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Protective effect of 18b-glycyrrhetinic acid on lipid peroxidation and antioxidant enzymes in experimental diabetes

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ABSTRACT

Chronic hyperglycemia in diabetes is a major causative factor of free radical generation, which further leads to many secondary diabetic complications via the damage to cellular proteins, membrane lipids, nucleic acids and eventually to cell death. Recently we reported the antidiabetic effect of 18ß-glycyrrhetinic acid on diabetic rats. This study was focused on the protective effect of 18ß-glycyrrhetinic acid on lipid peroxidation, activities of both enzymatic and non-enzymatic antioxidants and histopathological examination of pancreas in diabetic rats. Diabetes was induced in adult male albino rats of the Wistar strain, weighing 180–200 g, by administration of streptozotocin (40 mg/kg of body weight) intraperitoneally. The oxidative stress was measured by plasma and tissue lipidperoxidative marker (thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes) levels, non-enzymatic antioxidants (reduced glutathione, vitamin C and vitamin E) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and Glutathione-S-transferase) activities. The increased lipid peroxidation level with reduction in reduced glutathione, vitamin C and vitamin E and decreased enzymatic activities were the salient features observed in diabetic rats. On the other hand, oral administration of 18ß-glycyrrhetinic acid (100 mg/kg day) for 45 days, resulted in a significant reduction in lipid peroxidation level coupled with increased activities of both enzymatic and non-enzymatic antioxidants when compared to diabetic rats. Administration of 18ß-glycyrrhetinic acid in diabetic rats minimized the changes and near normal morphology of pancreas was observed in histopathological examination. 18ß-glycyrrhetinic acid is having a good antioxidant property, as evidenced by increased antioxidants status and decreased lipid peroxidation, which reflects the protective effect of 18ß-glycyrrhetinic acid from the risk of diabetic complications.

Key words: Lipid peroxidation; antioxidants; 18ß-glycyrrhetinic acid; diabetes

INTRODUCTION

Diabetes mellitus, characterized by hyperglycemia, is the most common serious metabolic disorder that is considered to be one of the five leading causes of death in the world. Various studies have shown that diabetes mellitus is associated with oxidative stress, leading to an increased production of reactive oxygen species, including superoxide radical, hydrogen peroxide, and hydroxyl radical or reduction of antioxidant defense system. Implication of oxidative stress in the pathogenesis of diabetes mellitus is suggested not only by oxygen free radical generation but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose, impaired antioxidant enzyme, and formation of peroxides [1]. Lipid peroxidation is a key marker of oxidative stress. It is a free radical-induced process causing oxidative deterioration of polyunsaturated fatty acids that eventually results in extensive membrane damage and dysfunction [2]. The significant extent of lipid peroxidation products that was measured as thiobarbituric acid reactive substances has been reported in diabetes. The formation of reactice oxygen species was prevented by an antioxidant system that included non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants, and reactive oxygen species–scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxides.

Since the synthetic drugs have undesirable side effects or contraindications, the World Health Organization has recommended the evaluation of traditional plant treatments for diabetes. Natural plant drugs are frequently considered to be less toxic with lower side effects than synthetic ones. Licorice (*Glycyrrhizza glabra* L.) and its main water-soluble constituent glycyrrhizin (GL), a pentacyclic triterpene derivative of the L-amyrin type (oleanane), have been widely used as an antidote, demulcent and as a folk medicine for generations in Asia and Europe, and it is currently used as a flavoring and sweetening agent in food products. After oral administration or intravenous injection, glycyrrhizin has been shown to be hydrolyzed by the glucuronidase in intestinal bacteria to its active principal aglycone, 18ß-glycyrrhetinic acid, which is then absorbed into the blood [3]. Glycyrrhizin and 18ß-glycyrrhetinic acid have been shown to possess several beneficial pharmacological activities, which include an antiulcerative effect [4] anti-inflammatory activity [5], direct and indirect antiviral activity [6], interferon inducibility [7], and an antihepatitis effect [8,9,10]. In addition, 18ß-glycyrrhetinic acid can delay the development of autoimmune disease [11] and decrease body fat mass [12]. Recent studies indicate that glycyrrhetinic acid enhanced glucose-stimulated insulin secretion and induced mRNA expression of insulin receptor substrate-2, pancreas duodenum homeobox-1, and glucokinase [13].

