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EFFECTS OF RICE BRAN OIL AND GAMMA-ORYZANOL ON ANTIOXIDANT DEFENSE IN STREPTOZOTOCIN NICOTINAMIDE INDUCED DIABETIC RATS

C.Komal*, C. Bhushan, B. Gauri

* National Institute of Food Technology Entrepreneurship and Management, (NIFTEM) Kundli, Haryana, India.

Gian Sagar Medical College and Hospital, Banur, Punjab, India.

Mata Gujri College, Fatehgarh Sahib, Fatehgarh Sahib, Punjab, India.

ABSTRACT

Rice bran oil (RBO), a unique cooking oil is produced from the pericarp and germ of *Oryza sativa* seeds. Oryzanol is a mixture of sterol esters of ferulic acid and is known to have several health benefits. Male albino wistar rats were rendered hyperlipidemic by feeding high fat high cholesterol diet and diabetic by single intraperitoneal injection of freshly prepared streptozotocin [60 mg /kg b.w.] along with nicotinamide (NA) (120 mg/kg, i.p). Animals were supplemented for a period of 42 days with Rice Bran Oil (RBO) [15%] singly; blends of rice bran oil and oryzanol (RBO-OZ-100) with RBO (15%) and OZ at 100mg/Kg body weight and Oryzanol (OZ-100) singly at 100mg/Kg body weight. Glibenclamide (5mg/kg body weight) was used as a standard reference drug. Serum and tissues (liver kidneys and pancreas) were investigated for its antidiabetic, antihyperlipidemic and antioxidative efficacy in rats. High fat-high cholesterol feeding (HFHC) and STZ-NA induced diabetes resulted in significant increase in blood glucose, lipid – lipoprotein fractions and oxidative stress biomarkers in serum and tissues of rats. Treatment with RBO and oryzanol significantly ($p \leq 0.05$) restored the biochemical indices near to normal. The effect of RBO-OZ-100 and OZ-100 was better than glibenclamide. Results suggest that RBO and OZ efficiently regulated blood glucose; ameliorated lipid abnormalities associated with diabetes and improved the antioxidant enzymatic status in STZ-NA diabetic rats by virtue of bioactive compounds. The therapeutic potential of RBO and OZ can be used as preventive and protective therapy against the detrimental effects of diabetes and associated hyperlipidemia.

KEYWORDS: Rice Bran Oil, Streptozotocin, Diabetes mellitus, hyperlipidemia, Oxidative stress, antioxidant enzymes.

INTRODUCTION

Diabetes mellitus (DM); a metabolic disorder resulting from defects in insulin secretion or insulin action or both resulting in hyperglycaemia with disturbances in metabolism of carbohydrates, protein and fats. It is one of the major leading causes of morbidity and mortality worldwide. The incidence has increased dramatically in last decades mainly due to the “Nutrition Transition” and sedentary life style (Hu, 2011). Persistent hyperglycaemia alters our natural defense mechanism resulting in increased generation and inefficient scavenging of free radicals ensuing in imbalanced redox status at cellular and tissue levels (Pitocco et al., 2013). Various drugs used in the treatment of diabetes achieve transiently regulated euglycemia but fail to prevent lipid and lipoprotein alterations, ultimately, exposing the diabetics to cardiovascular complications. These drugs, besides being expensive exert adverse effects. There is a dire need for evaluating the natural compounds having dual functionality for hyperlipidemia and diabetes with no side effects. Concurrently bioactives identified from cereal grains and its by- products are gaining importance and present an exciting opportunity for the development of new types of therapeutics for the treatment of chronic diseases (Esa et al., 2013). This has accelerated the global effort to harness and harvest those cereal grains that bear substantial amounts of potential bioactive compounds showing multiple beneficial effects in combating diabetes and the related complications without causing side effects.

Rice bran oil (RBO), a unique cooking oil is produced from the pericarp and germ of *Oryza sativa* seeds. It constitutes about 10% of rough rice grain and contains 18-22% oil. It is the only oil which besides having balanced fatty acid composition and ideal PUFA/SFA and fair linoleic acid (LA)/ alpha linoleic acid (ALNA) ratio (Ghafoorunissa,

1994); also contains three categories of natural antioxidants i.e. tocopherols, tocotrienols and oryzanol. High content of these antioxidants impart higher oxidative stability and longer shelf life as compared to other edible oils (Raghuram and Rukmini, 1995). It has high unsaponifiable fraction (1.5-2.6%) in contrast to other refined vegetable oils that contains only 0.3-0.9% (Rong et al., 1997). It contains oleic acid (38.4%) as 2-oleate, linoleic acid (34.4%) and α -linolenic acid (2.2%) as unsaturated fatty acid and palmitic (21.5%) and stearic acid (2.9%) as saturated fatty acid (Edwards and Radcliffe, 1994; Radcliffe et al., 1997). Epidemiological evidences have attributed, the cholesterol lowering property of RBO, to the presence of oryzanol, a unique component in the oil, which is not found in any other edible oil and also to some other components present in unsaponifiable matter (Rong et al., 1997; Seetharamaiah and Chandrasekhara, 1989; Sharma and Rukmini, 1987).

Oryzanol is a mixture of sterol esters of ferulic acid. The main sterols esterified are cycloartenol and 24-methylene cycloartenol (4,4'-dimethylsterols) and β -sitosterol and campesterol (4-desmethylsterols). Oryzanol is known to have several health benefits like, it is effective in treating a broad range of gastro intestinal disorders including stress induced gastric and duodenal ulcers, neuroendocrinological, anabolic and dermatological disorders (Cicero and Gaddi 2001); to treat nerve imbalance and disorders of menopause (Ha et al., 2006); inhibits platelet aggregation induced by ADP and totally inhibited aggregation induced by collagen (Seetharamaiah et al., 1990b) and have anticarcinogenic properties (Tamagawa et al., 1992). Studies have reported oryzanol has a hypolipidemic effect possibly either by increasing fecal excretion of cholesterol and its metabolites or by suppressing the HMG Co.A reductase activity (Wilson et al., 2007; Seetharamaiah and Chandrasekhara, 1990a). The study was carried out to evaluate the antioxidative potential of RBO and OZ singly and in blends in experimentally induced diabetes and hyperlipidemia in albino rats

MATERIALS AND METHODS

Chemicals

All the chemicals used in the study were of analytical grade, procured from Sigma, Merck, BDH and Qualigens. Glibenclamide; Nicotinamide (NA) were obtained from Sigma-Aldrich, St. Louis, USA. Diagnostic kits for the estimation of serum and hepatic biomarkers were purchased from Erba Mannheim (Transasia Bio-Medicals Ltd. Daman, India). RBO was provided from A.P Solvex Ltd, Dhuri, India. Gamma oryzanol was purchased from Qingdao Reach International Inc, China.

Experimental Animals

The study protocol was approved by Institutional Animal Ethics Committee (IAEC) of the University constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Healthy male albino wistar rats of 6 weeks age (50.2 ± 2.86 g) were procured from the small animal house of Chaudhary Charan Singh Haryana Agriculture University Hissar (CCSHAU), India. Each animal was housed individually in the polypropylene cage with sterilized wood chip bedding in a specific pathogen free animal house room under the constant environmental condition with 12 hour light and dark cycle, $22 \pm 1^\circ\text{C}$ temperature; $50 \pm 10\%$ relative humidity and artificial illumination between 06:00 and 18:00 hrs. Animals were given the standard pellet diet (Hindustan Liver Ltd., India) and water *ad libitum* during acclimatization period of 1 week. The diet contained 20% protein, 5% protein, 5% fat and 5% fibre, 60% carbohydrates and 10% mixture of vitamins and minerals. The diet served as normal fat diet (NFD) for control group. High fat high cholesterol diet (HFHC) was formulated incorporating 84.5% NFD; 15% coconut oil (w/w) and 0.5% cholesterol (w/w).

