Linoleic AcidALA appears to have a negligible effect on plasma TG level. Responses of plasma TG levels to OA- or LA-rich diets have been reported previously, but with no consistent and/or evident pattern (32). At the same time, changes seen in serum TG levels in the present study could not be explained by the fatty acid composition of CO.

One of the limitations of the current study was not measuring other indices of antioxidant properties (total antioxidant capacity) of the experimental vegetable oils.

In summary, the present study indicated that RBO, GSO, and CO had a hypolipidemic effect and improved oxidative stress. Moreover, this study showed that GSO played a more effective role in reducing hyperlipidemia compared to RBO and CO. Yet, further studies are recommended to be conducted on the issue.

Acknowledgements

This article was extracted from Narges Alinejad's M.Sc. thesis and was financially supported by Shiraz University of Medical Sciences (proposal No. 87-4247). Hereby, the authors would like to express their appreciation towards the vice-chancellor for research affairs of Shiraz University of Medical sciences for financially supporting the study.

Authors' Contribution

All authors took part in data collection, data analysis, preparing the draft of the manuscript, and final edition of the manuscript for submission.

Financial disclosure

There is no financial disclosure.

Funding/Support

Shiraz University of Medical Sciences, as the sponsor of the project, supported all stages of the research.

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