We earlier reported the antidiabetic and hypolipidemic effect of 18ß-glycyrrhetinic acid in streptozotocin-diabetic rats [14, 15]. Considerable clinical and experimental evidence now exists to suggest an involvement of free radical mediated oxidative processes in the pathogenesis of diabetic complications. In the present study we have determined the repercussion of diabetes on the defense system against oxidative stress in streptozotocin-diabetic rats and also studied the influence of the treatment with 18ß-glycyrrhetinic acid on the lipid peroxidative markers and antioxidant system.

MATERIALS AND METHODS

Animals

Male albino (9 week-old) rats of Wistar strain with a body weight ranging from 180 to 200 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air conditioned room (25±1 °C) with a 12 h light/12 h dark cycle. Feed and water were provided ad libitum to all the animals. The study protocols were approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Proposal number: 459), Annamalai University, Annamalainagar.

Chemicals

Streptozotocin and 18ß-glycyrrhetinic acid were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All other chemicals used in this study were of analytical grade obtained from E. Merck or HIMEDIA, India.

Experimental induction of diabetes

The animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. Streptozotocin-injected animals exhibited hyperglycemia within a few days. Diabetes in streptozotocin rats was confirmed by measuring the fasting blood glucose (by glucose oxidase method) 96 h after injection with

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streptozotocin. The animals with blood glucose above 235 mg/dL were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into five groups of six animals each as given below. 18ß-glycyrrhetinic acid and glibenclamide were dissolved in 5% dimethyl sulfoxide and administered post orally by intragastric intubations, once in a day in the morning for 45 days.

Group I : Normal (5% dimethyl sulfoxide only)
Group II : Normal+18ß-glycyrrhetinic acid (100 mg/kg body weight)
Group III : Diabetic control (5% dimethyl sulfoxide only)
Group IV : Diabetic + 18ß-glycyrrhetinic acid (100 mg/kg body weight)
Group V : Diabetic + glibenclamide (600 µg/kg body weight).

After 45 days of treatment, the 12 h fasted animals were anaesthetized between 08.00 a.m and 09.00 a.m, using ketamine (24 mg/kg body weight, intramuscular injection) and sacrificed by cervical dislocation. Blood was collected in tubes with ethylenediaminetetra acetic acid for the estimation of plasma lipid peroxidation and antioxidants. Tissue was sliced into pieces and homogenised in appropriate buffer in cold condition (pH 7.0) to give 20% homogenate. The homogenate were centrifuged at 1000 rpm for 10 min at 0 °C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Biochemical analysis

The estimation of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes was done by the methods of Niehaus and Samuelson (1968) [16], Jiang et al. (1992) [17] and Klein (1970), [18] respectively. The levels of vitamins C and E and reduced glutathione were estimated by the methods of Roe and Kuether (1943), [19] Baker et al. (1980) [20] and Ellman (1959), [21] respectively. The activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase were measured by the methods of Kakkar et al. (1978) [22], Sinha. (1972) [23] Rotruck et al. (1973) [24] and Habig et al. (1974), [25] respectively.

Tissue sampling for histopathological study

For histopathological study, three rats from each group were perfused with cold physiological saline, followed by formalin (10% formaldehyde). Pancreas were excised immediately and fixed in 10% formalin.

Statistical analysis

Values are given as means ± S.D. for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at $p = 0.05$ and the values not sharing a common superscript differ significantly.

RESULTS

Tables 1, 2 and 3 show the levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes, respectively in the plasma and tissues of diabetic rats. Diabetic rats had elevated levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes and on treatment with 18ß-glycyrrhetinic acid or glibenclamide decreased these parameters significantly.

