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RESEARCH ARTICLE

Protective effect of dietary Ginger extract alone or in combination with Rosiglitazone and Glimepiride on hepatotoxicity and oxidative stress in streptozotocin-induced diabetes in rats

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Abstract

The objective of this study was undertaken to evaluate the protective effect of Ginger extract either alone or in combination with Rosiglitazone and Glimepiride on serum Glucose, Triglycerides, Cholesterol ,AST, ALT, γ-GT, as well as liver Malondialdehyde, Catalase (CAT), Superoxide Dismutase (SOD), reduced Glutathione (GSH) and lysosomal enzymes ;Acid phospahtase (ACP), β-D-galactosidase (β-GAL) and N-acetyl-β-D-glucosaminidase (β-NAG), in streptozotocin-induced diabetic rats. The animals were divided into seven groups: control normal animals (CN), control untreated diabetic animals (CD) in which experimental hyperglycemia were induced by single intraperitoneal injection of streptozotocin (40mg/kg body mass). The other 5 diabetic groups were treated orally for 30 days with Rosiglitazone (D/Rosi) (0.8 mg/kg b.w), Glimepiride (D/Glim) (0.8 mg/kg of b.w), Ginger extract (D/G)(500 mg/kg of body mass), combination of Glimepiride and Ginger extract (D/Glim+G) and combination of Rosiglitazone and Ginger (D/Rosi+G) starting 24 hours after streptozotocin injection. The antioxidant effect of the Ginger extract was compared with Rosiglitazone and Glimepiride, well-known hypoglycaemic drugs. The diabetic rats exhibited lowered hepatic GSH content and CAT, SOD activities associated with elevated levels of hepatic MDA, liver functions enzymes and lysosomal enzymes as compared with normal rats. In contrast ginger treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress, liver functions enzymes, lysosomal enzymes and hepatic damage. Ginger extracts showed an encouraging hypoglycemic, hypolipaemic, as well as antioxidant properties and could be considered as a valuable candidate in the reversal of the complication of diabetes.

Introduction

Diabetes mellitus, a leading non-communicable disease with multiple etiologies, is considered as one of the five leading causes of death in the world (Zimmet, 1999]. The chronic hyperglycaemia of diabetes is associated with damage, dysfunction and failure of various organs, e.g. kidneys, retina, heart, liver, peripheral and central nervous system, over the long term (Fajans *et al*., 1997). Oxygen free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins [Maritim *et al*., 2003**]**. Some reports indicate that diabetic complications are associated with overproduction of free radicals and accumulation of lipid peroxidation by-products [Palanduz *et al*., 2001]. Enhanced oxidative stress has been well documented in both experimental and human diabetes mellitus [Baynes, 1991]. Lysosomal enzymes have generated much interest due to their role as indicators of oxidative stress. Lysosomal enzymes catalyze hydrolytic cleavage of glycosidic bonds of glycosaminoglycans, glycoproteins and glycolipids **(**Belfiore *et*

al., 1972]. Hence study of antioxidant and lysosomal enzymes is of great relevance during diabetes and also other stress related diseases.

Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the disease. An appropriate dose of streptozotocin was used to induce experimental diabetes. Streptozotocin selectively destroyed pancreatic *β*-cells, resulting in hypoinsulinemia (Szkudelski, 2001).

Rosiglitazone is an insulin-sensitizing oral agent in the thiazolidinedione class, which improves the sensitivity of tissues to insulin and reduces insulin resistance and is being used to treat patients with type-II diabetes mellitus (Pospisilova, 2001). The drug binds to peroxisome proliferator-activated receptor gamma in liver, muscle and adipose tissue. Insulin sensitivity, pancreatic beta cell functions and surrogate markers of cardiovascular risk factors are significantly improved by rosiglitazone (Wagstaff and Goa, 2002).

Glimepiride is a sulfonylurea used as the drug of choice in the treatment of type II diabetes. The hypoglycemic activity of Glimepiride relied on its ability to enhance insulin release and action (Bando and Yamada, 2001).

An increased reactive oxygen species and insufficient antioxidant activity are associated with diabetes mellitus, which is mainly responsible for diabetic pathogenesis. Natural products and their active principles, as sources for new drug discovery and treatment of diseases, have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. *Zingiber officinale Roscoe*, commonly known as Ginger, is one of the commonly used spices around the world **(**Ajith *et al*., 2007**).** Ginger contains active phenolic compounds that have antioxidant properties, hypolipidemic, antioxidant and hepatoprotective properties **(**Ahmed *et al*., 2008**).** Combination therapy often takes advantage of complementary effects of different agents. This medicinal herb is considered to be an excellent candidate for oral therapy as it is effective, non-toxic and without serious side effects. The chemical constituents of Ginger are gingerols, shogaols, some phenolic ketone derivatives, volatile oils, alkaloids, saponins and flavonoids. Ginger extracts (ethanolic, methanolic and chloroform) have antioxidative properties and scavenge superoxide anion and hydroxyl radicals (He *et al*., 1998**).**

In the present study, attempts have been made to reduce oxidative stress of diabetes by ginger, without compromising its antidiabetic effects, and also to explore whether this treatment can restore the altered antioxidant defense system in the liver of STZ-induced diabetic rats.

Materials and methods:

Drugs and Chemicals

STZ was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA, stored at 2–4 °C and protected from sunlight. Rosiglitazone maleate was purchased from Glaxo Smith Kline (GSK) Beecham Pharmaceuticals, Egypt; and Glimepiride was purchased from Hoechst AG, (Sanofi Aventis Company) Frankfurt am Main, Germany.

All other chemicals and reagents used in the study were of analytical grades and were obtained from standard commercial suppliers.