Induction of Diabetes Mellitus to Experimental Animals

Rats were rendered diabetic by single intraperitoneal injection of freshly prepared streptozotocin (60mg kg^{-1}) in 0.1 M citrate buffer (pH 4.5) in a volume of 1ml kg^{-1} body weight (Siddique et al., 1987). The control group received 1 ml citrate buffer as vehicle. Nicotinamide (NA) was dissolved in normal saline and administered (120 mg/kg , i.p) 15 min before STZ (Masiello et al., 1998) The animals had free access to food and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. After 48-hours of STZ administration, blood glucose was evaluated in overnight fasting rats. The rats having blood glucose levels between a range of 200-300 mg/dl were considered to be diabetic and were selected for the study. Experimental dietary regime was started on the third day after STZ- NA induced diabetes and continued for a period of six weeks.

Preparation of Glibenclamide

Glibenclamide (GLM), an oral hypoglycaemic drug has been used since decades for the management of diabetes mellitus (Schmid-Antomarchi et al., 1987) Glibenclamide (5mg/kg body weight) was dissolved in dimethyl sulfoxide (DMSO) before administering intraperitoneally. Studies have shown that glibenclamide has the potential to counteract the reactive oxygen species mediated oxidative stress (Aboonabi et al., 2014; Elmali et al., 2005; Obata et al., 1998).

Acute Toxicity Study

Acute oral toxicity was performed as per organisation for Economic Cooperation and Development (OECD) guidelines 423 (2001). After the dietary supplementation of experimental diets, animals were observed individually for general behavioural at least once during the first 30 minutes and periodically during the first 24 hours with special attention given during first four hours and daily thereafter for a period of 14 days. At the end of the experimental period, all survivors were sacrificed to examine gross changes in their vital organs.

Experimental Diets

Animals were randomly divided into six groups of similar average body weight. The feeding regime was carried for a period of six weeks and animals were given different dietary treatments as: NC (n=6), fed NFD and served as normal control; Diabetic rats (n=30) were randomly divided into five groups of six animals each, reared on HFHC diets as under: DC, served as diabetic control; DC-RBO; fed RBO (15%); DC-RBO-OZ-100; fed RBO (15%) + γ -Oryzanol (OZ) (100mg/kg body weight). DC-OZ-100; fed γ -Oryzanol (OZ) (100mg/kg body weight); DC-GLM; administered Glibenclamide (GLM) (5mg/kg body weight). Basal and experimental diets were isoenergetic (~3600C) and were freshly prepared weekly in a pathogen free sterilized room (Reeves et al; 1993) and were stored at -20°C.

Biochemical Assays

The blood samples were collected from tail tip vein of experimental animals periodically for fasting blood sugar. For other biochemical parameters the animals were bled from retroorbital plexus at the end of feeding schedule and the sample was collected in heparinized prechilled vials, centrifuged at 3000g at 4°C for 10 minutes to obtain serum. At the end of experimental period, the animals were sacrificed by cervical decapitation. The organs (liver, pancreas and kidneys) were removed, freed of adhering tissues, washed with ice cold isotonic saline, blotted dry and weighed. A small part of the tissues were excised, minced and was used for enzyme activity assay and other biochemical evaluation. The remaining tissues were stored at -80 °C for further biochemical analysis.

Biochemical analysis of serum

Biochemical indices viz. fasting blood glucose (FBG) was determined by means of one touch ultra glucometer (Johnson & Johnson Company, USA) with compatible blood glucose strips (Henry, 1984). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Anderson et al., 1993). Glycosylated haemoglobin (HbA_{1c}) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan (1985). Serum total cholesterol (TC), triglycerides (TG) and HDL-cholesterol were estimated by using diagnostic kits (Erba Mannheim Transasia Bio-Medicals Ltd. Daman, India). VLDL and LDL-cholesterol were calculated as per Friedewald's equation (1972): VLDL-C = TG/5 and LDL-C = TC - (HDL-C + VLDL-C). Atherogenic index was calculated by the following formula: AI = (Total-C - HDL-C)/HDL-C. Antiarterial hardness index was calculated as LDL-C/HDL-C. Serum glutamate pyruvate transaminases (SGPT) and serum glutamate oxaloacetate transaminases (SGOT) were measured spectrophotometrically by utilizing the method of Reitman and Frankel (1979). Gamma glutamyl transferase activity was measured by the Rosalki and Rau Method (1972).

Biochemical analysis of Tissue Homogenates

For enzyme activity assay, 0.8-1.0g of tissue (liver, pancreas and kidneys) was minced and homogenized in 10 times its volume of 0.2M/L tris HCl (pH=8.0) containing 0.5M/L CaCl₂ using Potter Elevehjem apparatus at 0-4°C using motor driven Teflon pestle rotated at 3000rpm. The homogenate was centrifuged at 10000g for 30 minutes at 4°C and 3/4th of the volume was carefully drawn using Pasteur's pipette. The supernatants were stored at -80°C until analysis. Enzyme assay involved, lipid peroxidation (TBARS) (Ohkawa et al., 1979), reduced glutathione (GSH) (Sedlak & Lindsey, 1968) and antioxidant enzymes viz. glutathione peroxidase (GSHPx) (Neches et al., 1968); catalase (CAT) (Luck, 1971) and superoxide dismutase (SOD) (Kono, 1978). The supernatant was extracted and used for the estimation of liver glycogen (Montgomery, 1957) and protein content using Bio-Rad protein assay kit and BSA as standard.

STATISTICAL ANALYSIS

Results are expressed as Mean \pm Standard Error of Mean (SEM) of 6 rats. Statistical analysis involved Analysis of Variance (ANOVA- one-way). Tukey's post-hoc multiple comparison test was carried out using SPSS (version 16.0) and student's 't'-test using Sigma Plot (version 8.0). The values at $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The results revealed that all the animals survived the entire length of the experimental period indicating that the tested dose did not cause any mortality or toxic symptoms in the rats. There was no apparent change in behaviour, physical appearance between the treated and untreated animals. The faeces were dry and dark indicative of normal functioning of gastrointestinal tract. Upon sacrificing the animals, the visceral organs did not show any pathological abnormality.

Effect on Nutritional Parameters

The effect of experimental diets on nutritional parameters is shown in Table 1. The STZ-NA treatment resulted in loss of body weight and depletion of reserves in all the experimental animals. The DC group revealed a significant loss (60.8%) in body weight as compared to NC group (71.6%). Supplementation of experimental diets counteracted the effect with the highest increase in DC-RBO-OZ-100 (69.8%). Weight loss is a prominent feature in diabetes as fats and proteins are excessively metabolized for gluconeogenesis (Jayasri et al., 2009). Improved weight gain in treated animals indicates probable restoration of lipid and protein metabolism and reversal of gluconeogenesis which has been confirmed in previous studies (Ahmad et al., 2014; Kumar et al., 2013). The experimental diets reduced the relative organ weights (liver, kidney and pancreas) of treated animals as compared to the DC group. The diabetic rats showed a marked increase in relative liver weight probably due to increased glycogenolysis and gluconeogenesis. Relative Food consumption (RFC) and food efficiency ratio (FER) did not show any marked difference (data not shown) among the treated groups. The differences in the nutritional parameters between the treated groups and normal control group were not significant indicating that the RBO and OZ had no toxic effect at the given dose. The resumption in the nutritional parameters in treated animals indicates a marked improvement in carbohydrate and lipid metabolism.

