Indian Journal of Experimental Biology
Vol. 51, February 2013, pp. 129-138

Glycyrrhizin ameliorates insulin resistance, hyperglycemia, dyslipidemia and oxidative stress in fructose-induced metabolic syndrome-X in rat model

Rajarshi Sil, Doel Ray[#] & Abhay Sankar Chakraborti*

Department of Biophysics, Molecular Biology and Bioinformatics, University College of Science, University of Calcutta, 92 Acharyya Prafulla Chandra Road, Kolkata 700 009, India

Received 26 June 2012; revised 8 October 2012

This study investigates if glycyrrhizin, a constituent of licorice (*Glycyrrhiza glabra*) root, is able to treat the complications (insulin resistance, hyperglycemia, dyslipidemia and oxidative stress) of metabolic syndrome. Metabolic syndrome was induced in rats by feeding a fructose-enriched (60%) diet for six weeks, after which single dose of glycyrrhizin (50 mg/kg body weight) was administered intraperitoneally. Different biochemical parameters from blood were estimated during three weeks after treatment. Then the rats were sacrificed to collect skeletal muscle tissue. Glycyrrhizin reduced the enhanced levels of blood glucose, insulin and lipids in metabolic syndrome group. Increased advanced glycation end products of hemoglobin, glycohemoglobin, hemoglobin-mediated iron release and iron-mediated free radical reactions (arachidonic acid and deoxyribose degradation) in metabolic syndrome were inhibited by glycyrrhizin treatment. Reduced activities of enzymatic antioxidants (superoxide dismutase and catalase) and elevated oxidative stress markers (malonaldehyde, fructosamine, hemoglobin carbonyl content and DNA damage) in metabolic syndrome were reversed to almost normal levels by glycyrrhizin. The decreased levels of peroxisome proliferator activated receptor γ (PPARγ) and glucose transporter 4 (GLUT4) proteins in skeletal muscle of metabolic syndrome group were elevated by glycyrrhizin, indicating improved fatty acid oxidation and glucose homeostasis.

Keywords: Metabolic syndrome, High fructose diet, Glycation, Insulin resistance, Peroxisome proliferator activated receptor γ, Glucose transporter 4

Metabolic syndrome-X is one of the important health problems in the present world. It is a combination of clinical abnormalities including insulin resistance, hyperglycemia, dyslipidemia, hypertension and obesity. The syndrome increases the risk of cardiovascular diseases and type 2 diabetes[1]. It also leads to non-alcoholic fatty liver disease interfering with normal liver function[2]. Development of oxidative stress, an imbalance in prooxidant-antioxidant status, is the final mediator by which metabolic syndrome contributes to different pathological complications[3].

Fructose, a highly lipogenic nutrient, is a contributor to nearly all of the classic manifestations of metabolic syndrome. Nowadays increased fructose consumption through high fructose corn syrup present in soft drinks, juice beverages and pre-packaged food increases the risk of this syndrome[4]. Long-term intake of a fructose-enriched diet increases the risk of insulin resistance. Insulin resistance, in which the insulin level is not reduced but its function is affected, plays a key role in the development of metabolic syndrome[5]. Insulin resistance causes reduced glucose uptake and utilization by skeletal muscle and adipose tissues, due to reduced level of glucose transporter 4 (GLUT4), which is associated with insulin-regulated glucose transport[6]. Increased cellular lipid content due to reduced mitochondrial and peroxisomal β-oxidation also reduces insulin sensitivity in metabolic syndrome[7]. Cellular β-oxidation depends on peroxisome proliferator activated receptor γ (PPARγ), which is a ligand activated transcription factor belonging to the nuclear receptor superfamily. It regulates fatty acid oxidation in peroxisome and mitochondria. PPARγ expression level decreases in metabolic syndrome[8]. Therefore, improvement of GLUT4 and PPARγ levels may be a potential target in the treatment of this syndrome.

In recent years, scientists are in search of easily available, inexpensive therapeutics having minimum side effects for the better treatment of diseases. For these, phytochemicals from different herbal sources

*Correspondent author
Telephone: 033-2350-8386
E-mail: ascbmbg@caluniv.ac.in; abhay_chakraborti@yahoo.co.in
[#]Present address: National Institute of Plant Genome Research, New Delhi 110 067, India

are being increasingly used. Licorice (*Glycyrrhiza glabra*) is a widely used medicinal plant. Glycyrrhizin, a triterpene saponin, is the major constituent of licorice root. It has long been studied for its medicinal activity. Takii *et al.*[9] have shown the antidiabetic effect of glycyrrhizin in genetically diabetic KK-A$^{\gamma}$ mice. Recent studies have reported the curative effect of glycyrrhizin[10] or its metabolite, 18β-glycyrrhetinic acid[11-13] in streptozotocin-induced type 1 diabetes mellitus in rat model. Glycyrrhizin has also been shown to upregulate total PPARγ expression in different tissues of normal rats[14]. Eu *et al.*[15] have shown that glycyrrhizin increases insulin sensitivity in high fat diet-induced obese rats. These findings have prompted us to undertake the present study to find the effect of glycyrrhizin on fructose-induced metabolic syndrome with respect to insulin resistance, hyperglycemia, dyslipidemia and oxidative stress as well as the expression of GLUT4 and PPARγ.

Materials and Methods

Materials—Arachidonic acid, deoxyribose, dinitrophenyl hydrazine (DNPH), dithiothreitol (DTT), ferrozine, glycyrrhizin, histopaque 1077, malonaldehyde (MDA), nitroblue tetrazoloium (NBT), phenylmethylsulfonylfluoride (PMSF), pyrogallol, sephadex G100, thiobarbituric acid (TBA) and protease inhibitor cocktail were purchased from Sigma-Aldrich, USA. Rat insulin ELISA kit and glycohemoglobin (GHb) estimation kit were purchased from DRG Diagnostic, Germany, and Transasia Biomedicals, India, respectively. Kits for estimating blood glucose, total cholesterol (TC), triglyceride (TG) and high-density lipoprotein-cholesterol (HDL-C) were purchased from Span Diagnostics, India. Primary and secondary antibodies and western blot Luminol reagent were purchased from Santa Cruz Biotechnology, USA. Other chemicals used in this study were of analytical grade and obtained from SRL and E. Merck, India.