Table 1. Effect of 18ß-glycyrrhetinic acid on thiobarbituric acid reactive substances in the plasma and tissues of STZ-diabetic rats

Groups	Plasma TBARS (mmol/dL)	Tissue TBARS (mmol/100 g wet tissue)		
		Liver	Kidney	Heart
Control	0.23 ± 0.02^{a}	0.85 ± 0.07^{a}	1.44 ± 0.08^{a}	0.60 ± 0.05^{a}
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	0.20 ± 0. 01^{a}	0.83 ± 0.06^{a}	1.40 ± 0.11^{a}	0.59 ± 0.04^{a}
Diabetic control	0.38 ± 0.02^{b}	3.09 ± 0.29^{b}	3.90 ± 0.31^{b}	1.82 ± 0.12^{b}
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	0.29 ± 0.02^{c}	1.47 ± 0.10^{c}	1.90 ± 0.14^{c}	0.78 ± 0.05^{c}
Diabetic + glibenclamide (600 µg /kg BW)	0.25 ± 0.02^{ac}	0.93 ± 0.07^{d}	1.57 ± 0.11^{d}	0.66 ± 0.06^{a}

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 2. Effect of 18ß-glycyrrhetinic acid on lipid hydroperoxides in the plasma and tissues of STZ-diabetic rats

Groups	Plasma LOOH (mmol/dL)	Tissue lipid hydroperoxide (mmol/100 g wet tissue)		
		Liver	Kidney	Heart
Control	10.05 ± 0.82^{a}	74.42 ± 7.12^{a}	67.85 ± 5.97^{a}	74.76 ± 7.02^{a}
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	9.52 ± 0.92^{a}	70.23 ± 5.97^{a}	65.90 ± 5.83^{a}	73.80 ± 5.83^{a}
Diabetic control	22.73 ± 1.05^{b}	121.42 ± 10.10^{b}	166.66 ± 13.29^{b}	146.42 ± 11.73^{b}
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	12.09 ± 1.03^{c}	85.71 ± 7.82^{c}	81.33 ± 7.05^{c}	86.23 ± 7.37^{c}
Diabetic + glibenclamide (600 µg /kg BW)	11.35 ± 0.97^{d}	79.19 ± 5.83^{a}	72.61 ± 7.15^{a}	80.90 ± 6.35^{ac}

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 3. Effect of 18ß-glycyrrhetinic acid on conjugated dienes in the plasma and tissues of STZ-diabetic rats

Groups	Plasma Conjugated dienes (mmol/dL)	Tissue conjugated dienes (mmol/100 g wet tissue)		
		Liver	Kidney	Heart
Control	0.69 ± 0.04^{a}	72.07 ± 6.54^{a}	19.34 ± 1.92^{a}	42.16 ± 3.20^{a}
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	0.65 ± 0.05^{a}	68.12 ± 4.93^{a}	18.17 ± 1.30^{a}	39.44 ± 2.31^{a}
Diabetic control	0.93 ± 0.07^{b}	106.42 ± 9.09^{b}	36.85 ± 2.45^{b}	69.79 ± 5.45^{b}
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	0.76 ± 0.05^{c}	87.01 ± 6.27^{c}	25.17 ± 1.47^{c}	53.07 ± 4.37^{c}
Diabetic + glibenclamide (600 µg /kg BW)	0.72 ± 0.04^{ac}	80.84 ± 7.35^{d}	22.19 ± 1.54^{c}	47.19 ± 3.56^{a}

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 4. Effect of 18ß-glycyrrhetinic acid on vitamin C in the plasma and tissues of STZ-diabetic rats