Effect on Plasma Insulin and glucose

Fig.1 shows significant ($p \leq 0.05$) decrease in plasma insulin levels (3.7 ± 0.28 g/dl) with concomitant increase in blood glucose (413.4 ± 3.79 mg/dl) in STZ-NA induced diabetic control rats, as compared with the normal control ones (16.9 ± 0.31 g/dl and 82.1 ± 2.54 mg/dl) (Fig. 2). The supplementation of RBO; OZ and Glibenclamide to diabetic rats increased the plasma insulin levels and reduced glucose levels significantly ($p \leq 0.05$). The increasing and decreasing trend of plasma insulin and blood glucose in the treated groups was comparable with that of glibenclamide with the maximal increase of 76% in plasma insulin (from 3.7 ± 0.28 to 15.8 ± 0.28 g/dl) and 70% reduction in blood glucose (from 364.2 ± 8.34 to 122.5 ± 10.49 mg/dl) as compared to diabetic control. The diabetogenic effect may be due to increased glycogenolysis; gluconeogenesis or due to the irreversible destruction of β cells of islets of langerhans resulting in insufficient secretion of insulin (Guyton, 2000). Streptozotocin (STZ); a cytotoxic compound causes necrosis of pancreatic β -cells by facilitating its uptake via GLUT2, induces DNA damage thereby increasing activity of poly(ADP-ribose) polymerase (PARP-1) which in turn depletes intracellular nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP). Nicotinamide (NA) inhibits PARP-1 activity, preventing the depletion of NAD^+ and ATP in cells thus protecting the destruction of insulin-secreting cells against STZ. Studies have shown that the combined administration of STZ and NA results in stable diabetes with moderate hyperglycaemia with reduced insulin secretion and stores (Su et al., 2006; Masiello et al., 1998). In our study RBO and OZ exhibit insulinotropic action probably due to stimulation of β cells thus restoring their function and showing a protective effect. Glibenclamide, acts in a similar manner and potentiates the release of insulin by pancreatic cells which has been confirmed in previous studies (Sharma and Garg, 2009; Gerich, 1989).

Effect on Glycogen and Glycated Haemoglobin

A significant ($p \leq 0.05$) decrease was observed in hepatic glycogen content (46.2%) in DC group in comparison to NC group (from 45.4 ± 0.21 to 24.4 ± 0.34 g/g wet tissue) as evident from Fig. 2. However treatment with experimental diets significantly increased the glycogen content. Glibenclamide treated rats indicated a marked improvement in glycogen content (59.6%) followed by DC-RBO-100 (42.5%) and DC-OZ-100 (41.3%). The restored glycogen levels are indicative of antiglycaemic effect of the supplemented diets as glycogenesis involve restoration of the insulin levels which have been confirmed in previous studies (Chauhan et al., 2012; Chauhan et al., 2010; Sharma and Garg, 2009). Moreover, the restored levels of insulin promotes glycogenesis by stimulating glycogen synthase and inhibiting

glycogen phosphorylase (Kumar et al., 2013; Guyton 2000). Moreover insulin promotes protein synthesis, prevents degradation of proteins, henceforth muscle wasting. It facilitates the amino acids to be utilized for protein synthesis rather than acting as substrates for gluconeogenesis. Our study demonstrated a marked reduction ($p \leq 0.05$) in plasma protein levels while the effect was partially overshadowed by supplemented diets. HbA1c is a marker for protein glycation in diabetes mellitus and is directly proportional to blood glucose. The glycated haemoglobin showed 14.4% increase in the DC group from NC ones (4.3%), where as the levels of glycated haemoglobin declined markedly in treated animals indicating the hypoglycaemic effect of RBO and oryzanol (Table 2).

Effect on Blood Oxidative Stress Biomarkers

Serum transaminases (SGPT and SGOT) are indicators of liver function and STZ; free radicals and oxidative stress can lead to hepatic damage, thereby increasing their activity. Studies have shown that the enzymatic activity increases in diabetes owing to hepatic damage or malfunctioning of β - cells resulting in inadequate secretion of insulin. (Chauhan et al., 2012; Chauhan et al., 2010, Whitehead et al., 1999). Treatment with RBO and OZ restored the transaminases activity to near normal levels (Table 2).

Gamma glutamyl transferase (GGT) plays a key role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and detoxification of drugs and xenobiotics (Courtay et al., 1992). Elevated [serum](#) GGT activity generally occurs in diseases of the liver, biliary system, and pancreas. GGT facilitates the continuous availability of cellular GSH, thus strengthens antioxidant defense system (Lim et al., 2004; Forman et al. 1997). Results reveal that GGT activity considerably increased ($p \leq 0.05$) in DC group (4.8 ± 1.56 IU/L) as compared to NC group (0.9 ± 0.01 IU/ L) indicative of increased generation of ROS and oxidative stress (Table 2). Supplementation of RBO and OZ tended to neutralize the effect probably by scavenging the free radicals. Previous studies confirms the inverse relationship between antioxidants and GGT activity (Aboonabi et al., 2014; Ramesh and Pugalendi, 2006)

Effect on Serum lipid and lipoprotein fractions

The lipid- Lipoprotein fractions (Fig.3) of the DC group revealed a significant increase in TC, TG, PL, LDL-C, VLDL-C (52.6%; 51.3%;31%;70.9%;51.3% with a significant decrease in HDL-C (11%). A decreasing trend was observed in serum lipids and lipoprotein fractions (LDL-C and VLDL-C) of treated animals reared on supplemented diets with RBO, oryzanol and glibenclamide. On the contrary the HDL-C levels significantly improved in treated animals. Administration of RBO and OZ to experimental animals singly or in blends resulted in improvement of hyperglycaemia which further attenuated hyperlipidemia over the feeding regime period of 42 days.

The maximal decrease in TC (55.5%), TG (52.7%) and VLDL-C (52.7%) levels was in group supplemented with blends of RBO and OZ-100 mg/kg b.w. as compared to DC group Animals supplemented singly with OZ-100 mg/kg b.w and glibenclamide showed a similar decrease in phospholipids (34.5% and 34.6% respectively) and LDL-C (81.7% and 81.9% respectively). In contrast the HDL -C levels showed marked improvement in DC-OZ-100 group (42%) followed by DC-GLM group (39.6%).

Persistent hyperglycaemia and insulin deficiency alters fat metabolism causing increased mobilization of fats from adipose tissue and lipolysis. Furthermore, the activation of enzymes lipoprotein lipase (LP) and lecithin acyl-cholesterol transferase (LCAT) results in decrease in HDL-C levels owing to in increased levels of TG; VLDL-C and LDL-C. The results are in accordance with the previous research findings (Mooradian et al., 2008; Howard, 1987). The lipid- lipoprotein fractions were brought to normal in RBO and OZ treated rats indicating increased plasma insulin levels, thereby promoting glucose utilization and inhibiting fat mobilization from fat depots. The results are correlated with earlier studies showing an ameliorative effect with RBO and its bioactive compounds (Wang et al., 2014; Cheng et al., 2010; Wilson et al., 2007).

Atherogenic index and antiarterial hardness significantly ($p \leq 0.05$) increased in diabetic control rats. The effect was however, neutralized by supplementing RBO, OZ and glibenclamide in treated animals (Fig. 3). The most striking effect was observed in groups supplemented with oryzanol singly (81.7% and 87.1%) and glibenclamide (81.5 % and 87%) as compared to the STZ-NA untreated rats. Studies have clearly demonstrated that these indices are used as predictors for CVD risk rather than TC or HDL-C alone (Chou et al., 2009; Ridker et al., 2005). The experimental diets improved the overall lipoprotein pattern thus reducing the risk of CVD and complications associated with diabetes.

Effect on Oxidative stress Biomarkers

In comparison to NC group, STZ-NA treated diabetic rats showed marked increase in TBARS levels with a concomitant decrease in GSH levels in tissue homogenates (liver, pancreas and renal) indicating excess production of free radicals (FR) and reactive oxygen species (ROS). Inclusion of RBO and OZ singly or in blends to diets of experimental animals reversed the effect and significantly ($p \leq 0.05$) decreased TBARS levels and increased GSH levels. As evident from the Fig. 4 (A) the maximum decrease in hepatic (45.1%), and pancreatic (19.9%) TBARS levels was observed in the group fed blends of RBO and OZ. The renal TBARS levels showed the maximum decrease in DC-OZ-100 group (43.6%) as compared to DC group. The decline observed in the pancreatic and renal TBARS levels among the treated groups reared either singly or in blends of RBO and oryzanol was not significant. The GSH content in hepatic, pancreatic and renal homogenates markedly improved in supplemented groups with the highest levels restored in glibenclamide supplemented group (34.3 ± 2.16 ; 32.6 ± 1.92 and 33.5 ± 1.37 mg/g) respectively as shown in Fig. 4 (B).

