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Full Length Research Paper

Protective effect of *Pedicularis decora* Franch root extracts on oxidative stress and hepatic injury in alloxan-induced diabetic mice

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Pedicularis decora (P. decora) **has been known to have antioxidative effect, but the effect of its successive extracts has not been well studied. Our objective was to investigate the effect of *n*-butanol and water extracts of *P. decora* Franch root on the oxidative stress and hepatic injury in alloxan-induced diabetic mice. Diabetes was induced in ICR mice by a single alloxan injection (150 mg kg^{-1} b. w., i.p.). After diabetes was induced, the mice were treated with *n*-butanol and water extracts of *P. decora* Franch root, α-tocopherol and saline for three weeks. Treatment with α-tocopherol, *n*-butanol and water extract of *P. decora* Franch root caused considerable gain in body and liver weight, glucose, liver glycogen and serum insulin concentration. The two extracts of *P. decora* Franch and α-tocopherol were found to show hepatoprotective effect by lowering down the contents of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol and triglyceride and elevating HDL cholesterol in serum in alloxan induced diabetic mice. Treatment with the two extracts of *P. decora* Franch and α-tocopherol significantly lowered oxidative stress biomarkers such as lipid peroxidation (LPO) levels in serum and in liver tissue, nitric oxide (NO) levels in serum and catalase (CAT) activities in liver tissue against alloxan treated mice. Reduced glutathione (GSH) and hepatic enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST) as well as serum total antioxidant activity (TAA) were significantly increased by treatment with the two extracts and α-tocopherol, against alloxan treated mice. Based on these results, it was observed that α-tocopherol, *n*-butanol and water extract of *P. decora* Franch root administration could counteract alloxan induced the diabetic symbols and inhibit oxidative stress and hepatic injury. Additionally, *n*-butanol extract was more pronounced on the parameters studied of the oxidative stress and hepatic injury. The *n*-butanol extract is more protective than water extract suggests that *n*-butanol extract may better ameliorate the harmful effects in alloxan-induced mice.**

Key words: *Pedicularis decora* Franch, *n*-butanol extract, water extract, hepatic injury, oxidative stress.

INTRODUCTION

Oxidative stress is involved in the pathogenesis of diabetes complications in animals or humans (Baynes and Thorpe, 1999). Further, excessive oxidative stress causes exceeding free radicals product especially reactive oxygen species (ROS). ROS has been known to produce cellular and tissue injury through covalent binding, DNA strand breaking, lipid peroxidation (LPO) and augment fibrosis. This oxidative damage is an important etiological factor implicated in several human diseases such as cancer, inflammatory disorders, as well as in the ageing processes (Sirnivasan et al., 2007; Paradies et al., 2011). However, antioxidants such as antioxidant enzymes and nonenzymatic antioxidants, which provide protection to living organisms, have

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reduced the potential deleterious effect of ROS (Kayali and Tarhan, 2006).

Diabetes mellitus is a chronic metabolic disorder and is always accompanied with an increased generation of free radicals especially ROS (Okutana et al., 2005). Alloxan is a hydrophilic compound and a commonly used chemical to generate diabetic animal models in the laboratory. Alloxan is catalyzed by thioredoxin (thioltransferase), or the nicotinamide adenine dinucleotide phosphate: cytochrome P-450 reductase to dialuric acid. In these reactions, H_2O_2, •OH and superoxide radical are generated via iron catalyst (Mathews and Leiter, 1999). Therefore, it is generally accepted that alloxan can function as a potent generator of oxidative stress exhibited excessive production of ROS and is key to its role as a diabetogen (Roja et al., 2005; Zhang et al., 2009).

In recent years, the identification of plant products with antioxidant property has received considerable attention. As a folk medicinal herb, *Pedicularis decora* Franch (Family: *Orobanchaceae*) is used in traditional Chinese medicine as tonics for the treatment of general debility, collapse, exhaustion, spontaneous sweating, seminal emission and senility and to invigorate the mind and the circulation of blood (Wang et al., 1996). Previous studies have shown that *P. decora* Franch crude extract had an antioxidant effect in the identical model of alloxan-induced diabetic mice (Yang et al., 2001). The present study was undertaken to investigate the effect of *n*-butanol and water extracts of *P. decora* Franch on the oxidative stress and hepatic injury in alloxan-induced diabetic mice.

MATERIALS AND METHODS

Chemicals

Alloxan tetrahydrate was purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade and were purchased locally.

Plant material and extracts

P. decora Franch root was collected in December 2008, at Taibai Mountain, in the region of Shaanxi province, China and authenticated through Institute of Botany, Chinese Academy of Sciences, Beijing, China. A voucher specimen of the root is deposited in the School of Life Science and Technology, Xi'an Jiaotong University, China.

