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Original Article

Antioxidant Effect of Aqueous Extract of *Cynodon Dactylon* in Streptozotocin Diabetic Rats

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Abstract

Background: *Cynodon dactylon L. pers* is one of the plants with applied medicinal value.

This study aimed to examine the effect of *C. dactylon (L.)* aqueous extract on Malondialdehyde (MDA) levels and catalase (CAT) activity in liver and kidney of diabetic rats.

Materials and Methods: In this experimental study, 30 adult male rats were divided into five groups (n=6). Control rats were injected with physiological saline and the others were made diabetic by the injection of streptozotocin (STZ; 70 mg/kg, i.p.). The third, fourth and fifth diabetic groups were given the oral aqueous extract of *C. dactylon* at different doses (50, 250 and 500 mg/kg) for 4 weeks. At the end of the study, blood glucose, MDA concentration and CAT activities of kidney and liver tissues were determined.

Results: The activity of CAT of diabetic rats treated with the 500 mg/kg extract was increased significantly compared to the untreated diabetic rats ($P<0.05$). Treatment with extract resulted in reduction of MDA in comparison to diabetic group.

Conclusion: Aqueous extract of *C. dactylon (L.)* could be effective in decreasing diabetic complication and this effect is attributed to the antioxidant activity of the plant.

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Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or receptor insensitivity to endogenous

insulin. While exogenous insulin and other medications can control many aspects of diabetes, assorted complications affecting the vascular system, kidney, Liver and peripheral nerves are common and extremely costly in terms of longevity and quality of life (1).

Oxidative stress is the presence of active oxygen species (ROS) is excess of the available antioxidant buffering capacity. These reactive oxygen species can damage proteins lipid and DNA altering organism of structure and function (2). Oxidation of lipids in plasma lipoprotein and cellular membranes is associated with the development of vascular disease in diabetes. Oxygen derived free radicals and reactive oxygen species interact with lipid bilayer of cell membrane resulting in lipid peroxidation (3). Malonaldehyde (MDA) is a stable and a product of lipid peroxidation (3).

Antioxidant defense mechanisms are important for the protection of cells and tissues against oxidative damage. The major endogenous antioxidant enzyme-systems in the mammalian body include superoxide dismutase (SOD), catalase (CAT), selenium-dependent glutathione peroxidase (GSHPx-Se), glutathione peroxidase (GSHPx) and glutathione reductase (GSHR) (4). Many studies have shown that its concentration is considerably in diabetes mellitus, correlating with poor glycemic control. Many herbal such as: *Rhus coriaria*, *Cinnamomum zelanicum*, *Cynodon dacttylon*, *Hypericum perforatum* and onion known antidiabetic effects and use in patient treatment (5, 6).

The *Cynodon dactylon* (Family: Poaceae) commonly known as "*Doob*; Hindi" "*Aroogum pillo*; Tamil", "*Garike*; Telulgu" and "*Bermuda grass*; English" in India is a creeper. It is a weed and has been regarded to possess varied medicinal properties (7). The aqueous extract of the rhizome is used as antiinflammatory, diuretic, antiemetic, antidiabetic and blood purifying agent (8, 9). According to the importance of medicinal plant and due to the least side effect, In the present study, the authors tried to show the protective effects of the extract of *Cynodon dactylon* on tissues oxidative damages in diabetic rats. At the end of the study, MDA concentration and CAT activities of kidney and liver tissues were determined.

Materials and Methods

Plant collection and extract preparation

Rhizome of C. dactylon was collected from the campus of the University of Urmia, Iran. The plant was identified by an expert botanist, and a voucher specimen was kept in the herbarium of agricultural faculty of Urmia University, Urmia, Iran. The Rhizome of plant was washed thoroughly with water and air-dried at 25C for 7 days in absence of sunlight. The dried plants were thoroughly ground to a powder. Then 10 g of dry powder was mixed with 100 mL of distilled water for 2 days at room temperature. The resulting dark brown extract was cooled and filtered through Whatmann No. 1 filter paper and concentrated at 55–60C in Rotavapor (BÜCHI Labortechnik AG Meierseggstrasse, Flawil, Switzerland) under reduced pressure (10). 15.7 g of the dry extract was obtained from every 100 g of powder.

