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Research Paper

Ameliorative effects of glycyrrhizin on streptozotocin-induced diabetes in rats

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Abstract

Objectives Glycyrrhizin is the main water-soluble constituent of the root of liquorice (*Glycyrrhiza glabra*). The study investigates the effect of glycyrrhizin on streptozotocin (STZ)-induced diabetic changes and associated oxidative stress, including haemoglobin-induced free iron-mediated oxidative reactions.

Methods Male Wistar rats were grouped as normal control, STZ-induced diabetic control, normal treated with glycyrrhizin, diabetic treated with glycyrrhizin and diabetic treated with a standard anti-hyperglycaemic drug, glibenclamide. Different parameters were studied in blood and tissue samples of the rats.

Key findings Glycyrrhizin treatment improved significantly the diabetogenic effects of STZ, namely enhanced blood glucose level, glucose intolerant behaviour, decreased serum insulin level including pancreatic islet cell numbers, increased glycohaemoglobin level and enhanced levels of cholesterol and triglyceride. The treatment significantly reduced diabetes-induced abnormalities of pancreas and kidney tissues. Oxidative stress parameters, namely, serum superoxide dismutase, catalase, malondialdehyde and fructosamine in diabetic rats were reverted to respective normal values after glycyrrhizin administration. Free iron in haemoglobin, iron-mediated free radical reactions and carbonyl formation in haemoglobin were pronounced in diabetes, and were counteracted by glycyrrhizin. Effects of glycyrrhizin and glibenclamide treatments appeared comparable.

Conclusion Glycyrrhizin is quite effective against hyperglycaemia, hyperlipidaemia and associated oxidative stress, and may be a potential therapeutic agent for diabetes treatment.

Keywords diabetes mellitus; free iron; glycyrrhizin; haemoglobin; oxidative stress; streptozotocin

Introduction

Diabetes mellitus is characterized by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action. The uncontrolled disease may lead to serious complications namely, retinopathy, nephropathy, neuropathy, cardiovascular disease, etc.[1] Diet restrictions, exercise, oral anti-hyperglycaemic medicines and insulin are the main modes of treatment for diabetes. A lot of synthetic drugs have been used in the treatment of diabetes mellitus but many of them have side effects and develop drug resistance. In recent years, major research has been going on to find appropriate antidiabetic agents from herbal sources such as *Allium cepa*,[2] *Pterocarpus marsupium*,[3] *Gymnema sylvestre*,[4] etc.

Liquorice (*Glycyrrhiza glabra* L.) is a widely used medicinal plant,[5,6] and has been used for ages in herbal therapy in India for curing inflammatory responses and bacterial and viral diseases. Glycyrrhizin is the main water-soluble constituent of liquorice root. On hydrolysis, it releases two molecules of D-glucuronic acid and the aglycone, glycyrrhetinic acid. Both glycyrrhizin and 18β-glycyrrhetinic acid have been shown to possess several beneficial pharmacological actions, including antiviral activity,[7] anti-hepatotoxic activity[8] and protection against autoimmune disorders[9].

The anti-hyperglycaemic effect of glycyrrhizin in genetically diabetic KK-A^y mice has been reported by Takii *et al*.[10] Ko *et al*.[11] have reported that glycyrrhetinic acid, the aglycone of glycyrrhizin, stimulates glucose-induced insulin secretion in isolated pancreatic islets. In a recent study, Kalaiarasi and Pugalendi[12] have shown that glycyrrhetinic acid treatment enhances plasma insulin level and reduces the levels of gluconeogenic enzymes in

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liver and kidney tissues of streptozotocin (STZ)-induced diabetic rats. The aim of the present study is to determine the effects of glycyrrhizin on STZ-induced diabetic changes such as hyperglycaemia, hyperlipidaemia and histological abnormalities of kidney and pancreas and some oxidative stress parameters.

Materials and Methods

Chemicals

Glycyrrhizin (monoammonium salt), STZ, Sephadex G-100, arachidonic acid, 2,4-dinitrophenylhydrazine, thiobarbituric acid (TBA), malondialdehyde, hydroxylamine hydrochloride, ferrozine (monosodium salt), pyrogallol, nitroblue tetrazolium, 1-deoxy-1-morpholino-D-fructose (DOMF), ethidium bromide, bovine serum albumin (BSA), hematoxylin, eosin and phloxine B were purchased from Sigma Chemical Company (St Louis, USA). Glibenclamide was purchased from Prudence Pharma Chemicals (Ankaleshwar, India). Rat insulin ELISA kit and glycohaemoglobin kit were purchased from DRG Diagnostic (Frauenbergstr, Germany) and Eagle Diagnostic (Texas, USA), respectively. Glucose, cholesterol and triglyceride estimation kits were purchased from Span Diagnostics Ltd (Mumbai, India).

Maintenance of animals

For animal experiments, prior approval was obtained from the Institutional Animal Ethics Committee, and the experiments were carried out in accordance with internationally accepted norms, monitored by the committee. Male Wistar rats, 100–120 g, were maintained at 24–26°C, 60–80% relative humidity and on a 12-h light–dark cycle and were fed a standard rat chow and allowed free access to water.

Experimental design

The rats were maintained into two groups – normoglycaemic and diabetic. Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg body weight) in 0.01 M citrate buffer, pH 4.5. The normoglycaemic rats received an equal volume of the buffer. The glucose concentration in blood taken from the tail vein was determined by using the glucose oxidase/peroxidase method.[13] About two weeks after the STZ injection, stable fasting blood glucose levels at ≥200 mg/dl were considered diabetic and treatments were started.