Groups	Plasma Vitamin C (mg/dL)	Tissue Vitamin C (µg/mg protein)		
		Liver	Kidney	Heart
Control	2.19 ± 0.20^{a}	0.88 ± 0.06^{a}	0.79 ± 0.06^{a}	0.47 ± 0.04^{a}
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	2.26 ± 0.18^{a}	0.92 ± 0.07^{a}	0.81 ± 0.08^{a}	0.52 ± 0.04^{a}
Diabetic control	0.82 ± 0.06^{b}	0.42 ± 0.04^{b}	0.50 ± 0.05^{b}	0.23 ± 0.02^{b}
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	1.78 ± 0.13^{c}	0.79 ± 0.07^{c}	0.70 ± 0.05^{a}	0.39 ± 0.03^{c}
Diabetic + glibenclamide (600 µg /kg BW)	1.94 ± 0.08^{d}	0.83 ± 0.07^{a}	0.74 ± 0.06^{a}	0.44 ± 0.04^{a}

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 5. Effect of 18ß-glycyrrhetinic acid on vitamin E in the plasma and tissues of STZ-diabetic rats

Groups	Plasma Vitamin E (mg/dL)	Tissue Vitamin E(µg/mg protein)		
		Liver	Kidney	Heart
Control	1.99 ± 0.13^{a}	5.84 ± 0.42^{a}	3.81 ± 0.27^{a}	3.86 ± 0.26^{a}
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	1.92 ± 0.10^{a}	5.93 ± 0.36^{a}	3.92± 0.15^{a}	3.94 ±.0.28^{a}
Diabetic control	3.03 ± 0.26^{b}	3.76 ± 0.11^{b}	1.58 ± 0.09^{b}	1.80 ± 0.09^{b}
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	2.35 ± 0.19^{c}	4.68 ± 0 .39^{c}	2.96 ± 0.11^{c}	2.63 ± 0.10^{c}
Diabetic + glibenclamide (600 µg /kg BW)	2.19 ± 0.13^{d}	4.95 ± 0.40^{d}	3.53 ± 0.25^{a}	3.28 ± 0.25^{d}

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

The levels of vitamin C, vitamin E and reduced glutathione in the plasma and tissues of diabetic rats are given in table 4, 5 & 6, respectively. Diabetic rats showed increased level of vitamin E in plasma and decreased levels of vitamin C and reduced glutathione in the plasma and tissues. Treatment with 18ß-glycyrrhetinic acid or glibenclamide prevented the above changes in diabetic rats and improved towards normal levels.

Tables 7, 8, 9 & 10 show the activities of catalase, superoxide dismutase

Table 6. Effect of 18ß-glycyrrhetinic acid on reduced glutathione in the plasma and tissues of STZ-diabetic rats

Groups	Plasma GSH (mg/dL)	Tissue reduced glutathione (µg/mg protein)		
		Liver	Kidney	Heart
Control	30.04 ±1.98[a]	13.12 ± 1.25[a]	12.04 ± 0.94[a]	10.03 ± 0.93[a]
Control + 18ß-glycyrrhetinic acid (100 mg/kg BW)	32.00 ± 2.95[a]	13.52 ± 1.17[a]	12.17 ± 1.19[a]	10.22 ± 0.98[a]
Diabetic control	15.37 ±1.28[b]	07.93 ± 0.54[b]	06.81 ± 0.44[b]	05.16 ± 0.45[b]
Diabetic + 18ß-lycyrrhetinic acid (100 mg /kg BW)	23.04 ±1.15[c]	11.60 ± 1.04[c]	10.63 ± 0.93[c]	8.82 ± 0.74[c]
Diabetic + glibenclamide (600 µg /kg BW)	27.66 ± 2.59[ac]	12.71 ± 1.18[d]	11.48 ± 1.06[d]	9.45 ± 0.59[d]

Values are given as means ± S.D. from six rats in each group.
Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).