Animals

Adult male wistar rats, weighing 200-250 g were obtained from animal house of the National Organization for Drug Control & Research (NODCAR), Giza, Egypt. The rats were housed in clean polypropylene cages having 8 rats per cage and maintained under temperature controlled room (27 ± 2 ^{0}C) and exposed to a 12-h light/dark cycle. The rats were given standard pellets diet (Lipton rat feed, Ltd., and Pune) and water *ad libitum* throughout the experimental period. Experimental design and animal handling were done according to the guidelines of the Egyptian Society of Neuroscience and the Ethical Committee of the faculty of Pharmacy, Cairo University, for Animal Use.

Induction of Diabetes in Rats

Type II diabetes was induced in rats by a single intraperitoneal (i.p) injection of a freshly prepared solution of streptozotocin at a dose (40 mg/kg.bw) (Eskandari *et al.*, 2004**)**.Diabetes in rats was identified by measuring fasting blood glucose concentrations 48 h after injection of STZ. Rats with a blood glucose level above 200 mg dl^{-1} were selected for experiments.

Plant material collection

Ginger rhizomes ,was obtained from Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Egypt, and cleaned, air dried in shade and then grinded to fine powder before extraction. Such powdered samples were kept in dark bottles for further investigation. The aqueous ethanol extract of ginger was prepared according to the method described previously (Ajith *et al*., 2007). Dose equivalent to 500 mg (Abdel samad *et al*., 2006) of the crude extract per kg body weight was calculated and suspended in 1%, v/v Gum Acacia solution for the experiment.

Administration of Ginger extract, Glimepiride, and Rosiglitazone

The different drugs were dissolved in 1 % Gum Acacia solutions just before administration. Rosiglitazone, Glimepiride at a dose of 0.8 mg/kg body weight which is corresponding to the human max therapeutic dose, either alone or in combination with Ginger extract at a dose of 500 mg/kg respectively, and Ginger extract alone were administered to STZ-induced diabetic rats, by gavage technique for 30 days.

Experimental design

The rats were divided into 7 groups, each group consists of 8 rats—**Group I**: Normal Control; **Group II**: diabetic control untreated animals; **Group III**: diabetic animals given Rosiglitazone; **Group IV**: diabetic animals given combination of Rosiglitazone+Ginger; **Group V**: diabetic animals given Glimepiride; **Group VI**: diabetic animals given combination of Glimepiride+Ginger; **GroupVII**: diabetic animals given Ginger extract.

Sample collection

At the end of the experimental period, the animals were fasted overnight, and then blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at (3000 rpm, 20 min & 4 ^{0}C) for separation of serum. Then the animals were sacrificed by cervical decapitation (between 9:00 and 11:00 am) and the liver tissues were excised at 4 ^{0}C. The liver tissues were washed with ice-cold saline, and were rapidly removed, weighed and divided into three parts (liver homogenate, cytosolic fraction, lysosomal fraction), and then immersed in liquid nitrogen and immediately stored at -80 ^{0}C for further biochemical analysis. Liver homogenates were prepared according to the method described by (El-Demerdash *et al*., 2005) and used for the estimation of TBARS, reduced GSH, and CAT. Liver cytosolic fraction was used for estimation of SOD, and the liver lysosomal fraction was prepared according to the method of (Tanaka and Iizuka, 1968) and used for estimation of ACP, *β*-NAG and *β*-GAL.

Preparation of liver lysosomal fraction

Liver lysosomal fraction was prepared according to the method of (Tanaka and Iizuka , 1968)].1 gm of liver tissue was homogenized in 3 ml of 0.25 M sucrose buffer (pH 7.4). After homogenization the volume were adjusted to 6 ml with sucrose buffer. The homogenates were then centrifuged (820 xg, 15 min, 4 ^{0}C). The supernatant was separated and the sediment washed and recentrifuged under the same condition, then the supernatant was separated and added to the first supernatant. The whole lysosomal fractions were prepared by centrifuging the combined supernatant (14,000 xg, 15 min, 4 ^{0}C). The sediment was washed and resuspended in 0.25 M sucrose buffer and this step was repeated three times for isolating pure lysosomal fraction. After washing and purification, the sediment was resuspended in 0.25 M sucrose buffer to give 1 g liver weight per 1.25 ml sucrose buffer.

Biochemical assays

Estimation of Diabetic Markers, lipid profile and liver enzymes

Serum Glucose, Cholesterol, Triglycerides, Serum aminotransferases; (ALT) (AST) and (γ-GT) levels were determined using Spectrophotometric assay kits (***CinnaGen Inc, IRAN***).

Estimation of Oxidative stress markers and antioxidant enzymes

SOD activity was assayed in the cytosolic fraction by the method of **(**Marklund and Marklund**,** 1974) at 420 nm for 1 min on a UV-Vis Unicam spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the autooxidation of pyrogallol by 50%, which is equal to 1 U per milligram of protein. CAT activity was determined at room temperature by using **(**Aebi, 1984] and absorbance of the sample was measured at 240 nm for 1 min in a UV-Vis Unicam spectrophotometer.

The concentration of reduced GSH was measured as described by (Ellman, 1979**)** modified by (Ahmed *et al*., 1991). The extent of lipid peroxidation was estimated as the concentration of thiobarbituric acid reactive product TBARS by using the method of (Buege and Aust, 1978].The quantity of lipid peroxides is expressed as nmol TBARS equivalents/mg protein. All the enzyme activities were expressed per mg protein and the tissue protein was estimated according to the method of (Lowry *et al*., 1951) using bovine serum albumin (BSA) as a standard.

The activity of liver lysosomal enzymes (ACP, *β*-NAG, and *β*-GAL) was measured according to the method described by **(**Van Hoof and Hers, 1968**)** with slight modification by [Younan and Rosleff**,** 1974).

Statistical Analysis

All of the data are expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Duncan's post hoc test as appropriate using a computer-based fitting program (SPSS/17). Differences were considered to be statistically significant when $p < 0.05$.

