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Hepatoprotective effect of aqueous and pure extracts of raisin in alloxan-induced diabetic rats

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Abstract. The present study was conducted to evaluate the possible antioxidant effects of raisin aqueous and pur extract of "Karkni" on liver histology, antioxidant enzymes activities and lipid peroxide levels in liver of diabetic and normal rats. 125 mg/kg of pure and aqueous extract of "Karkni" were orally administered daily to alloxan-diabetic rats for 4 weeks. Alloxan- induced diabetic rats showed significant increases in the levels of total protein and malondialdehyde (MDA), glutathione peroxidase (GPx), catalase (CAT) and reduced glutathione (GSH) levels were significantly decreased compared to normal rats ($p < 0.05$). Also, no significant effects on glutathione peroxidase (GPx) activity in diabetic rats were observed. The changes of the above parameters to their normal levels after 4 weeks of treatment were observed mainly with the pure extract. The results suggesting that pure extract suppresses the liver tissue inflammation attenuate lipid peroxidation and can be helpful in reducing liver damage caused by alloxan-induced diabetes.

Keywords: "Karkni", alloxan, liver, antioxidant enzymes, MDA

1. Introduction

In the past few years, research on polyphenols has remarkably expanded and is constantly reporting interesting biological activities of these compounds. Due to the participation of oxidative processes in the onset and development of degenerative diseases, much attention has been paid to the antioxidant properties of polyphenols [1]. Raisins have been a favorite food since 1490 BC due to their nutritive value and high micronutrients content [2]. Raisins rank among the highest in the concentration of total phenolic compounds and have the highest levels of total antioxidant activity among solid fruit products. Despite carbohydrate content, some research suggests that raisins has been shown to confer antioxidant protection, reduce markers of inflammation and lower circulating levels of oxidized LDL [3, 4]. Also Browning reaction products (BRPs) in raisin have been reported to prevent or retard oxidation reactions in lipid systems [5]. More, polyphenols provided by raisins may interfere with cholesterol absorption [6] and can decrease hepatic cholesterol concentrations [7]. Diabetes is a world-wide chronic metabolic disorder affects relatively high

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percentage of population (20.8 million people in the United States and 48 million in Europe). It is characterized by hyperglycemia caused by dysfunction in carbohydrate, protein and fat metabolism either because the insulin produced by the body is insufficient, or because cells do not respond properly to the insulin that is produced [8]. Diabetes is associated with long term damage and failure of various organs such as eyes, kidneys, liver, nerves, heart...etc [9]. The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continuous supply to organs that require a glucose energy source [10]. Diabetes mellitus and advanced liver disease are associated with each other more frequently than expected by chance, and such an association carries a significant risk of morbidity and mortality [11] and some researchers proved herbal extracts are known to prevent the oxidative damages, inflammation, fibrosis and cirrhosis in liver [12] and restored antioxidant enzyme activity in diabetic rats [13]. Since few studies has been completed on the antioxidant activity of raisin extracts, the aim of the present study, was to determine the potential beneficial antidiabetic effects of two type of "Karkni" extract (aqueous and pure extract) during 4-week on alloxan-Induced diabetic rats.

2. Materials and methods

2.1. Plant material

2.1.1. Aqueous extract

Dried fruit was extracted with distilled water by grinding with a mortar and pestle. It was incubated for 24 h and filtered using a Buckner funnel and Whatman's No. 1 filter paper. It was the filtrate that was administered to the animals in the course of this study fresh for a maximum of two days after which fresh extract was prepared. Pure extract After simple extraction, the water fraction was mixed with equal volume of ethyl acetate in a separatory funnel to yield the water and ethyl acetate fraction. The mixture was stirred vigorously and allowed to stand until there was complete separation of two phases: an upper organic phase and a lower aqueous phase. Organic phase was filtered through paper filter to remove insoluble substances. The ethyl acetate was evaporated by a vacuum rotary evaporator and the pure polyphenols were stored for analysis.