Fig.4 (C, D, E) demonstrates the enzymatic antioxidant activities viz. GSHPx, CAT and SOD in tissue homogenates of controls and treatment groups respectively. A significant ($p \leq 0.05$) reduction in the enzymatic activities was observed in STZ-NA administered rats as compared to the NC group. However, treatment with RBO, OZ and glibenclamide nullified the effect and restored the enzymatic activities to near normal. The GSHPx activity was higher in pancreatic and renal tissues with maximum activity in DC-OZ-100 group (36.9%) and DC-RBO-OZ-100 group (38.2%) respectively. The effect was more pronounced in RBO and OZ treated groups as compared to glibenclamide treated group. This may be because of synergistic action of various bioactive compounds in contrast to the purified compound of the drug.

A similar trend was observed in catalase activity (Fig 4.D) in the hepatic, pancreatic and renal tissue with the highest values in DC-OZ-100 group (243.4 ± 1.28 ; 351.6 ± 1.41 and 329.2 ± 1.57 μ moles of H_2O_2 decomposed/minute/mg protein). The antioxidative potential of RBO and OZ in blends and OZ singly was better than the standard glibenclamide drug (153.5 ± 2.12 ; 254.9 ± 1.57 ; 237.6 ± 1.25 μ moles of H_2O_2 decomposed/minute/mg protein).

The activity of SOD improved among the treated groups, however the increase was not significant in hepatic and renal tissues. A marked increase in enzymatic activity in pancreatic tissue was observed in glibenclamide treated group (15.1%) followed by DC-RBO-100 group (9.4%) in comparison to DC group (Fig. 4 E).

The biological systems have been equipped with non-enzymatic and enzymatic antioxidants that provides natural defense mechanisms to counteract free radicals and oxidative stress. However, studies have shown that oxidative stress induced by hyperglycemia and associated hyperlipidemia exhausts the antioxidant defense causing oxidative damage to cell membranes, loss of cellular functions making them more susceptible to lipid peroxidation. Similar results have been reported in earlier studies (Chatuphonpraser, 2014; Sharma et al., 2010; Kędziora-Kornatowska, et al., 2000; Hussain; 2002 and; Kakkar et al., 1995).

Furthermore, studies have demonstrated that imbalance between prooxidants and antioxidants results in inactivation of antioxidants due to glycation which further disturbs the cellular redox status (Moemen et al., 2014; Choudhuri et al. 2013; Shin et al., 2006; Kawamura et al., 1992). This compromised antioxidant potency promote diabetes and further lead to diabetic complications (Kędziora-Kornatowska, et al., 2000). The bioactive compounds in RBO and oryzanol act as antioxidants thereby scavenging free radicals thus strengthening the antioxidant defense (Wang et al., 2014, Shakib et al., 2014).

CONCLUSION

RBO and OZ possess significant antihyperglycemic, antihyperlipidemic and antioxidative properties thereby strengthening our defense system. Consumption of RBO and OZ can delay the onset and would help in alleviation of associated diabetic complications including the prevention of the development of atherosclerosis and other cardiovascular diseases. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients, and evaluate the potential value of RBO and OZ for the management of diabetes and hyperlipidemias.

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REFERENCES

- [1] Aboonabi , A., Rahmat , A., Othman, F.,Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats, Toxicology Reports1, pp.915–922, 2014.
- [2] Ahmad, M., Prawez, S., Sultana, M., Raina, R, Pankaj, N.K.,Verma, P.K., Rahman, S., Anti-Hyperglycemic, Anti-Hyperlipidemic and Antioxidant Potential of Alcoholic-Extract of *Sida cordifolia* (Areal Part) in Streptozotocin-Induced-Diabetes in Wistar-Rats, [Proceedings of the National Academy of Sciences, India Section B: Biological Sciences](#), vol. 84, Issue 2, pp. 397-405, June 2014,
- [3] Anderson, L., Dinesen, B., Jorgensen, P.N., Poulsen, F., Roder, M.E., Enzyme immune assay for intact human insulin in serum or plasma, *Clinical Chemistry*, vol. 39, pp. 578-582, 1993.
- [4] Chatuphonprasert, W., Lao-ong, T., Jarukamjorn, K., Improvement of superoxide dismutase and catalase in streptozotocin–nicotinamide-induced type 2-diabetes in mice by berberine and glibenclamide, vol. 52, No. 4, pp. 419-427, April 2014.
- [5] Chauhan, K., Sharma S., Chauhan, B., Bajaj, G., Biochemical evaluation of lipid and oxidative stress modulating effects of neutraceuticals. *Inventi Impact Neutraceuticals*, vol.1, no.2, pp.44-50, 2010
- [6] Chauhan, K., Sharma, S., Rohatagi, K., Chauhan, B., Antihyperlipidemic and antioxidative efficacy of *Catharanthus roseus* Linn (Sadabahar) in Streptozotocin induced diabetic rats, *Asian Journal of Pharmaceutical and Health Sciences*, vol. 2, no. 1,pp. 235-243, 2012.
- [7] Cheng, H.H, Ma, C.Y., Chou, T.W., Chen, Y.Y., Lai, M.H., Gamma-oryzanol ameliorates insulin resistance and hyperlipidemia in rats with streptozotocin/nicotinamide-induced type 2 diabetes. *Int J Vitam Nutr Res*, vol 80, no.(1), pp. 45-53, January 2010.
- [8] Chou, T.W., Ma, C.Y., Cheng, H.H., Chen, Y.Y., Lai, M.H., A rice bran oil diet improves lipid abnormalities and suppress hyperinsulinemic responses in rats with streptozotocin/nicotinamide-induced type 2 diabetes, *J Clin Biochem Nutr*, vol.45, pp.29–32, 2009.
- [9] Choudhuri, S., Dutta, D., Chowdhury, I.H., Mitra, B., Sen, A., Mandal, L.K., Mukhopadhyay, S., Bhattacharya, B., Association of hyperglycemia mediated increased advanced glycation and erythrocyte antioxidant enzyme activity in different stages of diabetic retinopathy, *Diabetes Res Clin Pract*, vol.100, no.3, 376-384, June 2013.
- [10] Cicero, A.F.G., Gaddi, A. Rice bran oil and gamma –oryzanol in the treatment of hyperlipoproteinaemias and other conditions, *Phytother Res*, vol. 15, pp. 277-289, 2001.
- [11] Courtay, C., Oster, T., Michelet, F., Visvikis, A., Diederich, M., Wellman, M. et al.,Gamma-glutamyltransferase: nucleotide sequence of the human pancreatic cDNA. Evidence for a ubiquitous gamma-glutamyltransferase polypeptide in human tissues, *Biochemical Pharmacology*, vol. 43 , no. 12, pp. 2527–2533, June 1992.
- [12] Edwards, M.S., Radcliffe, J.D., A comparison of the effect of rice bran oil and corn oil on lipid status in the rat. *Biochem Arch*, vol. 10, pp.87-94, 1994.
- [13] Elmalí, E., Altan, N., Bukan, N., Effect of the sulphonylurea Gliben- clamide on liver and kidney antioxidant enzymes in streptozotocin- induced diabetic rats, *J Drug Res Dev* vol.5, pp. 203–208, 2005.
- [14] Esa, N.M., Ling, T.B., Peng, L.S. By-products of Rice Processing: An Overview of Health Benefits and Applications, *J Rice Res*, vol. 1, no.107, 2013.
- [15] Forman, H.J., Liu, R.M., Tian, L., Glutathione cycling in oxidative stress, *Lung Biol Health Dis*, vol. 105, pp. 99–121, 1997.
- [16] Friedewald, W.T., Levy, R.I., Fredrickson, D.S., Estimation of the concentration of LDL-C in plasma, without use of the preparative ultracentrifuge, *Clinical Chemistry*, vol.18, pp. 499-502, 1972.
- [17] Gerich JE. Oral hypoglycemic agents, *New Eng J Med*, vol. 321, pp.1231-1245, 1989.
- [18] Ghafoorunssia, Dietary lipids and heart disease- the Indian context. *Natl Med J India*, vol.7, no.6, 270-276, 1994.
- [19] Guyton AC, Hall JE. *Textbook of medical physiology*, 10 ed. Philadelphia: Saunders WB, pp. 810-818, 2000.