Results

Plasma glucose levels

Table (1) showed that FPG levels of the diabetic untreated rats were markedly increased and amounted to 335% of the normal values. Treatment of the diabetic rats with either Rosi, Rosi+G, Glim, or Glim+G partially reversed the increase in FBG levels to reach to 141%, 136%, 152%, and 130% of the normal values, respectively, with no significant difference between the four treated groups. However, Ginger extract treatment to the STZ-diabetic rats decreased the elevated FBG levels of the diabetic control rats to values amounted to 249% of normal control values, but still significantly higher than the normal values and the other treatments.

Serum Total Cholesterol and Triglycerides Levels

Streptozotocin-induced diabetic rats showed significant increases in the levels of serum triglyceride and total cholesterol, registering increases of 206% and 195%, respectively, at the end of the experiment compared to the normal controls (CN).Treatment of STZ-diabetic rats with different drugs was accompanied by a significant reduction of serum triglycerides and total cholesterol levels as compared to STZ-CD rats. Finally and most importantly, combinations of Rosi+G and Glim+G were the only treatments regimen that succeeded to return back the values of the measured parameters near the normal control values. Ginger, taken alone, showed significant decrease in the serum total cholesterol and triglycerides levels observed in diabetic control rats.

Serum liver function enzymes, AST, ALT and γ-GT activities

Table (2) depicted the changes of serum enzymes activities. The diabetic control groups exhibited a significant elevation of serum enzymes activities (AST, ALT, and GGT) which reached to 351%, 340%, and 287% as compared with the normal control groups, respectively. Treatment of the diabetic rats with the other drugs except ginger extract group succeeded to lower the serum enzymes activities (AST, ALT, and GGT) to values near the normal controls. Addition of Ginger to the treatment regimens Rosi or Glim caused a marked decrease in serum AST, ALT, and GGT activities estimated to be about 175%, 173%, 157% and 143%, 156%, 157% of the normal controls, respectively. It should be pointed out that serum AST, ALT, and GGT activities were not significantly different between the diabetic groups treated with Rosi+G and Glim+G .Ginger supplementation to the STZ-diabetic rats caused slight decrease in the elevated serum AST, ALT, and GGT activities of the diabetic control rats to values amounted to 295%, 281% and 234%, of normal control values, respectively, but still significantly higher than the normal values.

Hepatic antioxidant markers Catalase and SOD activities, GSH levels and lipid peroxidation marker (MDA)

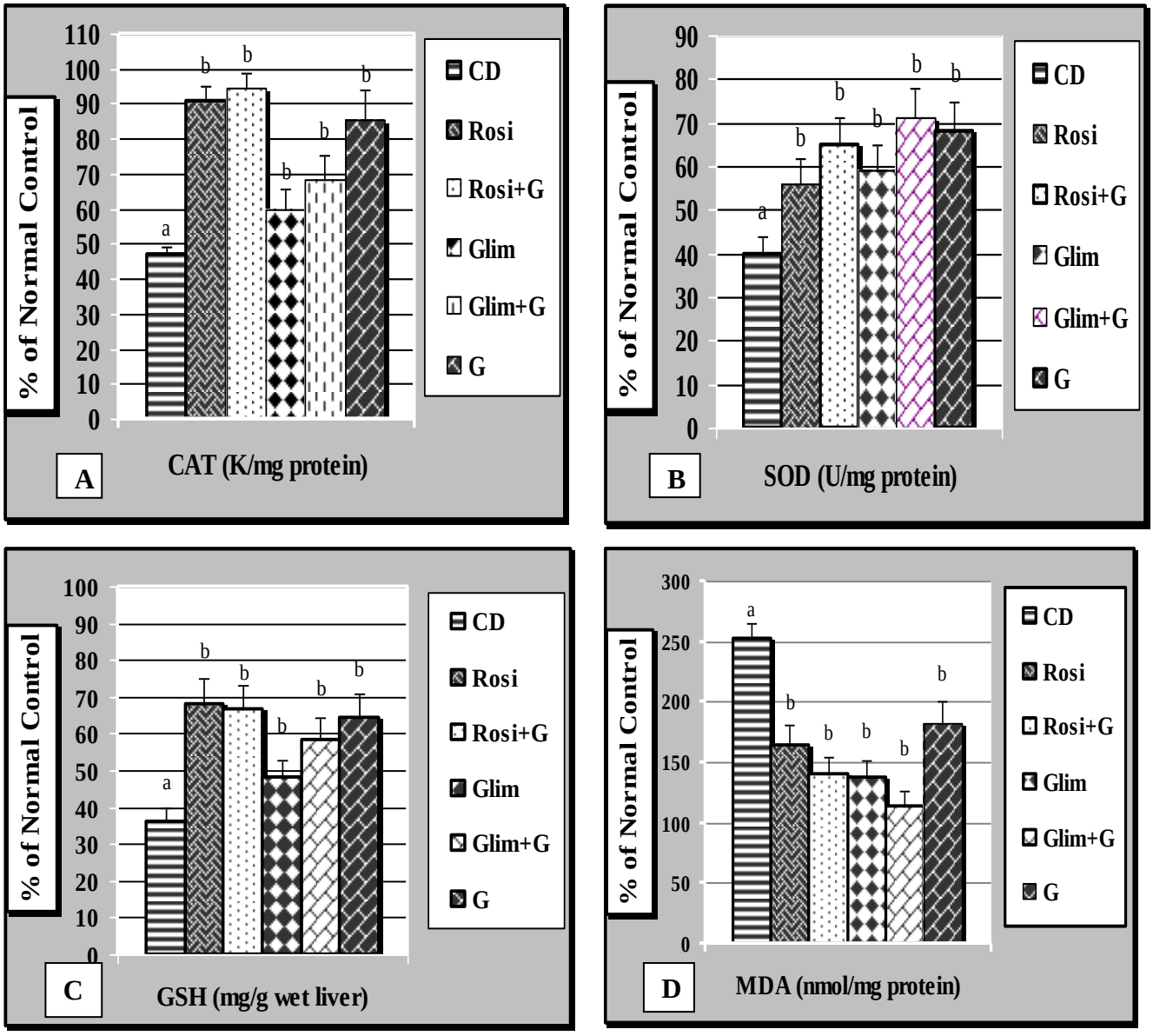
The results in Fig (1) revealed that the hepatic TBARS level of the STZ-induced diabetic rats were markedly increased and amounted to 252% of the normal control value. A significant decrease in the level of TBARS was observed in the diabetic groups treated with either Rosi, Rosi+G, Glim, or G as compared with the normal controls (165%, 140%, 138%, 182%, respectively, $p < 0.05$). However in these groups, the TBARS levels were still higher than the normal control group. On the other hand, the addition of ginger extract with Glim to the diabetic rats showed the lowest reduction of TBARS level as compared with the other groups.

