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Research Article

Antihyperlipidemic Activity of Various Combinations of Rice Bran Oil and Safflower Oil on Triton WR– 1339 Induced Hyperlipidemia in Rats

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Abstract

Numerous studies investigating the effect of rice bran oil (RBO) on lipid metabolism and oxidation in rats have given interesting but often contrasting results. Therefore, the current study was initiated to investigate the anti-hyperlipidemic potential of the various combinations of safflower oil (SO), a polyunsaturated fatty acid (PUFA) rich vegetable oil and RBO in Triton WR-1339-induced acute hyperlipidemia in wistar rats.

Various combinations of RBO and SO were evaluated for anti-hyperlipidemic activity by estimating serum triacylglyceride (TG), total cholesterol (TC), very low density lipoprotein-cholesterol (VLDL), low-density lipoprotein-cholesterol (LDL) and high-density lipoprotein-cholesterol (HDL) levels with atorvastatin as the reference standard. The degree of protection was also assessed by measuring the levels of various hepatic anti-oxidant enzymes.

Administration of [RBO+SO (7:3)] for 30 days demonstrated the maximum depression in the TG and VLDL levels and the maximum elevation in HDL and HDL/ TC ratio along with a significant increase in the level of hepatic anti-oxidant enzymes.

The findings indicate that [RBO+SO (7:3)] possesses the potential to lower plasma lipid concentrations and might be of therapeutic benefit in the treatment of hyperlipidemia. However further studies are warranted to confirm its mechanisms for the higher anti-hyperlipidemic effect as compared to the other combinations.

Key words: Rice bran oil, safflower oil, Triton WR-1339, hyperlipidemia, rats.

INTRODUCTION

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions that account for most morbidity and mortality among middle-aged and older adults. Epidemiological studies have identified several lifestyle or behavioral, genetic, or metabolic factors that affect the concentrations of the various plasma lipoproteins, leading to atheromatous disease [1]. Some additional reports have further demonstrated that increased formation of free radicals/reactive oxygen species (ROS) and concomitant depletion of antioxidants contribute to cardiovascular disease (CVD) progression [2].

The use of currently available allopathic hypolipidemic drugs has been impaired by numerous side effects, severe contraindications and extravagant cost and this has further necessitated the search for suitable alternatives [3].

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In recent years, rice bran oil (RBO) has been in steady demand as a ''healthy oil'' in Asian countries, particularly in India on account of its effectiveness as other vegetable oils in lowering plasma cholesterol levels from a number of animal and human studies [4, 5]. In certain cases, there are evidences of more prominent lowering of plasma cholesterol by RBO as compared to other commonly used vegetable oils rich in linoleic acid [5]; an effect that can be attributed to its rich unsaponifiable fraction mainly composed of sterols such as γ-oryzanol, triterpene alcohols and perhaps tocotrienols [5,6]. Furthermore, it has also been demonstrated that inclusion of RBO in the diet improves the anti-oxygenic potential and ensures protection against oxidative stress [7].

Safflower oil (SO), a polyunsaturated fatty acid (PUFA) rich vegetable oil, on the other hand, has been reported to significantly reduce plasma cholesterol and triglyceride levels, thereby minimizing the incidence of heart attacks [8,9]. Kinobeon A, isolated from cultured cells of safflower has been shown to have significant inhibitory activity against lipid peroxidation in rat liver microsomes and claimed to have effective superoxide radical scavenging activity [10].

Previous reports implicate the beneficial effects of combination of RBO and SO in various proportions in various experimental animal models; however, there is scarcity of *in vivo* experiments that can exemplify the distinct proportions of individual oil that gives the maximum beneficial effect in hyperlipidemia. It has been asserted that the micronutrients of the RBO unsaponifiable fraction in combination with the high linoleic acid content of SO, acts synergistically to lower the serum cholesterol level [11]. In addition, there is an enhanced possibility of increasing the cost/efficacy ratio by blending RBO with other less expensive vegetable oil such as SO that is rich in polyunsaturated fatty acid [12].

Therefore, the present study was designed to evaluate and optimize the anti- hyperlipidemic activity of various combinations of RBO and SO on triton WR 1339 induced acute hyperlipidemic rats.