- [20] Ha, T.Y., Ko, S.N., Lee, S.M., Kim, H.R., Chung, S.H., Kim, S.R., Yoon, H.H., Kim, I.H., Changes in neutraceutical lipid components of rice at different degrees of milling, *Eur J Lipid Sci Technol*, vol.108, pp.175-181, 2006.
- [21] Henry, J., *Clinical diagnosis and management by laboratory methods*. 17ed. Saunders WB, pp.1433, 1984.
- [22] Howard, B.V., Lipoprotein metabolism in diabetes mellitus, *J Lipid Res*, vol. 28, pp. 613-628, 1987.
- [23] [Hu](#), F.B., Globalization of Diabetes :The role of diet, lifestyle, and genes, *Diabetes Care*, vol.34, no. 6, pp. 1249–1257, June 2011
- [24] Hussain, H.E.M.A., Hypoglycemic, hypolipidemic and antioxidant properties of combination of curcumin from *Curcuma longa*, Linn, and partially purified product from *Abroma augusta*, Linn. In streptozotocin induced diabetes, *Ind J Clin Biochem*, vol.17, pp. 33-43, 2002.
- [25] Jayasri, M.A., Radha, A., Mathew, T.L. α -Amylase and α -glucosidase inhibitory activity of *Costus pictus* D. DON in the management of diabetes. *J Herb Med Toxicol*, vol. 3, pp. 91–94, 2009.
- [26] Kędziora-Kornatowska, K. Z., Luciak, M., Paszkowski, J., Lipid Peroxidation and Activities of Antioxidant Enzymes in the Diabetic Kidney: Effect of Treatment with Angiotensin Convertase Inhibitors, *IUBMB Life*, vol. 49, pp. 303–307, 2000.
- [27] Kakkar, R., Kalra, J, Mantha, S. V., Prasad, K., Lipid peroxidation and activity of antioxidant enzymes in diabetic rats, *Mol Cell Biochem*, vol. 151, pp. 113-119, 1995.
- [28] Karunanayake, E.H., Chandrasekharan, N.V., An evaluation of a colorimetric procedure for the estimation of glycosylated haemoglobin and establishment of reference values for Sri Lanka, *Journal National Science Council Sri Lanka*, vol.13, pp.235-258, 1985.
- [29] Kawamura, N., Ookawara, T., Suzuki, K., Konishi, K., Mino, M., and Taniguchi, N., Increased glycated Cu,Zn-superoxide dismutase levels in erythrocytes of diabetics with insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* vol.74, pp.1352–1354, 1992.
- [30] Kono, Y., Generation of superoxide radical during autooxidation of hydroxylamine and an assay for SOD, *Archives Biochemistry Biophysics*, vol.186, pp.189, 1978.
- [31] **Kumar, V., Ahmed, D., Gupta, P.S., Anwar, F., Mujeeb, M.**, Anti-diabetic, anti-oxidant and anti-hyperlipidemic activities of *Melastoma malabathricum* Linn. leaves in streptozotocin induced diabetic rats, *BMC Complementary and Alternative Medicine*, vol. 13, no. 222, pp. 1-19, 2013.
- [32] Lim, J.S., Chun, B.Y. Kam, S., Jacobs, D.R., Lee, D.H., Inverse associations of gamma glutamyltransferase with serum antioxidants, *Free Rad Biol Med*, vol.37, no. 7, pp. 1018–1023, 2004.
- [33] Luck, H., Catalase, In: *Methods of enzymatic analysis*. Berameyer Hansulrich (eds). New York: London Academic Press, pp.855, 1971.
- [34] Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G., Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide, *Diabetes*, vol.47, pp. 224–229, 1998.
- [35] Montgomery, R., Determination of glycogen. *Archives Biochemistry Biophysics*, vol.67, pp.378 – 386, 1957.
- [36] Mooradian, A.D., Haas, M.J., Wehmeier, K.R., Wong, N.C., Obesity-related changes in high density lipoprotein metabolism, *Obesity*, vol.16, pp. 1152-1160, 2008.
- [37] Necheles, T.F., Bolas, T.A., Allen, D.M., Erythrocyte glutathione peroxidase deficiency and hemolytic disease of the newborn infant, *Journal of Paediatrics*, vol.72, no.3, pp.31, (1968).
- [38] Obata T., Yamanaka Y., Glibenclamide, an antagonist of ATP-sensitive K⁺ channels, blocks free radical generation in the rat myocardium, *Neurosci Lett*, vol.257, pp. 57–59, 1998.
- [39] OECD, Guidelines of testing of chemicals, 423 acute oral toxicity (acute toxic class method), 2001.
- [40] Ohkawa, H., Oshishi, N., Yagi, K., Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction, *Annals of Biochemistry*, vol. 95, pp.351, 1979.
- [41] [Pitocco](#), D., [Tesauro](#), M., [Alessandro](#), R., [Ghirlanda](#), G. [Cardillo](#), C., Oxidative Stress in Diabetes: Implications for Vascular and Other Complications, *Int J Mol Sci*, Vol. 14, no.11, pp.21525–21550, November 2013.
- [42] Radcliffe, J.D., Imrhan, V. A., Hsueh, A.M., Serum lipids in rats fed diets containing rice bran oil or high linoleic acid safflower oil, *Biochem Arch*, vol.13, pp. 87-95, 1997.