The results in Fig (1) revealed that the hepatic level of GSH level and activities of SOD and CAT of the STZ-induced diabetic rats were markedly decreased. Treatments of diabetic rats with different drugs significantly reversed the decrease in the level of GSH, and SOD, CAT activities towards the normal control values, however, in these treated groups, the enzymatic activities were still significantly lower than the normal values. Treatment of diabetic rats with ginger , showed significant increases in SOD, CAT, activities and GSH level, and a decrease in MDA level, which reflects gradual restoration of the antioxidant enzyme systems to near-normal values.

Hepatic lysosomal enzymes activity

The results obtained in the present study as in table (3) showed a significant increase in hepatic lysosomal enzymes activities, ACP, *β*-NAG, and *β*-GAL, of the diabetic untreated rats which reached to 135%, 185%, 162%, respectively, as compared to normal control values. However, all treatments improved these hepatic lysosomal enzymes activities toward near normal control values with different extents. Moreover, it should be pointed out that the most effective treatment regimen is combination of Rosi+G, and Glim+G.

Fig (1) (A, B,C&D):Changes in hepatic activities of (A) catalase (CAT), (B) superoxide dismutase (SOD), and hepatic content of (C) reduced glutathione (GSH), (D) malondialdehyde (MDA) in rats treated with Rosiglitazone (Rosi), Glimepiride (Glim), Ginger (G) alone or combined with either Rosiglitazone (Rosi+G), or Glimepiride (Glim+G) in STZ-induced diabetic rats in comparison with their normal controls (CN).
The values presented as percentage of normal control ±S.E of eight rats in each group.
(a) Significantly different from the normal control group;
(b) Significantly different from the diabetic untreated control group;
P<0.05(using one way ANOVA followed by Duncan's post-hoc test).



Discussion

Diabetes is possibly the world's fastest growing metabolic disease, and as knowledge of the heterogeneity of this disorder increases, so does the need for more appropriate therapies (Bandyopadhyay, 2004**)**. Diabetes is a pathologic condition, resulting in severe metabolic imbalances and non-physiologic changes in many tissues, where oxidative stress plays an important role in the etiology [Baynes and Thorpe, 1997]. STZ causes diabetes by rapid depletion of beta cells of Islets of Langerhans which leads to reduction in the insulin release (Gupta *et al.,* 2004)**.** Hyperglycemia causes oxidative damage by generation of ROS (Mohamed *et al.,* 1999) leading to the development of diabetic complications (Nathan *et al*., 2005).

In the current study, we observed significant increase in blood glucose levels in diabetic rats **(**Table 1). This may be due to the destruction of pancreatic beta cells by STZ, reinforcing the fact that, STZ induces diabetes, probably through the generation of oxygen free radicals (Gupta *et al*., 2004]. The elevation of glucose in STZ-treated rats might be due to an oxidative stress produced in the pancreas due to a single strand break in pancreatic islets DNA (Yamamoto *et al*., 1981**).** Moreover, hyperglycemia of STZ-treated rats could be explained due to impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues which are primarily due to insulin resistance (Leibowitz, 1990**).**

Treatment of diabetic rats for 30 days with all treatments except Ginger extract reversed the increase in FBG to reach near of the normal values.

Rosiglitazone corrected the disturbed glucose homeostasis possibly by enhancing insulin sensitivity in liver, adipose tissue, and muscle, through increasing insulin receptor kinase activity, insulin receptor phosphorylation, and the number of insulin receptors or hepatic glucose metabolism (Balfour and Plosker, 1999**)**. Moreover, many of the glucoregulatory effects of thiazolidinediones (TZDs) are mediated via reduced systemic and tissue lipid availability **(**Balfour and Plosker, 1999**).** Another mechanism could be TZD–stimulating effect on the peroxisome proliferator-activated receptor (PPAR-γ), which increases glucose transporter 4 (GLUT-4) production and translocation and, consequently, migration of GLUT-4 to the cell surface, leading finally to the uptake of glucose (Malinowski and Bolesta, 2000**).**

On the other hand, the antihyperglycemic effect of Glimepiride is due to drug induced stimulation of glucose transport into the cells as proposed by (Bähr *et al*., 1995**)** or to potentiation of the insulin-stimulated glucose transport mediated possibly by protein kinase C, which stimulates intracellular translocation of GLUT-4 to the plasma membrane (Muller and Wied, 1993). A new aspect for the antihyperglycemic effect of glimepiride is that it directly binds to PPAR-γ and stimulates the transcriptional activity of this receptor **(**Fukuen *et al*., 2005). Moreover, Glimepiride's effect may be related to the secretion of *β*-cell insulin, thus elevating the level of insulin (Srinivasan *et al.,* 2005**).**

The main mechanism of the hypoglycemic activity of glimepiride is connected with the stimulation of receptor proteins of beta cells of the pancreatic islets and the stimulation of insulin secretion, which ultimately leads to a decreased blood glucose level (Lebovitz and Melander, 1997) **.**It has also been found that glimepiride may directly influence the process of glucose utilization by stimulating the activity of glycolysis, glycogenogenesis and lipogenesis pathways in liver, as well as inhibition of the gluconeogenesis path **(**Beck-Nielsen *et al*., 1998**).**

Studies have also showed that Glimepiride affects the translocation of glucose transporters GLUT1 and GLUT4 from the cytoplasmatic store to the cell membrane of skeletal muscle and liver cells (Muller and Wied, 1993)**.** It is also suggested that Glimepiride may act through a mechanism reducing the insulin-resistance of peripheral tissues and increasing the number of insulin receptors (Feinglos and Lebovitz, 1978).