2.2. Experimental animals

Male Wistar rats (Central Pharmacy, Tunisia), weighing 200–250 g were housed under standard environmental conditions (23°C, 55 ± 5% humidity and a 12 h light/dark cycle) and maintained with free access to water and a standard diet ad libitum. Experimental diabetes was induced in rats by intraperitoneally injection of alloxan monohydrate at a dose of 120 mg/kg. After 2 weeks, animals having blood glucose level of 200 mg/dl and above were considered diabetic. The rats so induced were divided into 5 groups of 8 animals per group. Group 1; Normal treated rats (Control). Group 2; Diabetic control rats. Group 3; Diabetic rats given 125 mg/kg of the aqueous extract of "Karkni" extract (D+AE). Group 4; Diabetic rats given 125 mg/kg of the pure extract of "Karkni" (D+PE). Oral administration to the animals was done once every 24 hours. The animals were fasted overnight for 12 hours. Body weight, food and water intakes of the rats were monitored daily. After completion of treatment period, liver tissues were removed, ground and preserved for analysis.

All the breeding phases and all experiments were carried out in compliance with the rules of the Tunisian Society for the Care and Use of Laboratory Animals. All experiments were conducted at the animal facilities of the faculty of Medicine, Monastir; with the approval of the Faculty of Medicine Ethics committee (19 September 1994) according to protocol number 94-1939.

2.3. Experimental procedure

2.3.1. Histology sampling

Tissues were excised and immediately fixed in 10 % formaldehyde (pH 6.9) and embedded in paraffin wax for sectioning at 5 mm. Hematoxylin and eosin (H&E)-stained histology slides were subsequently analyzed.

2.3.2. Antioxidant enzyme

In liver tissue, superoxide dismutase activity was measured at 450 nm by testing the ability of superoxide dismutase (SOD) to inhibit the reduction of nitroblue tetrazolium by superoxide [14]. Glutathione peroxidase (GSH-Px) activity was determined according to the method of Floche and Gunzler (1984) at 412 nm using cumene hydroperoxide as substrate. Glutathione reductase (GSSH) activity was evaluated at 340 nm by measuring the decrease in NADPH absorbance in the presence of oxidized glutathione [15]. One unit of enzyme reduces 1 μmol of oxidized glutathione per min at pH 7 at 25°C. Catalase (CAT) activity was assayed in tissues by measuring the rate of decomposition of hydrogen peroxide at 240 nm [16]. Protein concentrations were measured according to the method of Lowry et al. [17] using bovine serum albumin as standard. The extent of lipid peroxidation was determined as the concentration of thiobarbituric acid reactive substances (TBARS), according to the method of Buege and Aust [18].

2.4. Statistical analyses

Data were subjected to one-way analysis of variance for means of comparison, and significant differences were calculated according to Tukey multiple range test. Data are reported as means ± standard error. Differences at $p < 0.05$ were considered statistically significant. SPSS (version 11.0) was used to perform the statistical analysis.

3. Results

3.1. Body and liver weight

The effects of the karkni extract on body and liver weight of rats were shown in Table 1. In the present study, alloxan-induced diabetic rats showed significant ($p < 0.05$) increased body weight level compared to control rats 241,19 and 234,31 g respectively (Table 1). After 4 weeks, a significant increase in body weight was observed in diabetic group (241.19/265.60 g). In the same period, the addition of 125 mg/kg of aqueous and pure extract of "Karkni" did not improve weight gain of rats compared with the diabetic group. Concerning water and food intake, significant reduction ($p < 0.05$) was observed in (D+AE) and (D+PE) groups. Liver weight expressed as a percentage of body weight. After 4 weeks, a slight increase in liver weight was observed in diabetic rats vs healthy controls.

3.2. Histological examinations

In the control group, known healthy liver histology was seen (Fig. 1A). But the microscopical appearance of liver of alloxan -Induced diabetic rat showed signs of inflammation (Fig. 1C); degenerative changes in the hepatocytes represented by disorganization of the hepatic cords, congestion of the central veins with mild hepatocellular necrosis and the sinusoids were infiltrated by mild nonspecific inflammatory cells. Theses alterations were remarkably in the liver sections of the rats that received aqueous extract of "Karkni" (Fig. 1D) and completely suppressed in the rats that received pure extract of "Karkni" (Fig. 1B) for 4 weeks.