- [43] Raghuram, T.C., Rukmini, C., Nutritional significance of rice bran oil. *Ind J Med Res*, vol.102, pp.241-244, 1995.
- [44] Ramesh, B., Pugalendi, K.V., Impact of umbelliferone (7-hydroxycoumarin) on hepatic marker enzymes in streptozotocin diabetic rats, *Indian J Pharmacol*, vol. 38, no. 3, pp. 209-210, 2006.
- [45] Reeves, P.G., Nielsen, F.H., Fahey, G.C. Jr., AIN-93 Purified diets for laboratory rodents: Final report of the American institute of nutrition adhoc writing committee on the reformulation of the AIN-76 a rodent diet, *Journal of Nutrition*, vol.123, pp.1939-1951, 1993.
- [46] Reitman, Frankel., *Am J Clin Pathol* 1957; 28: 56-63. Quoted in: Haoks Physiological Chemistry. Oser BL (ed) New Delhi: Tata McGraw Hill Publishers, 1125-1127, 1979.
- [47] Ridker P.M., Rifai, N., Cook, N.R. Buring, J.B.G.E., Non-HDL Cholesterol, Apolipoproteins A-I and B100, Standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women, *JAMA*, vol. 294, No. 3, pp. 326-333, , 2005.
- [48] Rong, N., Ausman, L.M., Nicolosi, R.J., Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters, *Lipids*, vol.32, pp.303-309, 1997.
- [49] Rosalki, S.B., Rau, D., Serum g-glutamyl transpeptidase activity in alcoholism, *Clin Chim Acta*, vol.39, pp. 41-47, 1972.
- [50] Schmid-Antomarchi, H., de Weille, J., Fosset, M., Lazdunski, M., The antidiabetic sulphonylurea Glibenclamide is a potent blocker of the ATP-modulated K⁺ channel in insulin secreting cells, *Biochem. Bio- phys Res Commun*, vol.146, pp. 21-25, 1987.
- [51] Sedlak, J., Lindsey, R.H., Estimation of total protein bound and non protein sulphhydryl groups in tissues with Ellman's reagent, *Annals of Biochemistry*, vol. 25, pp. 192, 1968.
- [52] Seetharamaiah, G.S., Chandrasekhara, N., Effect of oryzanol on cholesterol absorption and biliary and fecal bile acid in rats, *Ind J Med Res*, vol. 92, pp. 471-475, 1990a
- [53] Seetharamaiah, G.S., Chandrasekhara, N., Studies on hypocholesterolemic activity of rice bran oil, *Atherosclerosis*, vol.78, pp.219-223, 1989.
- [54] Seetharamaiah, G.S., Krishnaktha, T.P., Chandrasekhara, N. Influence of oryzanol on platelet aggregation in rats, *J Nutr Sci Vitaminol*, vol.36, no.3, pp. 291-297, 1990b.
- [55] Shakib, M.C., Gabriel, S., Gabriel, G., Rice Bran Oil Compared to Atorvastatin for Treatment of Dyslipidemia in Patients with Type 2 Diabetes, *Journal of Medical Sciences*, vol, 15, Issue 2, no. 1:95-102, March 2014.
- [56] Sharma, N., Garg, V., Paul, A., Antihyperglycemic, antihyperlipidemic and antioxidative potential of *Prosopis cineraria* bark. *Indian Journal of Clinical Biochemistry*, vol.25 no. 2, pp. 193-200
- [57] Sharma, N. and Garg, V., Antidiabetic and antioxidant potential of ethanolic extract of *Butea monosperma* leaves in alloxan induced diabetic mice, *Indian J Biochem Biophys*, vol.46, pp.99 – 105, 2009.
- [58] Sharma, R.D., Rukmini, C., Hypocholesterolemic activity of unsaponifiable mater of rice bran oil. *Ind J Med Res*, vol.85, pp 278-281, 1987.
- [59] Shin, A.H., Oh, C.J., Park, J.W., Glycation-induced inactivation of antioxidant enzymes and modulation of cellular redox status in lens cells, *Arch Pharm Res*, vol.29, no.7, pp. 577-581, July 2006.
- [60] Siddique, O., Sun, Y., Lin, J.C., Chien, Y.W., Facilitated transdermal transport of insulin, *Journal of Pharmaceutical Science*, vol. 76, pp.341- 345, 1987.
- [61] Su, H.C. Hung, L. M. Chen, J.K., Resveratrol, a red wine antioxidant, possesses an insulin-like effect in streptozotocin-induced diabetic rats, *Am J Physiol Endocrinol Metab*, vol. 290, pp. E1339-E1346, 2006.
- [62] Tamagawa, M., Otaki Y., Takahashi, T., Otaka, T., Kimura, S., Miwa, T., Carcinogenicity study of gamma-oryzanol in B6C3F1 mice, vol. 30, no.1, pp.49-56, January 1992.
- [63] Wang, Y., Li, Y., Sun, A., Wang, F., Yu, G., Hypolipidemic and antioxidative effects of aqueous enzymatic extract from rice bran in rats fed a high-fat and -cholesterol diet, *Nutrients*, vol 6, pp. 3696-3710, 2014.
- [64] Whitehead, M.W., Hawkes, N.D., Hainsworth, I., Kingham, J.G., A prospective study of the causes of notably raised aspartate transaminase of liver origin, *Gut*, vol. 45, pp. 129-133, 1999.
- [65] Wilson, T.A., Nicolosi, R.J., Woolfrey, B., Kritchevsky, D., Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters, *J Nutr Biochem*, vol. 18, pp. 105-112, 2007.

Table 1. Effect of Experimental Diets on Nutritional Parameters in STZ-NA Diabetic Rats

Treatment	Liver	Kidney	Pancreas	Body Weight Gain
	g/kg body weight			(%)
NC	130.9±0.57	27.4±0.12	1.4±0.01	71.68
DC	158.2±0.84 ^a	39.0±0.28 ^a	1.1±0.03 ^a	60.87 ^a
DC-RBO	135.8±0.37 ^b	30.0±0.14 ^{NS}	1.3±0.01 ^{NS}	68.40 ^b
DC-RBO-OZ-100	149.1±0.29 ^b	30.3±0.54 ^{NS}	1.4±0.01 ^{NS}	69.82 ^b
DC-OZ-100	139.4±0.47 ^b	31.9±0.71 ^b	1.5±0.04 ^b	68.92 ^b
DC-GLM	148.3±0.81 ^b	30.0±0.19 ^{NS}	1.5±0.01 ^b	69.12 ^b

Values are Mean ± SEM of 6 rats in each group
 Group NC is compared with Groups DC
 Other Treatment groups are compared with DC
^aP≤0.05 : Significantly different from NC
^bP≤0.05 : Significantly different from DC
 NS: Non Significant

Table 2. Effect of Experimental Diets on Blood Biomarkers in STZ-NA Diabetic Rats

Treatment	HBA ₁ C	SGOT*	SGPT*	GGT #	Total Protein
	(%)	IU/L	IU/L	IU/L	(g/dl)
NC	4.3	73.5±1.13	25.2±2.57	0.9±0.01	7.2±0.15
DC	14.4 ^a	121.4±1.25 ^a	60.1±3.25 ^a	4.8±1.56 ^a	4.5±0.19 ^a
DC-RBO	5.3 ^b	98.8±1.07 ^b	32.4±3.05 ^b	1.7±0.02 ^b	5.1±1.01 ^{NS}
DC-RBO-OZ-100	4.9 ^b	82.3±1.24 ^b	29.4±2.81 ^b	1.0±0.04 ^b	7.1±1.21 ^b
DC-OZ-100	4.5 ^b	81.5±1.08 ^b	28.3±2.15 ^b	0.9±0.07 ^b	6.8±0.48 ^b
DC-GLM	4.2 ^b	79.3±2.01 ^b	29.6±3.12 ^b	0.8±0.01 ^b	6.7±0.57 ^b

Values are Mean ± SEM of 6 rats in each group
 *µmol of pyruvate liberated per hour
 # µmol of p-nitroanilidine liberated per minute
 Group NC is compared with Groups DC
 Other Treatment groups are compared with DC
^aP≤0.05 : Significantly different from NC
^bP≤0.05 : Significantly different from DC
 NS: Non Significant

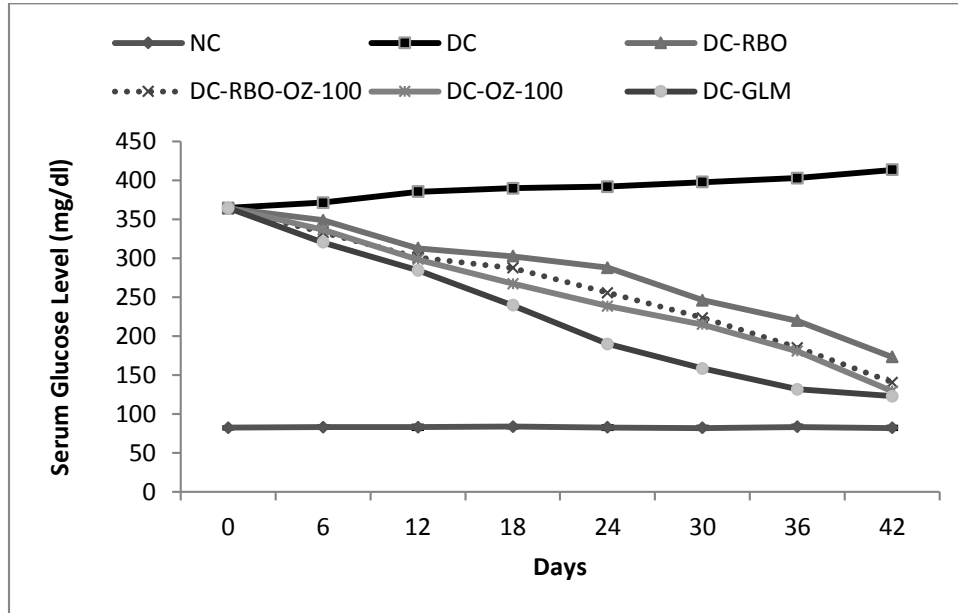


Fig. 1. Effect of Experimental Diets on Serum Glucose in STZ-NA Diabetic Rats
Values (Mean ± SEM) of 6 rats in each group