Diabetic rats were divided into three groups:

Group DC – diabetic control rats were administered with 50 mM phosphate buffer saline, pH 7.4.

Group DT – diabetic rats treated with glycyrrhizin. Glycyrrhizin, dissolved in PBS, was administered by one intraperitoneal injection at a dose of 100 mg/kg body weight.

Group DTG – diabetic rats treated with glibenclamide. Glibenclamide was fed along with diet at a dose of 8 mg/kg body weight every day for five weeks.

Normoglycaemic rats were divided into two groups:

Group NC – normal control rats received the placebo.

Group NT – normal rats treated with glycyrrhizin as described in Group DT.

Each group was composed of eight rats.

Collection of serum, haemoglobin and tissue samples

Blood was collected from the tail lateral vein without and with heparin for estimation of enzymes and other parameters, respectively. For estimation of serum insulin level, blood samples were drawn from the retro-orbital plexus using heparinized glass capillary tubes after the rat was properly anaesthetized. Serum was separated from blood samples by centrifugation at 1600*g* for 15 min at 4°C. Haemoglobin was isolated and purified from red blood cells (RBC) by using Sephadex G-100 column chromatography,[14] and its concentration was measured from the Soret absorbance using extinction coefficient, ε_{415nm} as 125/mM/cm (monomer basis). After five weeks of glycyrrhizin treatment, different groups of rats were sacrificed by cervical dislocation for collection of pancreas and kidney. The organs were dissected out and small pieces of tissues (2–3 mm thick) were collected for histological examination.

Biochemical estimations

For the intraperitoneal glucose tolerance test (IPGTT), rats fasted overnight were injected intraperitoneally with a sterile solution of 20% glucose at a dose of 2 g/kg body weight. Tail blood was collected before (0 min) and 30, 60, 90 and 120 min after glucose administration and glucose was estimated.

Glycohaemoglobin in whole blood and insulin level in serum were measured using commercially available kits following the manufacturer's direction.

Cholesterol and triglyceride levels in serum were estimated by using estimation kits based on the methods of Wybenga *et al*.[15] and McGowan *et al*.,[16] respectively.

Superoxide dismutase (SOD) and catalase activity was assayed according to the methods of Marklund and Marklund[17] and Aebi,[18] respectively, in serum samples of rats fasted overnight. Serum protein was measured following the method of Lowry *et al*.[19] using BSA standard.

The serum malondialdehyde level was measured from TBARS formation following the method of Yagi.[20] Serum fructosamine (Amadori product) was measured by the nitroblue tetrazolium reduction assay according to Johnson *et al*.[21] The formazan derivative formed was estimated from a standard curve of DOMF.

Free iron in haemoglobin was measured according to the method of Panter,[22] and was calculated from a standard curve using a standard iron solution. The carbonyl content in haemoglobin was measured using 2,4-dinitrophenylhydrazine according to the method of Levine *et al*.[23] and the product formed was calculated using molar absorption coefficient 22 000/M/cm at 370 nm.

Haemoglobin-mediated DNA (plasmid) breakdown was estimated essentially following a method described previously.[24] Different forms of DNA were separated by agarose (1%) gel electrophoresis and visualized by ethidium bromide staining.

Histological studies

After five weeks of glycyrrhizin treatment, different groups of rats were sacrificed for collection of pancreas and kidney.

Pancreas and kidney tissues were fixed in Boiun's fluid and 10% formalin, respectively, and were dehydrated in a series of ethanol solutions (70, 80, 90, 100%, v/v). The tissue samples were processed by using routine paraffin techniques and embedded in paraffin wax. The samples were then sectioned (5 μm) and pancreatic sections were stained with Gomori's chrome alum hematoxylin-phloxin B and kidney sections were stained with Haris hematoxylin-eosin. Stained sections were examined and photographed with light microscope (Olympus).

Statistical analysis

Statistical significance of the results (mean ± SEM) was determined using unpaired two-tailed Student's *t*-test and one-way analysis of variance. Difference of means of the parameters of the groups (NC vs DC, DC vs DT and DC vs DTG) was found to be significant at $P < 0.05$ and $P < 0.01$. The significance of similarity of means of the parameters of the groups (NC vs NT and DT vs DTG) was tested at 1% level.

Results

Effect of glycyrrhizin on hyperglycaemia and intraperitoneal glucose tolerance test in diabetic rats

Treatments with glycyrrhizin and glibenclamide were started after two weeks of STZ administration. Fasting blood glucose levels obtained after two weeks of glycyrrhizin or glibenclamide treatment are presented in Figure 1a. Glucose levels increased significantly in diabetic rats (DC) with respect to normal rats (NC). Although glycyrrhizin had no effect on blood glucose concentrations of normal treated rats (NT), its effect on blood glucose of diabetic rats (DT) was clearly evident. One week after single administration of glycyrrhizin in diabetic rats, the glucose level became normal (not shown), and was maintained thereafter. A similar effect was found with glibenclamide, which was provided with food everyday in diabetic rats (DTG) throughout the experiment.

IPGTT was performed three weeks after treatment with glycyrrhizin or glibenclamide. DC rats exhibited glucose intolerant behaviour in comparison with control rats (NC) (Figure 1b). Blood glucose level in diabetic rats treated with glycyrrhizin (DT) or glibenclamide (DTG) returned to almost normal levels 120 min after glucose injection. No difference was noted between glucose tolerance curves of normal control group (NC) and glycyrrhizin-treated control group (NT).

