Hypolipidemic effect of oryzanol concentrate and low temperature extracted crude rice bran oil in experimental male wistar rats

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Abstract Blends of refined groundnut oil (GNO) and oryzanol concentrate having 3, 5, and 10% oryzanol in the blend, and a rice bran oil (RBO) which had retained all the nutrients such as oryzanol, tocopherols and tocotrienols and the unsaponifiable matter components of crude oil (GWF RBO) were prepared. Weanling rats were fed with diet containing the oil blends/rice bran oil at 10% level for 60 days and then dissected. The lipid profiles in serum, liver were investigated and the cholesterol levels were marginally reduced (7–16% in serum, 10–14.5% in liver) in rats fed oryzanol containing diet. RBO, GWF RBO containing diets showed a reduction of serum cholesterol by 14%, 15% respectively when compared to those fed with GNO. Serum and liver lipid analysis also showed significant change in TG concentration in rats fed blended oils containing oryzanol compared to the rats given GNO. Histology of liver and kidneys did not show changes. These studies indicated that oryzanol has an effect in lowering serum and liver cholesterol and shows antiatherogenic properties when incorporated into groundnut oil.

Keywords Groundnut oil · Cholesterol lowering effect · Hypolipidemic effect · Rice bran oil · Gum and wax free rice bran oil · Oryzanol · Vegetable oil blends

Introduction

There is an increased incidence of coronary heart disease (CHD) and mortality in South East Asian population. Dietary lipids are known to play an important role in the modulation of CHD (Janus et al. 1996). In India, dietary habits, especially the fat consumption, vary according to geographical region and availability of fat. Each oil used for cooking is unique in its fatty acid composition and also in its minor components.

Traditional cooking oil used in India includes groundnut, coconut, sesame, mustard, safflower and sunflower oil, while newer sources include rice bran oil, palmolein and soybean oil (Kuriyan et al. 2005). Groundnut oil contains 13% palmitic acid, 38% oleic acid, 41% linoleic acid, and small amounts of other saturated fatty acids (Gunstone 1996). Rice bran obtained during polishing of rice contains 18% to 22% oil (Cicero and Derosa 2005). Rice bran oil is extracted from the germ and bran layers of brown rice and contains both saponifiable and unsaponifiable compounds. The saponifiable fraction consists primarily of triglycerides and smaller amounts of diglycerides, monoglycerides, free fatty acids, waxes, glycolipids, and phospholipids (Orthoefer 1996). The oil contains mainly oleic acid (38.4%), linoleic acid (34.4%), α -linolenic acid (2.2%) as unsaturated fatty acids, and palmitic (21.5%) and stearic (2.9%) acids as saturated fatty acids (Kuriyan et al. 2005). The unsaponifiable components of RBO include phytosterols, tocopherols, tocotrienols and oryzanol. Studies have suggested that oryzanol is primarily responsible for the hypolipidemic effects of rice bran oil. Rice bran oil containing oryzanol added to rat diets was shown to lower plasma cholesterol levels compared to rats given a diet containing rice bran oil devoid of oryzanol (Seetharamaiah and Chandrasekhara 1988; Tabassum et al. 2005). Studies supporting the findings that oryzanol is an

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active component of rice bran oil with hypolipidemic qualities needs to be exploited for pharmaceutical utility as well (Michelle 2003). Oryzanol is a class of nonsaponifiable lipids of rice bran oil (RBO) consisting of group of ferulic acid esters of triterpene alcohol and plant sterols (Rong et al. 1997).

The oil extracted from rice bran has shown cholesterol lowering effects in both animals (Sharma and Rukmini 1986) and humans (Raghuram et al. 1989). The nutritional benefits of rice bran oil have been reviewed recently (Gopala Krishna 2002). Health aspect of oryzanol indicates that it has antioxidant property and quenches free radicals. It also has cholesterol lowering property, reduce elevated serum thyroid stimulating hormone (TSH) level in hypothyroid patients. It is also used as an ergogenic aid for strength/power by athletes. Gamma oryzanol is also used for the treatment of hyperlipaemia (Patel and Naik 2004). We have recently developed a process for preparing oryzanol concentrate from rice bran oil. New products based on traditional foods such as bisibelebath and upma were developed incorporating oryzanol concentrate (Baby Latha et al. 2011). It would be beneficial to supplement the commonly used vegetable oils with various amounts of oryzanol concentrate and evaluate the effects of feeding such oryzanol rich oils on serum and tissue lipids.