Our results clearly showed that Ginger extract effectively lowers serum glucose levels in diabetic rats. However, it should be noted that serum glucose levels in ginger-treated diabetic rats did not reach normal levels at the used dosage. A similar result was reported by (Akhani *et al*., 2004) in their study on the effects of ginger juice in STZ-induced diabetic rats.

In addition**,** Ginger extract has shown effective glycaemic control properties in diabetic rats. The mechanisms underlying these actions are associated with the inhibition of key enzymes controlling carbohydrate metabolism and increased insulin release/sensitivity, resulting in enhanced glucose uptake in peripheral adipose and skeletal muscle tissues (Li *et al*., 2012**).** The prominent lipid lowering effects of Ginger also contribute to improving the insulin resistant condition. It was reported that, in diabetic rats, [6]-gingerol showed a protective effect on pancreatic *β*-cells and restored the plasma insulin level .This action of Ginger may involve interaction of gingerols and shogaol with 5-HT3 receptor ion channel complex by binding to a modulatory site distinct from the serotonin binding site (Chakraborty *et al*., 2012**)**.

The protective effect of Ginger against diabetic complications was found to be correlated with the phenolic contents of gingerols and shogaol in these extracts (Chakraborty *et al*., 2012) Ginger extract, with antioxidative characteristic,

since it can scavenge superoxide anion and hydroxyl radicals (Krishnakantha and Lokesh, 1993**),** ameliorated the effect produced by STZ-induced diabetic rats.

The administration of Ginger to STZ diabetic rats reduced blood glucose levels, in accordance with earlier reports [Shanmugam *et al*., 2010**)** which further strengthen the antidiabetogenic action of Ginger extract.

Lipids play an important role in cardiovascular disease, by modifying the composition, structure and stability of cell membranes. Altered lipid metabolism is considered to accelerate the development of atherosclerosis, a major risk factor in myocardial infarction (Slater and White**,** 1996**).** Hypertriglyceridemia and hypercholesterolemia have been previously reported to occur in diabetic rats (Tunali and Yanardag, 2006**)** and the significant increases observed in STZ diabetic rats in the present results were in accordance with these studies. We observed a marked improvement in plasma lipid profile by all treatments as compared to diabetic controls.

In the present study, total lipids were increased in serum of STZ-diabetic rats as compared with the normal ones at the end of the experiment. Our results are in accordance with the finding of (Lalhlenmawia *et al.*, 2007**)** who recorded a marked increase of total lipids in serum and liver of STZ-diabetic rats. Several investigators, however, recognized that insulin deficiency in STZ -diabetic animals brings about an enhanced breakdown of fat (Rawi *et al*., 1998**)** increase in mobilization of free fatty acids from the peripheral depots (Kumar *et al*., 2010] and consequence of the uninhibited actions of lipolytic hormones (glucagon and catecholamines) on the fat depots **(**Ravi *et al*., 2005**).** Excess fatty acids in the serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins **(**Bopanna *et al*., 1997**).**

In association with these reportedly diabetes-induced changes in carbohydrate metabolism, various studies have demonstrated a general elevation in almost plasma lipid fractions that often coexist with hyperglycemia (Mazhar *et al*., 2005**)**. Insulin can affect the adipocytes by inhibiting lipase enzyme and hence inhibits lipolysis and promoting storage of triglycerides in adipocytes (Zaahkouk, 2001**)**. Thus, insulin lack in diabetes enhances hydrolysis of triglycerides into diglycerides, unesterified fatty acids and free glycerol (Ebara *et al*., 1994**)**.These fatty acids may diffuse out of the cells or may be re-esterified into triglycerides for storage or secretion of VLDL. Because insulin is known to suppress VLDL secretion, the lack of this suppression by deficient insulin secretion in diabetes may lead to hypertriglyceridemia, which possibly can mediate the mechanism by which diabetes causes hyperlipidemia (Saudek and Eder, 1979**)**. As the concentration of triglycerides increases in the circulation, this increases the hydrolysis of triglycerides from VLDL, yielding LDL. These particles carry most of the cholesterol in the blood and are cleared mainly by the hepatic LDL receptor, which are increased by insulin **(**Hassan, 2007**).** Consequently, the clearance of LDL can be delayed resulting in hypercholesterolemia. In other words, the reduction in the rate of cholesterol removal from circulation appears responsible for hypercholesterolemia recognized in diabetic states **(**Hassan, 2007**).**

The administration of Rosiglitazone to STZ diabetic rat resulted in marked decrease of serum triglyceride, and cholesterol as compared to diabetic controls. These results could be attributed to the ability of Rosiglitazone to mediate its action on lipid profile via up-regulation of gene expression of both hormone-sensitive lipase and lipoprotein lipase, thus increasing insulin responsiveness in lipolysis and lipogenesis (Teruel *et al*., 2005**).** Besides, Rosiglitazone induces fatty acid transport protein and fatty-acid- binding proteins, possibly through its action on PPAR-γ1, leading subsequently to utilization of glucose, instead of fatty acid, as the source of energy (Chakrabarti *et al*., 2002**)**. In addition, the drug allows the re-esterification of fatty acids, thus lowering fatty acids' release into the plasma (Teruel *et al*., 2005**)**, and decreases HMG-CoA reductase activity, leading to a hypocholestrolemic effect (Guo and Zhou, 2004**)**.