Effect of glycyrrhizin on insulin and glycohaemoglobin levels in diabetic rats

Induction of diabetes resulted in significant reduction in serum insulin level compared with that in normal rats (Figure 2a). The levels improved significantly in diabetic rats after two weeks of treatment with glycyrrhizin (DT) or glibenclamide (DTG). However, normal levels of insulin were not restored by either treatment. The glycohaemoglobin level is an important indicator in diabetic condition, and its level was measured after six weeks of diabetes induction (Figure 2b). The level was found to be significantly higher in diabetic rats (DC) than in normal animals (NC). Diabetic treated rats (DT) after four weeks of glycyrrhizin administration, showed a significantly reduced glycation level of haemoglobin. Glibenclamide treatment (DTG) for the same period was also effective in significantly inhibiting glycation

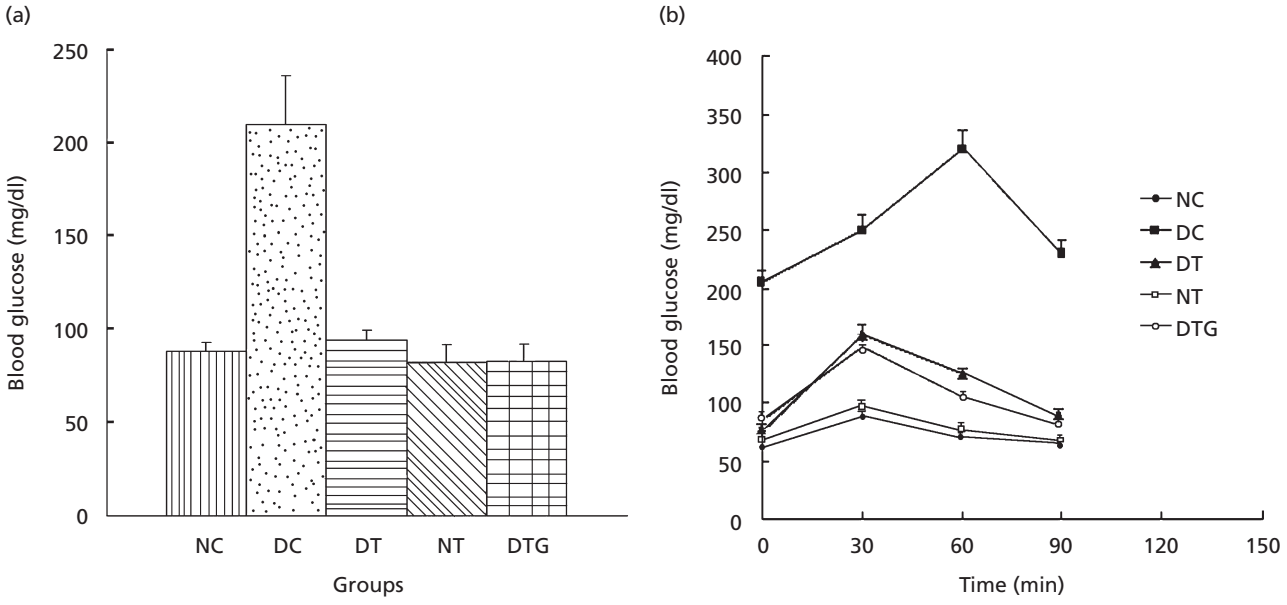

Figure 1 (a) Blood glucose levels in different groups of rats – normoglycaemic (NC, normal control), diabetic (DC, diabetic control administered with 50 mM phosphate buffer saline, pH 7.4) and treated (NT (normal rats treated with glycyrrhizin), DT (diabetic rats treated with glycyrrhizin), DTG (diabetic rats treated with glibenclamide)). Experiments were done after two weeks of treatment. Results are mean ± SEM of eight experiments in each group ($n = 8$). $P < 0.05$ for NC vs DC and for DC vs DT, NT and DTG. (b) Glucose tolerance curves of different groups of rats after three weeks of treatment. Results are mean ± SEM of five experiments ($n = 5$).