Table 7. Effect of 18ß-glycyrrhetinic acid on the activity of catalase in the tissues of STZ-diabetic rats

Groups	Catalase (U*/mg protein)		
	Liver	Kidney	Heart
Control	81.10 ± 5.65[a]	40.73 ± 2.45[a]	51.41 ± 3.87[a]
Control +18ß-glycyrrhetinic acid (100 mg/kg BW)	84.24 ± 6.79[a]	43.74 ± 1.99[a]	54.64 ± 4.99[a]
Diabetic control	49.97 ± 4.41[b]	19.71 ± 1.12[b]	30.01 ± 3.04[b]
Diabetic+18ß-glycyrrhetinic acid (100 mg /kg BW)	68.65 ± 4.59[c]	32.56 ± 3.17[c]	42.94 ± 3.86[c]
Diabetic + glibenclamide (600 µg /kg BW)	75.22 ± 5.68[d]	37.41 ± 3.38[a]	47.40 ± 4.55[d]

Values are given as means ± S.D. from six rats in each group. Values not sharing a common superscript differ significantly at p < 0.05. Duncan's Multiple Range Test (DMRT).U* = µmol of hydrogen peroxide consumed/minute

Table 8. Effect of 18ß-glycyrrhetinic acid on the activity of superoxide dismutase in the tissues of STZ-diabetic rats

Groups	Superoxide dismutase (U*/mg protein)		
	Liver	Kidney	Heart
Control	9.65 ± 0.63[a]	14.62 ± 1.08[a]	5.39 ± 0.39[a]
Control +18ß-glycyrrhetinic acid (100 mg/kg BW)	9.02 ± 0.78[a]	14.30 ± 1.12[a]	5.51 ± 0.51[a]
Diabetic control	4.52 ± 0.30[b]	6.91 ± 0.52[b]	2.61 ± 0.23[b]
Diabetic +18ß- glycyrrhetinic acid (100 mg /kg BW)	7.89 ± 0.62[c]	12.14 ± 1.19[c]	4.71 ± 0.39[c]
Diabetic + glibenclamide (600 µg /kg BW)	8.91 ± 0.77[d]	13.51 ± 1.20[ac]	5.06 ± 0.49[a]

Values are given as means ± S.D. from six rats in each group.Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).U* = Enzyme concentration required for 50% inhibition of NBT reduction/minute.

Table 9. Effect of 18ß-glycyrrhetinic acid on the activity of glutathione peroxidase in the tissues of STZ-diabetic rats

Groups	Glutathione peroxidase (U*/mg protein)		
	Liver	Kidney	Heart
Control	11.03 ± 1.01[a]	8.39 ± 0.72[a]	6.15 ± 0.46[a]
Control +18ß-glycyrrhetinic acid (100 mg/kg BW)	10.85 ± 1.02[a]	8.18 ± 0.77[a]	6.01 ± 0.60[a]
Diabetic control	4.96 ± 0.33[b]	4.04 ± 0.42[b]	3.19 ± 0.25[b]
Diabetic +18ß-glycyrrhetinic acid (100 mg /kg BW)	9.46 ± 0.77[c]	6.30 ± 0.44[c]	5.11 ± 0.43[c]
Diabetic + glibenclamide (600 µg /kg BW)	10.37 ± 0.73[a]	7.66 ± 0.53[d]	5.86 ± 0.49[d]

Values are given as means ± S.D. from six rats in each group.Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT).U*= µmol of GSH utilized/minute

Table 10. Effect of 18ß-glycyrrhetinic acid on the activity of glutathione-S-transferase in the tissues of STZ-diabetic rats

Groups	Glutathione-S-transferase (U*/mg protein)		
	Liver	Kidney	Heart
Control	6.94 ± 0.69[a]	5.81 ± 0.37[a]	5.40 ± 0.46[a]
Control +18ß-glycyrrhetinic acid (100 mg/kg BW)	7.12 ± 0.34[a]	6.03 ± 0.54[a]	5.52 ± 0.50[a]
Diabetic control	3.01 ± 0.30[b]	2.96 ± 0.14[b]	2.24 ± 0.15[b]
Diabetic +18ß- glycyrrhetinic acid (100 mg /kg BW)	5.43 ± 0.47[c]	4.89 ± 0.44[c]	4.88 ± 0.43[c]
Diabetic + glibenclamide (600 µg /kg BW)	6.11 ± 0.53[a]	5.57 ± 0.53[a]	5.06 ± 0.49[a]

Values are given as means ± S.D. from six rats in each group.Values not sharing a common superscript differ significantly at $p < 0.05$. Duncan's Multiple Range Test (DMRT). U* = µg of CDNB conjugate formed/minute

glutathione peroxidase and glutathione-S-transferase in the tissues of diabetic rats. Diabetic rats had decreased activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the tissues and treatment with 18ß-glycyrrhetinic acid or glibenclamide increased the activities significantly.