In the present study, we examined the effect of feeding blends of refined groundnut oil with oryzanol concentrates, RBO and GWF RBO and studied the effect of feeding these oils on the lipid profiles of rats in comparison to rats fed diets containing GNO without supplementation of oryzanol. GWF RBO being the crude oil and considered to have better oryzanol content than commercial RBO was also used for the study. In addition we also fed a group of rats with GWF RBO which has better oryzanol content than commercial RBO.

Materials and methods

Chemicals

GNO, RBO were purchased from a local supermarket. Oryzanol concentrate was prepared by using a new process (Indian patent pending). GWF RBO was prepared in the laboratory by extracting oil from rice bran followed by solvent dewaxing and degumming. Cholesterol, dipalmitoyl phosphatidylcholine, and BF_3 in methanol were purchased from Sigma chemical Co. (St. Louis, MO, U.S.A). choline chloride, magnesium oxide, DL-methionine, manganese carbonate, ferric citrate, calcium phosphate, potassium citrate monohydrate, sodium selenite, zinc carbonate, cupric carbonate, potassium sulphate and vitamins were purchased from Himedia Laboratories (Mumbai, India). Ferric chloride and ammonium thiocyanate were purchased from Qualigen

Fine Chemicals Ltd., (Mumbai, India). Casein was purchased from Nimesh Corporation (Mumbai, India). All solvents used were of analytical grade.

Preparation of blended oils The Groundnut oil containing oryzanol was prepared by adding different amounts of concentrates to provide oryzanol equivalent to 3, 5, and 10% in GNO. The blends were prepared by mixing of pre-weighed oryzanol concentrate containing 17% of oryzanol with required amounts of groundnut oil at 65 ± 1 °C using mechanical stirrer at a mixing speed of 180 rpm for 15 min. The oryzanol incorporated blends were cooled to room temperature and stored at 4 °C till used for experiments.

Preparation of gum and wax free rice bran oil The gums and wax free rice bran oil (GWF RBO) was prepared by extracting the oil at 5 °C from rice bran by soaking bran in hexane (1:3 w/v) at 5 °C for 24 h, followed by decantation of the hexane extract. Three more such extractions using a solvent to bran ratio of 3:1 and a soaking period of 8 h was carried out. The decanted extracts were pooled, desolventized and then the oil obtained was freed of solvent residue by desolventizing under vacuum in a vacuum oven for 24 h at 40 °C and 400 mm vacuum. The oil had traces of gums and wax and hence was designated as GWF RBO and was used as such in our studies.

Experimental animals The experimental protocol was approved by the institutional animal ethics committee. Male Wistar rats weighing 37 ± 3 g were grouped into 6 groups (four rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, in an approved animal house facility at the Central Food Technological Research Institute in Mysore, India. The animals were given AIN-76 (American Institution of Nutrition) diet containing native oils (GNO, RBO and GWF RBO) whereas remaining 3 groups received diets containing blended oils of GNO and oryzanol concentrate to contain 3, 5 and 10% oryzanol. The animals were fed for a total period of 60 days. Animals were given fresh diet daily, and left over diets were weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The animals had free access to food and water throughout the study. After 60 days of feeding, rats were fasted overnight and sacrificed under diethyl ether anesthesia. Blood was drawn by cardiac puncture, and serum was separated by centrifuging at 2000 rpm for 20 min at 4 °C. The liver was removed and rinsed thoroughly with ice-cold saline, blotted, weighed and stored at -20 °C until analyzed.

Animal diet composition The following were the ingredients used in the basal diets (g/100 g): Sucrose 60 g, casein 20 g, cellulose 5 g, mineral mix 3.5 g, vitamin mix 1 g, choline

chloride 0.2 g, methionine 0.3 g, fat 10 g (Anonymous 1977). The control group of animals were fed with AIN-76 diet containing GNO and the experimental groups were fed with diets containing GNO with 3, 5, 10% oryzanol and RBO, GWF RBO.

