Research articles

Effect of oryzanol concentrate on hypolipidemic properties and antioxidant enzymes of liver in high fat fed and low STZ induced-male Wistar rats

G. Bhaskaragoud[1], Pallavi Chatterjee[1], G. Suresh Kumar[1]

[1]Department of Biochemistry, Central Food Technological Research Institute, Mysore- 570 020, India.

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Corresponding author: **Suresh Kumar G.** Email: sureshg@cftri.res.in

ABSTRACT

Introduction and Aim: Oryaznaol is a sterylferulate, present mainly in riceban oil and plays major role lipid metabolism. In the present study, the effect of rice bran oil concentrate (RC) was evaluated for its antioxidant and hypolipedemic property in liver of high fat fed diabetic (HD) rats.

Materials and Methods: Animals were made diabetic by feeding high fat diet with a low STZ and analyzed for various parameters including antioxidant enzymes in liver both in control, and RC treated groups (Rice bran oil concentrate 0.1 & 0.3%).

Results: The results clearly indicated that blood glucose was increased in diabetic group when compared to non-diabetic groups. Blood glucose level was reduced in RC (0.1 and 0.3% doses) treated animals. The content of liver triglycerides, cholesterols, and phospholipids were altered in HD group and were significantly reversed in treated groups. Antioxidant activities of SOD, GPx, and catalase were found to be high in the control and treated groups when compared to HD group. Liver lipid peroxidation and Glucose tolerance were found to be higher in HD group when compared to ND group, whereas upon treatment dose dependent reduction was observed.

Conclusion: Hence, the RC concentrate has been found to have health beneficial effects particularly in liver by ameliorating antioxidant enzymes.

Keywords: Ricebran oil; oryzanol concentrate; diabetes; antioxidant enzymes.

Abbreviation: ROS: Reactive oxygen species; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; CAT: Catalase; STZ: Streptozotocin; TG: Triglyceride; TC: Total cholesterol; PL: Phospholipid; RBO: Rice bran oil; RC: Ricebran oil concentrate/ricebran oil concentrate for control; RD: Ricebran oil concentreate for diabetic; GNO: Ground nut oil. PV; Peroxide value; FFA: Free Fatty Acid.

INTRODUCTION

Diabetic mellitus is a chronic disorder of carbohydrate metabolism characterised by chronic hyperglycemia due to deficiency of insulin or its improper functions. Hyperglycemia is known to produce oxidative stress that leads to enhanced production of mitochondrial reactive oxygen species (ROS). ROS which create cellular damage by the glucose oxidation ability have been associated in the pathogenesis of diabetes mellitus (1, 2). Prediabetes, i.e. a phase characterised by impaired fasting glucose, impaired glucose tolerance or both as well as closely associated obesity eventually development of complications due to oxidative induced metabolic disorder (3, 4). Oxygen free radicals such as superoxide ($O_2^{\bullet -}$), hydrogen peroxides (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) are involved in the pathophysiology of ischemia/reperfusion injury and atherosclerosis. Oxidation of lipids in plasma lipoproteins and cellular membranes is associated with the development of vascular disease in diabetes. Superoxide dismutase (SOD) is the antioxidant enzyme so that it catalyses the dismutation of the highly reactive superoxide anion (O_2) to less reactive species hydrogen peroxide H_2O_2 (5). Peroxide can be destroyed by CAT or GPx reactions.

In mammals, hydrogen peroxide is detoxified by CAT and GPx. Catalase protects cells from hydrogen peroxide produced within them. Even though CAT is not necessary for some cell types under normal conditions, it performs a vital role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (6).

The changes in lipid profile, and production of free radicals by oxidative damage appears to be involved in the pathogenesis of long-term complications in both types of diabetes, in animals and humans. This is due to increased concentration of lipid peroxidation products and the accumulation of advanced glycosylation end products, and glycoxidation products

in tissue proteins that cause cellular and tissue damage (7, 8).