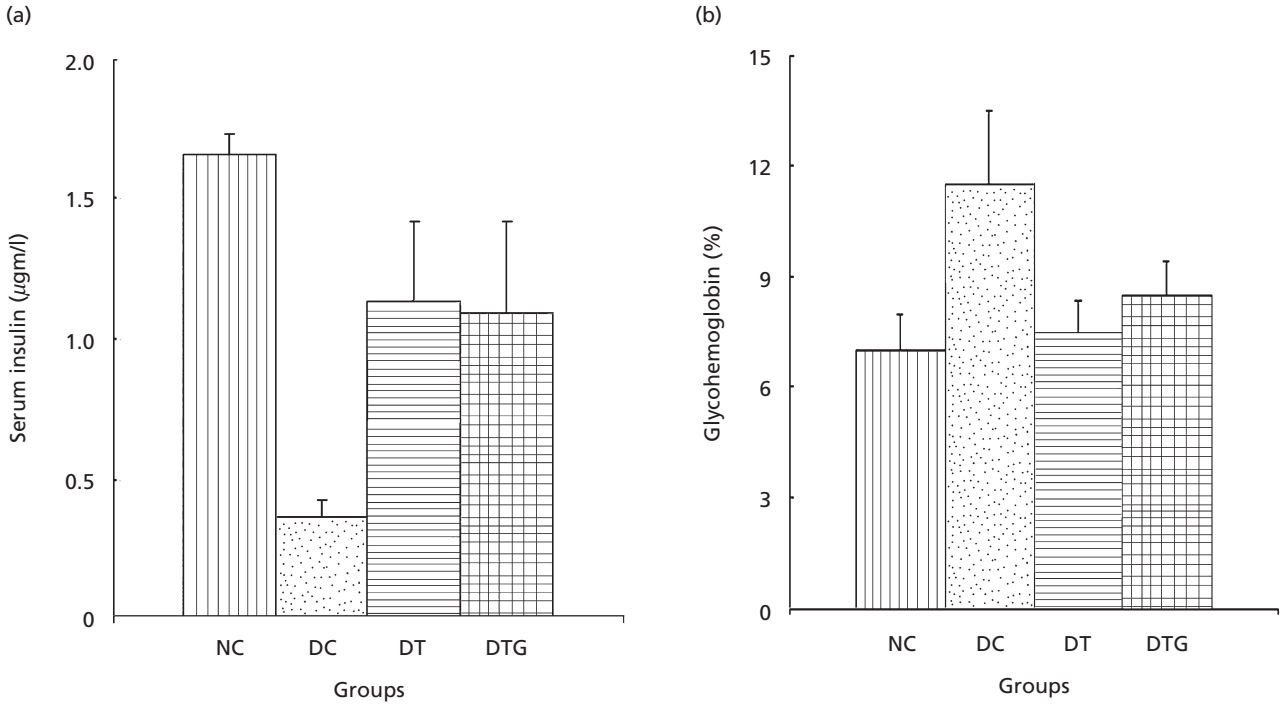

Figure 2 (a) Serum insulin level of different groups of rats after two weeks of treatment. Results are mean ± SEM of five experiments ($n = 5$). $P < 0.01$ for NC (normal control rats) vs DC (diabetic control rats); $P < 0.05$ for DC vs DT (diabetic rats treated with glycyrrhizin) and DTG (diabetic rats treated with glibenclamide). (b) Glycohaemoglobin levels after four weeks of treatment. Results are mean ± SEM of eight experiments ($n = 8$). $P < 0.05$ for NC vs DC and for DC vs DT.

of haemoglobin. The treatments did not exhibit any effect on either insulin or glycohaemoglobin level in normal rats (NT) (not shown).

Effect of glycyrrhizin on histology of pancreas and kidney of diabetic rats

Histological examination of pancreatic sections revealed a significant decrease in the diameters of pancreatic islets as well as count of islets and number of cells per islet of diabetic rats (DC) in comparison with control group (NC), as shown in Figure 3a(ii) and 3a(i), respectively. These parameters were considerably improved in glycyrrhizin-treated rats (DT) (Figure 3a(iii)). Kidney sections of diabetic rats (Figure 3b(ii)) showed diffused mesangium and thicker basal membrane of glomeruli in comparison with those of normal rats (Figure 3b(i)). Treatment of diabetic rats with glycyrrhizin caused a lesser degree of mesangial matrix increment and basal membrane thickening (Figure 3b(iii)). Glibenclamide treatment also lessened the abnormality in the morphology of both pancreas and liver tissues of diabetic rats (not shown).

Effect of glycyrrhizin on serum cholesterol and triglyceride levels in diabetic rats

Serum cholesterol and triglyceride levels appeared to be significantly higher in diabetic rats (DC) than in normal rats (NC) (Figure 4a and 4b, respectively). After two weeks of treatment with glycyrrhizin, both cholesterol and triglyceride levels were normalized. Almost similar results were obtained after two weeks of daily supply of glibenclamide to diabetic rats (DTG). The levels were almost similar in NC and NT groups of rats.

Effect of glycyrrhizin on antioxidant enzymes and oxidative stress markers in diabetic rats

Antioxidant enzyme activity (serum SOD and catalase) and oxidative stress markers (serum malondialdehyde, fructosamine and haemoglobin carbonyl contents) in all groups of rats were measured (Table 1). Induction of diabetes in rats (DC) caused a significant decrease in serum SOD and catalase activity compared with the normal level (NC). The antioxidant enzyme activity increased significantly in diabetic rats after two weeks of glycyrrhizin treatment (DT). The antioxidant enzyme activity in normal rats (NC) and normal treated rats (NT) was almost similar.

An increase in the level of serum malondialdehyde, an index of free radical formation, was evident in diabetic rats (DC). Two weeks after glycyrrhizin treatment, the diabetic rats (DT) exhibited a significant reduction in serum malondialdehyde level. Similar treatment of normal rats (NT) also reduced the malondialdehyde level to some extent.

The serum fructosamine (Amadori product) level, a measure of early protein glycation, was significantly higher in diabetic rats (DC) than in normal rats (NC). Fructosamine was measured after seven weeks of diabetes induction. The level was normalized after five weeks of glycyrrhizin treatment in