Table 1
Effect of "Karkni" extract on body weight, liver weight, food and water intake in alloxan-induced diabetic rats

	Control rats	Diabetic rats	D+AE	D+PE
Initial BW (g)	234.31 ± 6.42^{a}	241.19 ± 19.2^{b}	246.18 ± 23.65^{c}	243.77 ± 20.97^{c}
Final BW (g)	260.41 ± 1.73^{c}	265.60 ± 2.87^{d}	258.51 ± 10.51^{b}	241.68 ± 32.18^{a}
Liver (g/100 gBW)	2.92 ± 0.05^{a}	3.23 ± 0.5^{a}	2.83 ± 0.18^{a}	2.63 ± 0.78^{a}
Food intake (g/d)	53.18 ± 11.40^{a}	78.99 ± 19.47^{d}	76.46 ± 14.50^{c}	72.25 ± 20.63^{b}
Water intake (mL/d)	76.52 ± 8.95^{a}	161.22 ± 21.98^{d}	132.65 ± 17.35^{b}	129.98 ± 41.45^{b}

Values with different superscript letters (a. b. c. d) indicate significant differences amonggroups at $p < 0.05$ by Duncan's multiple range test. C. control; D. rats with Alloxan-induced diabetes; D+AE. rats treated with 125 mg/kg/day of aqueous extract; D+PE. rats treated with 125 mg/kg/day of pure extract.