MATERIALS AND METHODS

Drugs and Chemicals

RBO and SO were purchased from the A.P Organics Ltd, Dhuri (Punjab), India and S.K. Oil Industries, Jalgaon, Maharashtra, India respectively. Triton WR-1339 was purchased from Sigma-Aldrich Chemie GMBH. Atorvastatin was obtained as a gift sample from Torrent Research Center, Gandhinagar, Gujarat, India. All diagnostic kits were purchased from Lab Care Diagnostics Pvt. Ltd., India. All solvents and reagents were either of HPLC grade or analytical reagent grade and were obtained from commercial sources.

Chemical test for the identification of fixed oils

RBO and SO, being fixed oils, their presence was confirmed by the chemical test for glycerine, which is produced by their hydrolysis using sodium hydroxide and sodium hydrogen sulphate [8].

Evaluation of physicochemical parameters of SO and RBO

Both RBO and SO were analyzed for various physicochemical parameters, including density, viscosity [13], boiling point [14], acid value [15], saponification value [15] and ester value [15].

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, Ahmadabad as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Healthy adult wistar rats of either sex (250-300 gm weight) were selected for the study. Animals were maintained at 22 ± 2^{o}C and kept in well ventilated animal house under natural photoperiodic

condition in polypropylene cages with free access to food and water *ad libitum*. During the period of experiment the animals were fed with the Amrut Rat Diet supplied by Pranav Agro Industry Ltd, Pune, Maharashtra, India. Animals were acclimatized for one week before starting the experiment.

Treatment protocols

Rats (n=48) were randomized into the eight groups as mentioned below:

Group 1 (Normal Control): Received normal saline throughout the experimental period.

Group 2 (Triton WR 1339 Control): Saline per day for 30 days and on the 31st day intra-peritoneal injection of Triton WR 1339 (300 mg/kg in saline) was given.

Group 3 (Triton WR 1339 + RBO): Treatment was given with RBO (7 ml/kg, p.o.) for 30 days and on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg, in saline) was given.

Group 4 (Triton WR 1339 + SO): Treatment was given with SO (7 ml/kg, p.o.) for 30 days & on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg in saline) was given.

Group 5 [Triton WR 1339 + (RBO + SO, 8:2)]: Treatment was given with combination of RBO with SO (8:2, 7 ml/kg, p.o.) for 30 days and on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg in saline) was given.

Group 6 [Triton WR 1339 + (RBO + SO, 7:3)]: Treatment was given with combination of RBO with SO (7:3, 7 ml/kg, p.o.) for 30 days and on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg in saline) was given.

Group 7 [Triton WR 1339 + (RBO + SO, 5:5)]: Treatment was given with combination of RBO with SO (5:5, 7 ml/kg, p.o.) for 30 days and on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg in saline) was given.

Group 8 (Triton WR 1339 + Atorvastatin): Treatment was given with standard drug atorvastatin (2 mg/kg in 0.5% CMC suspension, orally) for 30 days and on the 31st day, intraperitoneal injection of triton WR 1339 (300 mg/kg in saline) was given.

After 24 h of triton injection, the blood was collected from the retro- orbital plexus of the rat for the estimation of lipid profile and animals were sacrificed using excess dose of ether. The liver was isolated and stored in a deep freezer at -20˚C temperature for estimation of oxidative stress parameters.

Collection of serum

The blood samples were withdrawn from retro-orbital plexus under light ether anaesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was kept at 4°C until used.

Parameters assessed in serum

In vitro quantitative determinations of the activity of total cholesterol (TC), serum triacylglyceride (TG), low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL) concentration in serum were carried out using standard enzymatic kits (Lab Care Diagnostics, India). The concentration of very low density lipoprotein-cholesterol (VLDL) was estimated as previously proposed [16]. Various other coronary disease risk factors such as atherogenic index (A.I.), and HDL/TC ratio were also evaluated as per previously reported methods [17].

Isolation of liver

All the animals were euthanasiously sacrificed by cervical dislocation. Liver was collected and was blotted free of blood and tissue fluids. Then it was weighed on balance and the relative weight was calculated.

Preparation of the liver homogenate for enzyme assay

Liver, kept in cold conditions (pre-cooled in inverted petridish on ice) was removed. It was cross chopped with surgical scalpel into fine slices and was chilled in the cold 0.25 M sucrose, quickly

blotted on a filter paper. The tissue was minced and homogenized in 10 mM Tris-HCl buffer, pH 7.4 (10% w/v) with 25 strokes of tight teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for other enzymes assays.