Fatty acid composition of oils and tissue samples Fatty acid composition of native, blended oils was determined as their fatty acid methyl esters as per AOCS O.M.No. Ce 2–66 (AOCS 2002) by using Gas chromatograph (Model GC-14A, Shimadzu Corporation, Kyoto, Japan) equipped with data processor (model CR-4A) and a flame ionization detector and fitted to a stainless steel column (3 m length $\times$ 0.55 mm i.d.), packed with 15% diethylene glycol succinate (DEGS) supported on 60–80 mesh chromosorb WAW and operated under the following conditions: Column temperature 180 °C, injector temperature 220 °C, and detector temperature 230 °C, nitrogen as carrier gas at a flow rate of 50 ml/min, hydrogen gas at 30 ml/min, and air pressure at 450 ml/min. The individual fatty acids separated were identified by comparing their retention time with reference standards and quantified using data processor (model CR-4A).

The methyl esters of fatty acids present in the lipids fraction of tissues (serum, liver, heart) were prepared using BF₃/MeOH (Morrison and Smith 1964) after saponification of the lipids and the fatty acid composition was determined as indicated above for oils using gas chromatograph.

Analysis of tissue lipid profile Serum lipids were extracted according to Bligh and Dyer (1959). Methanol and chloroform were added to the serum separately in proportions of 2:2 and mixed well, and the extract was filtered using Whatman no.1 filter paper. The filtrate was allowed to settle, and the lower chloroform layer was separated and used for further analysis.

Liver lipid was extracted by the method of Folch et al. (1957). One gram of liver was homogenized with 1 ml of 0.74% potassium chloride, and 20 ml of chloroform and methanol (2:1, by vol) was added and homogenized. The mixture was left overnight and filtered through a Whatman no.1 filter paper. 3 ml of 0.74% potassium chloride was added and mixed well. The solution was allowed to stand at room temperature. The upper aqueous layer was removed carefully, and then lower phase was washed with 3 ml of chloroform/methanol/water (3:48:47, by vol) mixture. The chloroform extract was used for lipid analysis.

Serum and liver cholesterol levels were quantified using the method of Searcy and Bergquist (1960). An aliquot from the chloroform extract of lipid was dried under a stream of nitrogen followed by the addition of ferric chloride-acetic acid reagent. After mixing thoroughly, it was left at room temperature for 15 min. Then, concentrated sulphuric acid

(1 ml) was added, mixed immediately on a vortex, and left at room temperature in the dark for 45 min. The color intensity of the clear solution was determined in the spectrophotometer at 540 nm. Cholesterol levels in biological samples were estimated from the standard curve generated using standard AR grade cholesterol procured from Sigma.

Triglyceride (TG) were estimated by the method of Fletcher (1968), using tripalmitin as reference standard (30–300 μ g range). An aliquot of the chloroform extract was evaporated under nitrogen and redissolved in isopropanol. TG purifier was added, mixed and centrifuged and supernatant was collected. To the supernatant, 0.6 ml of 5% potassium hydroxide in isopropanol:water (2:3, by vol) was added and kept at 65 °C for 15 min. To this 1 ml of sodium metaperiodate (12 ml of 0.025 M stock solution of sodium metaperiodate in 1 N acetic acid and 20 ml of isopropanol was mixed and made upto 100 ml with 1 N acetic acid) was added and mixed, and 0.5 ml of acetyl acetone solution was added, mixed and incubated at 50 °C for 30 min. The color intensity was measured at 405 nm in a spectrophotometer (Shimadzu, model 1601A).

Phospholipids (PLs) were estimated by ferrous ammonium thiocyanate method (Stewart 1980) using calibration curve of dipalmitoyl phosphatidyl choline as the reference standard. The lipid extract in chloroform was evaporated under nitrogen and redissolved in 2 ml of chloroform. To this 2 ml of ferrous ammonium thiocyanate was added and vortexed well. Following the phase separation, absorbance of chloroform layer was measured at 488 nm using spectrophotometer.

Oryzanol estimation Oryzanol was estimated by addition of 10 ml of hexane to 0.01 g of lipid sample extracted from liver sample as mentioned earlier. The optical density of the samples were measured at 314 nm using spectrophotometer (UV-1601, UV-VIS spectrophotometer, Shimadzu Corporation) and oryzanol content calculated using extinction coefficient value of 358.9 (Gopala Krishna et al. 2006).

Histology of liver and kidneys Tissues from liver and kidneys were fixed in 10% neutral buffered formalin and 6 μ m thick sections were prepared from paraffin embedded blocks. This was followed by hematoxylin and eosin staining for microscopic observations (Lillie 1965).