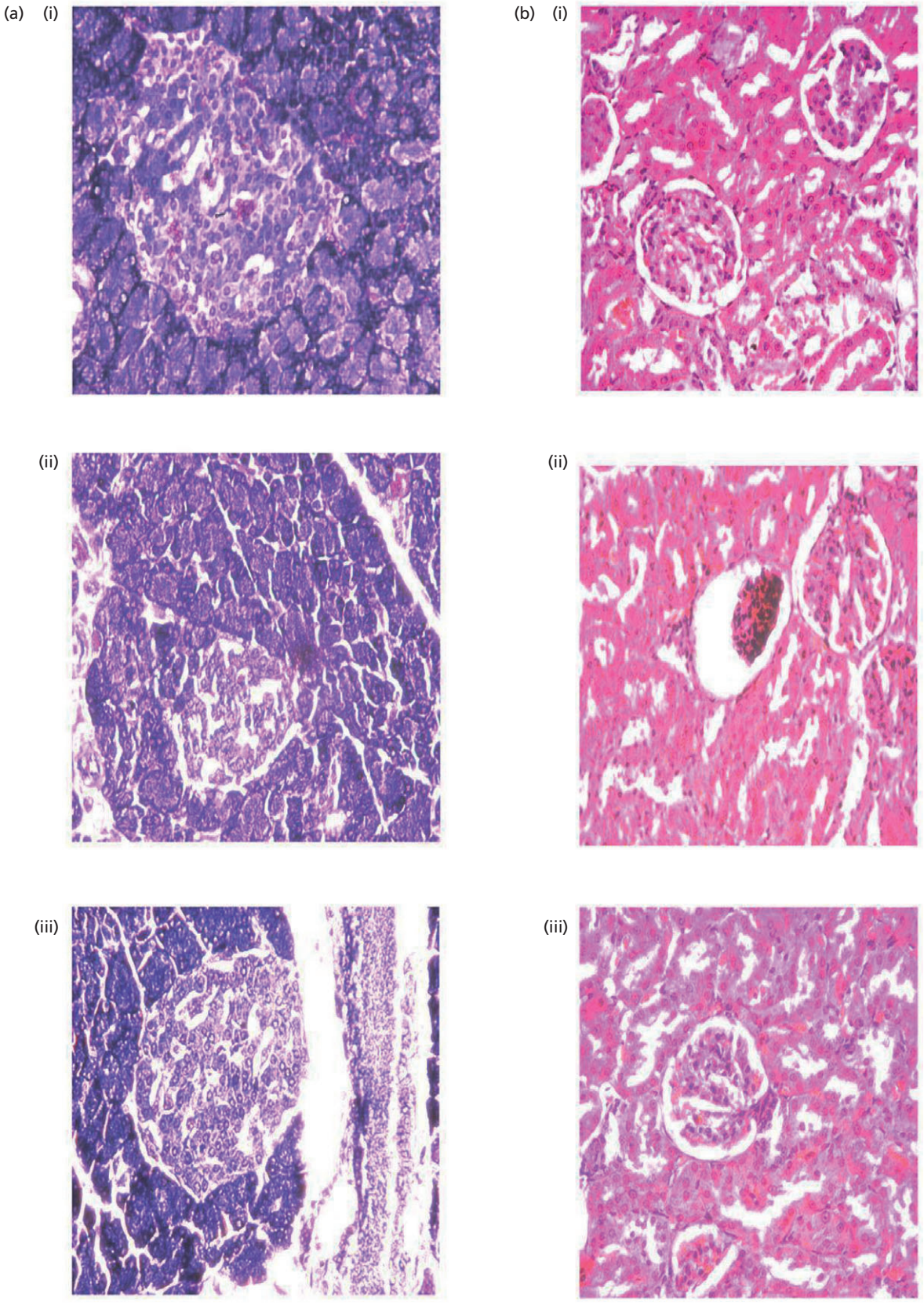

Figure 3 Representative sections of histological examinations of (a) pancreas and (b) kidney tissues of different groups of rats: (i) NC (normal control), (ii) DC (diabetic control) and (iii) DT (diabetic rats treated with glycyrrhizin) (Magnification 40×).