The current production of rice bran oil in the world is about 705,000 tonnes, of which 500,00 tonnes is produced in India using rice bran (9). In recent years health, a beneficial effect of rice bran oil is credited as of its fatty acid profile (higher percentage of unsaturated fatty acids) also fat-soluble molecules in particularly "Oryzanol" ranging between 0.7 and 3%. Oryzanol is a group of steryl-ferulates (esters of sterols and ferulic acid), an active principle of rice bran oil. Many studies are suggesting the oryzanol positive effect as hypolipidemic, hypocholesterolemic through intervention in the lipid and cholesterol metabolisms (10-12). Studies on the antioxidant effect of γ-oryzanol (7) in the amelioration of type 2 diabetes have shown to reduce lipid profile, and increase insulin sensitivity (13).

The current work focuses on the hypoglycemic effect of rice bran oil concentrate on STZ induced diabetes and its role in reducing antioxidant and liver lipid profile.

MATERIALS AND METHODS

Chemicals

GNO, casein and starch were obtained from a local market, Mysore. Mixed FAME standards from Supelco (USA). Boron trifluoride-methanol and cholesterol was procured from Sigma-Aldrich Chemicals (St Louis, USA). Mineral mix, choline chloride, DL-Methionine and bile salts were purchased from Sisco Research Laboratories (SRL), Mumbai. Commercially available Agappe kits (TC, HDL cholesterol, LDL cholesterol and TG estimation) were used for the analysis of serum lipid profile. Solvents and other chemicals used were of analytical grade reagents. Streptozotocin was purchased from Sigma- Aldrich Chemicals (St. Louis, USA). All other chemicals and reagents used were of analytical grade.

Nutraceutical content, physicochemical analysis and fatty acid composition of oils

Rice bran oil concentrate also named as oryzanol concentrates (Patent- 740/DEL/2005 A) were procured from CSIR-CFTRI, Mysuru and used for the study. Rice bran oil (RBO), Groundnut oil (GNO), vanaspathi were purchased locally and tested for their quantity of nutraceuticals, quality by analysing free fatty acids (FFA), peroxide value (PV; 14, 15) and fatty acid composition (16).

Analysis fat soluble bioactives by HPLC

Oryzanol: Total γ-Oryzanol in oil samples were determined using HPLC (model LC-10A VP Shimadzu Corporation, Kyoto, Japan) as described by (16), coupled with C18 column from Purospher® STAR (MERCK) and UV detector with the detector wavelength (λ) of 325nm, mobile phase containing acetonitrile: methanol: isopropyl alcohol (50:45:5, v/v/v) and γ-oryzanol as standard.

Total carotenoids: β-Carotene was quantified by reverse phase HPLC (17) equipped with a photodiode array detector (PDA) and fitted with C_{18} column, detector set at 450nm. The mobile phase consisted of acetonitrile: methanol: dichloromethane (60:20:20, v/v/v) with 0.1% ammonium acetate. β-Carotene was used as a reference standard.

Total tocopherol: Total tocopherol quantification was performed using HPLC (18) coupled with silica column and fluorescence detector (FLD). Following conditions were used: $\lambda\ Ex_{290nm}$, $\lambda\ Em_{330nm}$ as excitation and emission nm, the mobile phase consisting hexane: isopropanol (99.5:0.5, v/v), and α-tocopherol has been used as the standard. About 5 to 50 mg of oil/concentrate samples were used for HPLC analysis.

Animals and diet composition

Male Wistar rats [OUTB-Wstar, IND-cft (2C)] (Rattus norvegicus) were used in the entire study as per the guidelines of the institutional ethical committee. All the project protocols involving animals were approved by the Institutional Ethical Committee of CSIR-CFTRI. Animals of about 100g were procured and carried out the experiments in the animal house facility of CFTRI, Mysore, under the standard conditions with 12h light-dark cycle and had free access to food and water. Animals were divided into two main groups, control and diabetic group. The control groups were further subdivided to normal diet fed control (NC), high fat-fed control (HC), 0.1% rice bran oil concentrate fed control (0.1% RC) and 0.3% rice bran oil concentrate fed control (0.3% RC) with n=6. Similarly, diabetic animals were grouped on normal diet fed diabetic (ND), high fat fed diabetic (HD), 0.1% rice bran oil concentrate fed diabetic (0.1% RD) and 0.3% rice bran oil concentrate fed diabetic (0.3% RD) with n=10. Diet composition followed was AIN-76 and for high-fat diet (30%) preparation, starch was replaced with vanaspati. Rice bran oil concentrate was mixed with diet, 0.1 and 0.3% oryzanol containing RC incorporated into the oil before preparation. Diabetes was induced by intraperitoneal injection of low dosage of streptozotocin (30 mg/ Kg bodyweight). After a week, animals were tested for blood glucose level using glucose strips with Accu-Chek meters. After eighth week of the experiment, animals were sacrificed (euthanasia) and liver tissue, and blood samples collected for various analysis.