Animals—Animals were maintained in accordance with the regulations specified and monitored by the Institutional Animal Ethics Committee of the Department of Biophysics, Molecular Biology and Bioinformatics, University of Calcutta, Kolkata (Registration number: 935/c/06/CPCSEA, 30.06.2009). Male Wistar rats, weighing 90-100 g body weight were obtained from university approved animal supplier. They were housed in an animal room under controlled conditions of 12 h light/dark cycle at 26-28 °C and 60-80% RH.

Toxicity study of glycyrrhizin—To find the toxic effect of glycyrrhizin on normal rats, several toxicity markers were checked. Rats were treated intraperitoneally (ip) with different single doses of glycyrrhizin (50, 100, and 200 mg/kg body weight in 50 m*M* potassium phosphate buffer, *p*H 7.4) with five rats in each group. Rats were supplied normal diet and water *ad libitum*. No significant hair loss or differences in hair colour, food intake, water intake, body weight gain, and behaviour were observed for next 4 weeks. Different biochemical parameters namely, serum alanine transaminase, alkaline phosphatase, xanthine oxidase, aspartate transaminase activities and reduced glutathione content of liver were estimated according to the standard methods[16-20]. No considerable change was found in the parameters between normal and treated rats, indicating no toxicity up to the dose of 200 mg/kg body weight (unpublished data). In the present experiments, glycyrrhizin was administered at a dose of 50 mg/kg body weight to find its effect.

Experimental design—The experimental design is presented in Scheme 1. Total 24 rats were divided primarily into two groups and were maintained as follows:

Normal control group (NC): The animals (n = 8 rats) received a control diet and water *ad libitum*.

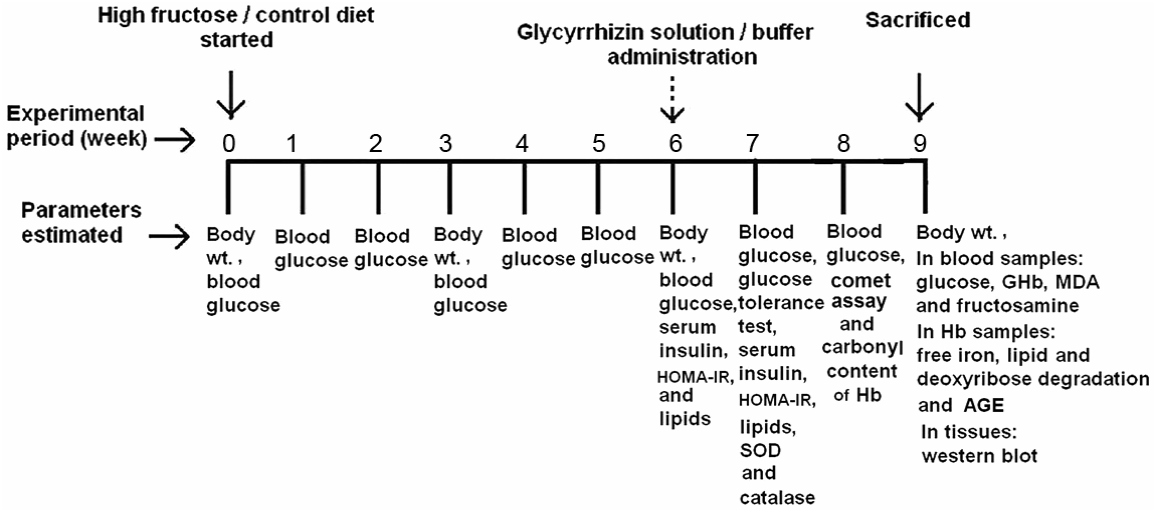
High fructose diet group (HFD): The animals (n = 16 rats) received a fructose-enriched diet and water *ad libitum*.

The diet composition is given in Table 1[21]. Each rat was supplied with 10 g freshly-prepared diet during daytime. After six weeks, the levels of fasting blood glucose, insulin, TC and TG appeared to be significantly higher ($P < 0.01$) in HFD group of rats in comparison with those of NC group of rats, indicating induction of metabolic syndrome in HFD rats. On day 43, HFD rats were treated with glycyrrhizin solution or buffer as follows:

HFDT group: HFD rats (n = 8 rats) were treated with single injection (ip) of glycyrrhizin solution in 50 m*M* potassium phosphate buffer, *p*H 7.4, at a dose of 50 mg/kg body weight.

HFD group: HFD rats (n = 8 rats) were similarly treated with only buffer.

Both HFD and HFDT groups of rat were still on the fructose-enriched diet until sacrificed after three weeks of glycyrrhizin treatment.



Scheme 1—Schematic representation of the experimental design. It shows the parameters tested at different time intervals in different groups of rat. Control diet was supplied to NC group and fructose-enriched diet was supplied to HFD and HFDT groups. Buffer was administered in HFD group and glycyrrhizin solution was administered in HFDT group of rats.

Collection of serum, hemoglobin and tissue— Blood samples (fasting) were collected from tail vein without and with heparin for estimation of enzymes and other parameters, respectively. For estimation of serum insulin level, blood was drawn from the retro-orbital plexus. Serum was separated by centrifugation at 3,500 *g* at 4 °C for 10 min. Hemolysates were prepared from erythrocytes after washing with normal saline (0.9% NaCl) and hypotonic lysis with distilled water. Hemoglobin (Hb) was isolated from hemolysate by centrifugation at 17,500 *g* at 4 °C for 15 min and purified by sephadex G100 column pre-equilibrated with 50 m*M* potassium phosphate buffer, *p*H 7.4. The concentration of Hb was measured from the soret absorbance with molar extinction coefficient 125 mM^{-1} cm^{-1} (monomer basis)[22]. Rats were sacrificed by cervical dislocation. Quadricep muscle tissue was dissected out immediately, washed with ice-cold phosphate buffer saline, *p*H 7.4 and preserved at -80 °C for Western blot experiments done within next three weeks.