The root of *P. decora* Franch was dried at room temperature and crushed into a coarse powder. The powdered roots (800 g) were extracted with 95% ethanol three times at room temperature. The ethanolic root extract was filtered using Whatmann filter paper no. 1 and evaporated to dryness over a water bath at 50 to 60°C. The ethanolic extract yielded a dark yellowish sticky semisolid, weighing 126 g. This crude extract was dissolved in *n*-ButOH – H_2O (1:1) and successively given the *n*-ButOH and water extracts. The solvent was completely removed by rotary vacuum evaporator. The *n*-ButOH and water extracts yielded yellowish and dark umber semisolid residues, weighing 32 and 49 g, respectively. The extracts were preserved in a refrigerator for further use.

Animals and experiment design

ICR mice (18 to 22 g) were purchased from the Experimental Animal Center of Xi'an Jiaotong University, Shaanxi Province, China. The mice were bred in our animal facility with controlled temperature (18 to 22°C), humidity (50 to 60%) and photoperiod (12:12h light/dark cycle). Animals were fed with a commercial balanced diet (Xi'an Ltd., Shaanxi) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals prepared by Xi'an Jiaotong University.

Seven days after acclimatization, for the diabetes induction, mice were kept on fasting prior to alloxan injection. Alloxan tetrahydrate was freshly dissolved in saline and maintained on ice prior to use. Four groups of the animals were treated with a single injection of alloxan (150 mg/kg b.w., i.p.), whereas the control animals received a single intraperitoneal injection of the same volume of saline. The animals with glucose levels >11.1 mmol/L were considered diabetic after 1 week of alloxan injection.

All the mice were divided into five groups with ten mice each. The previous control mice served as Group I and were orally gavaged with the normal saline alone (0.9% NaCl, 0.1 ml per day p.o.) for three weeks. The previous four groups of diabetic animals were further divided into the following groups: Group II animals served as model group and received an equal volume of saline alone (0.9% NaCl, 0.1 ml per day p. o.); Group III animals were orally gavaged with standard antioxidant, α-tocopherol (25 mg/kg b. w., p. o.) for three weeks. According to the previous study, the crude ethanolic extract (15 g/kg b. w., p. o.), had an antioxidant effect at the same dose of alloxan-induced diabetic mice (Yang et al., 2001). So, Groups IV and Groups V animals were orally gavaged with *n*-ButOH extract (15 g/kg b. w., p. o.) and with water extract (15 g/kg b. w., p. o.), respectively. The four groups were also treated for three weeks.

Preparation of blood and tissue homogenate

Blood samples were collected from the overnight fasted mice at the end of the study and serum was separated after centrifuged at 4000 rpm at 4°C for 10 min. After blood sample collection, the liver of the mice were removed and immediately frozen in liquid nitrogen and kept at – 80°C for further study. The liver tissues were homogenized in 50 mM phosphate buffer solution (pH 7.4) using a tissue Homogenizer at 4°C. The supernatant was collected after centrifuged at 1,000 g for 5 min and stored at – 80°C for further analyses.

Assay of LPO and antioxidant biomarkers and glycogen in liver

LPO was assayed according to the method of Wright et al. (1981). The reaction mixture, in a total volume of 1.0 ml, contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml of tissue homogenate (10 %, w/v), 0.2 ml ascorbic acid (100 mM), 0.02 ml ferric chloride (100 mM) and was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by the addition of 1.0 ml trichloro acetic acid (10%, w/v). Followed by adding 1.0 ml thiobarbituric acid (0.67%, w/v) and all the tubes were placed in a boiling water bath for 20 min. At the end, the tubes were shifted to an ice-bath and centrifuged at 2500 g for 10 min. The amount of malonaldehyde formed in each of the samples was assessed by measuring the optical density of the supernatant at 532 nm against a reagent blank.

Superoxide dismutase (SOD) activity was determined by the method described by Misra and Fridovich (1972). Assay mixture

contained 0.05 M carbonate buffer (pH 10.2), 0.1 mmol EDTA, 30 mmol epinephrine and 0.05 % acetic acid. Reaction was started by the addition of tissue homogenate and absorbance changes were measured at 480 nm for 4 min. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which was equivalent to one unit and was expressed in terms of units/mg protein.

Catalase (CAT) activity was assayed using the method described by Claiborne (1986). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate (pH 7.0), 19 mM H_2O_2, and a 100^{-1} sample. The reaction was initiated by the addition of H_2O_2 and the absorbance changes were measured at 240 nm (25°C) for 30 s. Results are expressed as K of CAT activity per gram protein. Glutathione peroxidase (GPx) activity was measured according to with a slight modification. The reaction mixture contained GSH, NaN_3-PBS pH = 6.5, H_2O_2, trichloroacetic acid (TCA), Na_2HPO_4, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance at 423 nm was monitored on a UV-220 spectrophotometer at 37°C. One unit of enzyme activity was defined as the amount of enzyme that oxidized 1mol/l of GSH per min per mg of protein (Hafeman et al., 1973).

Reduced glutathione (GSH) content of tissues was determined by using the method described by Jollow et al. (1974). The assay is based on the development of a stable color complex with 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB). The color was read at 412 nm. Glutathione-s-transferase (GST) activities were determined as described by Habig and Jakoby (1974). The reaction mixture contained 0.1 M Na phosphate (pH 6.5), 30 mM GSH, 30 mM 1-chloro-2, 6-dinitrobenzene and hepatic tissue homogenate. The optical density was measured at 340 nm with a spectrophotometer.