Histopathological changes

Fig 1. Pancreas (H&E, 100X)

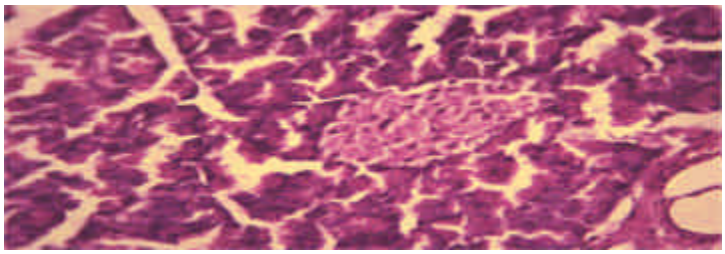

Fig 1a. Normal pancreatic islet cells

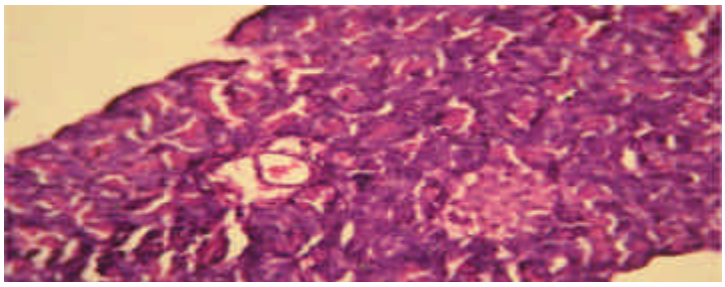

Fig 1b. Rats treated with 18ß-glycyrrhetinic acid showing normal islets cells

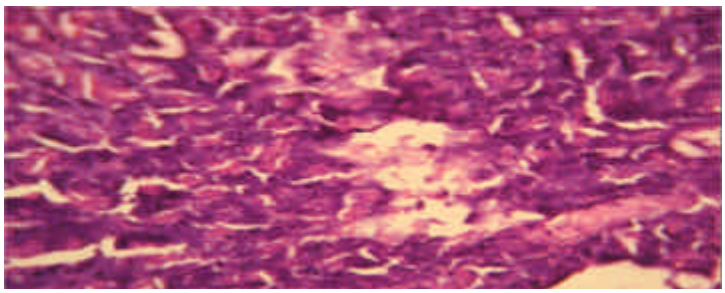

Fig 1c. Diabetic control rats showing fatty infiltration and shrinkage of islets cells

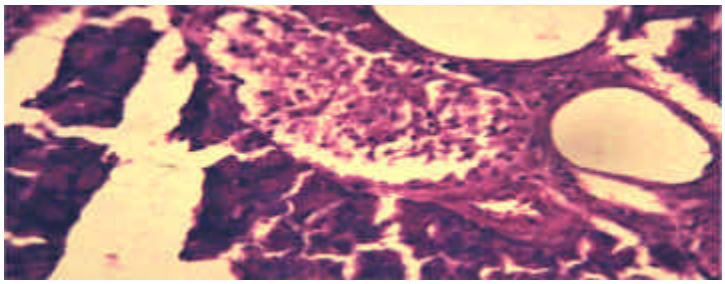

Fig 1d. Diabetic rats treated with 18ß-glycyrrhetinic acid showing reduced fatty infiltration and normal islets cells

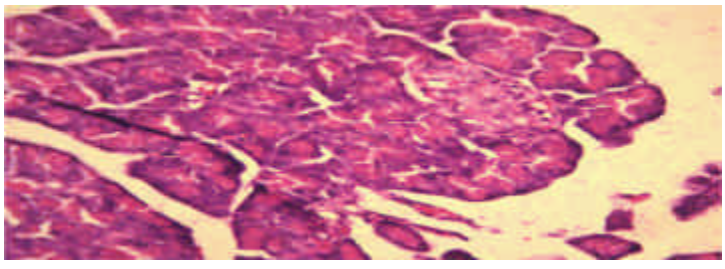

Fig 1e. Diabetic rats treated with glibenclamide showing no fatty changes and normal pancreatic islets