Blood glucose, GHb, insulin, homeostasis model of assessment-insulin resistance (HOMA-IR) index, lipids and intraperitoneal glucose tolerance test (*IPGTT*)—Blood glucose was estimated following the conventional glucose oxidase/peroxidase method by using the assay kit[23]. Serum insulin, TC, TG, HDL-C and GHb were measured using commercial assay kits according to the manufacturer's protocols. Low-density lipoprotein-cholesterol (LDL-C) was estimated according to the Friedewald equation[24]. Very low-density lipoprotein-cholesterol (VLDL-C) was obtained by subtracting both HDL-C and LDL-C from TC. HOMA-IR index was calculated according to the formulae of Matthews *et al*[25]. For glucose tolerance test, a sterile solution of 20% glucose was injected (ip) at a dose of 2 g/kg body weight to overnight fasting animals. Blood was collected to estimate glucose from tail vein before (0 min) and 30, 60, 90 and 120 min after injection.

Table 1— Composition of diets (g/100g)

Ingredients	Control diet	High-fructose diet
Corn starch	60	--------
Fructose	--------	60
Casein	20	20
Methionine	0.7	0.7
Groundnut oil	5	5
Wheat bran	10.6	10.6
Salt	3.5	3.5
Vitamin	0.2	0.2

Superoxide dismutase (SOD), catalase, MDA and fructosamine in serum— SOD and catalase activities were assayed according to the methods of Murklund and Murklund[26] and Beers and Sizer[27] using pyrogallol and hydrogen peroxide, respectively. Protein content was measured following the method of Lowry *et al.*[28] using bovine serum albumin as the standard. MDA level was measured as TBA reactive substance[29]. For fructosamine (Amadori product), NBT was used to detect the formazan formed[30].

Free iron level in Hb, Hb-mediated lipid peroxidation and deoxyribose degradation— Free iron in Hb was measured by reaction with ferrozine according to the method of Panter[31]. Hb-mediated lipid (arachidonic acid) peroxidation was estimated

according to the method of Sadrzadeh *et al.*[32] with modifications as described before[33]. The values were corrected for endogenous TBA reactive substance present in arachidonic acid. Hb-mediated deoxyribose degradation was assayed spectrofluorimetrically following the method of Gutteridge[34].

Carbonyl content and advanced glycation end products (*AGEs*) *in Hb*— Carbonyl content in Hb was measured using DNPH according to the method of Levine *et al*[35]. AGEs in Hb were estimated spectrofluorimetrically from fluorescence emission at 440 nm after exciting Hb samples at 370 nm[36].

Single cell gel electrophoresis (comet assay) of lymphocytes— Lymphocytes were isolated from blood using histopaque 1077 and were resuspended in phosphate buffer saline, *p*H 7.4. Comet assay and scoring were performed according to the method of Singh *et al.*[37] and Collins *et al.*[38], respectively. Photomicrograph of ethidium bromide-stained cells was taken in Olympus BX51 fluorescence microscope with Evolution VF cool CCD camera at 200 magnifications.

Western blotting experiment with quadricep muscle tissue— Quadricep muscle tissue was homogenized with ice-cold lysis buffer containing 25 m*M* Tris HCl, *p*H 7.5, 250 m*M* NaCl, 1% Triton X-100 (v/v), 1 m*M* DTT, 1 m*M* PMSF and protease inhibitor cocktail and the supernatant was collected by centrifugation at 20,000 *g* at 4 °C for 30 min[39]. Proteins were separated by SDS-PAGE (10%) and western blot experiments were performed using mouse monoclonal antibody for β-actin (1:500 dilution) and GLUT4 (1:250 dilution) and goat polyclonal antibody for PPARγ (1:250 dilution). Band intensities were quantified by densitometry using Biorad Geldoc apparatus with Quantity One software.

Statistical analysis— Results were expressed as mean±SD obtained for *n* experiments; *n* represents the number of rats. Statistical significance was determined by AcaStat 6 software using unpaired Student's *t*-test and $P < 0.05$ was considered to be significant.

Results and Discussion

Metabolic syndrome parameters: effect of glycyrrhizin—Several metabolic syndrome parameters namely, fasting blood glucose, serum insulin, lipid profile and body weight were measured in NC and HFD group of rats. Six weeks of fructose-enriched diet led to significant increase in fasting blood glucose, serum insulin, and HOMA-IR index in HFD rats in comparison with those in NC rats (Table 2). At week six, serum levels of TC, TG, LDL-C and VLDL-C were significantly higher and HDL-C was significantly lower in HFD group than the respective levels in normal rats. High fructose diet induced significant increase in body weight in HFD group in comparison with weight gain by NC group having normal diet (Table 3). The weight gain, abnormal lipid profile together with high blood glucose, serum insulin and HOMA-IR index of HFD group of rats demonstrated the onset of metabolic syndrome induced by fructose-enriched diet. The results are consistent with previous reports of other groups[4,8].

The metabolic syndrome parameters were measured at different intervals after glycyrrhizin treatment of HFD rats (HFDT) and compared them with respective levels of HFD and NC group of rats.

Table 2— Metabolic syndrome parameters of different groups of rat after six weeks of high fructose diet feeding

[Values are mean ± SD from each group]

Groups	Blood glucose (mg/dL)	Insulin (μg/L)	HOMA -IR	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
NC	80.6 ± 5.8	0.82 ± 0.09	5.54 ±0.81	64.7 ± 7.5	72.5 ± 9.8	35.8 ± 4.2	14.4 ± 3.4	14.6 ± 2.0
HFD	160.5 ±7.8 [a]	2.14 ± 0.22 [a]	21.17 ± 3.33 [a]	131.8 ± 10.4 [a]	163.0 ± 11.3 [a]	13.3 ± 3.2 [a]	76.4 ± 13.5 [a]	32.7 ± 1.5 [a]

For insulin and HOMA-IR, $n = 6$ rats and for other parameters, $n = 8$ rats.
[a] $P < 0.01$ *versus* NC.