Parameters assessed in the liver homogenate

The activities of anti-oxidant enzymes like reduced glutathione and the extent of lipid peroxidation were assayed in the liver homogenate as per previously established methods [18, 19, 20].

Statistical analysis

All the values are expressed as mean ± S.E.M. Statistics was applied using GraphPad Prism 5.0 version. Statistical significance between all the groups was carried out using one way ANOVA analysis followed by Tukey's multiple comparison test. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Chemical tests for identification of fixed oil

Since RBO and SO are fixed oils, a clear blue solution was obtained in the confirmatory test which was performed using sodium hydroxide, thereby indicating the presence of glycerine in the oils. A subsequent test using sodium hydrogen sulphate evoked a pungent odour confirming the presence of glycerine in the oils.

Physicochemical parameters of RBO and SO

The results of the physicochemical tests for RBO and SO are depicted in **Table 1**.

Effects of RBO, SO & their combinations on lipid profile

The effects of RBO, SO & their various combinations on lipid profile are elucidated in **Fig.1 to Fig.5**. Administration of triton WR 1339 (300 mg/kg, i.p.) resulted in a significant increase in the serum levels of TC, TG, LDL and VLDL and a significant decrease in HDL after 24 h when compared to normal control animals.

Table 1: Physicochemical parameters of rice bran oil (RBO) and safflower oil (SO)

S. No.	Parameters	Values obtained for RBO	Values obtained for SO
1	Density	0.84 gm/ml	0.87 gm/ml
2	Viscosity	60.4 centipoise	39.5 centipoise
3	Boiling point	105-110˚C	255-260˚C
4	Acid Value	6.11	4.82
5	Saponification Value	183.72	192.14
6	Ester Value	177.61	187.32

Administration of RBO (7 ml/kg, p.o.) produced a significant decrease in the levels of TC, TG, LDL and VLDL and a significant increase in the level of HDL as compared to the triton WR 1339 control group.

Administration of SO (7 ml/kg, p.o.), on the other hand, produced a significant decrease in the levels of TC and LDL as compared to the triton WR 1339 control group. However, a non significant decrease in the level of TG and a non significant increase in the level of HDL were observed with SO (7 ml/kg, p.o.) as compared to the triton WR 1339 control group.

Administration of RBO+SO (8:2) (7 ml/kg, p.o.) produced a significant decrease in the levels of TC, TG, LDL and VLDL and a significant increase in the level of HDL as compared to the triton WR 1339 control group. It also produced a significant decrease in the TC, TG and VLDL levels, a non-significant decrease in the LDL level and a non-significant increase in the HDL level as compared to SO treated group. When compared to the RBO treated group, it produced a significant decrease in the LDL level, a non-significant decrease in the TC and TG level and a non-significant increase in the HDL level.

Administration of RBO+SO (7:3) (7 ml/kg, p.o.) produced a significant decrease in the levels of TC, TG, LDL and VLDL and a significant increase in the level of HDL as compared to the triton WR 1339 control group. It also produced a significant decrease in the TC level, a non-significant decrease in the TG, LDL and VLDL levels and a non-significant increase in the HDL level as compared to the RBO treated group. When compared to the SO treated group, it produced a significant decrease in the TC, TG and VLDL levels, a non-significant decrease in the LDL level and a significant increase in the HDL level. The maximum depression in the TG and VLDL levels and the maximum elevation in HDL levels were evident with RBO+SO (7:3) when compared to RBO+SO (8:2) and RBO+SO (5:5) combinations.

Administration of RBO+SO (5:5) (7 ml/kg, p.o.) produced a significant decrease in the levels of TC, TG, LDL and VLDL and a significant increase in the level of HDL as compared to the triton WR 1339 control group. It also produced a significant decrease in the TC level, a non-significant decrease in the TG, LDL and VLDL levels and a non-significant increase in the HDL level as compared to the RBO treated group. When compared to the SO treated group, it produced a significant decrease in the TC, TG and VLDL levels, a non-significant decrease in the LDL level and a non -significant increase in the HDL level.