Glutathione reductase (GR) activity was assayed by the method of Carlberg and Mannervik (1975). The assay system consisted of 1.65 ml sodium phosphate buffer (0.1 M; pH 7.4), 0.1ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1 mM), 0.1mL NADPH (0.1 mM) and 0.05 ml supernatant in a total mixture of 2 ml. The enzyme activity was quantified by measuring the disappearance of NADPH at 340 nm at 30 s intervals for 3 min. The activity was calculated using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1} and expressed as nanomoles NADPH oxidized/min/mg protein.

Nitric oxide (NO) release can be determined spectrophotometrically by measuring accumulation of nitrites, based on the method of Hortellano et al. (1995). In short, NO is oxidased to form nitrate and nitrite. Nitrate is reduced to nitrite by NADPH in the presence of the enzyme nitrate reductase. Then the Griess reagent (1% sulfanilic acid, 0.1% 1-naphthol, 5% H_3PO_4) was used for colorimetric determination. The absorbance was read at 540 nm and sodium nitrite was used as standard.

Total antioxidant activity (TAA) was determined by the method of Koracevic et al. (2001). This assay measured capacity of biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) formed from sodium benzoate, under the influence of ROS. The optical density was measured at 532 nm and 1 mmol/l uric acid was used as standard.

Glycogen contents were determined as previously described by Lo et al. (1970). Briefly, partial liver samples were collected immediately, cut, weighed, and boiled for 30 min in 1 ml of 30% KOH saturated with Na_2SO_4. Once tissues were completely digested, glycogen was precipitated with 2 ml of 95% ice-cold ethanol and incubated on ice for 20 min. Tubes were spun for 30 min at 500 g. The pellets were resuspended in 1 ml H_2O, and 1 ml of 5% phenol was added. A colorimetric reaction was obtained by adding 5 ml of sulfuric acid. After incubation on ice for 30 min, absorbance was determined at a wavelength of 490 nm.

Biomarkers of blood assay

Glucose was determined in the serum based on the hexokinase method (Bernd and Benno, 1973). Briefly, glucose-6-phosphate, formed as a product of the hexokinase catalyzed, was made to react with NDA^+ to generate NADH, which has absorption maxima at 340 nm. γ-Glutamyl transferase (GGT) was estimated as described by Rosalki and Rau (1972) in which the p-nitroaniline liberated by the enzyme in the presence of L-γ-glutamyl-*p*-nitroanilide, produces a yellow colour, which was estimated spectrophotometrically at 410 nm.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method of Wooten (1964). The method is based on the ability of the enzymes to form pyruvate, which reacts with 2, 4-dinitrophenylhydrazine in hydrochloric acid. The hydrazone thus formed turns into an orange complex in alkaline medium, which was measured at 540 nm.

Alkaline phosphatase (ALP) was measured according to King (1965). Disodium phenyl phosphate was catalyzed by ALP (at pH 10) and the phenol was liberated. Then reaction with 4-aminoantipyrine (4-AAP), ferripotassium cyanide and the absorbance measurement at 500 nm was used for quantization. Lactate dehydrogenase (LDH) assay conditions were based on those described earlier (Bass et al., 1969). The assay medium for LDH contained 50 mM Tris-HCl buffer pH 7.5, 0.15 mM NADH and 1 mM pyruvate (omitted for control). The absorptivity of NADH at 340 nm was 6.22 µM/ cm.

Total cholesterol was measured in the serum based on the CHOD-PAP method (Trinder, 1969). In this method, cholesterol was oxidased to hydrogen peroxide (H_2O_2) when catalyzed by cholesterol oxide (CO) in the presence of oxygen (O_2). Then, H_2O_2 was made to react with N-ethyl-N-(3-sulfopropyl)-manisidine (ESPAS) and 4-aminoantipyrine (4-AA-P) to generated red quinone imine compound, which was measured spectrophotometrically at 570 nm. In addition, triglyceride was assayed in the serum based on the GPO-PAP method (Trinder, 1969). Similary, the method is also based on the final product of quinone imine compound in the presence of H_2O_2. HDL-cholesterol estimation was based on the immunoinhibition method, enzymatic color test (Huang et al., 1997). The serum sample was diluted 10-fold with phosphate-buffered saline by the analyzer. The reaction mixture contained R_1 (antibody reagent) and R_2 (cholesterol reagent) components. The absorbance measurement at 600 nm was used for quantization.

Concentration of insulin was measured in serum using a commercially available radioimmunoassay (RIA) kit (Germany). The intra-day precision of the assay was estimated at 5.4 to 6.0% (Herbert et al., 1965).

Statistical analysis

Results are expressed as mean ± S.D. For comparisons between the groups, data were tested by one-way ANOVA; $p < 0.05$ was considered statistically significant.