Table 3— Body weight of different groups of rat

[Values are mean ± SD from each group]

Groups	Initial body weight (g)	Body weight (g) after 6 weeks of control/fructose diet feeding	Body weight (g) after 3 weeks of buffer/ glycyrrhizin administration (total 9 weeks)		Body weight gain (g) in 9 weeks
NC ($n = 8$)	91.7 ± 6.5	103.7 ± 8.3	118.6 ± 5.0		26.4 ± 4.7
HFD ($n = 16$)	92.2 ± 8.7	122.6 ± 6.7	HFD ($n = 8$)	151.4 ± 7.3	58.9 ± 7.4 [a]
			HFDT ($n = 8$)	134.3 ± 5.0	40.3 ± 7.5 [b]

[a] $P < 0.01$ *versus* NC; [b] $P < 0.05$ *versus* HFD.

The dose of glycyrrhizin used in the present study (50 mg/kg body weight) was much less than the LD_{50} dose (0.84 g/kg body weight, ip)[40]. The existing reports suggest that ip administration of glycyrrhizin in rats at a dose of 100 mg/kg body weight caused no toxicity[41,42]. No toxic effect of glycyrrhizin was found up to administration of 200 mg/kg body weight (unpublished data). After one week of treatment with glycyrrhizin, fasting blood glucose level decreased significantly in the treated group, as compared with HFD group (Fig. 1a). For next two weeks, HFDT group exhibited slow increase in blood glucose level, which might be due to continuous feeding of high fructose diet. However, still there was a significant difference in blood glucose levels between HFD and HFDT groups after three weeks of treatment. After one week of glycyrrhizin treatment, insulin level and HOMA-IR index decreased significantly in HFDT group, as compared with HFD group (Fig. 1b and 1c), indicating increased insulin sensitivity in the treated group. Glucose tolerance behaviour, as indicated by IPGTT done after one week of glycyrrhizin treatment, appeared almost normal in HFDT group of rats in comparison with the normal and abnormal tolerant profiles in NC and HFD groups, respectively (Fig. 1d). Increased serum levels of TC, TG, LDL-C and VLDL-C and decreased level of HDL-C in HFD rats reverted significantly towards respective normal levels after one week of treatment with glycyrrhizin, (Fig. 2). Glycyrrhizin treatment was also effective in lowering body weight gained by fructose-enriched diet (Table 3), probably by increasing fatty acid

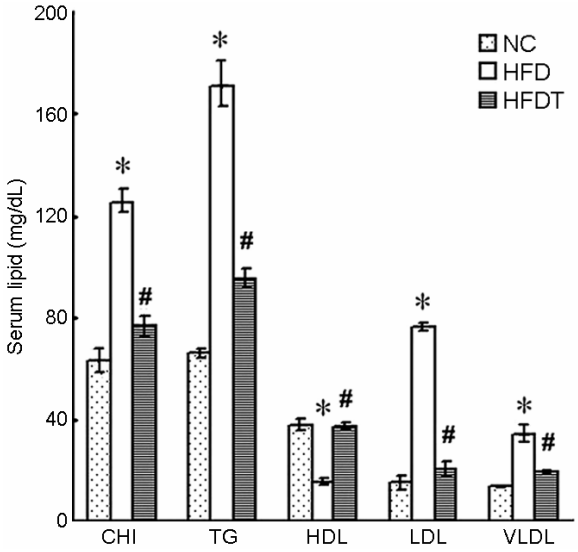


Fig. 2— Serum lipid profiles of different groups of rat. The levels of TC, TG, HDL-C, LDL-C and VLDL-C were estimated after one week of glycyrrhizin treatment. The results are mean ± SD, n = 8 in each group. * $P < 0.01$ *versus* NC; # $P < 0.01$ *versus* HFD.

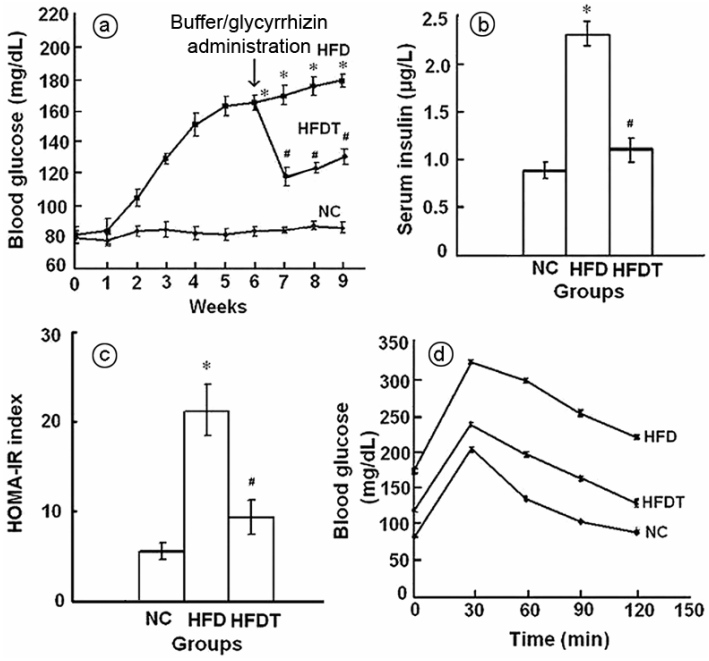


Fig. 1— Blood glucose and insulin level during experimental period. (a) Change of blood glucose with time before and after glycyrrhizin treatment. n = 8 in each group. (b) Serum insulin level of different groups of rat after one week of glycyrrhizin treatment. n = 6 in each group. (c) HOMA-IR index of different groups of rat after one week of glycyrrhizin treatment. n = 6 in each group. (d) Glucose tolerance curves of NC, HFD and HFDT rats after one week of glycyrrhizin treatment. n = 5 in each group. The results are mean ± SD. * $P < 0.01$ *versus* NC; # $P < 0.01$ *versus* HFD.

oxidation and thereby decreasing total lipid storage in body. Increased insulin sensitivity and reduction in body weight in glycyrrhizin-treated high fat diet-induced obese rats have been reported by Eu *et al*[15].