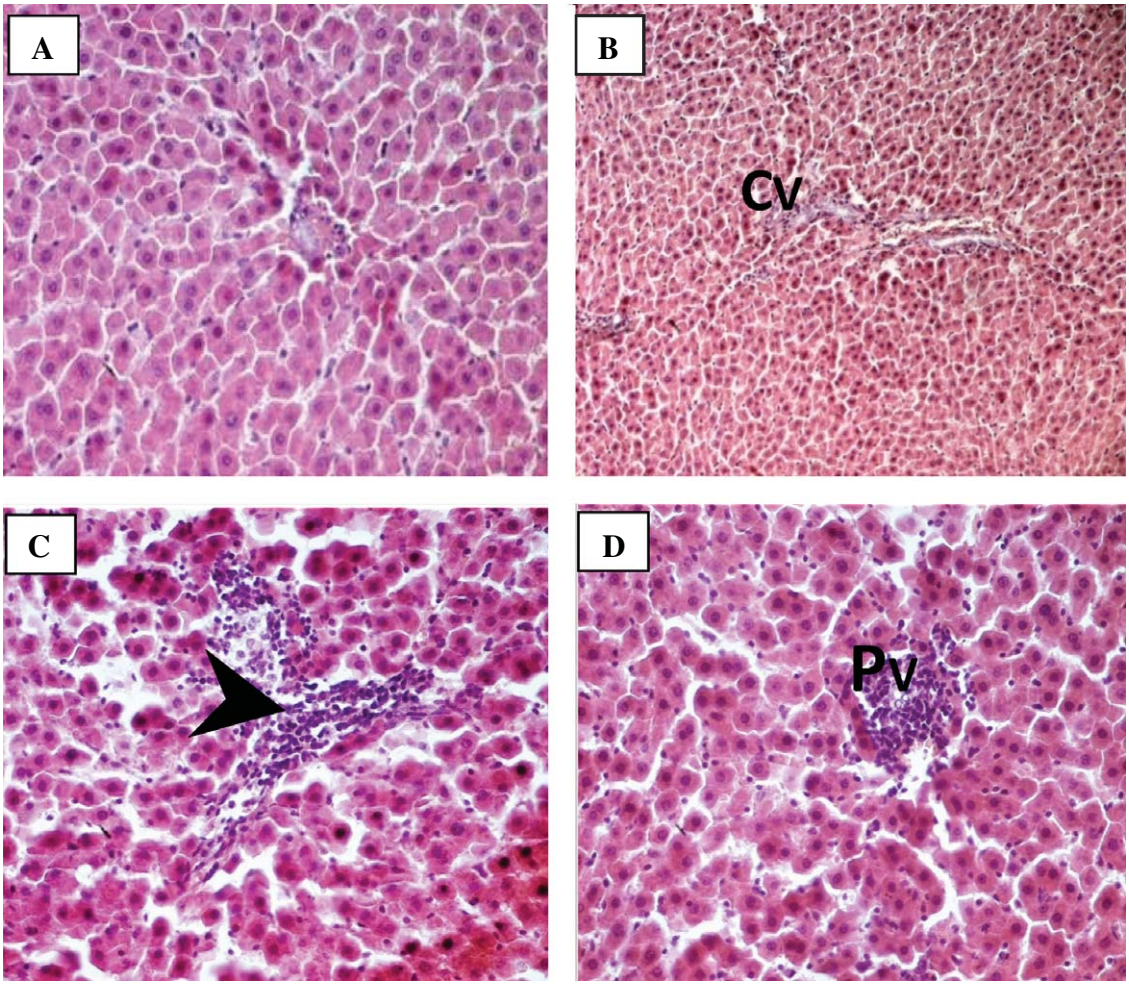

Fig. 1. Photomicrographs of sections of liver section of control and experimental rats. (A): Section of liver tissue from a control liver showing narrow sinusoidal spaces (thick arrows) and well formed hepatic cords (arrow head) (H&E, ×100). (B): Section of liver tissue from rat receiving the pure extract of raisin showing the hepatocytes radiating from the central vein (Cv) and separated from each other by equal-sized blood sinusoids containing kupffer cells (arrow) (H&E, ×100). (C): Section of liver tissue from an alloxan-induced diabetic rat showing lymphoid infiltration of central vein and severe dilatation of sinusoids (arrow) (H&E, ×100). (D): Section of liver tissue from an alloxan-induced diabetic rat receiving the aqueous extract of raisin showing lymphoid infiltration of the portal vein (Pv) and moderate dilatation of sinusoids (arrow) (H&E, ×100).

3.3. Protein and lipid peroxidation

Data are shown in Fig. 2. There was a significant elevation in tissue MDA in diabetic rats as compared with normal rats ($p < 0.05$). Administration of "Karkni" extract significantly decreased MDA in liver mainly with pure extract of "Karkni" (0.08 mmol MDA/mg protein). However, a significantly increased of protein level was also observed in diabetic group but it was restored in (D+AE) and (D+PE) groups (Fig. 3).

3.4. Antioxidant enzymes: SOD, CAT, GSH and GPx

During diabetes, there are a significant reduction in the activities of GSH, and CAT and compared with control group ($p < 0.05$) (Figs. 4B; 5A respectively). The effects of "Karkni" extract at doses 250 and 375 mg/kg were

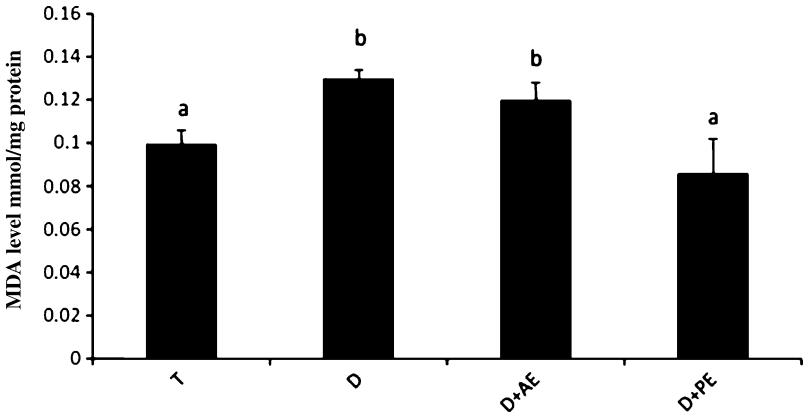

Fig. 2. Change of MDA level in rats with alloxan-induced diabetes over 4 weeks. Values with different superscript letters (a, b, c) indicate significant differences among groups at $p < 0.05$ by Duncan's multiple range test C, control; D. rats with Alloxan-induced diabetes; D+AE. rats treated with 125 mg/kg/day of aqueous extract; D+PE. Rats treated with 125 mg/kg/day of pure extract.