RESULTS

Effect of the *n*-butanol and water extracts of P. decora Franch on body weight, blood glucose and serum insulin levels in alloxan-induced diabetic mice

The changes of body weight, blood glucose and serum insulin are given in Figure 1. Administration of alloxan to the mice resulted in significant ($p < 0.001$) increase of glucose concentration and significant ($p < 0.001$) decrease of insulin concentration as well as body weight when compared with Group I mice. Treatment with

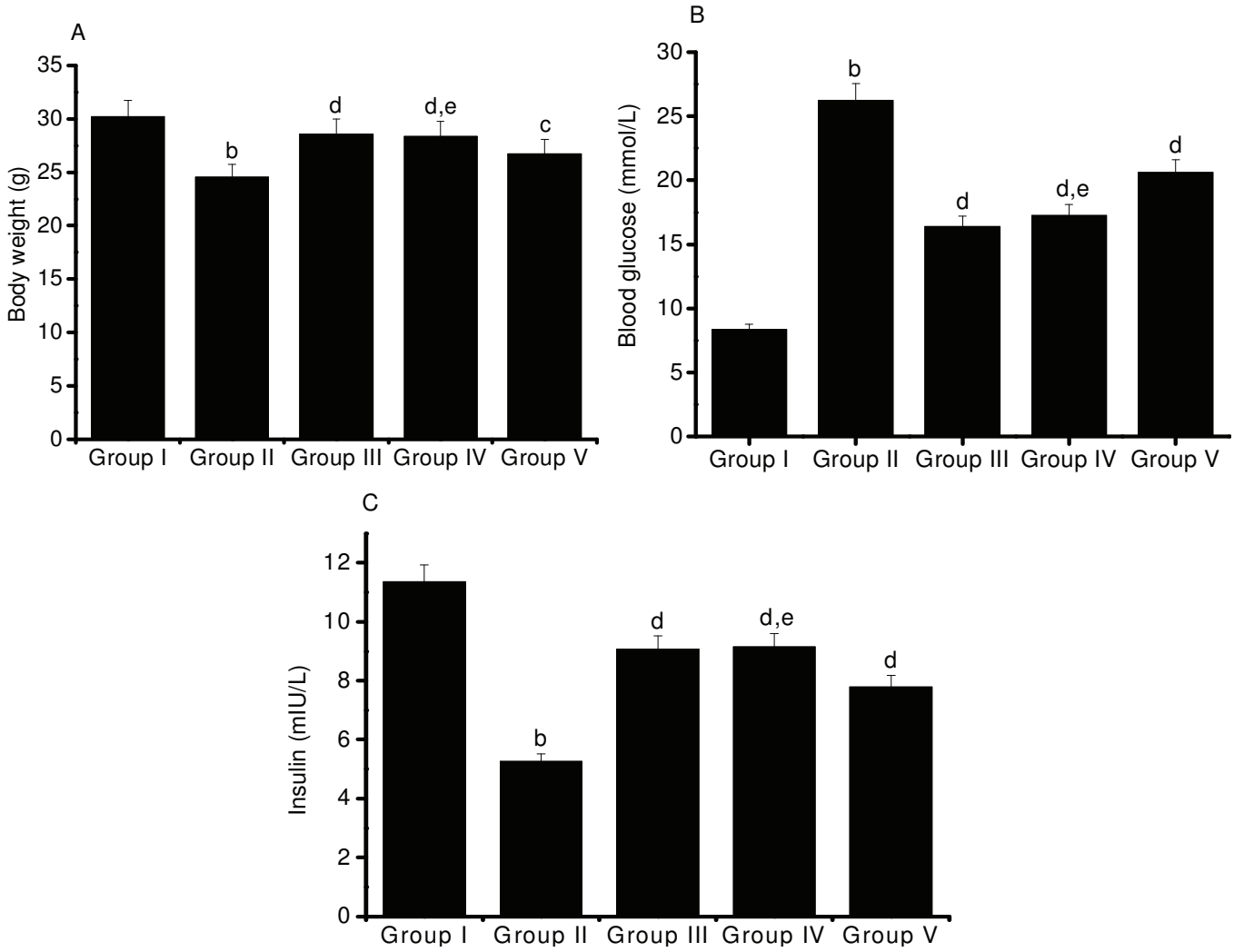

Figure 1. Effect of body weight, blood glucose and serum insulin in mice. The values are the mean ± SD of 10 mice per group. [b] P < 0.001 Group II compared with Group I, [c] P < 0.01 Groups III-V compared with Group II, [d] P < 0.001 Groups III-V compared with Group II, [e] P < 0.05 Group V compared with Group IV.

n-butanol and water extracts of *P. decora* Franch root, α-tocopherol caused a significant ($p < 0.001$) decrease in the blood glucose concentration and a significant ($p < 0.001$) increase in the serum insulin concentration and a significant ($p < 0.01$ and $p < 0.001$) increase in body weight when compared to Group II mice. Increased insulin concentration, body weight and decreased glucose concentration was significantly ($p < 0.05$) pronounced in *n*-butanol extract supplemented mice (Group IV) when compared to water extract treated mice (Group V).