Statistical analysis The experiments were carried out in quadruplicate and each sample analysed in duplicate to get eight set of data. The results were analysed by analysis of variance (Fischer 1970). Data were expressed as mean $\pm$ S.D. Analysis of variance was employed to evaluate the differences between the groups. A difference of $p < 0.05$ was considered to be significant.

Results and discussion

The present study was undertaken to evaluate the effects of blended oils containing GNO with different amounts of oryzanol on profile of serum and liver lipids. GNO contains unsaturated fatty acids to an extent of about 80%. Lesser known oils like RBO that are used in India are also rich in unsaturated fatty acids (Gopala Krishna et al. 2006). Rice bran oil in addition provides oryzanol.

Effect of dietary lipids on growth parameters The amount of diet consumed by rats fed on different dietary lipids was comparable. The average food intake was 13.2 ± 0.4 g/rat daily (combined mean $\pm$ SD in all groups, Table 1). There was no significant change in the food efficiency ratio measured by gain in body weight to the amount of food consumed. There were no significant changes in the weights of liver, heart in the groups fed dietary fats containing GNO with those given different concentrations of oryzanol and groups fed with RBO and GWF RBO.

Fatty acid composition of dietary lipids Analysis of the dietary lipids showed that GNO-containing diets and diets containing GNO with oryzanol concentrate at different levels had saturated fatty acids (SAFAs) to an extent of $24.5 \pm 0.6\%$. RBO containing diets had $24 \pm 1.7\%$ of SAFAs. The percentage of monounsaturated fatty acids (MUFA%) for GNO groups was $42.4 \pm 0.5\%$ and for RBO, GWF RBO fed groups contained $42.7 \pm 0.5\%$ MUFA. PUFA% for GNO groups was $32.7 \pm 0.5\%$ and RBO, GWF RBO diets contained $33.3 \pm 2.1\%$ respectively (Table 2). The SFA/MUFA/PUFA ratio of all the oil blends was almost similar. One of the objectives of this study was to prepare oil blends containing GNO with different amounts of oryzanol and to evaluate its effects in comparison with those given RBO and GWF RBO. This was achieved by blending GNO with three different concentrations of oryzanol concentrate. These oil blends were then fed to rats for a period of 60 days. The most active compound contributing to the improved lipid

profiles in wistar rats was Oryzanol. This was our main focus for carrying out the study.

Effects of native oils & blended oils on lipid parameters in serum The type of fat given to animals influenced serum lipid levels. Rats fed GNO had serum total cholesterol concentration of 77.6 mg/dl while those fed GNO with oryzanol at concentrations of 3, 5, 10% showed serum cholesterol of 65.1, 70.5 and 72 mg/dl respectively. Thus GNO with oryzanol at 3%, 5%, 10% showed 16%, 9% and 7% reduction in serum cholesterol concentration and RBO, GWF RBO fed rats showed 5% and 14% reduction in serum cholesterol concentration in comparison to rats fed a diet containing GNO. GWF RBO oil fed group showed a reduction of 10% in serum cholesterol in comparison to RBO. The TG was decreased by 30% in rats given GNO with oryzanol at 10% level and those rats fed RBO, GWF RBO showed a reduction in TG by 21.5% and 27.1% in comparison to those given GNO. GWF RBO showed a reduction in TG by 7% compared to those fed GNO. Phospholipids (PLs) level was decreased by 31% in rats fed with GNO containing oryzanol at 3% level and 37% in rats fed GNO containing oryzanol at 5% and a reduction of 40% in rats fed with GNO containing oryzanol at 10% level and rats fed GWF RBO showed a reduction of 23% in PLs compared to that given GNO. GWF RBO oil fed groups showed a reduction of 24% in PLs level in comparison to those fed RBO (Table 3).

Effects of native oils and blended oils on lipid parameters in liver Rats fed GNO had total cholesterol concentration of 5.2 mg/g while those fed GNO containing oryzanol at 3, 5, 10% levels showed cholesterol concentration of 4.4, 4.6, 4.5 mg/g. Rats given RBO, GWF RBO showed cholesterol concentration of 4.5 and 4.4 mg/g respectively. Thus rats given GNO with oryzanol at 3, 5, and 10% showed 14.5, 10 and 12% reduction in liver cholesterol whereas those fed RBO, GWF RBO showed 13.8 and 15% reduction in liver cholesterol concentration in comparison to rats given native