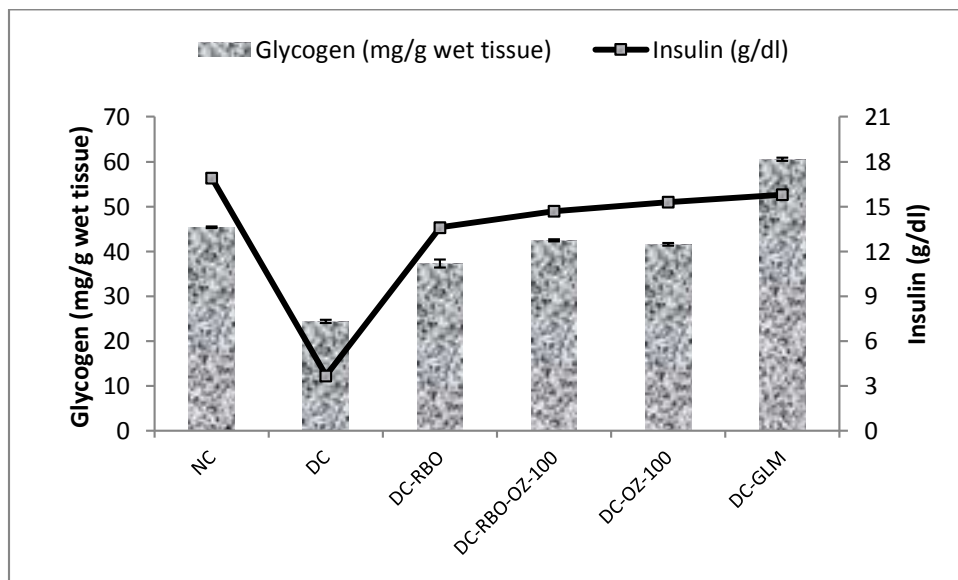


Fig. 2. Effect of Experimental Diets on Combined Changes in Plasma Insulin and Hepatic Glycogen in STZ-NA Diabetic Rats Values (Mean ± SEM) of 6 rats in each group

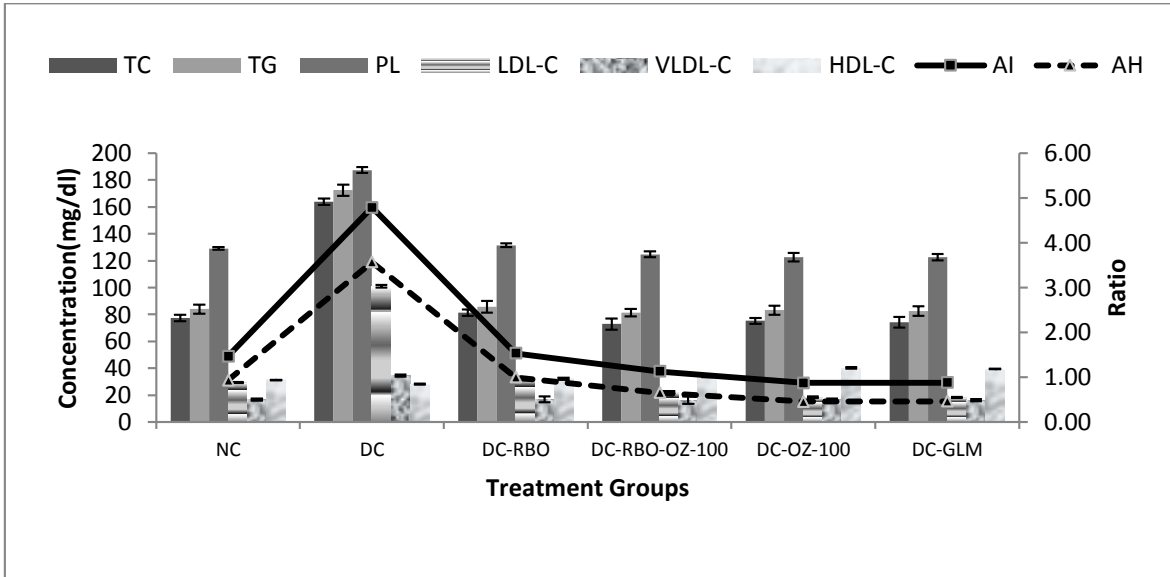


Fig. 3. Effect of Experimental Diets on Combined Changes in Serum Lipid- Lipoprotein fractions, Atherogenic Index and Antiarterial Hardness in STZ-NA Diabetic Rats Values (Mean± SEM) of 6 rats in each group

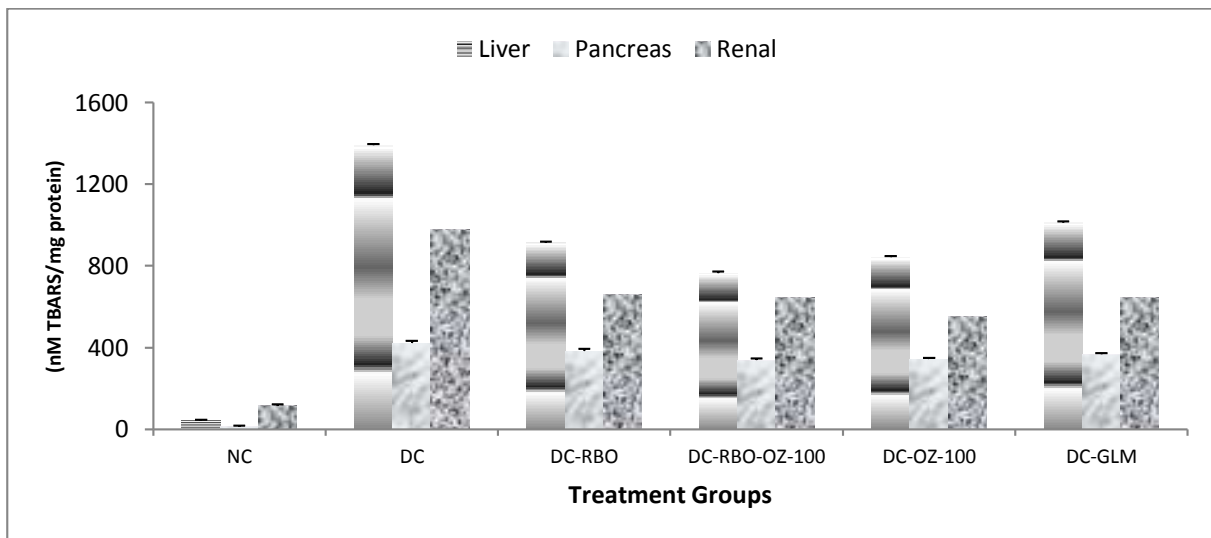


Fig. 4(A) Lipid Peroxidation Status (TBARS)