Effect of the *n*-butanol and water extracts of P. decora Franch on serum and liver LPO, serum NO and TAA levels in alloxan-induced diabetic mice

As shown in Figures 2 and 3, administration of alloxan caused a significant ($p < 0.001$) increase LPO and NO levels in mice when compared with Group I mice. The *n*-butanol and water extracts of *P. decora* Franch root, α-tocopherol showed significant ($p < 0.001$) decrease in LPO and NO when compared to alloxan treated mice (group II). Additionally, the *n*-butanol extract (Group IV) showed significant ($p < 0.05$) decrease in LPO and NO when compared to the water extract treated group (Group V). To determine total antioxidant status of all animals, TAA was assessed. It was found to be diminished drastically in alloxan treated animals ($p < 0.001$) when compared with Group I. An increase was evident ($p < 0.001$) in this parameter after treatment with the two extracts of *P. decora* Franch root and α-tocopherol when compared to Group II. The *n*-butanol extract (Group IV) led to a significant ($p < 0.05$) elevating of serum TAA when compared to the water extract treatment group (Group V).

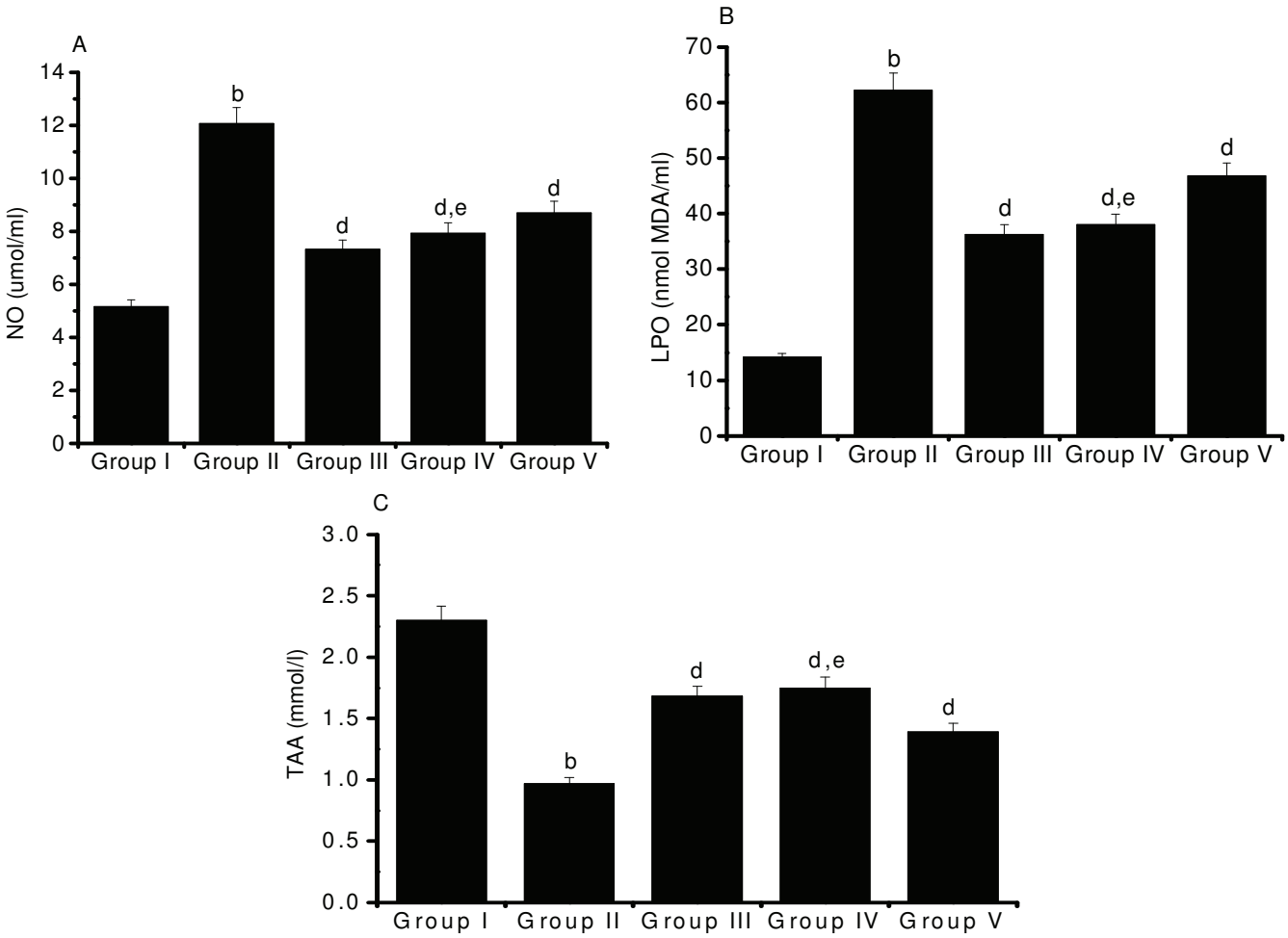

Figure 2. Effect of LPO and TAA, NO in serum. The values are the mean ± SD of 10 mice per group. [a] P < 0.01group II compared with Group I; [b] P < 0.001 Group II compared with Group I; [c] P < 0.01 Groups III-V compared to Group II; [d] P < 0.001 Groups III-V compared to group II; [e] P < 0.05 denotes a significant difference between group V and group IV.