Table 1 Body weight and organ weights of rats fed with diet containing GNO and GNO with Oz 3, 5, 10% and RBO, GWF RBO

Oil samples	Food intake (g/rat) daily	Total gain in body wt. (g)	FER	Liver wt. (g/100 g body wt.)	Heart wt (g/100 g body wt.)
GNO	12.8 ± 1.00^a	238.0 ± 28.5^a	0.3 ± 0.01^a	3.5 ± 0.28^a	0.3 ± 0.02^a
GNO Oz3%	12.7 ± 1.15^a	227.0 ± 36.0^a	0.3 ± 0.02^a	3.4 ± 0.46^a	0.3 ± 0.02^a
GNO Oz5%	13.5 ± 0.60^a	237.5 ± 14.0^a	0.3 ± 0.01^a	3.6 ± 0.24^a	0.3 ± 0.03^a
GNO Oz10%	13.0 ± 0.63^a	221.0 ± 15.5^a	0.3 ± 0.01^a	3.5 ± 0.22^a	0.3 ± 0.03^a
RBO	13.9 ± 0.51^a	238.5 ± 15.0^a	0.3 ± 0.02^a	3.4 ± 0.17^a	0.3 ± 0.02^a
GWF RBO	13.1 ± 1.34^a	240.0 ± 25.0^a	0.3 ± 0.01^a	3.5 ± 0.28^a	0.3 ± 0.03^a

Values are mean $\pm$ SD ($n=4$ rats); FER (Food efficiency ratio) calculated by dividing total gain in body weight to food intake, GNO Refined Groundnut oil, GNO Oz oryzanol RBO Rice bran oil, GWF RBO Gum and wax free rice bran oil. Values in the same column with common letters are not significantly different at $p < 0.05$

Table 2 Fatty acid composition (%) of diet containing GNO and GNO with Oz 3, 5, 10% and RBO, GWF RBO

Fatty acid (relative area%)	GNO	GNO Oz3%	GNO Oz5%	GNO Oz10%	RBO	GWF RBO
C14:0	0.3±0.1 ^a	0.2±0.1 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.4±0.15 ^a	0.3±0.13 ^a
C16:0	20.5±1.1 ^a	22.0±1.1 ^a	22.5±1.2 ^a	23.0±1.5 ^a	23.4±1.2 ^a	21.2±1.7 ^a
C18:0	3.0±1.3 ^a	2.2±0.8 ^a	1.7±0.63 ^a	1.7±0.7 ^a	1.4±1.2 ^a	1.3±0.63 ^a
C18:1	41.8±0.8 ^a	44.0±2.1 ^a	42.6±1.6 ^a	42.8±1.8 ^a	43.1±1.4 ^a	42.4±0.9 ^a
C18:2	32.2±0.7 ^a	32.0±0.8 ^a	31.0±0.8 ^a	31.6±1.2 ^a	31.0±1.4 ^a	32.7±0.8 ^a
C18:3	1.0±0.1 ^a	nd	1.8±0.7 ^a	0.6±0.5 ^a	0.8±0.1 ^a	2.1±1.3 ^a
SFA	23.7	24.4	24.5	25.0	25.2	22.7
MUFA	41.8	44.0	42.6	42.8	43.1	42.4
PUFA	33.2	32.0	32.8	32.2	31.8	34.8
SFA/MUFA/PUFA	23.7:41.8:33.2	24.4:44:32	24.5:42.6:32.8	25.04:42.8:32.2	25.2:43.1:31.8	22.7:42.2:34.8

nd not detected, GNO Refined Groundnut oil, Oz oryzanol, RBO Rice bran oil, GWF RBO Gum and wax free rice bran oil. Values are mean±SD (n=4), values in the same row with common letters are not significantly different at $p<0.05$

GNO. RBO in comparison to GWF RBO did not show any significant difference in cholesterol concentration. The TG was found to be reduced by 16, 23 and 32% in rats given GNO containing 3, 5 and 10% oryzanol whereas rats given RBO, GWF RBO showed a reduction of TG by 31.5 and 46% in comparison to rats fed diets containing GNO. GWF RBO showed a reduction of 21% in TG in comparison to those fed RBO. The PLs levels were reduced by 8.5%, 14% and 8% in rats fed GNO containing oryzanol at 3, 5, 10% in comparison to those fed GNO. GWF RBO showed a reduction of 10% in phospholipids level in

comparison to those fed GNO. GWF RBO showed a significant difference of 6% in comparison to those fed with RBO (Table 4).