Oxidative stress markers in blood samples of metabolic syndrome group of rats: effect of glycyrrhizin— Free radical scavenging enzyme (SOD and catalase) activities in serum were reduced and serum MDA and fructosamine levels were increased significantly in HFD rats, compared to those in normal rats (Table 4). Glycyrrhizin treatment significantly enhanced the levels of antioxidative enzymes and reduced MDA and fructosamine levels in HFDT group of rats, thereby indicating antioxidant function of the herbal agent. SOD and catalase activities were assayed after one week and MDA and fructosamine levels were determined after three weeks of glycyrrhizin treatment.

Since metabolic syndrome exhibits increased blood glucose level, GHb level was measured in blood samples of different groups of rat. The level increased significantly in HFD group, in comparison with the NC group, and reverted to the near normal level after three weeks of treatment with glycyrrhizin (Table 5). The lowering of blood glucose level due to treatment with the herbal agent may have reduced the GHb level.

Both *in vitro*[33,44,45] and *in vivo*[10,46] studies suggest that GHb is a source of catalytic iron causing oxidative stress in hyperglycemic condition. Ferrozine-detected free iron level was estimated in Hb samples of different groups of rat and was found to increase significantly in HFD rats in comparison with the normal level (Table 5). H_2O_2 is known to react with ferrous iron to form hydroxyl radical[47], which, in turn, causes free radical reactions. Oxidative reactions namely, arachidonic acid and deoxyribose breakdown were assayed in presence of H_2O_2 and Hb samples of different groups of rat. Both these reactions were significantly increased with Hb samples of HFD group than with those of normal group, indicating enhanced Hb-catalyzed oxidative reactions in metabolic syndrome (Table 5). However, glycyrrhizin treatment effectively lowered free iron level and both arachidonic acid and deoxyribose breakdown reactions, as demonstrated with Hb samples of HFDT group of rats. Sen *et al.*[10] have also reported similar effect of glycyrrhizin on streptozotocin-induced diabetes in rats. Thus both in type 1 diabetes and high fructose-induced metabolic syndrome, glycyrrhizin treatment interrupts the glycation cascade, preventing the potential pathological consequences.

Metal-catalyzed oxidation may cause covalent modification of proteins by introducing carbonyl

Table 4— Effect of glycyrrhizin on free radical scavenging enzymes (SOD and catalase) and oxidative stress markers (MDA and fructosamine) in serum of different groups of rat

[Values are mean ± SD from each group]

Groups	SOD (Units/min/mg protein)	Catalase (Units/min/mg protein)	MDA (Fluorescence intensity in arbitrary unit/mg protein)	Fructosamine (μmol/mg protein)
NC	0.956 ± 0.117	0.255 ± 0.032	11.5 ± 2.2	142.2 ± 21.5
HFD	0.378 ± 0.118 [a]	0.137 ± 0.015 [a]	48.8 ± 7.6 [a]	464.9 ± 37.2 [a]
HFDT	0.658 ± 0.044 [b]	0.217 ± 0.011 [b]	28.5 ± 4.8 [b]	257.7 ± 23.3 [b]

Serum enzymes were assayed after one week and serum MDA and fructosamine levels were assayed after three weeks of glycyrrhizin treatment.
For each parameter, $n = 7$ rats.
[a] $P < 0.01$ *versus* NC; [b] $P < 0.01$ *versus* HFD.

Table 5— GHb, free iron in Hb and Hb-mediated oxidative reactions in different groups of rat

[Values are mean ± SD from each group]

Groups	GHb (%)	Free iron in Hb (μg/g Hb)	Arachidonic acid peroxidation (μmol of TBA reactive substance formed/h/mg Hb)	Deoxyribose degradation (Fluorescence intensity in arbitrary unit/mg Hb)
NC	5.90 ± 0.63	96.7 ± 14.9	15.6 ± 6.8	235.3 ± 38.3
HFD	10.07 ± 1.04 [a]	244.1 ± 26.1 [a]	60.3 ± 10.0 [a]	778.2 ± 62.7 [a]
HFDT	6.77 ± 0.34 [c]	147.3 ± 9.0 [b]	25.3 ± 4.7 [b]	416.6 ± 54.6 [b]

The experiments were done after three weeks of glycyrrhizin treatment.
For GHb, $n = 6$ rats and for other parameters $n = 7$ rats.
[a] $P < 0.01$ *versus* NC; [b] $P < 0.01$ *versus* HFD; [c] $P < 0.05$ *versus* HFD.

groups into amino acid residues of proteins. Such oxidative modification is an index of oxidative stress. The extent of carbonyl formation was estimated in Hb samples isolated from different groups of rat. Carbonyl content of Hb samples of HFD rats were significantly higher in comparison with those of NC rats (Fig. 3a). Glycyrrhizin efficiently improved this stress condition by decreasing carbonyl content of Hb samples of HFDT rats. The results obtained with Hb after two weeks of treatment have been presented.

AGEs, formed by long-term glycation of proteins with reducing sugars, lead to free radical generation and oxidative stress. Compared to NC rats, AGE formation was higher in Hb of HFD rats (Fig. 3b). Glycyrrhizin treatment effectively decreased AGE content of Hb by maintaining reduced blood glucose level for three weeks in HFDT rats compared to HFD rats. The treatment thus helps to decrease oxidative stress in metabolic syndrome.