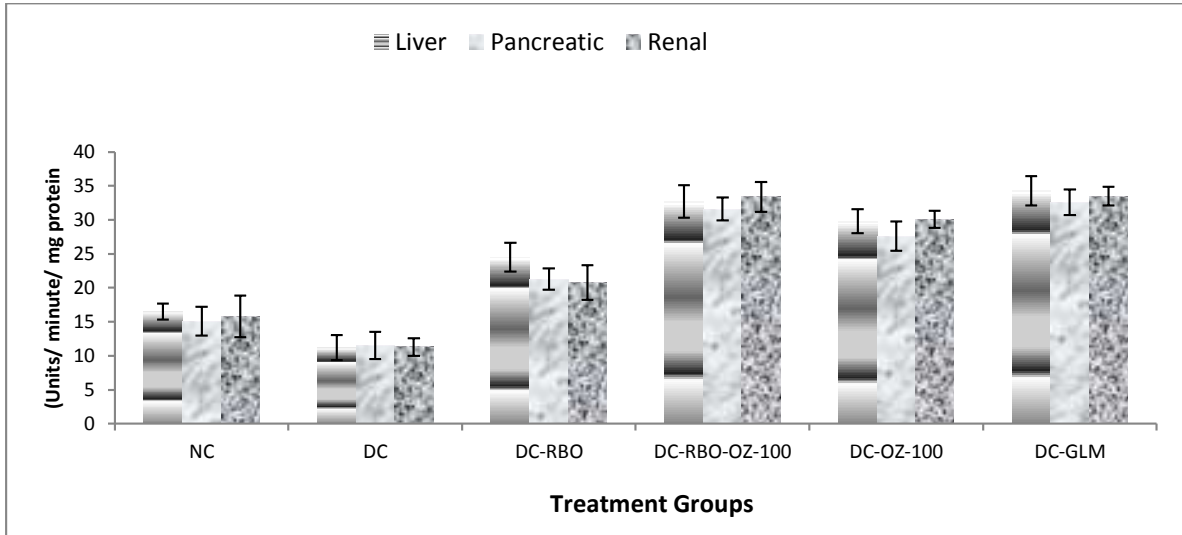


Fig. 4(B) Glutathione (GSH) levels

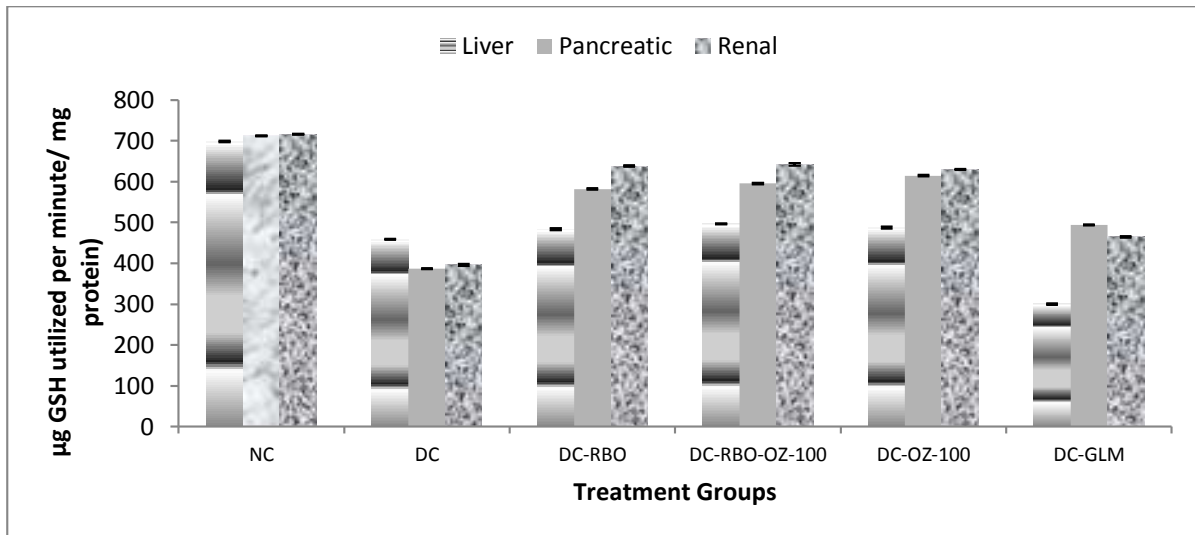


Fig. 4 (C) Glutathione Peroxidase (GSHPx) Activity

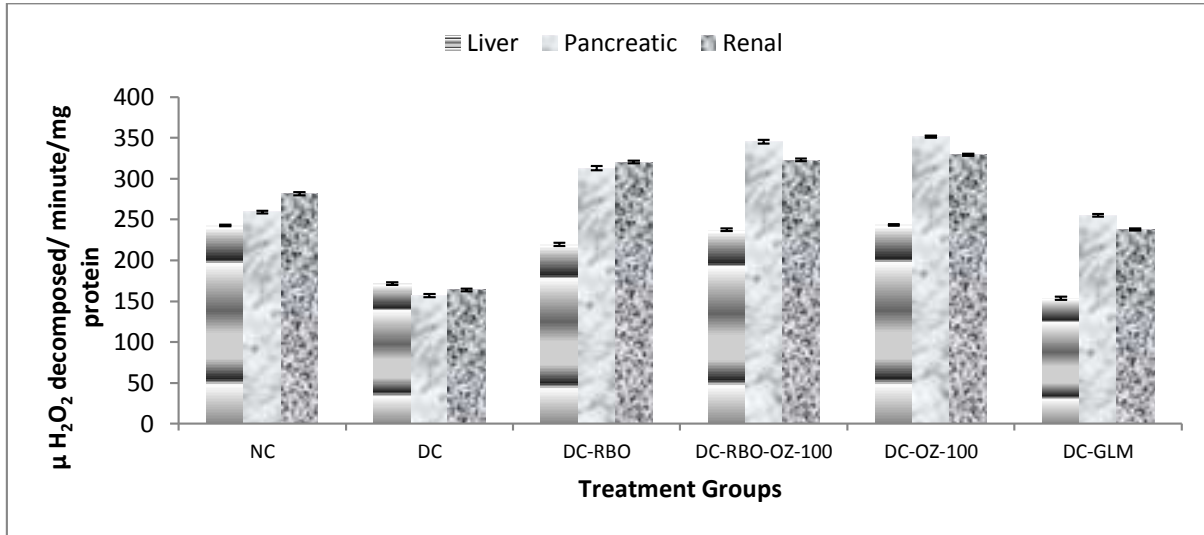


Fig 4 (D) Catalase (CAT) Activity

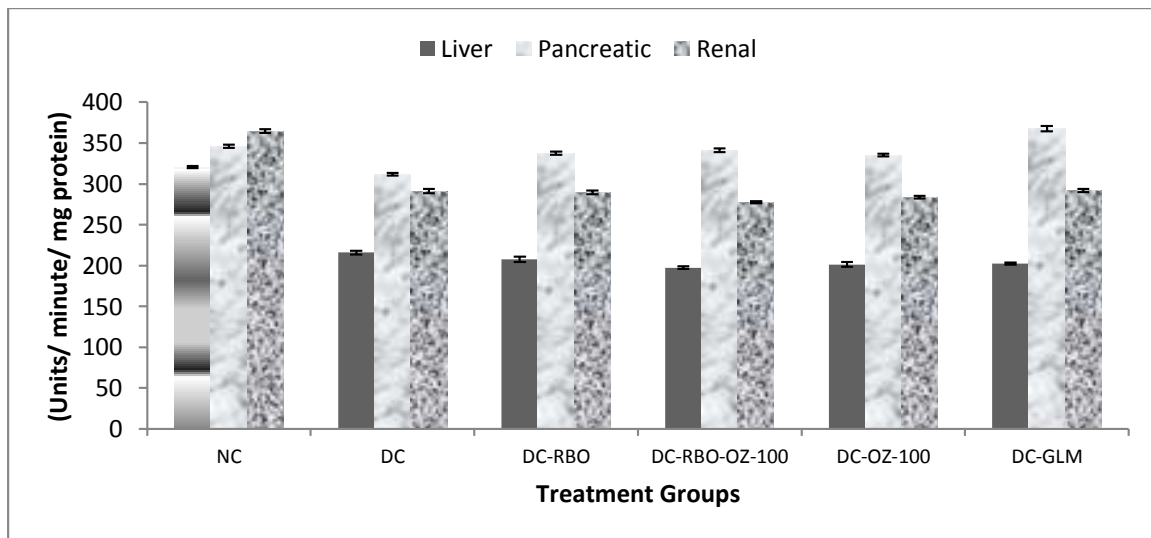


Fig. 4 (E) Superoxide Dismutase (SOD) Activity

Fig. 4. Effect of Experimental Diets on Oxidative Stress Biomarkers in STZ-NA Diabetic Rats

Values (Mean ± SEM) of 6 rats in each group