Oryzanol levels of liver samples Oryzanol in liver samples were estimated in rats fed with GNO and also in the groups fed with GNO containing oryzanol. Groundnut oil fed groups did not show the presence of oryzanol in liver. However, the animals fed with different concentrations of oryzanol incorporated into GNO showed presence of oryzanol to an extent of 0.6% (6 mg/g of liver). No differences

Table 3 Serum lipid profile (mg/dl) and fatty acid composition of rats fed with diet containing GNO and GNO with Oz at 3, 5, 10% and RBO, GWF RBO

	GNO	GNO Oz3%	GNO Oz5%	GNO Oz10%	RBO	GWF RBO
Parameters						
Cholesterol	77.6±4.5 ^a	65.1±2.9 ^b	70.5±6.5 ^a	72.1±2.2 ^a	74.0±4.4 ^a	66.4±6.4 ^b
Triglyceride	110.9±7.3 ^a	114.4±4.7 ^a	101.3±8.4 ^{ab}	77.4±8.0 ^c	87.1±3.0 ^{bc}	80.9±9.9 ^c
Phospholipid	230.0±19.9 ^a	158.8±13.6 ^b	142.9±17.8 ^{bc}	138.8±11.3 ^{bc}	154.8±13.3 ^{bcd}	117.8±11.6 ^c
Fatty acid (relative area%)						
C14:0	1.7±0.3 ^a	1.1±0.4 ^a	1.0±0.0 ^a	1.1±0.2 ^a	0.9±0.1 ^a	0.9±0.1 ^a
C16:0	29.2±3.5 ^a	25.8±0.3 ^a	27.8±0.9 ^a	28.6±1.0 ^a	26.9±0.7 ^a	28.9±0.3 ^a
C16:1	0.9±0.1 ^a	2.0±1.0 ^a	1.3±0.1 ^a	1.4±0.5 ^a	1.4±0.2 ^a	1.5±0.5 ^a
C18:0	8.2±0.6 ^a	6.4±0.6 ^a	6.8±0.8 ^a	7.8±2.3 ^a	6.2±0.4 ^a	6.1±1.4 ^a
C18:1	26.0±1.7 ^a	29.6±2.5 ^a	28.4±1.4 ^a	27.6±2.1 ^a	28.2±0.7 ^a	29.6±1.6 ^a
C18:2	18.2±1.4 ^a	18.4±3.5 ^a	16.7±0.6 ^a	16.9±1.6 ^a	17.9±0.8 ^a	17.5±1.0 ^a
C20:4	16.0±3.1 ^a	16.2±1.9 ^a	17.3±2.3 ^a	15.9±2.0 ^a	18.0±1.1 ^a	15.1±0.8 ^a
SFA	38.5	33.2	35.6	37.4	34.0	35.8
MUFA	26.9	31.6	29.7	29.0	29.6	31.1
PUFA	34.2	34.6	34	32.8	35.8	32.6
P/S ratio	0.80	1.04	0.90	0.80	1.05	0.90

Values are mean±SD (n=4 rats), values in the same row with common letters are not significantly different at $p<0.05$, GNO Refined Groundnut oil, Oz oryzanol, RBO Rice bran oil, GWF RBO Gum and wax free rice bran oil

Table 4 Liver Lipid Profile (mg/g Tissue), oryzanol content and fatty acid composition of rats fed with diet containing GNO with Oz 3, 5, 10% and RBO, GWF RBO