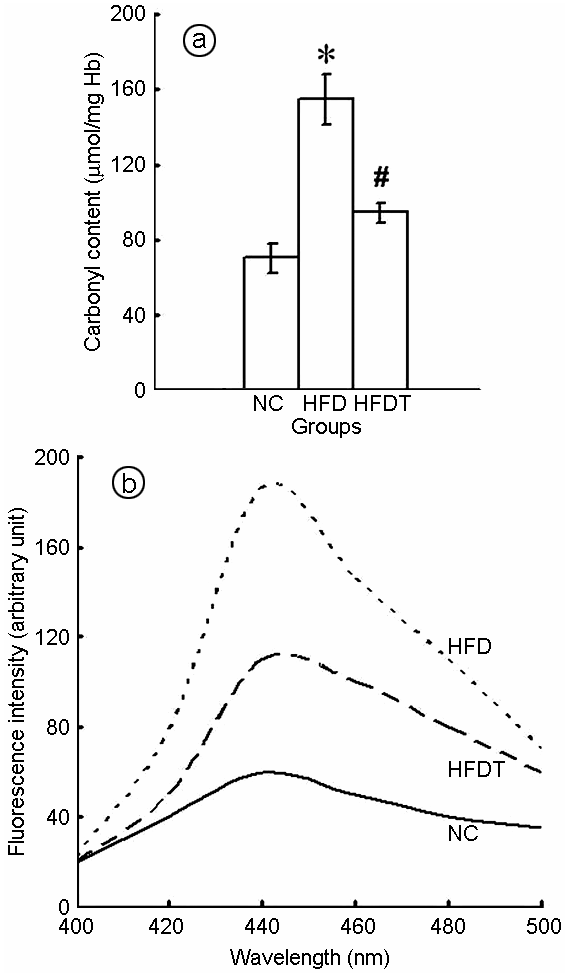


Fig. 3— Oxidative stress markers in Hb of different groups of rat. (a) Levels of carbonyl content in Hb samples from different groups of rat. The results are mean ± SD, $n = 7$ in each group. * $P < 0.01$ *versus* NC; # $P < 0.01$ *versus* HFD. Experiment was done after two weeks of glycyrrhizin treatment. (b) Representative fluorescence emission spectra (excitation 370 nm) showing extent of AGE formation in Hb samples (20 μM) from NC, HFD and HFDT groups of rat. This is the representative spectra of six independent experiments. Experiment was done after three weeks of glycyrrhizin treatment.

To study oxidative stress-mediated DNA damage, single cell gel electrophoresis (comet assay) of lymphocytes was done. Comet formation in the lymphocytes of HFD rats increased significantly in comparison with those of NC rats. However, glycyrrhizin treatment decreased comet formation, as shown in the lymphocytes of HFDT rats (Fig. 4), indicating reduced oxidative damage of DNA in the treated group. The experiment was done after two weeks of glycyrrhizin treatment.

Expression levels of GLUT4 and PPARγ in quadricep muscle extracts of metabolic syndrome group of rats: effect of glycyrrhizin— Western blot experiment (Fig. 5) revealed that the levels of GLUT4 and PPARγ decreased in quadricep muscle extracts of HFD rats, as compared with the levels of NC rats. However, glycyrrhizin treatment of HFD rats caused improvement and increased the proteins toward normal levels of expression. Shih *et al.*[8] have also reported that PPARγ and GLUT4 expressions decrease in skeletal muscle of fructose-fed rats having metabolic syndrome. On the other hand, the finding of Yin *et al.*[14] showing enhanced level of PPARγ in glycyrrhizin-treated normal rats is also in agreement with our observation in HFDT rats. Increased PPARγ expression enhances peroxisomal and mitochondrial β-oxidation, reducing cellular lipid content and insulin resistance[8]. Again, up regulation of PPARγ has been shown to increase GLUT4 expression in skeletal muscle[43], and is consistent with our result. Increased GLUT4 in HFDT rats, in turn, helps to improve cellular insulin sensitivity and glucose homeostasis in metabolic syndrome.

In conclusion, the present findings suggest the beneficial effect of glycyrrhizin against several complications of metabolic syndrome namely, insulin resistance, hyperglycemia, dyslipidemia and oxidative stress. Regulation of GLUT4 and PPARγ expression in skeletal muscle may be one of the mechanisms of glycyrrhizin action. However, it is not yet clear if glycyrrhizin administered intraperitoneally acts as such or through a hydrolyzed/metabolized product. Further studies are also necessary to elucidate the

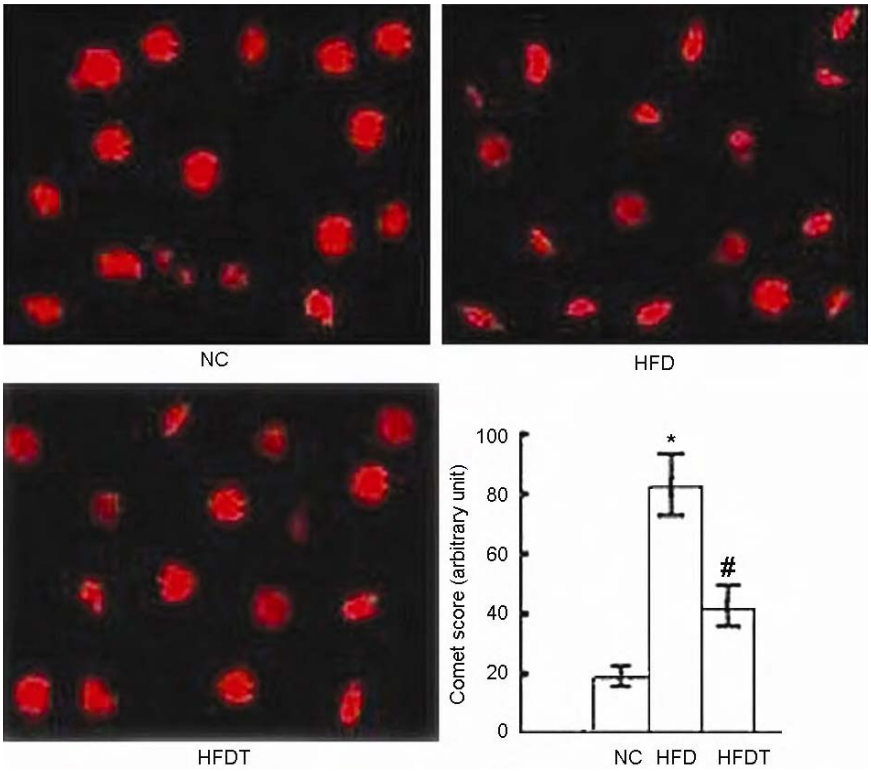


Fig. 4— Effect of glycyrrhizin treatment on DNA damage in lymphocytes. Single cell gel electrophoresis in lymphocytes showing comets (200 magnifications). Comet scores are shown on different groups of rat. The results are mean ± SD of six experiments in each case. * $P < 0.05$ *versus* NC; # $P < 0.05$ *versus* HFD. Experiment was done after two weeks of glycyrrhizin treatment.