Composition of AIN 76 diet

Composition	Normal Diet (g/kg)	High Fat Diet (g/kg)	0.1% RC/RD Diet(g/kg)	0.3% RC/RD Diet (g/kg)
Starch	653	453	451.98	449.92
Casein	200	200	200	200
Vitamin mix	10	10	10	10
Mineral mix	35	35	35	35
Choline chloride	2	2	2	2
DL-Methionine	0.5	0.5	0.5	0.5
GNO	100	100	96.92	90.78
Vanaspati	-	200	200	200
RC*	-	-	4.098	12.294

*Rice bran oil concentrate (RC) from rice bran oil

Biochemical parameters

Analysis of various parameters such as glucose, lipid, and proteins parameters in serum and tissues:

Serum glucose and tissue lipids were assayed by calorimetrically using the commercially available kits from Agappe Diagnostics Ltd. Protein estimation was carried out using the standard method of Lowry (11).

Glucose tolerance test (GTT):

Animals fasted overnight, next day morning GTT was conducted. In brief, 20% glucose in sterile saline water with a dose of 2 g/kg bodyweight was administered by orally to the diabetic and non-diabetic rats. Glucose levels were determined by collecting the blood samples from tail-vein at 0, 30, 60, and 120 min and samples measured by using the glucometer.

Antioxidant enzyme activity:

Super-oxide dismutase (SOD), was estimated by the xanthine-xanthine oxidase ferricytochrome c (X/XOD/Cyt c3+) method described by McCord andFridovich (19). Glutathione peroxidase (GPx), activity was measured by the method described by Flohe and Gunzler (20). Catalase, activity was carried out according to the method of Aebi (21). All the enzyme activites were expressed as units/mg protein.

Fecal sample preparation:

Fecal matter was collected 24 hours before sacrificing the animals. Fecal samples were lyophilized, 1g of fecal sample was weighed from each diet group, and total fat was extracted.

Extraction of fats from different sources:

Fat extraction was carried out by the method of Bhaskaragoud *et al.*, (16). Frozen tissues or fecal fats were homogenized in 0.74% KCl and agitated for a fixed time before elimination of the solid part. The homogenate from 1 g of tissue sample was diluted to 2:1 chloroform: methanol 20 times the volume of the tissue sample and kept overnight for extraction at 4°C. It was filtered through handmade filter paper. 3mL of 0.74% KCl was added to the crude extract and kept at room temperature. Then the upper aqueous layer was discarded. The interface was rinsed three times with pure upper phase, i.e., 3mL of chloroform: methanol: water in the ratio 0.1:1:0.5 and the lower phase were left undisturbed. Then the lowest phase was pipetted out carefully, dried using rotavapor. 1-1.5mL of chloroform was used to transfer the dried fat sample to 2mL vial; then chloroform samples were used for lipid analysis.

Lipid peroxides in liver:

Lipid peroxides in liver tissues were determined by the method (22). An aliquot of liver tissue was homogenised in 0.74% KCl, 20% acetic acid (pH 3.5), 8% SDS, 0.8% aqueous thiobarbituric acid were added to homogenate and mixed well. Incubated for 1hr in the water bath and left for cooling at room temperature. 3.0mL of n-butanol was added and mixed well, centrifuged at 3000 rpm for 10 minute. Butanol extract was measured by UV spectrophotometer at an absorbance of 532nm. Values were compared with standard 1,1,3,3-tetraethoxypropane.