Administration of standard drug atorvastatin (2 mg/kg, p.o.) produced a significant decrease in the levels of TC, TG, LDL and VLDL and a significant increase in the HDL level as compared to the triton WR 1339 control group.

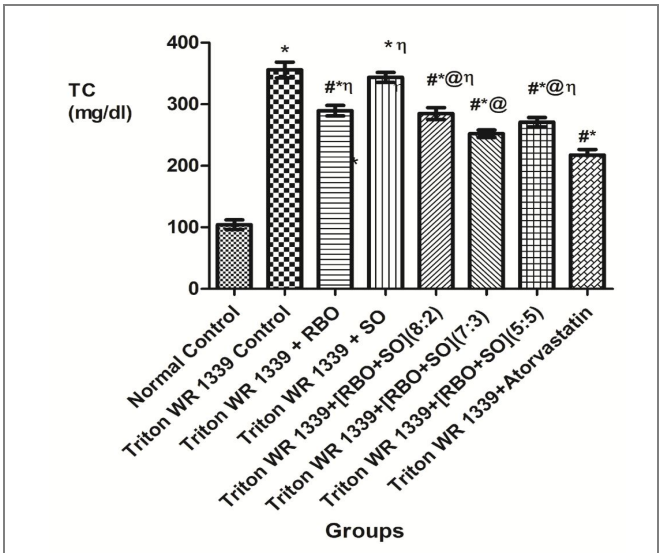

Fig.1. Effect of pre-treatment of various combinations of rice bran oil and safflower oil on total cholesterol (TC). Bars represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, P#< 0.05 compared with Triton WR-1339 control group, P@< 0.05 compared with safflower oil treated group, Pη< 0.05 compared with atorvastatin treated group.

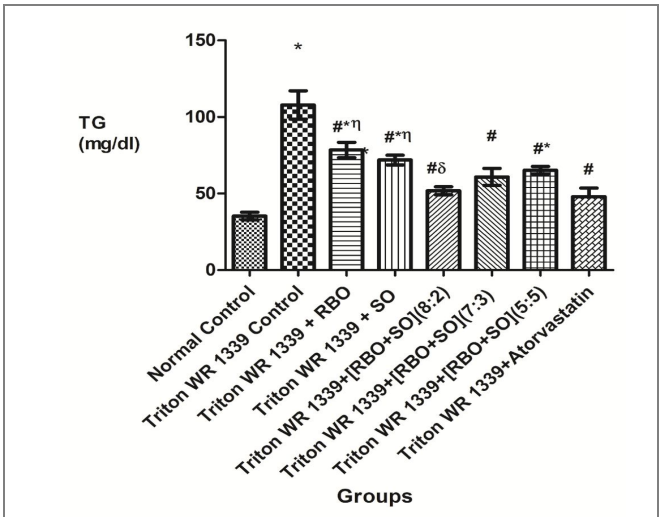

Fig.2. Effect of pre-treatment of various combinations of rice bran oil and safflower oil on triglyceride (TG) levels. Bars represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, $P^{\#}$< 0.05 compared with Triton WR-1339 control group, $P^{@}$< 0.05 compared with safflower oil treated group, P^{η}< 0.05 compared with atorvastatin treated group.

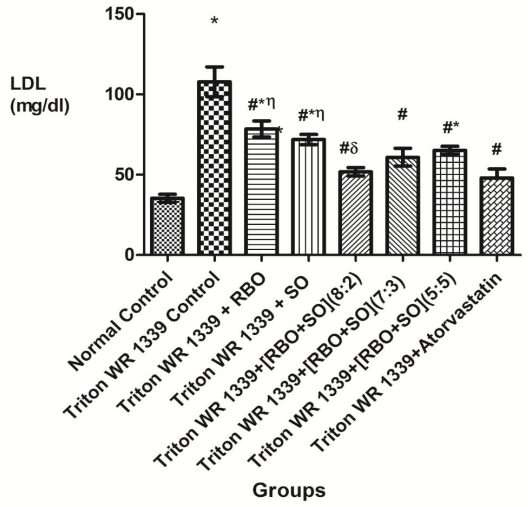

Fig.3. Effect of pre-treatment of various combinations of rice bran oil and safflower oil on low density lipoprotein cholesterol (LDL) levels. Bars represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, P#< 0.05 compared with Triton WR-1339 control group, P@< 0.05 compared with safflower oil treated group, Pη< 0.05 compared with atorvastatin treated group.