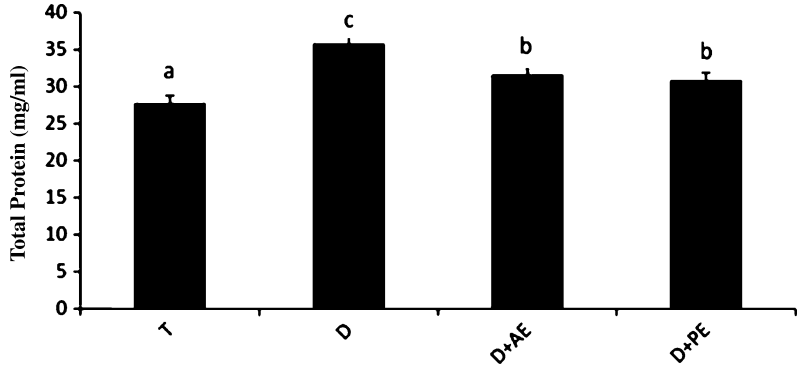

Fig. 3. Change of protein level in rats with alloxan-induced diabetes over 4 weeks. Values with different superscript letters (a, b, c) indicate significant differences among groups at $p < 0.05$ by Duncan's multiple range test C, control; D. rats with Alloxan-induced diabetes; D+AE. rats treated with 125 mg/kg/day of aqueous extract; D+PE. Rats treated with 125 mg/kg/day of pure extract.

significantly greater than that of 125 mg/kg. Also, significant increased effects on hepatic GPx activity was observed in diabetic group compared to control rats (Fig. 4A). But this activity was restored predominately with pure extract.

4. Discussion

The role of antioxidants in the maintenance of health and chemoprevention of disorders and diseases has received great attention [19]. Raisins are a concentrated source of carbohydrate. But same research showed that raisin polyphénols has demonstrated significant antioxidant anticarcinogenic, antiinflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal, and *in vitro* studies [9]. In the present study, we investigated the mechanism of antidiabetic effect of raisin and its beneficial effect in liver damage under diabetic conditions. Diabetic condition such as polyuria, polydipsia and polyphagia were commonly observed in diabetic rats, (D+AE), and (D+PE) groups compared to control rats. There are classical diabetes symptoms and this result was correlated with increased body weight in diabetic, (D+AE), and (D+PE) groups after administration of alloxan. After 4 weeks, The significant reduction in the quantity of food and volume of water intake observed in (D+C1) group (when compared