Statistical analysis

Values are expressed in means ± SE of 6 rats in control group and 8 rats in diabetic groups, and the differences between the average values were assessed using Analysis of Variance (ANOVA) by Duncan's multiple range test (DMRT). Statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Rice bran oil concentrate (RC) was containing oryzanol majorly and was found to be 25 folds higher than the natural rice bran oil. Whereas, other fat soluble components such as tocopherols and carotenoids were shown in trace amount. Fatty acid

composition of rice bran oil and its concentrate, ground nut oil and vanaspati are presented in Table 1. Free fatty acid in oryzanol concentrate was slightly higher than the RBO that could be due to the high level of oryzanol present in it (Table 1). The addition of oryzanol increase the FFA level was shown (4).

The characteristic feature of diabetes is chronic hyperglycemia. The present study showed that both diabetic (ND and HD) had more elevated blood glucose when compared to control groups (NC and HC). Treatment with rice bran oil concentrate (0.1 and 0.3%) reduced their blood glucose (261 mg/dL and 270 mg/dL) significantly when compared to non-treated high fat fed diabetic groups (329 mg/dL) (Table2). It was showed that the high fat fed with 0.5% oryzanol exhibited significantly lower blood glucose level compared with high fat fed diet group (7). Glucose tolerance test (Fig. 2.) result had been showed that, the level of glucose received high (413 mg/dL) in HFD group, and it not reduced immediately after 120 minute, whereas OFD (0.1% and 0.3%) obtained less (251 & 270 mg/dL) after 120 minutes. Earlier studies, reported that treatment of neonatal STZ induced T2DM rats with 0.3% chito-oligomersfor four wk showed a significant increase in glucose tolerance (8).

Table 1: Physico chemical properties, nutraceuticals content and fatty acid composition of oils used for animal experiments

Parameters studied	RBO	RC	GNO	Vanaspati
Nutraceuticals content %				
Oryzanol	1.44	25	Nil	Nil
Tocopherol	0.02	0.034	0.009	Nil
Carotinod	0.003	0.007	Nil	Nil
Physicochemico properties				
PV (meqO2kg^{-1})	0.02 ± 0.002^{a}	0.07 ± 0.010^{a}	0.02 ± 0.004^{a}	0.13 ± 0.08^{a}
FFA (% as oleic acid)	0.72 ± 0.016^{a}	5.07 ± 0.310^{c}	0.21 ± 0.37^{a}	1.95 ± 0.41^{b}
Fatty acid composition				
Palmitic acid (16:0)	7.2^{a}	6.5^{a}	8.5^{a}	55.2^{b}
Stearic acid (18:0)	2.1^{a}	3.96^{a}	3.51^{a}	4.2^{a}
Oleic acid (18:1)	43.4^{b}	44.2^{b}	40.5^{b}	24.8^{a}
Linolic acid (18:2)	31.2^{b}	32.0^{b}	28.5^{b}	2.1^{a}
Other fatty acids	15.6^{a}	13.3^{a}	18.6^{a}	13.5^{a}

All the analyses were carried out in triplicate and the average values were expressed mean ± SD. The significance of difference was calculated by Student's *t*-test and row not sharing a common superscript (a, b, c) were considered for significant at $p < 0.05$.

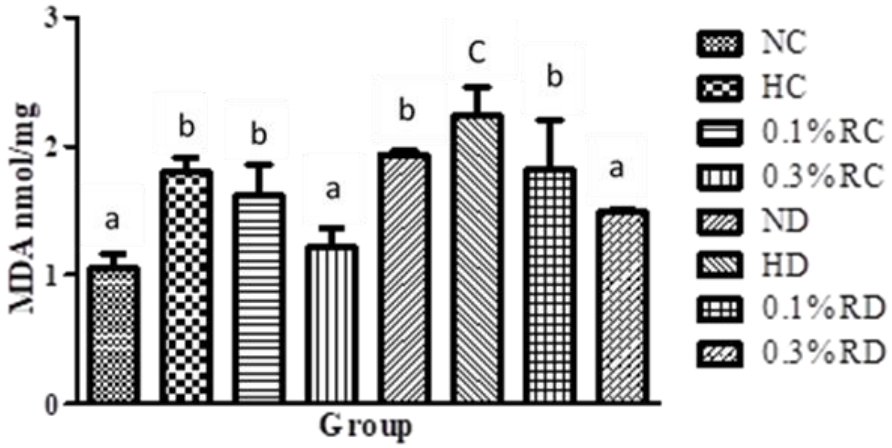


Fig. 1: Effect of Rice bran oil concentrate on liver lipid peroxide in control and diabetic rats.