The antilipidemic action of Glimepiride may reside in their ability to stimulate insulin secretion and action (Yassin and Mwafy, 2007**)**. On the other hand, improvement of the lipogenic profile is in agreement with **(**Greenfield *et al*., 1982**) ,** who pointed to a link between poor glycemic control in patients with type 2 DM and elevations in serum levels of TGs and that sulfonylurea-induced glycemic control is responsible for lipid profile improvement (Kasim *et al.*, 1986**)**. On the contrary, (Monnier *et al.*, 1995) attributed the drug's favorable effects on lipid metabolism to the increased production of LDL-C receptor by insulin, whose secretion is stimulated by Glimepiride therapy. Glimepiride-induced hypocholesterolemia is in harmony with previous findings [Santos *et al*., 1995; Kasim *et al.*, 1986). Despite the insulin stimulatory influence on the endogenous cholesterol synthesis from acetyl-CoA, this hormone tends to decrease the LDL-C concentrations through two effects: first, by diminution of the apo-B VLDL synthesis and, second, by the increase of LDL-C catabolism, demonstrating favorable effects of Glimepiride on lipid metabolism in animals with type 2 DM by reducing plasma TC and LDL-C.

Table 1 - Effects of treatment on FBG, Cholesterol and Triglycerides

Effects of Rosiglitazone (Rosi), Ginger (G),Glimepiride (Glim), and combination of Rosiglitazone with Ginger (Rosi+G), and combination of Glimepiride with Ginger (Glim+G) on serum blood Glucose, Cholesterol, and Triglycerides in STZ-induced diabetic rats in comparison with normal controls (CN).

Groups / *Parameters*	CN	CD	D/Rosi	D/Rosi+G	D/Glim	D/Glim+G	D/G
Glucose(mg/dl)	**89.47± 5.3**	**299.74[a] ±14.8**	**126.01[b] ±5.82**	**121.73[b] ±6.04**	**135.96[b] ±7.34**	**116.10[b] ±5.11**	**222.80[b] ±11.76**
Cholesterol (mg/dl)	**101.44± 6.22**	**198.22[a] ±8.16**	**140.89[b] ±4.77**	**121.78[b] ±5.14**	**127.37[b] ±4.42**	**118.79[b] ±4.83**	**147.93[b] ±5.93**
Triglycerides (mg/dl)	**74.05±4.76**	**152.53[a] ±6.92**	**117.73[b] ±4.85**	**99.99[b] ±5.55**	**132.16[b] ±4.77**	**106.44[b] ±5.18**	**130.72[b] ±5.93**

Values are means ±SEM of 8 rats.

(a) Significantly different from normal control group;

(b) Significantly different from diabetic untreated control group;

P<0.05(using one way ANOVA followed by Duncan's post-hoc test).

Table (2) -Effects of treatment on activities of liver enzymes

Effects of Rosiglitazone (Rosi), Ginger (G),Glimepiride (Glim), and combination of Rosiglitazone with Ginger (Rosi+G), and combination of Glimepiride with Ginger (Glim+G) on serum activities of AST (U/L), ALT (U/L), GGT (U/L) in STZ-induced diabetic rats in comparison with normal controls (CN).

Groups / *Parameters*	CN	CD	D/Rosi	D/Rosi+G	D/Glim	D/Glim+G	D/G
AST (U/L)	**38.02±2.65**	**133.40[a] ±7.04**	**78.32[b]±4.99**	**66.47[b]±4.24**	**72.61[b]± 3.26**	**54.19[b]± 2.56**	**112.16[b]±5.31**
ALT (U/L)	**40.13±3.06**	**136.50[a] ±5.13**	**85.91[b]±3.57**	**69.43[b]±3.62**	**80.05[b]± 3.77**	**62.61[b]± 4.18**	**112.70[b]±5.48**
GGT (U/L)	**43.70±4.19**	**125.56[a] ±5.31**	**74.49[b]±3.54**	**68.77[b]±2.94**	**75.80[b]± 3.79**	**68.51[b]± 3.04**	**102.33[b]±5.46**

Values are means ±SEM of 8 rats.

(a) Significantly different from normal control group;

(b) Significantly different from diabetic control untreated group;

p<0.05, (using one way ANOVA followed by Duncan's post-hoc test).

Table 3: Effects of treatment on activities of lysosomal enzymes.

Effects of Rosiglitazone (Rosi), Ginger (G) , Glimepiride (Glim) ,and combination of Rosiglitazone with Ginger (Rosi+G), and combination of Glimepiride with Ginger (Glim+G) on lysosomal levels of enzymes ACP (nmol/ml/hr), *β*-NAG (nmol/ml/hr) and *β*-GAL (nmol/ml/hr) in STZ-induced diabetic rats in comparison with normal controls (CN).

Groups / *Parameters*	CN	CD	D/Rosi	D/Rosi+G	D/Glim	D/Glim+G	D/G
ACP (nmol/ml/hr)	**1463.31±55.06**	**1968.52[a]±61.33**	**1556.23[b]±47.38**	**1541.84[b]±46.80**	**1659.79[b]±68.58**	**1530.08[b]±62.40**	**1565.00[b]±48.25**
***β*-NAG (nmol/ml/hr)**	**320.90±17.25**	**594.58[a]±24.02**	**428.11[b]±25.75**	**378.17[b]±22.11**	**447.24[b]±20.79**	**382.85[b]±15.88**	**419.47[b]±29.67**
***β*-GAL (nmol/ml/hr)**	**376.30±28.15**	**608.97[a]±19.39**	**435.14[b]±23.98**	**401.72[b]±26.57**	**454.64[b]±34.65**	**402.88[b]±23.39**	**422.95[b]±25.45**

Values are means ±SEM of 8 rats.