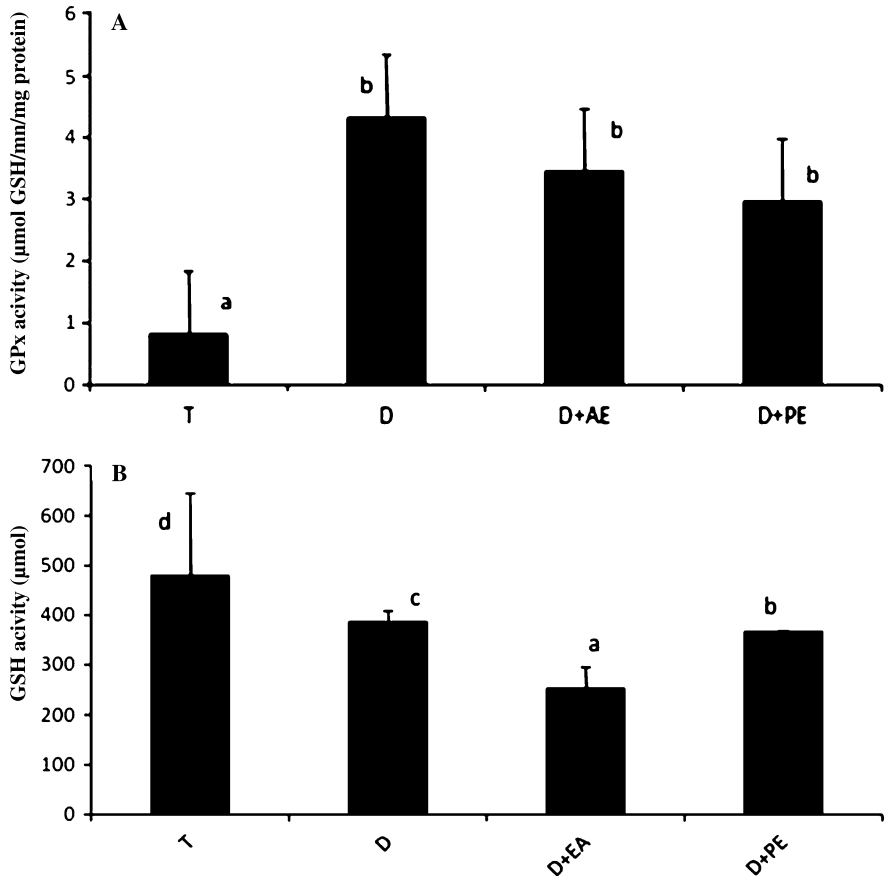

Fig. 4. Effect of "Karkni" extract treatment on GPx (A) and GSH (B) activity in diabetic rats. Values with different superscript letters (a, b, c) indicate significant differences among groups at $p < 0.05$ by Duncan's multiple range test. C control; D. rats with Alloxan-induced diabetes; D+AE. rats treated with 125 mg/kg/day of aqueous extract; D+PE. Rats treated with 125 mg/kg/day of pure extract.

to the Diabetic group) was explain by the role of "Karkni" extract which affect the neuro-endocrine regulation of food intake by the GI system. Any significant difference in liver weight between all the groups was observed (Table 1). On the contrary, Zafar and Naqvi [20] showed that significant increased liver weight in streptozotocin-induced diabetic rats. In our study, the histopathology of liver showed a sign of inflammation in the liver sections of diabetic rats. The results demonstrate that alloxan treatment increased the hepatic activity of cytochrome P450 in liver cells which cause lipid peroxidation and produces hepatocellular damage and enhanced production of fibrotic tissue [21]. This lipid peroxidation was associated with the progress of many diseases. For example, the oxidative modification of low density lipoprotein (LDL) and high density lipoprotein (HDL) has been accepted as an important initial event of atherosclerosis. The oxidized LDL contains multiple oxidation products of cholesteryl esters, phospholipids, and cholesterol and their breakdown products. The levels of these oxidation products are associated with the atherogenesis of oxidized LDL and the progress of atherosclerosis [22]. These findings of present study were in agreement with the findings of Das, Padayatti and Paulose [23] and Degirmenchi et al. [24] who showed dilatation of veins, loss of usual concentric arrangement of hepatocytes, liver fibrosis and decreased in glycogen activity in streptozotocin (STZ) induced rats. But The potentiation of liver toxicity by alloxan in rats was more striking than that of STZ [25] and liver function related parameters were more severe in alloxan model than that of STZ. But in our study, pure extract of "Karkni" showed strong protection against liver inflammation (Fig. 1D).The hepatoprotective effect can be explain by the present of same important phenolics compound which prevent the histopathological changes (inflammation) of liver associated with alloxan diabetes and its complications. Histopathology results were correlated with a significant