Values are expressed as mean ± SD and figure with different alphabets are significantly different ($p< 0.05$) (n = 6). Abbreviations are as in Table 2.

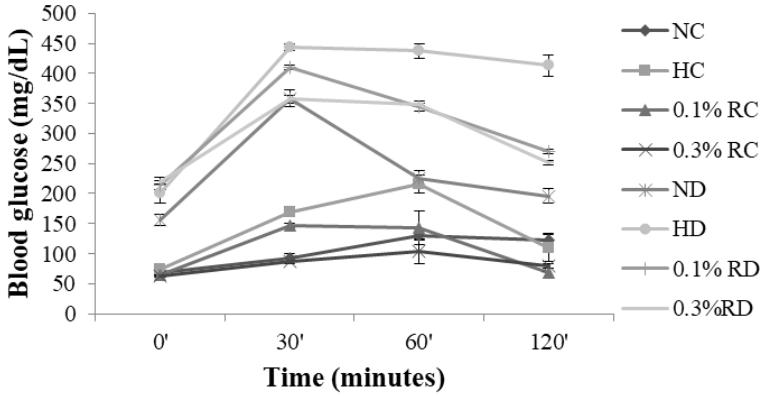


Fig. 2: Effect of Rice bran oil concentrate on OGTT in control and diabetic rats.

Values are expressed as mean ± SD and figure with different alphabets are significantly different (p< 0.05) (n = 6). Abbreviations are as in Table 2.

Our results revealed that oryzanol concentrate (0.1 and 0.3%) exhibited similar effects in alleviating HFD and STZ induced diabetes. These verdicts are in agreement with the findings (23), wherein ferulic acid and oryzanol used to treat the rodents.

The liver is a major organ in which most of the metabolism occurs and is affected by conditions like hyperglycemia. In the current study, the weight of the liver was found to be increased in HC than that of NC. Fat content in the high fat fed groups increased (213 mg/g) in the liver tissue which is three-fold higher than the control diet fed animals. However, in the diabetic animal, a content of fat was found more in high fat fed diet (41 mg/g) group than the normal diet groups. Treatment with rice bran oil concentrate at 0.1 and 0.3% reduced the fat content, but not significant between the treatments (Table 2). Similarly, cholesterol contents was found a high in HD group, treatment with different concentrates (0.1 and 0.3%) reduced at different extent (12). Phospholipids content was found decreased in liver tissues of HC control and HD diabetic and was increased after treatment with various concentration of oryzanol concentrate (Table 2). The fecal fat content was found more in animal treated with rice bran oil concentrate either in control and diabetic animals (Table 2). Our previous report showed the removal of bile acids from enterohepatic circulation is an important mechanism by which it restricts the accumulation of fat in the liver, as well as circulating lipids (16).

Prolonged hyperglycemia ends with severe complications via gerenrating more free radical and oxidative stress in cells. The antioxidant enzymes SOD, catalase and glutathione peroxidase (GPx) constitute a supportive team of defence against reactive oxidative stress and play a major role in alleviating cellular stress (7). In the present study both SOD, Catalase and GPx activities were reduced in the HD group. Hence, the generation of free radicals could be more. However, the enzyme activities were more in ND group. Treatment with 0.1 and 0.3% oryzanol treated groups, ameliorated their operations at different levels and was found to be significant when compared to HD group (Table 3). Earlier reports have shown similar results with oryzanol treatment wherein antioxidant properties of oryzanol may reduced the hyperglycemia (24).

The free radical (MDA or TBA reactant) was apparently more in both high fat fed groups of non-diabetic and diabetic animals, and these are ameliorated significantly in the RC treated groups (Fig. 1). In the previous report showed that oryzanol treated with high fat fed rat groups were reduced MDA level significantly (5, 6).