In our study, histopathological examination of diabetic pancreas (fig 1.) showed fatty infiltration and shrinkage of islet cells. Administration of 18ß-glycyrrhetinic acid showed reduced fatty infiltration and normal islet cells in pancreas, which supports the biochemical analysis.

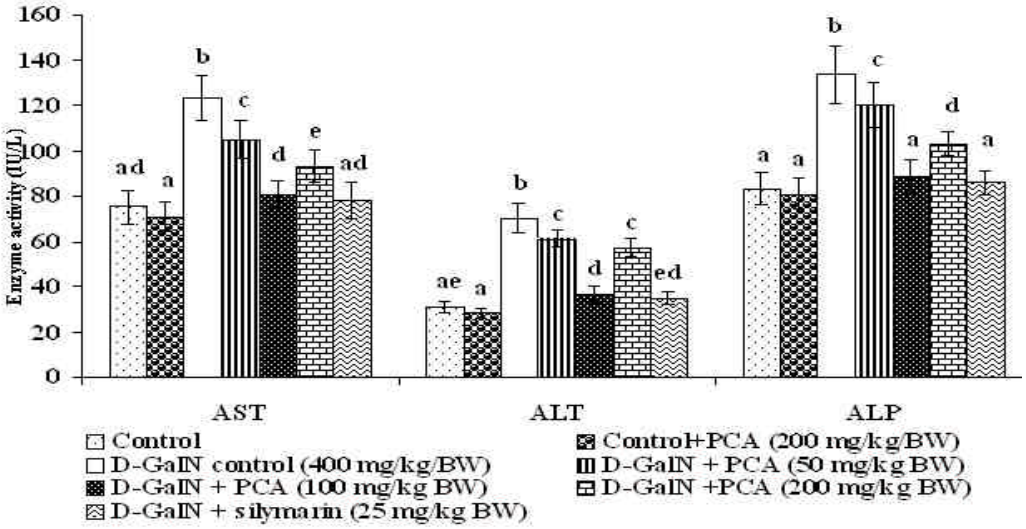

Fig. 2A Effect of PCA on AST, ALT, ALP in the serum of D-Galactosamine-hepatotoxic and control rats.

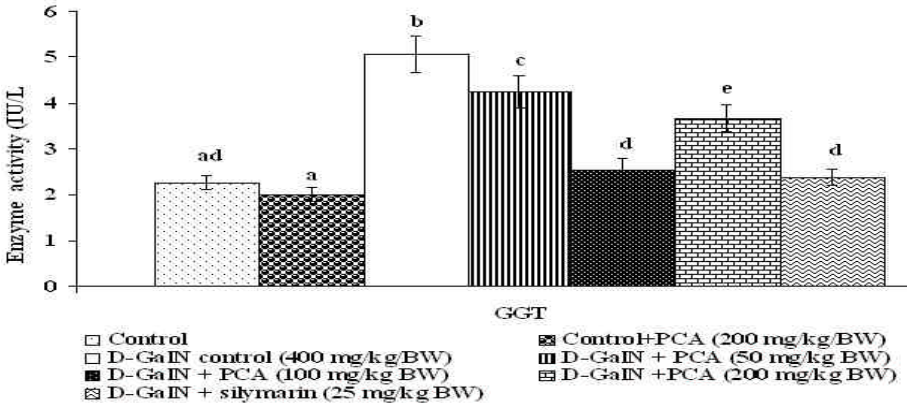

Fig. 2B Effect of PCA on GGT in the serum of D-Galactosamine-hepatotoxic and control rats.