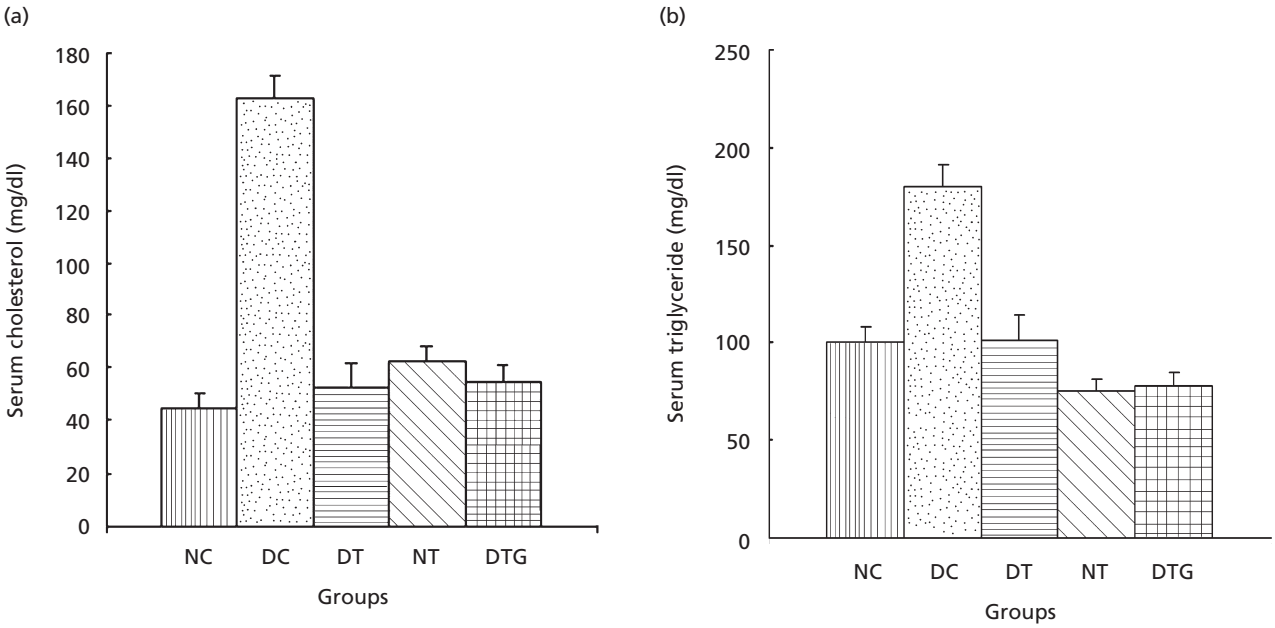

Figure 4 (a) Serum cholesterol levels in different groups of rats after two weeks of treatment. Results are mean ± SEM of eight experiments ($n = 8$). $P < 0.01$ for NC (normal control rats) vs DC (diabetic control rats) and for DC vs DT (diabetic rats treated with glycyrrhizin), NT (normal rats treated with glycyrrhizin) and DTG (diabetic rats treated with glibenclamide). (b) Serum triglyceride levels in different groups of rats after two weeks of treatment. Results are mean ± SEM of eight experiments ($n = 8$). $P < 0.05$ for NC vs DC and for DC vs DT, NT and DTG.

Table 1 Effect of glycyrrhizin on antioxidant enzymes and oxidative stress markers in normal and diabetic rats

Parameters	Groups				
	NC	DC	DT	NT	DT G
Serum SOD (units/mg protein)	0.34 ± 0.04	0.16 ± 0.02[a]	0.38 ± 0.05[c]	0.35 ± 0.04	0.33 ± 0.05[e]
Serum catalase (units/mg protein)	0.29 ± 0.02	0.07 ± 0.01[b]	0.28 ± 0.02[d]	0.30 ± 0.03	0.24 ± 0.02[f]
Serum malondialdehyde (arbitrary fluorescence units)	128 ± 15	255 ± 21[a]	100 ± 9[d]	110 ± 10	135 ± 14[e]
Serum fructosamine (μmol/mg protein)	222 ± 15	302 ± 25[a]	220 ± 13[c]	204 ± 15	235 ± 15[e]
Carbonyl content of haemoglobin (μmol/gm)	27 ± 2	40 ± 3[a]	22 ± 2[c]	23 ± 3	31 ± 3[e]

NC, normal control rats; DC, diabetic control rats; DT, diabetic rats treated with glycyrrhizin; NT, normal rats treated with glycyrrhizin; DTG, diabetic rats treated with glibenclamide. For NC vs DC: [a]$P < 0.05$, [b]$P < 0.01$; For DC vs DT: [c]$P < 0.05$, [d]$P < 0.01$; For DC vs DTG: [e]$P < 0.05$, [f]$P < 0.01$. All parameters (except fructosamine) were assayed two weeks after administration of a single dose of glycyrrhizin or after two weeks of daily treatment with glibenclamide. Fructosamine levels were determined after five weeks of treatment. Results are mean ± SEM ($n = 8$) of serum SOD, serum catalase, serum malondialdehyde, serum fructosamine and carbonyl content of haemoglobin.

diabetic rats (DT). The treatment also had some lowering effect on fructosamine level in normal rats (NT).

Carbonyl formation in proteins due to increased oxidation is another manifestation of oxidative stress. Carbonyl contents of haemoglobin samples isolated from normal, diabetic and treated groups have been shown. The content, measured after four weeks of diabetes induction, appeared to be significantly higher in diabetic rats (DC) than in normal rats (NC). Glycyrrhizin treatment in both diabetic and normal rats (DT and NT) efficiently reversed this stress condition.

All these parameters (serum SOD, catalase, malondialdehyde, fructosamine and carbonyl content of haemoglobin) in diabetic rats were significantly improved by glibenclamide treatment, as found in the DTG group of rats.

Effect of glycyrrhizin on iron release from haemoglobin and haemoglobin-induced oxidative reactions in diabetic rats

Ferrozine-detected iron levels in haemoglobin samples isolated from all the groups are presented in Figure 5a. The free iron level in the haemoglobin of diabetic rats (DC) was significantly higher than that of control rats (NC). The free iron level in the haemoglobin samples of glycyrrhizin-treated rats (DT) was found to be normalized after two weeks of treatment. Similar treatment of normal rats did not change the free iron level of haemoglobin. Glibenclamide treatment was also very effective in lowering the free iron level in diabetic rats (DTG).

To understand iron-mediated free radical reactions, haemoglobin-mediated DNA (plasmid) breakdown was