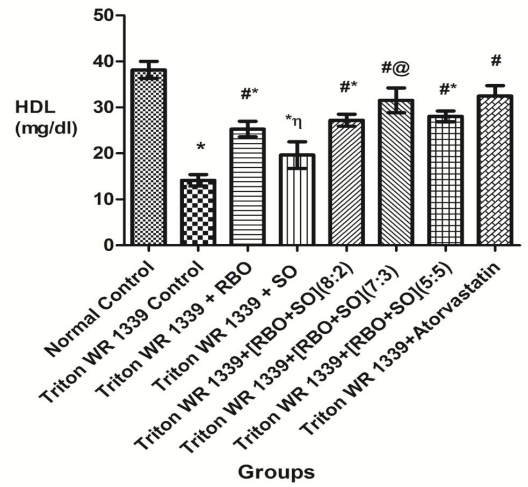

Fig.5. Effect of pre-treatment of various combinations of rice bran oil and safflower oil on high density lipoprotein cholesterol (HDL) levels. Bars represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, $P^{\#}$< 0.05 compared with Triton WR-1339 control group, $P^{@}$< 0.05 compared with safflower oil treated group, P^{η}< 0.05 compared with atorvastatin treated group.

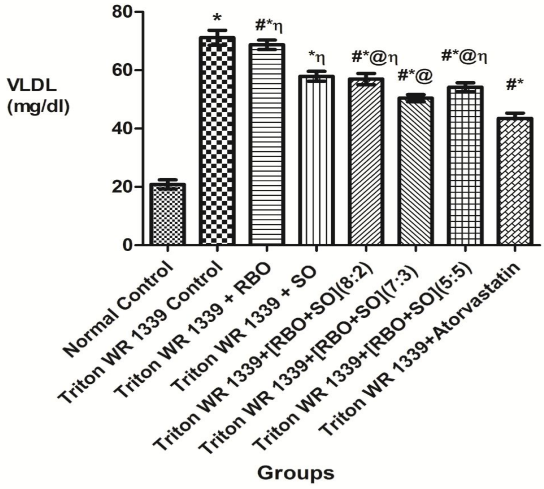

Fig.4. Effect of pre-treatment of various combinations of rice bran oil and safflower oil on very low density lipoprotein cholesterol (VLDL) levels. Bars represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, $P^{\#}$< 0.05 compared with Triton WR-1339 control group, $P^{@}$< 0.05 compared with safflower oil treated group, P^{η}< 0.05 compared with atorvastatin treated group.

Effects of RBO, SO & their combinations on atherogenic index and HDL/TC ratio (Coronary disease risk factors)

Atherogenic index (AI) was found to be significantly higher in the Triton WR 1339 group as compared to the normal control group. The other coronary disease risk predictor index: HDL/TC ratio in the Triton WR 1339 group was significantly lower as compared to the groups pre-treated with the individual oils and their different blends.

Administration of individual RBO and SO (both 7 ml/kg, p.o.) and all the other oil blends including the standard drug atorvastatin (2 mg/kg, p.o.) produced a significant decrease in the atherosclerotic index and a significant increase in the HDL/TC ratio as compared to the triton WR 1339 control group. However, administration of RBO+SO (8:2), RBO+SO (7:3) and RBO+SO (5:5) (7 ml/kg each, p.o.), produced a non-significant decrease in atherosclerotic index as compared to the RBO and SO treated group, whereas, administration of

RBO+SO in all the above combinations (7 ml/kg each, p.o.), produced a significant increase in HDL/TC ratio as compared to the SO treated group and a non-significant increase in HDL/TC ratio as compared to the RBO treated group **(Table 2)**. It was noteworthy to observe the maximum elevation in the HDL/TC ratio in the RBO+SO (7:3) treated group when compared to RBO+SO (8:2) and RBO+SO(5:5)combinations.

Table 2: Effects of RBO, SO and their combinations on Atherosclerotic index and HDL/TC level.