DISCUSSION

Numerous studies have demonstrated that oxidative stress is a key pathogenic factor in the development of diabetic complications. Oxidative stress induces the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides. However, endogenous antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) are responsible for the detoxification of the deleterious oxygen species [26]. In agreement with other studies [27, 28], we have observed a significant increase in the levels of lipid peroxidative markers in the plasma and tissues of diabetic rats and administration of 18ß-glycyrrhetinic acid to diabetic rats significantly has decreased the levels of these lipid peroxidative markers. This observation demonstrates the antiperoxidative and antioxidant effects of 18ß-glycyrrhetinic acid. In this respect, [29] also reported the 18ß-glycyrrhetinic acid ability to scavenge free radicals and inhibit lipid peroxidation.

Superoxide dismutase and catalase are the two major scavenging enzymes that remove radicals *in vivo*. Superoxide dismutase can catalyze dismutation of $O_2^{\cdot-}$ into H_2O_2, which is then deactivated to H_2O by catalase or glutathione peroxidase [30]. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which, in turn, generate hydroxyl radicals ($^{\cdot}OH$), resulting in initiation and propagation of lipid peroxidation. Glutathione peroxidase has a key role in enzymatic defense systems and reduces organic peroxides (H_2O_2, lipid or organic peroxides) into their corresponding alcohols. The decrease may be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes [31]. GSH-metabolizing enzymes, glutathione peroxidase and glutathione-S-transferase work in concert with glutathione in the decomposition of hydrogen peroxide and other organic hydroperoxides to non-toxic products, respectively, at the expense of reduced glutathione. Reduced activities of glutathione peroxidase and glutathione-S-transferase were due to inactivation of these enzymes by reactive oxygen species. 18ß-glycyrrhetinic acid augmented the activities of antioxidant enzymes in streptozotocin-treated rats by inhibiting lipid peroxidation. The ability of 18ß-glycyrrhetinic acid to enhance the levels of antioxidants along with its antilipid–peroxidative activity suggest that this compound might be potentially useful in counteracting free radical mediated injuries involved in the development of tissue damage caused by streptozotocin-diabetic rats.

Apart from the enzymatic antioxidants, nonenzymatic antioxidants such as vitamin C and E, reduced glutathione play an excellent role in preventing the cells from oxidative threats. Vitamin E is the most ancient antioxidant in the lipid phase [32]. In our study, vitamin E was increased in diabetic rats, which could be due to increased membrane damage by reactive oxygen species. Treatment with 18ß-glycyrrhetinic acid and glibenclamide brought vitamin E to near normal levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation.

Vitamin C and vitamin E are interrelated by recycling process [33]. Recycling of tocopheroxyl radicals to tocopherol is achieved with vitamin C [34]. In our study, vitamin C was decreased in diabetic rats as reported earlier [35]. The decreased level of ascorbic acid in diabetic rats may be due to either increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in glutathione level, since glutathione is required for the recycling of vitamin C [36]. Treatment with 18ß-glycyrrhetinic acid and glibenclamide significantly improved the vitamin C to near normal levels which could be due to decreased utilization.

Glutathione is a major non-protein thiol in living organisms which plays a

central role in co-ordinating the antioxidant defense process in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reaction [37]. Reduced glutathione functions as free radical scavenger and in the repair of free radical caused biological damage [38]. Reduced glutathione is required for the recycling of vitamin C and acts as a substrate for glutathione peroxidase and glutathione-S-transferase that are involved in preventing the deleterious effect of oxygen radicals. In our study, diabetic rats exhibited a decreased level of reduced glutathione which might be due to increased utilization for scavenging free radicals and increased consumption by glutathione peroxidase and glutathione-S-transferase. Treatment with 18ß-glycyrrhetinic acid and glibenclamide significantly improved reduced glutathione level in the plasma and tissues of diabetic rats which could be due to decreased utilization as lipid peroxidation is low.

Thus, 18ß-glycyrrhetinic acid is having a good antioxidant property, as evidenced by increased antioxidants status and decreased lipid peroxidation, which reflects the protective effect of 18ß-glycyrrhetinic acid from the risk of diabetic complications.

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