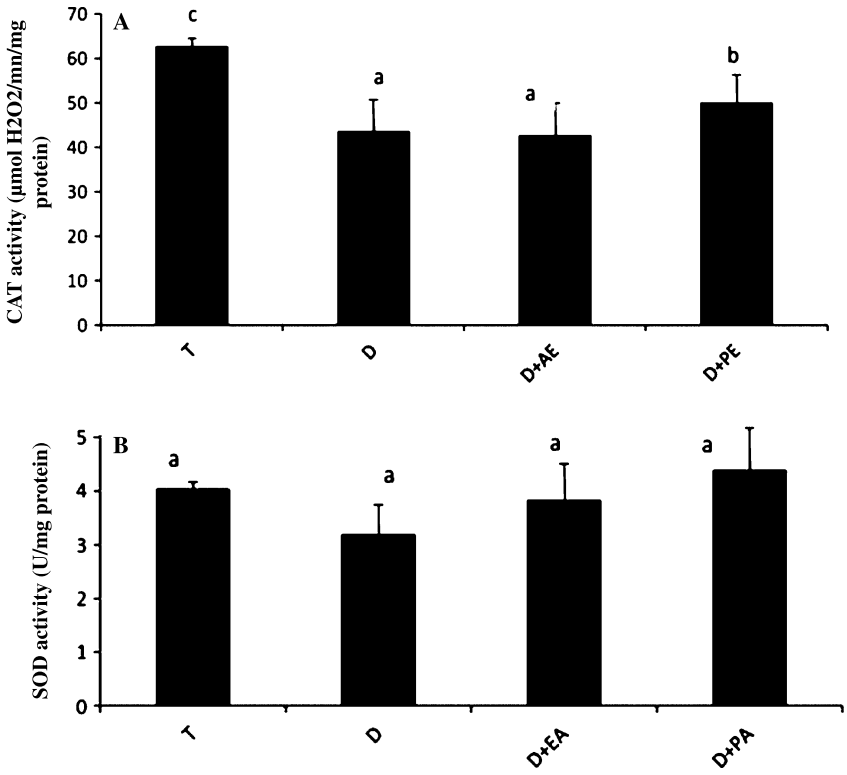

Fig. 5. Effect of "Karkni" extract treatment on CAT (A) and SOD (B) activity in diabetic rats.Values with different superscript letters (a, b, c) indicate significant differences among groups at $p < 0.05$ by Duncan's multiple range test. C control; D. rats with Alloxan-induced diabetes; D+AE. rats treated with 125 mg/kg/day of aqueous extract; D+PE. Rats treated with 125 mg/kg/day of pure extract.

elevation of MDA level in the liver of diabetic rats. It can be indicative of oxidative damage on mitochondrial and hepatocyte cell membrane [26]. But administration of pure extract of "Karkni" significantly lowered MDA level and it shows the preventive action of raisin on lipid peroxidation. Moreover, raisin was rich of terpenoids, phenolic acids, flavonoids, and other phenolic compounds [27]. It was possible that the benefit effect of "Karkni" extract is related to these components.

Niki added that many lipid peroxidation products are cytotoxic but it has been found that at sublethal concentrations they are capable of inducing adaptive response to enhance cell tolerance against forthcoming oxidative stress.Such adaptive response was observed for chemically stable lipid peroxidation products, such as HODE, lyso PC, hydroxycholesterol, and epoxycholesterol, as well as chemically active lipid peroxidation products such as a,b-unsaturated carbonyl compounds. The pretreatment of cells with sublethal concentrations of these lipid peroxidation products enhanced cytoprotective capacity against the subsequent oxidative stress by, for example, hydrogen peroxide, 6-hydroxydopamine, HPODE, and 7-hydroxycholesterol. Thus, it is clear that lipid peroxidation products may also exert both harmful and beneficial effects [22].

In our study, protein levels were significantly increased in liver. But, after the administration of "Karkni" extract, significantly decreased levels were noticed in (D+AE) and (D+PE) groups; 31.65 and 30,88 mg/ml respectively. This is confirming the beneficial effects of "Karkni" extract.

In the enzymatic antioxidant defense system, SOD is one of the important enzymes and scavenges the superoxide radi-radical by converting them to H_2O_2 and molecular oxygen [28]. The slight decrease in SOD activity in diabetic control rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme, which have been reported to occur in diabetes [29]. Also CAT as has been regarded as a major determinant of hepatic antioxidant status and catalyzes the reduction of hydrogen peroxides and protects the tissue from highly reactive hydroxyl radicals. The

decreased activities of CAT in liver of diabetic group can be due to the production of ROS such as superoxide ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) [30]. But after 4 weeks, activity of SOD and CAT were restored in (D+AE) and with high importance in (D+PE) groups and this action supports the protective effect of "Karkni" extract, despite the presence of carbohydrate, on above organs against free radical mediated damage. Alloxan-induced diabetic rats were observed to have decreased hepatic GSH levels in (D+AE) and (D+PE) (Fig. 4B) compared to normal rats. Also any changement was observed in (D+AE) and (D+PE) in GPx activity. The same result was observed by Anh, Jeon, Lee, Hwang, Lim, and Park [31] when they reported that increased SOD and catalase activities are observed in liver tissue after feeding grape seed extract. In contrast, Alía, Horcajo, Bravo and Goya [32] reported that antioxidant enzymes such as SOD, catalase, and glutathione content did not change, but that glutathione peroxidase activity increased after consumption of grape seeds and grape skins.

5. Conclusion

The present study evaluated the protective effects of "Karkni" extract in alloxan-induced diabetic rats. Oral administration of "Karkni" extract at a dose of 125 mg/kg for 4 weeks attenuate lipid peroxidation in liver tissue and restored the SOD, CAT, GPx activity in diabetic rats. But the results are more important with pure extract than aqueous extract of "Karkni".This implies that raisin it considered a great source of many polyphenolic compounds which can prevent or be helpful in reducing the complications of diabetes.

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