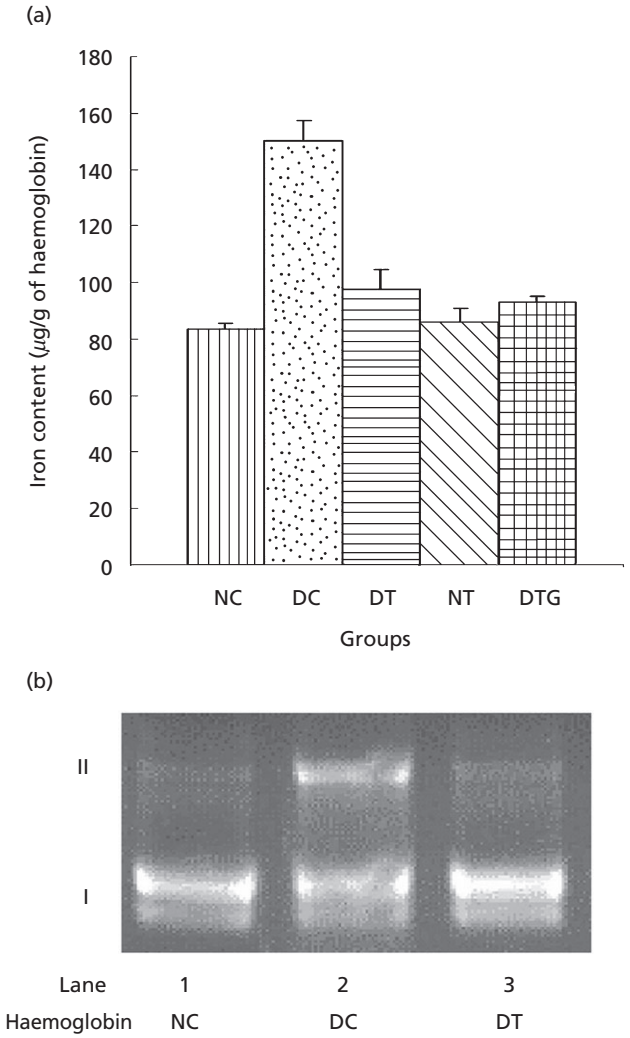

Figure 5 (a) Free iron levels in haemoglobin samples isolated from different groups of rats after two weeks of treatment. Results are mean ± SEM of five experiments ($n = 5$). $P < 0.05$ for NC (normal control rats) vs DC (diabetic control rats) and for DC vs DT (diabetic rats treated with glycyrrhizin), NT (normal rats treated with glycyrrhizin) and DTG (diabetic rats treated with glibenclamide). (b) Haemoglobin-mediated DNA (plasmid) breakdown after two weeks of treatment. Haemoglobin samples were isolated from NC, DC and DTG groups of rats. Similar results were obtained in three separate experiments.

studied. Haemoglobin samples were collected from rats treated for two weeks as well as respective control groups. DNA was incubated with a haemoglobin sample and subjected to agarose gel electrophoresis followed by ethidium bromide staining. Result of a representative experiment are shown in Figure 5b. DNA degradation was evident, with conversion of form I to form II as compared between normal haemoglobin-mediated reaction (lane 1) and diabetic haemoglobin-mediated reaction (lane 2). Forms I and II are intact supercoiled DNA and nicked DNA, respectively. DNA breakdown was significantly less in the presence of haemoglobin samples from the diabetic treated group of rats (DT), as compared between lanes 3 and 2. There was no appreciable DNA breakdown in the presence of haemoglobin samples of glycyrrhizin-treated normal rats (NT) or glibenclamide-treated diabetic rats (DTG) (not shown).

Discussion

Hyperglycaemia in diabetes causes different complications. The primary objective of all diabetic treatment is therefore to maintain the blood glucose concentration within the normal range. Here we have studied the effectiveness of glycyrrhizin, a major constituent of liquorice root, against the diabetogenic effects of STZ. The effect of glibenclamide, a well-known anti-hyperglycaemic drug, has been included for comparison though the doses and routes of administration of glycyrrhizin (single intraperitoneal injection at a dose of 100 mg/kg body weight) and glibenclamide (fed along with diet at a dose of 8 mg/kg body weight/day for five weeks) are significantly different. We have selected the modes of administration and doses from the existing reports using glibenclamide[25,26] and glycyrrhizin.[27,28] Repeated exposure to high-dose glycyrrhizin (240 or 480 mg/kg body weight, p.o.) has been shown to induce cytochrome P450 (CYP) 3A in Swiss Albino CD1 mice.[29] CYP activity is associated with drug metabolism. In a recent study, Hasegawa *et al*.[30] showed that insulin administration decreases the enhanced activity of CYP3A in STZ-induced diabetic rats and delays the elimination of nicardipine to the same level as that of the control rats. Studies on the effect of glycyrrhizin on CYP-catalysed drug metabolism and disposition are also necessary to understand the usefulness of the therapy.

The anti-hyperglycaemic effect of glycyrrhizin in genetically diabetic KK-A^y mice has been demonstrated by Takii *et al*.[10] Glycyrrhizin inhibits sodium glucose co-transporter-1 (S-Glut-1)-mediated glucose transport across the intestine. *G. radix* preparata extracts and glycyrrhetinic acid enhance glucose-stimulated insulin secretion and induce mRNA levels of insulin receptor substrate-2.[11] Ao *et al*.[31] have shown that 18α-glycyrrhizin exerts a synergistic effect on the action of glibenclamide in lowering blood glucose and increasing insulin levels in alloxan-treated diabetic rats. Recently, Kalaiarasi and Pugalendi[12] have reported that 18β-glycyrrhetinic acid decreases blood glucose level and enhances insulin secretion in STZ-induced diabetic rats. Our findings together with existing reports thus suggest that liquorice extract, glycyrrhizin and glycyrrhetinic acid possess anti-hyperglycaemic activity. However, it is not yet clear if glycyrrhizin administered intraperitoneally acts as such or through a hydrolysed/metabolized product. In the present communication, we have studied several parameters associated with hyperglycaemia to understand further the action of glycyrrhizin. As islet volume and number of islet cells (including β-cells) increase in glycyrrhizin-treated diabetic rats with respect to diabetic control rats, the possible mode of action of glycyrrhizin is the regeneration or sensitization of pancreatic β-cells that elevate serum insulin and thereby rectify hyperglycaemic condition. However, further demonstration of the functionality of β-cells in response to glycyrrhizin is necessary to understand the mode of action.