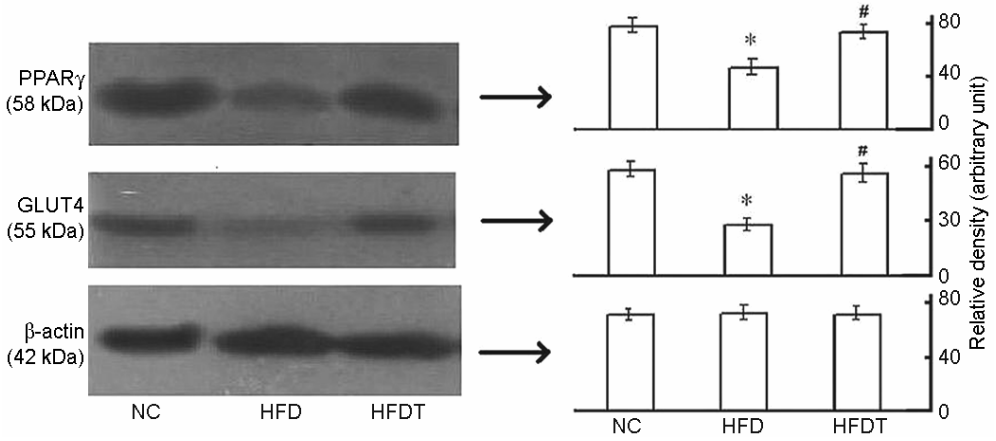


Fig. 5— Expression of PPARγ and GLUT4 in skeletal muscle of different groups of rat. Representative western blot and densitometry data depicting PPARγ and GLUT4 protein in the quadricep muscle of different groups of rat. The densitometry results are mean ± SD of six independent experiments. * $P < 0.05$ *versus* NC; # $P < 0.05$ *versus* HFD.

effect of glycyrrhizin on other underlying mechanisms of the syndrome affecting normal metabolism.

Acknowledgement

RS gets a Fellowship from the University Grants Commission, New Delhi, India. A part of the study was supported by a grant from the Council of Scientific and Industrial Research, New Delhi, India [grant No. 38(1129)/03/EMR-II]. Financial assistance from UGC-DSA and DST-FIST programme of the department is thankfully acknowledged.

References

1 Eckel RH, Grundy SM & Zimmet PZ, The metabolic syndrome, *Lancet,* 365 (2005) 1415.

2 Nyenwe EA, Baddorf SW, Waters B, Wan JY & Solomon SS, Nonalcoholic fatty liver disease and metabolic syndrome in hypopituitary patients, *Am J Med Sci*, 338 (2009) 190.

3 Delbosc S, Paizanis E, Magous R, Araiz C, Dimo T, Cristol JP, Cros G & Azay J, Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat, *Atherosclerosis*, 179 (2005) 43.

4 Dekker MJ, Su Q, Baker C, Rutledge AC & Adeli K, Fructose: A highly lipogenic nutrient implicated in insulin resistance, hepatic steatosis, and the metabolic syndrome, *Am J Physiol Endocrinol Metab*, 299 (2010) 685.

5 Gill H, Mugo M, Whaley-Connell A, Stump C & Sowers JR, The key role of insulin resistance in the cardiometabolic syndrome, *Am J Med Sci*, 330 (2005) 290.

6 Berger J, Biswas C, Vicario PP, Strout HV, Saperstein R & Pilch PF, Decreased expression of the insulin-responsive glucose transporter in diabetes and fasting, *Nature*, 340 (1989) 70.

7 Kuhlmann J, Neumann-Haefelin C, Belz U, Kalisch J, Juretschke HP, Stein M, Kleinschmidt E, Kramer W & Herling AW, Intramyocellular lipid and insulin resistance: a longitudinal *in vivo* 1H-spectroscopic study in Zucker diabetic fatty rats, *Diabetes,* 52 (2003) 138.

8 Shih CC, Lin CH, Lin WL & Wu JB, *Momordica charantia* extract on insulin resistance and the skeletal muscle GLUT4 protein in fructose-fed rats, *J Ethnopharmacol,* 123 (2009) 82.

9 Takii H, Kometani T, Nishimura T, Nakae T, Okada S & Fushiki T, Antidiabetic effect of glycyrrhizin in genetically diabetic KK-Ay mice, *Biol Pharm Bull*, 24 (2001) 484.

10 Sen S, Roy M & Chakraborti AS, Ameliorative effect of glycyrrhizin on streptozotocin-induced diabetes in rats, *J Pharm Pharmacol,* 63 (2011) 287.

11 Kalaiarasi P & Pugalendi KV, Protective effect of 18β-glycyrrhetinic acid on lipid peroxidation and antioxidant enzymes in experimental diabetes, *J Pharm Res,* 4 (2011) 107.

12 Kalaiarasi P, Kaviarasan K & Pugalendi KV, Hypolipidemic effect of 18β-glycyrrhetinic acid on streptozotocin- induced diabetic rats, *Eur J Pharmacol,* 612 (2009) 93.

13 Kalaiarasi P & Pugalendi KV, Antihyperglycemic effect of 18β-glycyrrhetinic acid, aglycone of glycyrrhizin, on streptozotocin-diabetic rats *Eur J Pharmacol,* 606 (2009) 269.