(a) Significantly different from normal control group;

(b) Significantly different from diabetic untreated control group;

$p<0.05$, (using one way ANOVA followed by Duncan's post-hoc test).

In the current study, the activities of liver function enzymes, namely, AST, ALT, and GGT, were increased significantly in STZ-diabetic untreated control rats (Table 2), as compared to normal control values, which are concomitant with the (Derosa *et al*., 2007**)** .These findings have further confirmed by [Hickman *et al*., 2008**;**Rawi *et al*., 1998**).** Eskander *et al*., (1995**)** found that liver was necrotized in diabetic rats. This elevation in enzymatic activities was attributed to their greater need for gluconeogenic substrates (Tanaka *et al*., 1988**)** and may be also due to the destructive changes in the hepatic cells as a result of toxemia **(**Kim *et al*., 2006; Rawi, 1995). On the other hand; other investigators have postulated that diabetes could induce defects in sarcolemmal enzymatic activities (Micheal *et al*., 1985**)** which lead finally to such effects.

Supporting our findings, it has been found that hyperglycemia (30 days) resulted in hepatolysis reflected by increased blood plasma aminotransferases as one of the consequences of diabetic complication **(**Mansour *et al*., 2002**)**. The increment of such markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream. The release of cellular enzymes reflects induction of intracellular stress, accompanied by non-specific alterations in the structural and functional characteristics of liver cell membranes altering their integrity and permeability (Ebenezar *et al.*, 2003).

In diabetes mellitus, increased hepatic glucose output may be derived either from glycogenolysis or from gluconeogensis or both (Rawi *et al*., 1998**)**. This can explain the marked increase of the gluconeogenic serum enzymes; Alanine transaminase (ALT), Aspartate transaminase (AST) and gamma glutamyl transferase (GGT); compared to those of the nondiabetic ones.

Our study demonstrated that, the administration of Rosi, Glim and Ginger extract resulted in the attenuation of liver injury induced by STZ treatment as indicated by the activities of ALT, AST and GGT. These results are in accordance with those of (Rawi *et al*., 1998), who found that the decrease of transaminases activities with treatment may be attributed to improved liver function with the return of gluconeogensis towards its normal rate. However, treatment of Ginger extract alone does not succeed to return back the serum enzymes levels to normal as compared to other groups.

Oxidative stress is known to play a pivotal role in development of diabetes. An imbalance of oxidant/antioxidant in favour of oxidants contributes to the pathogenesis of diabetes (Jin *et al*., 2008**)**. Several reports have shown the alterations in the anti-oxidant enzymes during diabetic condition (Preet *et al*., 2005**)**.The antioxidative defense system enzymes like SOD and CAT showed lower activities in diabetic untreated control rats.

In the current study, hepatic MDA levels were found to be significantly increased in diabetic rats associated with marked depletion of GSH levels as compared to normal controls. Depletion of hepatic GSH levels may be due to its enhanced oxidation, consumption in the detoxification of highly reactive peroxides, and its use in glutathione peroxidase reaction (Ugochukwu *et al.,* 2004**)**. GSH depletion could be also attributed to its decreased synthesis due to glycation of γ-glutamylcysteine synthetase or increased degradation of GSH under diabetic conditions [Altan *et al.,* 1994). Moreover, the accelerated polyol pathway [Jung *et al*., 2005) and the combined loss of hepatic glucose-6-phosphate dehydrogenase (G6PDH) [Sobngwi *et al*., 2005**)** and glutathione reductase (GR) activities under hyperglycemic conditions substantially impair the regeneration of GSH. In vitro studies have shown previously that GR is inactivated by glycation and is susceptible to oxidative damage [Barker *et al*., 1996**)**. We observed that the activity of hepatic SOD was significantly decreased in the diabetic untreated group that could be attributed to oxidative stress-induced inactivation, decreased protein expression, or as a result of non-enzymatic glycation of specific lysine residues in the enzyme (Siddiqui *et al*., 2005**).**

In our study, the activities of hepatic SOD and CAT decreased in diabetes group as reported earlier [Zhang *et al*., 2010**)** which could be due to inactivation caused by STZ-generated ROS. The decreased activities of SOD and CAT may be a response to increased production of H_2O_2 and $O_2^{-\circ}$ by the auto-oxidation of the excess of glucose and non-enzymatic glycation of proteins [Argano *et al.*, 1997)**. (**Pigeolet *et al*., 1990**)** have reported that the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. The decreased activity of SOD and CAT could also be due to their decreased protein expression levels in the diabetic condition, as recently reported in liver [Sindhu *et al.,* 2004**).**

Treatment of diabetes with Rosi, Glim, and Ginger had reversed the activities of these enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased LPO. Administration of Ginger to diabetic rats increased the activities of SOD and CAT and may offer protection to cells against oxidative stress by scavenging free radicals generated during diabetic condition (Mahesh *et al*., 2005). This may be due to the presence of many anti-oxidant compounds like gingerols, shogaols, phenolic and ketone derivatives, volatile oils, and flavonoids in ginger. These anti-oxidant compounds may modulate the anti-oxidant enzymes in diabetic rats [Manju and Nalini, 2005).].

We found a marked increase in hepatic MDA levels in diabetic rats compared to normal controls, indicating an increase in lipid peroxidation and a state of oxidative stress. Elevated MDA concentration associated with low GSH

content is the expected reflection of the imbalance between peroxidation and antioxidative processes in the diabetic rat livers that in turn caused oxidative stress. In this work, ginger extract showed the best antioxidant effect and succeeded to normalize liver antioxidants parameters.