	GNO	GNO Oz3%	GNO Oz5%	GNO Oz10%	RBO	GWF RBO
Parameters						
Cholesterol	5.2±0.2 ^a	4.4±0.4 ^b	4.6±0.2 ^{ab}	4.5±0.5 ^b	4.4±0.1 ^b	4.4±0.2 ^b
Triglyceride	8.3±1.7 ^a	7.0±0.6 ^{ab}	6.4±0.1 ^{ab}	5.6±1.1 ^{bc}	5.7±0.3 ^{bc}	4.5±0.6 ^c
Phospholipid	18.9±0.4 ^a	17.3±0.5 ^b	16.3±0.5 ^{bc}	17.4±0.1 ^{bcd}	18.1±0.3 ^{abd}	17.0±0.9 ^{bcd}
Oryzanol (mg%)	0.0	0.6±0.12 ^a	0.6±0.09 ^a	0.6±0.12 ^a	0.6±0.02 ^a	0.7±0.02 ^a
Fatty acid (relative area%)						
C14:0	0.4±0.1 ^a	0.6±0.1 ^{ab}	0.5±0.0 ^{abc}	0.8±0.1 ^{bd}	0.5±0.2 ^{abce}	0.7±0.1 ^{bcde}
C16:0	23.1±1.7 ^a	26.0±3.0 ^{ab}	25.5±1.4 ^{ab}	27.6±1.7 ^b	25.3±1 ^a	27.7±1.1 ^b
C16:1	1.1±0.1 ^a	3.3±2.5 ^a	2.2±0.2 ^a	2.2±0.7 ^a	2.0±0.5 ^a	2.1±1.0 ^a
C18:0	11.4±1.7 ^a	8.9±1.6 ^a	10.0±1.4 ^a	10.4±1.1 ^a	9.5±0.7 ^a	10.6±2.2 ^a
C18:1	28.1±3.8 ^a	33.0±4.0 ^a	33.0±3.2 ^a	31.3±2.3 ^a	29.7±3.2 ^a	28.2±4.2 ^a
C18:2	17.8±2.0 ^a	14.0±5.0 ^a	12.6±0.6 ^a	14.0±0.6 ^a	15.0±1.7 ^a	13.0±0.9 ^a
C20:4	17.6±2.8 ^a	14.0±2.5 ^a	15.7±1.4 ^a	15.4±2.6 ^a	16.8±1.4 ^a	17.4±2.7 ^a
SFA	35.0	35.5	36.0	38.7	35.3	39.0
MUFA	29.2	36.3	35.2	33.5	31.7	30.3
PUFA	35.4	28.0	28.3	29.4	31.8	30.4
P/S ratio	1.02	0.80	0.80	0.70	0.90	0.70

Values are mean±SD ($n=4$ rats), values in the same row with common letters are not significantly different at $P<0.05$, GNO Refined Groundnut oil, Oz oryzanol, RBO Rice bran oil, GWF RBO Gum and wax free rice bran oil

were observed in oryzanol content when rats were fed at 3, 5, 10% levels. Oryzanol content in RBO and GWF RBO was present to an extent of 1.4% and 1.8%. Animals given RBO and GWF RBO showed oryzanol to an extent of 0.6% (6 mg/g liver) and 0.7% (7 mg/g liver) (Table 2).

Studies on rice bran oil and groundnut oil have also shown that the serum total, free and esterified cholesterol level of rats maintained at a 10% RBO diet were lower than those maintained on a 10% Groundnut oil diet. Addition of 0.5% oryzanol to the diet containing rice bran oil further reduced the serum cholesterol. The

authors have concluded that the cholesterol lowering property of RBO might be due to oryzanol or other nonsaponifiable constituents present in RBO (Seetharamaiah and Chandrasekhara 1989).

With regard to these studies carried out we made an attempt to prepare oryzanol incorporated groundnut oil at different concentrations and compared the lipid profile and fatty acid profile of animals fed with these oils with rice bran oil fed animals. Results from our studies indicated that oryzanol incorporated groundnut oil showed better effects in comparison to rice bran oil.

Table 5 Fatty acid composition of heart samples of rats fed with diet containing GNO and GNO with Oz 3, 5, 10% and RBO, GWF RBO