Group	Atherosclerotic index (A.I)	HDL/TC
Normal control	0.92 ± 0.04	0.244 ± 0.018
Triton WR 1339 control (300 mg/kg, i.p.)	8.07 ± 1.20*	0.045 ± 0.003*
Triton WR 1339 (300 mg/kg, i.p.) + RBO (7 ml/kg, p.o.)	3.14 ± 0.27$^{\#}$	0.105 ± 0.008*$^{,\#,\eta}$
Triton WR 1339 (300 mg/kg, i.p.) + SO (7 ml/kg, p.o.)	3.91 ± 0.43*$^{,\#}$	0.071 ± 0.009$^{*,\eta}$
Triton WR 1339 (300 mg/kg, i.p.) + [RBO + SO] (8:2), (7 ml/kg, p.o.)	1.94 ± 0.19$^{\#}$	0.125 ± 0.008*$^{,\#,@,\eta}$
Triton WR 1339 (300 mg/kg, i.p.) + [RBO + SO] (7:3), (7 ml/kg, p.o.)	1.98 ± 0.25$^{\#}$	0.154 ± 0.010*$^{,\#,@}$
Triton WR 1339 (300 mg/kg, i.p.) + [RBO + SO] (5:5), (7 ml/kg, p.o.)	2.34 ± 0.11$^{\#}$	0.146 ± 0 .008*$^{,\#,@}$
Triton WR 1339 (300 mg/kg, i.p.) + Atorvastatin (2 mg/kg, p.o.)	1.57 ± 0.28$^{\#}$	0.180 ± 0.014*$^{,\#}$

Values represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, P$^{\#}$< 0.05 compared with Triton WR-1339 control group, P$^{@}$< 0.05 compared with safflower oil treated group, P$^{\eta}$< 0.05 compared with atorvastatin treated group.

Effects of RBO, SO & their combinations on lipid peroxidation and anti-oxidant level

Malondialdehyde (MDA) level was measured as an index of lipid peroxidation in the liver homogenate. Triton WR 1339 controlled animals showed a malondialdehyde level (nmol/mg protein) significantly higher than that observed in the normal control group.

Administration of individual RBO and SO (both 7 ml/kg, p.o.) produced a significant decrease in the MDA level as compared to the triton WR 1339 control group. RBO+SO (8:2), RBO+SO (7:3) and RBO+SO (5:5) (7 ml/kg each, p.o.) and standard drug atorvastatin (2 mg/kg, p.o.) produced a significant decrease in the MDA level as compared to the triton WR 1339 control group. However, when compared to the individual RBO and SO treated groups, RBO+SO (8:2), RBO+SO (7:3) and control. Administration of RBO (7 ml/kg, p.o.) produced a significant increase in the GSH level, while administration of SO (7 ml/kg, p.o.) produced a non-significant increase as compared to the triton WR 1339 control group. Administration of RBO+SO (8:2), RBO+SO (7:3) and RBO+SO (5:5) (7 ml/kg each, p.o.) and standard drug atorvastatin (2 mg/kg, p.o.) also produced a significant elevation in the GSH level as compared to the triton WR 1339 control group. However, administration of RBO+SO (8:2) and RBO+SO (7:3) (7 ml/kg each, p.o.) produced a non-significant increase and RBO+SO (5:5) (7 ml/kg each, p.o.) produced a significant increase in the GSH level as compared to the SO alone treated group. Furthermore, RBO+SO (8:2), RBO+SO (7:3) and RBO+SO (5:5) (7 ml/kg each, p.o.) produced a non-significant increase in the GSH level as compared to RBO alone treated group (**Table 3**).

DISCUSSION

Dietary advice has a significant role to play in normalizing abnormal serum lipids in high risk

cardiovascular diseases and may lead to a reduction in total serum cholesterol levels of the order of

Table 3: Effects of RBO, SO and their combinations on lipid peroxidation and anti-oxidant parameter