Hyperglycaemia induces nephropathy, a common diabetic complication, due to accumulation of proteins in the mesangial basal membrane, and by free radicals derived from

auto-oxidation of glucose or from advanced glycation end products.[32] Glycyrrhizin significantly reduces diabetes-induced abnormalities of kidney, probably through its anti-hyperglycaemic functions. However, semiquantitative analysis of the effect of glycyrrhizin on diabetic nephropathy is necessary to assess the extent of damage in diabetes and its control by the herbal agent.

Inadequate utilization of glucose in diabetes stimulates the mobilization of lipid stores in the organism, thus increasing cholesterol and triglyceride concentrations in blood.[33] Our findings on the hypolipidaemic effects of glycyrrhizin in diabetic rats are in agreement with those of Visavadiya and Narasimhacharya,[34] who reported that administration of liquorice root powder to hypercholesterolaemic rats for four weeks resulted in significant reduction in plasma and hepatic total lipids, cholesterol, triglycerides, plasma LDL and VLDL-cholesterol accompanied by significant increase in HDL-cholesterol levels.

Low activity of SOD and catalase in serum of diabetic rats may result from inactivation of the enzymes by H_2O_2 or by glycation.[35,36] The enhanced enzyme activity in glycyrrhizin-treated diabetic rats may be due to their reduced glycation. Free radicals react with lipids leading to lipid peroxidation. Malondialdehyde, a major product in this process, is used as an index of lipid peroxidation, which is enhanced by induction of diabetes and reduced by glycyrrhizin treatment, reflecting further the antioxidant activity of the herbal agent. Fructosamine is the product of protein glycation at an early stage, and it undergoes oxidative cleavage resulting in the formation of advanced glycation end products causing diabetic complications.[37] Glycyrrhizin normalizes serum fructosamine levels that had been enhanced by STZ. The treatment thus interrupts the glycation cascade, preventing the potential pathological consequences of diabetes.

Metal-catalysed oxidation may cause covalent modification of proteins by introducing carbonyl groups into amino acid residues of proteins.[23] Such oxidatively modified proteins are susceptible to degradation. Glycyrrhizin treatment is effective in preventing modification of haemoglobin in diabetic rats, which may be correlated with decreased glycohaemoglobin level in the treated group. We have shown earlier that human glycated haemoglobin, HbA_{1c} is more susceptible to oxidative modification than the normal species, HbA_0.[38] The antioxidative effects of glycyrrhizin appear to be comparable with, or better than, glibenclamide treatment, reflecting the therapeutic potency of the herbal agent.

Free radicals and oxidative stress have long been implicated in eliciting complications of the diabetic condition. However, the mechanism of increased formation of free radicals in diabetes is still not clear. Hyperglycaemia, protein glycation and glucose auto-oxidation have been suggested to induce free radical generation in diabetes.[39–41] Both in-vitro and in-vivo studies from our laboratory have suggested that glycation or fructation-induced modification of haemoglobin or myoglobin may be a source of catalytic iron and increased free radicals in the diabetic condition.[24,38,42–44]

The free iron level increases in haemoglobin samples of diabetic rats having a higher glycohaemoglobin level. Glycation weakens heme-globin linkage,[24,45] and facilitates heme release from the glycated heme protein.[45,46] H_2O_2 generation has been reported to increase in induced diabetes.[47] Moreover, H_2O_2 releases iron from haemoglobin[48,49], and induces more iron release from glycated or fructated heme proteins.[38,42,43] Glycyrrhizin or glibenclamide treatment in diabetic rats causes a substantial decrease in free iron level in haemoglobin samples, which may be associated with lowering glucose as well as glycohaemoglobin levels in diabetic rats.

The haemoglobin of diabetic rats degrades DNA (plasmid) more efficiently than that of normal control rats. Glycated heme proteins auto-oxidise more rapidly than nonglycated proteins,[38,42] thus generating more superoxide radicals (O_2^-), which may react with water to form H_2O_2. Haemoglobin of diabetic rats containing a higher level of glycohaemoglobin may therefore be a source of increased formation of O_2^- and H_2O_2. Free iron may then act as a source of $OH^\cdot$ radicals through the Fenton reaction: $Fe^{[2+]} + H_2O_2 \rightarrow Fe^{[3+]} + OH^- + OH^\cdot$.[49] Haemoglobin samples from diabetic rats may thus cause enhanced oxidative reactions, as shown by DNA degradation. Glycyrrhizin treatment is very effective in reversing the oxidative damage of DNA, which may be associated with lower levels of glycohaemoglobin and free iron in haemoglobin samples from diabetic treated rats. Deoxyribose degradation by haemoglobin of diabetic rats is also more efficient in comparison with that of normal rats, and glycyrrhizin or glibenclamide treatment significantly lowers the extent of deoxyribose degradation (data not shown). $OH^\cdot$ radicals specifically attack deoxyribose to yield a mixture of products, and its breakdown by haemoglobin is significantly inhibited by mannitol, an $OH^\cdot$ radical quencher, indicating the role of $OH^\cdot$ radicals in the oxidative reactions.

Conclusion

Glycyrrhizin is quite effective in combating hyperglycaemia and associated pathological complications such as hyperlipidaemia, abnormal histoarchitectures of different organs and oxidative stress including haemoglobin-induced iron-mediated free radical reactions. The effects of glycyrrhizin on diabetes-associated changes are almost comparable with those of glibenclamide, a standard antihyperglycemic drug, suggesting a possible use of the herbal agent as a drug to prevent complications of diabetes mellitus.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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