14 Yin CY, Ha TS & Kadir KA, Effects of glycyrrhizic acid on peroxisome proliferator-activated receptor gamma (PPARγ), lipoprotein lipase (LPL), serum lipid and HOMA-IR in rats, *PPAR Res,* (2010) doi: 10.1155/2010/530265.

15 Eu CH, Lim WY, Ton SH & Kadir KA, Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet-induced obese rats, *Lipids Health Dis,* (2010) doi: 10.1186/1476-511X-9-81.

16 Reitman S & Frankel S, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases, *Am J Clin Pathol*, 28 (1957) 56.

17 Thambidorai D & Bachawat BK, Purification and properties of brain alkaline phosphatase, *J Neurochem* 29 (1977) 503.

18 Bergmeyer HU, Gawehn K & Grassl M, Enzymes as biochemical reagents, in *Methods of Enzymatic Analysis* (Academic Press, New York) 1974, 507.

19 Amador E & Wacker W, Serum glutamic oxalacetic transaminase activity. A new modification and an analytical assessment of current assay techniques, *Clin Chem*, 8 (1962) 343.

20 Ellman GL, Tissue sulphydryl groups, *Arch Biochem Biophys*, 82 (1959) 70.

21 Nandhini AT, Balakrishnan SD & Anuradha CV, Taurine improves lipid profile in rats fed a high fructose-diet, *Nutr Res*, 22 (2002) 343.

22 Bhattacharyya J, Bhattacharyya M, Chakraborti AS, Chaudhuri U & Poddar RK, Structural organisations of hemoglobin and myoglobin influence their binding behaviour with phenothiazines, *Int J Biol Macromol,* 23 (1998) 11.

23 Trinder P, Determination of glucose in blood using a glucose oxidase with an alternative oxygen acceptor, *Ann Clin Biochem,* 9 (1969) 24.

24 Friedewald WT, Levy RI & Fredrickson DS, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, *Clin Chem,* 18 (1972) 499.

25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, *Diabetologia,* 28 (1985) 412.

26 Murklund J & Murklund G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay of super oxide dismutase, *Eur J Biochem*, 47 (1974) 469.

27 Beers RF & Sizer IW, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, *J Biol Chem,* 195 (1952) 133.

28 Lowry OH, Rosebrough NJ, Farr AL & Randal RJ, Protein measurement with the Folin phenol reagents, *J Biol Chem*, 193 (1951) 265.

29 Yagi K, Lipid peroxides and human diseases, *Chem Phys Lipids,* 45 (1987) 337.

30 Johnson RN, Metcalf PA & Baker JR, Fructosamine: a new approach to the estimation of serum glycosylation. An index of diabetic control, *Clinica Chimica Acta*, 127 (1982) 87.

31 Panter SS, Release of iron from hemoglobin, *Methods Enzymol*, 231 (1994) 502.

32 Sadrzadeh SM, Graf E, Panter SS, Hallaway PE & Eaton JW, Hemoglobin. A biologic fenton reagent, *J Biol Chem,* 259 (1984) 14354.

33 Kar M & Chakraborti AS, Effect off glycosylation on iron-mediated free radical reactions of hemoglobin, *Current Science,* 80 (2001) 770.

34 Gutteridge JMC, Iron promoters of the Fenton reaction and lipid peroxidation can be released from hemoglobin by peroxides, *FEBS Lett*, 201 (1986) 291.

35 Levine RC, Garland D, Oliver CN, Amiei A, Climent I, Lanz A, Ahn B, Shalleil SO & Stadtman ER, Determination of carbonyl content in oxidatively modified proteins, *Methods Enzymol*, 186 (1990) 464.

36 Wu JT, Tu MC & Zhung P, Advanced glycation end product (AGE): Characterization of the products from the reaction between D-glucose and serum albumin, *J Clin Lab Anal*, 10 (1998) 21.

37 Singh NP, McCoy MT, Tice RR & Schneider EL, A simple technique for quantitation of low levels of DNA damage in individual cells, *Exp Cell Res*, 175 (1998) 184.
38 Collins AR, Dusinka M, Franklin M, Somorovska M, Petrovska H, Fillion I, Pannayiotidis M, Raslova K & Vaughan N, Commet assay in human biomonitoring studies: Reliability, validation and application, *Environ Mol Mutagen*, 30 (1997) 139.
39 Lee YA, Cho EJ & Yokozawa T, Effects of proanthocyanidin preparations on hyperlipidemia and other biomarkers in mouse model of type 2 diabetes, *J Agric Food Chem,* 56 (2008) 7781.
40 Isbrucker RA & Burdock GA, Risk and safety assessment on the consumption of licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin, *Regulatory Toxicol Pharmacol*, 46 (2006) 167.
41 Yamamura Y, Santa T, Kotaki H, Uchino K, Sawada Y & Iga T, Administration-route dependency of absorption of glycyrrhizin in rats: intraperitoneal administration dramatically enhanced bioavailability, *Biol Pharm Bull*, 18 (1995) 337.
42 Zhai D, Zhao Y, Chen X, Guo J, He H, Yu Q, Yang J, Davey AK & Wang J, Protective effects of glycyrrhizin, glycyrrhetic acid and matrine on acute cholestasis induced by α-Napthyl Isothyocyanate in rats, *Planta Med,* 73 (2007) 1.
43 Guo L & Tabrizchi R, Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance, *Pharmacol Ther*, 111 (2006) 145.
44 Kar M & Chakraborti AS, Release of iron from hemoglobin - a possible source of free radicals in diabetes mellitus, *Indian J Exp Biol*, 37 (1999) 190.
45 Sen S, Kar M, Roy A & Chakraborti AS, Effect of nonenzymatic glycation on functional and structural properties of hemoglobin, *Biophys Chem*, 113 (2005) 289.
46 Roy M, Sen S & Chakraborti AS, Action of pelargonidin on hyperglycemia and oxidative damage in diabetic rats: Implication for glycation-induced hemoglobin modification, *Life Sci*, 82 (2008) 1102.
47 Halliwell B & Gutteridge JMC, Role of free radicals and catalytic metal ions in human disease, *Methods Enzymol,* 186 (1990) 1.