Group	Malondialdehyde (nmoles/mg of protein)	Reduced Glutathione (µg /mg protein)
Normal control	19.18 ± 4.12	74.33 ± 4.36
Triton WR 1339 control (300 mg/kg, i.p.)	54.79 ± 2.59*	31.38 ± 2.78*
Triton WR 1339 (300 mg/kg, i.p.) + RBO (7 ml/kg, p.o.)	37.29 ± 2.63$^{*,\#}$	39.85 ± 4.00 $^{*,\eta}$
Triton WR 1339 (300 mg/kg, i.p.) + SO (7 ml/kg, p.o.)	34.03 ± 1.52$^{*,\#}$	53.63 ± 6.31$^{\#}$
Triton WR 1339 (300 mg/kg, i.p.) + [RBO + SO] (8:2), (7 ml/kg, p.o.)	28.47 ± 2.52$^{\#}$	55.11 ± 5.83$^{\#}$
Triton WR 1339 (300 mg/kg, i.p.) + [RBO + SO] (7:3), (7 ml/kg, p.o.)	34.47 ± 2.82$^{*,\#}$	57.54 ± 4.92$^{\#}$
Triton WR 1339 (300 mg/kg, i.p.) +[RBO + SO] (5:5), (7 ml/kg, p.o.)	32.89 ± 3.42$^{*,\#}$	64.20 ± 5.01$^{\#,@}$
Triton WR 1339 (300 mg/kg, i.p.) + Atorvastatin (2 mg/kg, p.o.)	29.78 ± 4.41$^{\#}$	63.95 ± 3.56$^{\#}$

Values represent the means ± SEM (n=6). P*< 0.05 compared with normal control group, P$^{\#}$< 0.05 compared with Triton WR-1339 control group, P$^{@}$< 0.05 compared with safflower oil treated group, P$^{\eta}$< 0.05 compared with atorvastatin treated group.

3% to 6% [21, 22]. In similar lines, there are ample evidences to elucidate the hypocholesterolemic effects of RBO in relation to more commonly used vegetable oils. RBO is characterized by a relatively high content of non-fatty acid components, such as γ-oryzanol and tocotrienols that could participate in its hypocholesterolemic effects [23]. Moreover, epidemiological studies have revealed that vegetable oil such as safflower oil with abundant poly unsaturated fatty acids (PUFA) content can lower blood cholesterol and LDL level [24].

The non-ionic surfactant, triton WR-1339 has been used in lipid metabolism research to inhibit the removal of lipoprotein from the circulation [25]. Triton WR-1339 is a non-ionic detergent that prevents catabolism of triacylglycerol-rich lipoproteins by lipoprotein lipase and is commonly used for *in vivo* determination of TG production and VLDL secretion or clearance rate [26, 27]. In the present study, an intra-peritoneal injection of Triton-WR-1339 at a dose of 300 mg/kg was used to induce hyperlipidemia in rats. Significantly elevated serum levels of TC, TG, LDL and VLDL along with a decline in HDL levels were evident after 24 hrs as compared to the normal control group. These results demonstrate the feasibility of using triton WR-1339 to induce acute hyperlipidemia in rats. Atorvastatin (2 mg/kg, p.o.) was used in the study in order to understand how far RBO, SO and their various combinations are comparable to that of a standard drug.

The reduction in the TC level by RBO may be associated with the presence of phytosterols which act at the intestinal level by interfering with the absorption of the cholesterol from the gut [28]. The presence of cycloartenol, a triterpene alcohol with a similar structure to cholesterol, may compete with the binding sites of cholesterol, thereby sequestering cholesterol from the system. Moreover, tocotrienols, one of the essential components of RBO, are thought to inhibit the HMG-CoA reductase activity in the biosynthetic pathway of cholesterol [24].

The reduction in the TG level by RBO may be associated with the presence of triterpene alcohols and phytosterols which lower the circulating levels of cholesterol and TG due to the possible structural similarity of cycloartenol and cholesterol [5]. Another probable mechanism may involve an increase in the lipoprotein lipase (LPL) enzyme which is capable of breaking down plasma TGs of

TG-rich lipoproteins, including chylomicrons and VLDL [29].

The reduction in the LDL and increase in HDL level by RBO may be associated with the presence of ferulic acid (4- hydroxy-3–methoxy cinnamic acid) esters of triterpene alcohols and plant sterols [30]. The increase in HDL levels by RBO may also be attributed to the increase in lecithin cholesterol acyl transferase (LCAT), which plays a central role in cholesterol uptake by HDL particles from the peripheral tissues and facilitates maturation of HDL to cholesterol ester- rich HDL2 particles [31].