In this investigation, the concentrations of MDA in liver were decreased after treating diabetic rats with either Rosi , Glim or Ginger indicating the inactivation of LPO reactions and the decreased free radical generation. Studies have shown that there is a close relationship between the increase of free radicals, blood glucose and lipid peroxidation (LPO) in the progress of diabetes **(**Reddy *et al.*, 2005**)**. Diabetics usually exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes free radicals generation (Kamalakannan and Prince, 2006).

Moreover, in diabetic rats treated with Ginger, the activities of superoxide dismutase (SOD), catalase (CAT) increased in liver homogenate, which resulted in an increment of glutathione (GSH) level and a decrease of malondialdehyde (MDA) level. The protective effect of ginger was associated with decreased oxidative stress **(**Shanmugam *et al*., 2011). It was reported that zingerone, a metabolite from ginger, inhibited lipid peroxidation in rat liver microsomes (Reddy and Lokesh, 1992) and reduced the formation of advanced glycation end products (AGE) (Saraswat *et al*., 2009**)**.

Our findings revealed that simultaneous treatment for 30 days with Rosi, Rosi+G, Glim, Glim+G, and ginger extract remarkably prevent the oxidative stress in STZ-diabetic rats by virtue of their ability to restore hepatic lipid peroxidation levels and up regulation of detoxifying enzymes such as SOD, CAT and also the reduced glutathione (GSH) level.

Our results revealed that sustained hyperglycemia caused significant increase in leakage of the three lysosomal enzymes viz , N-acetyl-β-D-glucosaminidase (β-NAG), β-D-galactosidase (β-GAL) and acid phosphatase (ACP) in liver homogenate. Treatment with Rosi, Rosi+G , Glim, Glim+G, and ginger extract significantly attenuated the leakage of the three lysosomal enzymes.

Lysosomes are membrane-bound organelles with an acidic internal milieu containing hydrolytic enzymes for degradation of proteins, lipids, nucleic acids and saccharides. The lysosomal compartment has multiple functions and it may be indicator of adaptative abilities **(**HICKS, 1995**)**. It is well known that Lysosomes are especially sensitive to oxidative stress **(**Li *et al.*, 1998**)**. It was elucidated that hydroxyl radicals destabilize the lysosomal membranes and thereby cause leakage of lysosomal enzymes to the cytosol with ensuing cellular degeneration or even death (Öllinger, 2000**)**. The studies conducted by (Kołątaj *et al*., 1998) and Lombardo *et al.*, 1996) indicate, that the lysosomal system constitutes the defense lines of the cells against stressors and it responds to disturbances in cell homeostasis.

The major liver lysosomal markers are: Acid phosphatase (ACP), β-D-galactosidase (*β*-GAL) and N-acetyl-β-D-glucosaminidase (*β*-NAG) (Sheeler and Bianchi, 1987). Changes have been observed in the lysosomal enzymes activities in serum and tissues of both experimental animals and human diabetics (Gupta *et al*., 1999). Lysosomal enzymes are considered as markers of diabetic complications and also other stress related diseases **(**Kiffin *et al*., 2004**)**. Many studies have suggested direct connection between the lysosomal apparatus and insulin controlled metabolic pathways and a potential role for lysosomal enzymes as indicators of the metabolic complications during diabetes (Burlina *et al*., 1987).

Oxidative stress, a result of sustained hyperglycemia is one of the factors contributing to diabetic complications. Exposure of cells to high glucose generates glycated proteins, advanced glycation end products (AGE) and protein aggregates (Brownlee, 2005)**.** Advanced glycation end products (AGEs) formed by non-enzymatic glycation of reducing sugars such as glucose with amino groups of proteins/lipids/nucleic acids generate excessive amount of free radicals which in turn increases oxidative stress (Giacco and Brownlee, 2010) . The presence of modified proteins in the lysosomes may affect enzyme function directly, and/or lysosomal properties that result in changes in protein level and enzyme activities. Thus modified proteins, with or without concurrent oxidative stress, can increase the fragility of lysosomal membranes (Patschan and Goligorsky, 2008), and modulate intra-lysosomal pH **(**Ishibashi, 2006) which, in turn, may alter lysosomal enzyme activity as these enzymes typically require acidic pHs (Hideshima *et al.*, 2005).

Damage of the lysosomal membrane often results in cytosolic leakage of potent hydrolases which could cause intracellular havoc (Roberta *et al*., 2006). One of the factors responsible for damage could be due to oxidative stress which occurs during diabetes due to various reasons reported of which AGEs formation is one of the contributing factors (Kiffin *et al*., 2004**)**. Direct damage of the lysosomal membrane by reactive oxygen species during oxidative stress has been extensively reported (Kiffin *et al*., 2004**]**.

Abdel Gawad *et al*., (2005**)** assumed that such changes in marker lysosomal enzymes activities could be attributed to the variability in lysosomal membrane labilization, which affects the outward leakage of these enzymes. Lysosomal enzymes are known to be involved in cell death and tissue damage during diabetes (Fushimi *et al*., 1974].

Changes in oxidative stress biomarkers like CAT, SOD, lysosomal enzymes, etc, during diabetes are well documented. Antioxidant and lysosomal enzymes are important biomarkers for diabetes and, therapies targeting these enzymes would greatly ameliorate the complications of diabetes. Antioxidants indirectly prevent the leakage of lysosomal enzymes into cytosol and ameliorate the altered lysosomal enzyme activities during diabetes.

Conclusion

The present study demonstrated that ethanolic ginger extract has significant potential in the treatment of diabetes complications. It had significant hepatoprotective effects against diabetes-induced oxidative stress by augmenting host antioxidant defense mechanisms. Further study should be conducted to investigate the active component(s) of ginger responsible for the observed beneficial effects in the diabetic condition. We recommend administration of ginger extract as adjuvant with anti-diabetic drugs.

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