Effect of the *n*-butanol and water extracts of P. decora Franch on liver SOD, CAT, GPx, GST, GR activities and GSH levels in alloxan-induced diabetic mice

The activity of SOD, GPx, GST, GR and the content of GSH in liver is presented in Figure 3. Alloxan treatment caused significant ($p < 0.001$) decrease in the activity of SOD, GPx, GST, GR and the content of GSH when compared with control group (Group I). A significant increase in SOD, GST, GR, GSH ($p < 0.001$) and GPx ($p < 0.01$) was observed in the two extracts of *P. decora* Franch root and α-tocopherol treated mice when compared to alloxan treated group (Group II). A significant ($p < 0.05$) increase was observed in *n*-butanol extract administered group when compared to water extract treated group. On the contrary, significant ($p < 0.001$) elevation in CAT activities was observed in alloxan treated group (Group II) when compared with Group I. Treatment with the two extracts and α-tocopherol showed significant ($p < 0.001$) decrease in CAT activity when compared to Group II. In addition, this decrease was more ($p < 0.05$) pronounced in *n*-butanol extract treated mice liver.

Effect of the *n*-butanol and water extracts of P. decora Franch on serum biochemical values and liver glycogen and liver weight values in alloxan-induced diabetic mice

Serum activities of AST, ALT, ALP, GGT, LDH and concentrations of cholesterol, HDL cholesterol, and triglyceride are shown in Table 1. Alloxan administration resulted in significant rise in the levels of AST, ALT, AKP, LDH, GGT, cholesterol and triglyceride when compared to Group I. However, HDL cholesterol levels significantly reduced in alloxan treated animals when compared to Group I. Administrations of *P. decora* Franch root extracts and α-tocopherol were seen to lower significantly