On the other hand, the reduction in the TC level by SO may be associated with the presence of high amount of linoleic acid which belongs to the class of PUFAs [11]. It has been reported that safflower seed ethanolic extract possesses the potential to reduce acyl-CoA cholesterol acyl transferase (ACAT) activity, subsequently leading to lesser availability of cholesteryl ester for VLDL packing, thereby resulting in the reduction of plasma cholesterol [32]. SO enriched diet has also been claimed to decrease the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase mRNA expression, increase the hepatic LDL receptors and/or cyp7 mRNA expression [33]. The reduction in the LDL level by SO may be associated with the presence of PUFA which has been reported to increase the LDL apolipoprotein B (apoB) fractional catabolic rates (FCR) [34].

AI, one of the major risk factors of cardiovascular disease, indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the AI, the higher is the risk of the above organs for oxidative damage [35]. Administration of individual as well as different blends of RBO and SO provides a beneficial effect on lipid metabolism in regard to the reduction of AI. Similar results have been reproduced while investigating the hypolipidemic effect of other natural products [36]. Such an ameliorative action may be attributed to the lipid-lowering property of RBO and SO.

Furthermore, there exists an inverse correlation between an increased HDL/TC ratio and the development of atherosclerosis. In the present study, administration of various combinations of RBO and SO significantly improved the HDL/TC ratio, elucidating the beneficial effect of the combinations in preventing atherosclerosis incidence.

The high content of tocopherols and tocotrienols in RBO may improve the oxidative stability of the blends of RBO and SO. In addition to improving the plasma lipid profile, blending of RBO with SO can result in an economic advantage of lower prices [12].

Increased intracellular generation of reactive oxygen species (ROS) plays an important role in chronic inflammatory responses to atherosclerosis [37]. Under normal physiological conditions, there is a critical balance in the generation of oxygen free radicals and anti-oxidant defence systems. Impairment in the oxidant/antioxidant equilibrium due to hyperproduction of ROS provokes a situation of oxidative stress that induces molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases [38].

A lot of oxygenated compounds, particularly malondialdehyde (MDA) and conjugated dienes, are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids. MDA is an end product of peroxidative decomposition of polyenoic fatty acids in the lipid peroxidation process and its accumulation in tissue is indicative of the extent of lipid peroxidation [39]. Published studies have reported that serum MDA levels are elevated in subjects with hyperlipidemia and they decrease following dietary supplementation with anti-oxidants [40, 41].

Glutathione, a potent inhibitor of the neoplastic process, plays an important role in the endogenous anti-oxidant system. Reduced glutathione (GSH), a free radical scavenger, acts as an anti-oxidant by reacting with the free radicals and thus interrupting the propagation of new free radical species [42].

Treatment with RBO and SO and their various combinations showed a significant reduction in the level of lipid peroxidation (MDA) and a significant increase in the GSH levels as compared to the triton

WR 1339 control group, thereby emphasizing on the effective anti-oxidant property of both RBO and SO. The anti-oxidant potential of RBO may be due to the presence of γ-oryzanol, which has been shown to inhibit linoleic acid oxidation. [43, 44] and cholesterol oxidation [45]. Various components of γ-oryzanol, namely, cycloartenyl ferulate and 24-methylenecycloartanyl ferulate also act as anti-oxidants in methyl linoleate bulk and multiphase lipid systems and as radical scavengers [45]. The anti-oxidant activity of SO, on the other hand, may be attributed to the direct radical and lipid peroxide scavenging actions [46, 47], a possible synergistic interaction with other anti-oxidants [48], preventing oxidative attack on membrane lipids by sparing vitamin E [49] and inhibition of the action of lipoxygenases [50, 51].

CONCLUSION

The findings obtained from the current study reinforce the potential anti-hyperlipidemic activity of various combinations of RBO and SO in an acute hyperlipidemia model in rats, which may be attributed partially to their anti-oxidant activity. Although each blend of RBO and SO exerts a substantially beneficial effect on the serum lipid profile, maximum depression in TG and VLDL levels and maximum increase in HDL level and HDL/TC ratio was evident with RBO+SO (7:3), coupled with a significant increase in the level of hepatic anti-oxidant parameters. This promotes RBO+SO (7:3) combination as the optimum cholesterol lowering blend in rats, similar to that observed in the previous human experiments (11). The findings advocate further assessment to elucidate a more detailed cellular and molecular mechanism(s) of action for the antihyperlipidemic activity of the different blends of RBO and SO. The effect, although, could not be explicated by differences in fatty acid composition, RBO is presumed to have a substantial practical value in this regard.

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