Fatty acid (relative area%)	GNO	GNO Oz3%	GNO Oz5%	GNO Oz10%	RBO	GWF RBO
2:0	0.3±0.1 ^a	0.2±0.1 ^a	0.4±0.2 ^{ab}	0.6±0.3 ^{ab}	1.0±0.5 ^c	0.3±0.2 ^{ab}
C14:0	0.3±0.1 ^a	0.4±0.2 ^a	0.4±0.3 ^a	0.3±0.1 ^a	0.6±0.7 ^a	0.2±0.1 ^a
C16:0	17.0±1.0 ^a	16.7±1.1 ^a	18.6±1.0 ^{ab}	22.6±3.1 ^c	22.6±1.3 ^c	20.7±1 ^{bc}
C16:1	0.5±0.2 ^a	0.3±0.1 ^a	0.3±0.1 ^a	0.8±0.7 ^a	0.4±0.3 ^a	0.3±0.1 ^a
C18:0	15.0±1.0 ^a	16.3±0.4 ^a	17.0±1.4 ^a	13.5±3.6 ^a	15.0±0.7 ^a	17.0±2 ^a
C18:1	21.4±1.6 ^a	18.5±2.1 ^{ab}	20.6±3.0 ^{ab}	28.0±7.3 ^a	24.6±2.1 ^{ab}	23.3±3.1 ^{ab}
C18:2	23.7±1.5 ^a	24.5±1.2 ^a	21.1±1.1 ^{ab}	17.6±2.2 ^b	18.6±2.5 ^b	20.0±0.3 ^b
C20:4	20.7±2.5 ^a	23.0±5.0 ^a	21.3±0.8 ^a	16.2±4.3 ^a	16.8±1.8 ^a	18.0±1.5 ^a
SFA	33.0	34.0	36.4	37.0	39.2	38.2
MUFA	22.0	19.0	21.0	29.0	25.0	24.0
PUFA	44.4	47.5	42.4	34.0	35.4	38.0
P/S ratio	1.3	1.4	1.2	0.9	0.9	1.0

Values are mean±SD ($n=4$ rats), values in the same row with common letters are not significantly different at $p<0.05$, GNO Refined Groundnut oil, Oz oryzanol, RBO Rice bran oil, GWF RBO Gum and wax free rice bran oil

Fatty acid profile of tissue lipids The fatty acid profile of serum lipids of rats fed with different oil blends is given in Table 3. Linoleic acid (18:2) and arachidonic acid (20:4) content of rats fed GNO and GNO with different concentrations of oryzanol did not show any significant difference for all the samples. Similar result was also observed when fed RBO and GWF RBO. The linoleic acid concentration was approximately $45 \pm 2.1\%$ low in all groups in comparison with fatty acid composition of all starting oils

The liver lipids of rats fed GNO with oryzanol and GWF RBO showed lower linoleic acid in comparison to rats fed native GNO. Arachidonic acid percentage was also lowered similarly in all the groups (Table 4) compared to control group of rats fed native GNO.

The heart lipids of rats fed GNO with oryzanol at 10% showed significantly lower linoleic acid in comparison to rats fed native GNO. Arachidonic acid percentage was similar in all the groups (Table 5).

Histology Microscopic observations of liver and kidneys from rats of experimental groups did not show any significant change in tissue morphology. No significant pathological changes in tissue morphology of liver and kidneys from rats of experimental groups indicated nontoxic effect of oryzanol concentrate.

Although scientific evidence is limited, RBO is tenaciously believed to be a healthy vegetable oil. It exerts better hypocholesterolemic activity in comparison with other commonly used vegetable oils. It is characterized by a relatively high content of non-fatty acid components, some of which are known to have beneficial health effects. Components specific for RBO such as gamma-oryzanol and tocotrienols could be responsible for its hypocholesterolemic effects. In addition, studies have indicated that blending RBO with safflower oil, but not with sunflower oil, magnifies its hypocholesterolemic efficacy. This observation is of particular interest with regard to dietary intervention with RBO (Seetharamaiah and Chandrasekhara 1988). Gamma-oryzanol is one of the important components in RBO having this nutraceutical and can be useful in potential pharmaceutical, and cosmeceutical preparations. It is a mixture of ferulic acid esters of sterol and triterpene alcohols. It occurs in rice bran oil at a level of 1–2% and it also serves as a natural antioxidant (Sugano et al. 1999).

Studies have shown that consumption of RBO reduced cholesterol level in humans (Gerhardt and Gallo 1998). Studies have also been carried out by blending coconut oil (CNO) with rice bran oil/sesame oil (RBO/SESO) in which PUFA/SFA ratio was enhanced to 0.8–1.0 by blending of these oils (Reena and Lokesh 2007). Such oils showed hypolipidemic effects. Oliver (1987) has suggested that a diet with a P/S ratio of above 1.5 ameliorates coronary heart

disease. In the present study the serum and liver, cholesterol levels in rats fed GNO with different levels of oryzanol showed marginal changes from that observed in rats given native GNO the P/S ratio of oils used in these experiments were in the range of 0.07–1.05.

The present investigation has shown that feeding of rats with a diet containing oryzanol enriched groundnut oil brings down the level of serum cholesterol. This observation need to be validated in humans studies. Earlier experiments has shown hypocholesterolemic effects of oryzanol in humans (Raghuram et al. 1989).

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