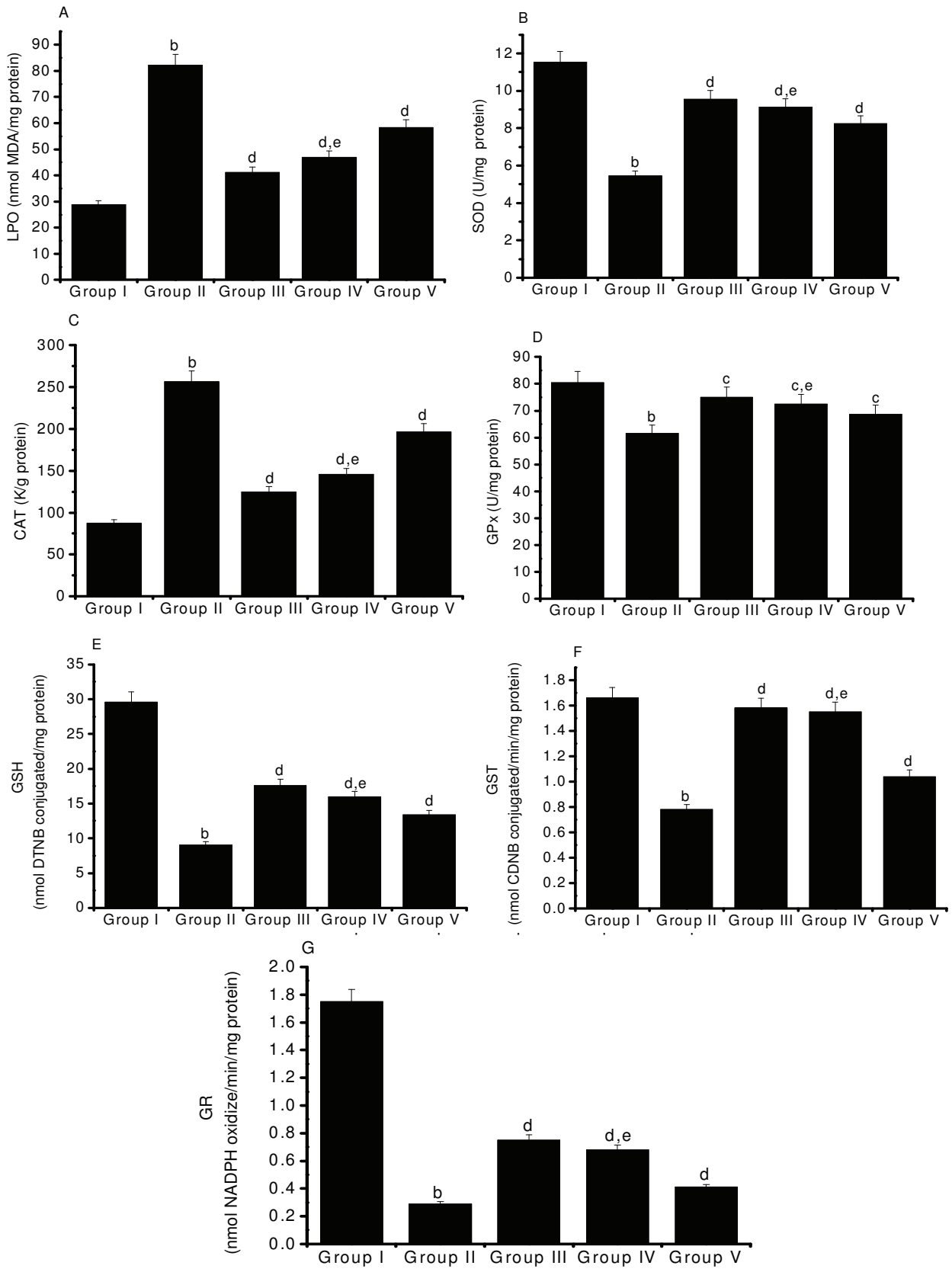

Figure 3. The effect of LPO and antioxidant enzymes in mice liver. The values are the mean ± SD of 10 mice per group. [b] $P < 0.001$ Group II compared with Group I; [c] $P < 0.01$ Groups III-V compared with Group II; [d] $P < 0.001$ Groups III-V compared with group II; [e] $P < 0.05$ denotes a significant difference between Groups V and IV.

Table 1. Effect of serum biochemical values, liver glycogen and weight in mice.

Parameters	Group I	Group II	Group III	Group IV	Group V
AST	12.9 ± 0.97	23.98 ± 1.28[b]	16.45 ± 1.24[f]	17.53 ± 1.31[f]	18.08 ± 1.26[f]
ALT	85.2 ± 12.4	195.1 ±17.8[b]	135.56 ± 15.02[f]	143.45 ±12.70[e]	158.24 ±15.73[d]
AKP	120.5 ± 30.7	261.6 ± 42.5[c]	150.1 ± 34.8[f]	148.7 ± 30.9[f]	178.6 ± 40.4[f]
LDH	126.5 ± 23.6	160.6 ± 40.5[a]	135.8 ± 32.1	141.6 ± 33.0	146.3 ± 34.2
GGT	7.75 ± 3.67	25.34 ± 7.34[b]	20.12 ± 5.09	21.53 ± 5.13	23.08 ± 4.57
Cholesterol	35.23 ± 8.9	90.67 ± 24.5[c]	45.16 ± 12.8[f]	48.45 ± 15.1[f]	57.67 ± 15.8[e]
HDL cholesterol	27.65 ± 8.6	12.55 ± 6.4[c]	21.32 ± 9.7[d]	22.47 ± 12.6[d]	18.65 ± 7.1
Triglyceride	180.34 ± 27.6	230.13 ± 50.9[a]	190.56 ± 28.9[d]	188.43 ± 27.7[d]	198.34 ± 30.2
Liver glycogen	2.24 ± 0.32	1.35 ± 0.29[c]	1.97 ± 0.36[f]	2.08 ± 0.31[f]	1.76 ± 0.26[e,h]
Liver weight	1.39 ± 0.18	1.05 ± 0.23[b]	1.29 ± 0.21[d]	1.26 ± 0.19[d]	1.13 ± 0.26[a]

the activities of AST, ALT ALP and the concentrations of cholesterol and triglyceride except the concentrations of triglyceride in water extract treated group when compared with Group II. Treatment with *n*-butanol extract and α-tocopherol were showed significant ($p < 0.05$) increase in serum HDL cholesterol when compared with alloxan treated mice.

In addition, liver glycogen and weight were also assayed as shown in Table 1. Alloxan administration resulted in significant decrease in liver glycogen and liver weight when compared to Group I. Administrations of *P. decora* Franch root extracts and α-tocopherol were seen to higher significantly the levels of liver glycogen and liver weight but not of liver weight in water extract treated group when compared with Group II. The *n*-butanol extract led to a significant ($p < 0.05$) increase in liver glycogen when compared with the water extract treatment group.

DISCUSSION

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia due to defects in insulin production and function. Alloxan is widely used in studies of experimental diabetes as this agent destroys pancreatic islet β-cells with a high specificity (Zhang et al., 2009). Uncontrolled diabetes leads to increased hepatic glucose output. The possible mechanism is that liver glycogen stores are mobilized and then hepatic gluconeogenesis is used to produce glucose (Duzguner and Kaya, 2007). Simultaneously, insulin deficiency also impairs nonhepatic tissue utilization of glucose. Therefore, alloxan induced diabetes is characterized by the drastic loss in body, liver, liver glycogen and in insulin as was seen in our results. Also, the increase in glucose was induced by alloxan. These changes show that our diabetic animals had major disease symptoms and agreed with these previous reports. Treatment with α-tocopherol, *n*-butanol and water extract of *P. decora* Franch root, however, caused considerable gain in body and liver weight, blood glucose, liver glycogen and serum insulin concentration. Further, the changes of liver glycogen suggest that the two extracts of *P. decora* Franch may act as an energy source similar to the traditional crops (Abbasi et al., 2010; Hussain et al., 1990). Recent reports have been indicated that persistent hyperglycemia causes increased production of oxidative stress in alloxan-induced diabetes (Bonnefont et al., 2000).