Table 2: Effect of rice bran oil concentrate on serum glucose, liver lipid profile and fecal fat content in control and diabetic rats

Groups	**Blood Glucose (mg/dL)**	**Liver tissue**					
		Weight (%)	**T.Fat* mg/g**	**TG* mg/g fat**	**TC* mg/g fat**	**PL* mg/100g**	**Fecal fat* mg/g**
NC	095 ± 03.0^{a}	2.6 ± 0.8^{a}	071.65^{b}	534.8^{c}	278.9^{a}	2.96^{a}	66.6^{c}
HC	121 ± 06.4^{b}	2.9 ± 0.7^{a}	213.25^{c}	670.6^{d}	296.0^{a}	2.41^{b}	67.4^{c}
0.1%RC	092 ± 12.3^{a}	2.7 ± 0.7^{a}	094.06^{b}	526.5^{c}	259.8^{a}	2.32^{b}	73.4^{d}
0.3%RC	089 ± 14.4^{a}	2.6 ± 0.9^{a}	085.05^{b}	550.2^{c}	259.8^{a}	2.60^{b}	94.1^{e}

ND	251 ± 09.7[c]	3.6 ± 0.4[a]	021.07[a]	294.8[a]	276.3[a]	1.24[a]	40.0[a]
HD	303 ± 12.1[c]	3.9 ± 0.9[a]	041.07[a]	725.5[e]	322.3[b]	1.38[a]	50.9[b]
0.1%RD	277 ± 13.9[c]	3.5 ± 1.0[a]	039.01[a]	306.9[a]	277.6[a]	2.03[b]	54.6[b]
0.3%RD	262 ± 09.6[c]	3.5 ± 0.8[a]	037.04[a]	455.8[b]	225.6[a]	1.66[a]	56.5[b]

NC:ND- Normal control group: Normal diabetic group,
HC:HD-High fat control group: High fat diabetic group,
0.1%RC:0.3%RC- High fat with RC fed treated control group,
0.1%RD:0.3%RD- High fat with RC fed treated diabetic group.

*****Values are the average pooled samples of each groups. The different superscripts in column are significantly different ($p< 0.05$)

Table 3: Effect of Rice bran oil concentrate on liver antioxidant enzymes of control and diabetic rats Glutathion peroxidase (GPx), Superoxide dismutase (SOD) and Catalase

	GPx U/mg protein	SOD U/mg protein	Catalase U/mg protein
NC	0.47±0.10[b]	1.81±0.51[b]	20.87±2.03[a]
HC	0.32±0.07[a]	1.08±0.32[a]	18.00±3.10[a]
0.1% RC	0.43±0.01[b]	1.54±0.51[a]	22.39±3.10[a]
0.3% RC	0.44±0.04[b]	2.27±0.42[c]	44.31±2.66[c]
ND	0.50±0.03[c]	2.25±0.64[c]	14.23±0.30[a]
HD	0.42±0.06[b]	1.34±0.31[a]	13.80±1.06[a]
0.1% RD	0.45±0.06[b]	1.86±0.51[b]	23.35±1.75[a]
0.3%RD	0.55±0.09[c]	1.79±0.21[b]	37.48±0.68[b]

Values of all groups are expressed as mean ± SD and that bearing different superscripts in column are significantly different ($p< 0.05$)

CONCLUSION

The present study shows that rice bran oil concentrate containing oryzanol-a unique nutraceutical as a major component attributed in reducing the hyperlipidemia, hyperglycemia, liver lipid profile, lipid peroxidation and glucose tolerance test in high fat fed and low STZ induced diabetic animals. Reduced liver weight clearly indicates reduced accumulation of cholesterol is due to mobilization cholesterol into bile acid. Interference of oryzanol or its hydrolysate may influence removal of excess dietary fat through fecal matter thus absorption and accumulation of fat in the liver is reduced thus normal lipid profile in serum. Furthermore, oryzanol reduces free radicals and normalizes antioxidant enzyme activities followed by increase in insulin sensitivity, and reduced hyperglycemia.

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