Hence, excessive ROS produced leads to oxidative damage and increased LPO. Our results showed a significant increase in LPO levels in alloxan-induced diabetic mice in serum and liver. SOD plays an important role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide, which in mammals is readily reduced to water principally by CAT and GPx. GPx plays a pivotal role in minimizing the oxidative stress. GPx and GST work in concert with GSH and decompose H_2O_2 and other organic hydroperoxides to non-toxic products. Glutathione, reduced form (GSH) is ubiquitous tripeptide thiol, is a vital intra/extra-cellular protective antioxidant against oxidative/nitrosative stress. GR is an enzyme responsible for its conversion back to the reduced state (Kayali and Tarhan, 2006; Okutana et al., 2005). Hence, the changes of these biomarkers is in accordance with the decrease in antioxidant state in the body as other reports (Ei-Missiry and Ei-Gindy, 2000; Meral et al., 2001). In the present study, administration of the two extracts and α-tocopherol significantly counteract the changes of oxidative stress biomarkers in alloxan induced mice thus preventing the accumulation of excessive oxidative stress. However, the increase in CAT activity in diabetic mice may be due to the fact that persistent hyperglycemia induced by alloxan leads to increased concentration of H_2O_2, which eventually causes induction of CAT activity. The decrease of CAT activity in the two extracts and α-tocopherol administration mice suggests a decrease in H_2O_2 concentration (Gumieniczek, 2003).

TAA indicates the ability to defend against free radical damage more precisely than measurement of individual

plasma antioxidants. Our results indicate that TAA levels were markedly decreased in diabetic group. Under diabetic conditions, free radicals such as O_2^- and NO are produced as a result of the induction of the glycation reaction in β-cells that have been affected by diabetic oxidative stress. Supporting that the nitrite–nitrate levels of diabetes were significantly increased compared to control group in our present study. NO interacts with O_2^- to form the highly reactive hydroxyl radical that leads to reactive oxidative damage under conditions of diabetes (Sepici-Dincel et al., 2007). In addition, NO targets intracellular antioxidative enzymes, resulting the loss of their function. Administration the two extracts of *P. decora* Franch root and α-tocopherol suggests a good antioxidant capacity and the free radical scavenging property in protecting against diabetic oxidative stress.

The liver is regarded as one of the central metabolic organs, regulating and maintaining homeostasis. The previous indices' changes attack the hepatic function as shown by an increase in the level of AST, ALT, AKP, LDH, GGT, cholesterol, triglyceride and the decrease in HDL cholesterol indices of liver damage which are also detected in human diabetes (Takaike et al., 2004). The abnormal high concentration of serum lipids is mainly due to the increase in mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production (Udayakumar et al., 2009). Thus, α-tocopherol and the two extracts from *P. decora* Franch root treatments exhibited hypocholesterolaemic and hypotriglyceridaemic effects while at the same time increasing the HDL- cholesterol. This may reduce the susceptibility of lipids to oxidation and stabilize the membrane lipids thereby reducing oxidative stress. In diabetic animals, the variations in the levels of AST, ALT and ALP are directly related to changes in metabolism in which the enzymes are involved. The increased activities of transaminases, which are active in the absence of insulin due to the availability of amino acids in the blood of diabetes and are also responsible for the increased gluconeogenesis and ketogenesis. Diabetes and hyperlipidaemia also cause cell damage by altering the cell membrane architecture, which results in enhanced activities of ALP in diabetic rats (Udayakumar et al., 2009). This suggested that alloxan treated diabetes caused lipid peroxide mediated tissue damage in the liver. The increase in the levels of these enzymes in diabetes may be as a result of the leaking out from the tissues and then migrating into the blood stream. The decrease in AST, ALT and ALP levels in α-tocopherol and the two extracts of *P. decora* Franch root treated groups indicates the protective effect on liver function.

The results showed the more pronounced effect on ameliorating the oxidative stress and hepatic injury induced by alloxan in *n*-BuOH extract of *P. decora Franch* root treatment mice. This may be the different chemical constituents of the two extracts. Therefore, isolation and characterization of the compounds in the two fractions should be further studied to elucidate the mechanism involved in alloxan induced animals. As a standard antioxidant, α-tocopherol was observed to have attenuating the oxidative stress as other reports (Srinivasan et al., 2007; Bansal et al., 2005).

Conclusion

In short, it could be concluded that the administration of α-tocopherol, *n*-butanol and water extract of *P. decora* Franch root may improve the altered body and liver weight, glucose, liver glycogen and insulin concentration in alloxan treated diabetic mice. The reduced levels of parameters of SOD, GST, GR, GSH, GPx and TAA in alloxan-induced mice were significantly increased by administration of α-tocopherol, *n*-butanol and water extract of *P. decora* Franch root. The two extracts and α-tocopherol significant effect in liver injuries as well as oxidative stress, resulting in reduced LPO, NO, CAT and serum biochemical paramaters including AST, ALT ALP, cholesterol and triglyceride as well as in elevated HDL cholesterol in alloxan treated mice. Additionally, *n*-butanol extract was more potent than the water extract on inhibiting the oxidative stress and liver injury impact.

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