Experimental animals:

Thirty healthy, male, Wistar rats weighing 180-230 g, were used in this study. They were housed under standard laboratory conditions of light, temperature and humidity. All animals were treated in accordance to the Principles of Laboratory Animal Care. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Urmia University. All Rats were fed a standard diet and water. The rats randomly divided into 5 experimental groups (n=6). Control rats (C) were injected with physiological serum the same volume of injection material. Group II rats (ND), were diabetic by injecting 70 mg/kgbw dose in intraperitoneal STZ (11). Group II contracted diabetes by being intraperitoneally injected 70 mg/kgbw STZ. The roots of third (DT_{50}), fourth (DT_{250}), and the fifth group (DT_{500}), in addition to the same treatment, were fed 50, 250 and 500 mg/kgbw of aqueous extract of *Cynodon dactylon*, respectively for 4 weeks.

Blood glucose determination

Blood samples were collected from the tail vein. Basal glucose levels were determined, using an automated blood glucose analyzer (Glucometer ACCUE CHECH). Sample collections were then made 72 h after STZ injection and blood glucose concentrations were determined. Rats with blood glucose concentrations above 200 mg/dl were declared diabetic and were used in the experimental group. Furthermore, after 28 days of treatment, the levels of blood glucose were determined. On the 28th day, (at the end of the treatment period), the rats were killed with diethyl ether. The liver and kidney tissues of each animal were removed, cleaned, dried and processed for biochemical measurements.

Measurement of liver and kidney tissue contents of MDA

At the end of the experimental period (after 4 weeks), all the rats were anesthetized and the liver and kidney tissue were removed. Each of the samples were homogenized (1:10) with Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in ice-cold 0.1 M potassium phosphate buffer (pH 7.4), and 1.15% KCl. The homogenate was centrifuged (10 min at 3000 RPM) and the supernatant was used for assays MDA levels (12). MDA concentration was determined by using the method described by Draper based on TBA (2-thiobarbituric acid) reactivity. Briefly, 2.5 mL of 10% trichloracetic acid and 0.5 mL of sample were added into tubes and mixed. After incubating for 15 min at 90°C and cooling with cold water the mixture was centrifuged at 3000 rpm for 10 min. Two milliliters of supernatant were taken and 1 ml of 0.675% TBA was added. The tubes were sealed and incubated at 90°C for 15 min and then cooled to room temperature. The optical density was measured at 532 nm by a spectrophotometer (13).

Measurement of liver and kidney CAT activity

The activity of CAT was determined by using the method described by Aebi. 1 ml H_2O_2 and 2 ml of the sample were added into tubes and mixed. Then the optical density was measured at 240 NM by a spectrophotometer (14).

Statistical analysis

One–way analysis of variance (ANOVA) and Tukey statistical test were used to compare these parameters in the study group. The results were expressed as mean±S.E.M (standard error of means). P-values less than 0.05 were considered significant.

Results

The results of the effect of aqueous extract of *Cynodon dactylon* on blood glucose levels of rats are presented in Fig. 1. STZ induced a significant increase of blood glucose in comparison with control group ($P<0.05$). Furthermore, compared diabetic group, in the extract-treated group (500 mg/kgbw) there was a significant decrease in blood contents of glucose ($P<0.05$).

In diabetic rats there was a significant decrease in CAT activity when compared to the control rats ($P<0.05$) (Fig. 2). Furthermore, the diabetic group treated with extract (500 mg/kgbw) showed a significant increase in CAT activity in kidney and liver tissues ($P<0.05$). MDA levels in diabetic rats were higher than in the control group (Table I). The administration of aqueous extract to diabetic rats did not significantly reduce levels of MDA, but a profound reduction of MDA levels was observed in diabetic group treated with 500 mg extract when compared to the diabetic control group.

TABLE I: The liver and kidney tissue contents of MDA in experimental groups.

MDA (µm/gr)	*Treatment*	
	MDA_K	MDA_L
C	./445±./129	./807±./263
ND	./555±./103	1.13±./167
DT_{50}	./534±./193	1.07±./173
DT_{250}	./463±./151	./95±./295
DT_{500}	./525±./168	./791±./152

Discussion

Streptozotocin (STZ) is toxic glucose analogues that preferentially accumulate in pancreatic beta cells via

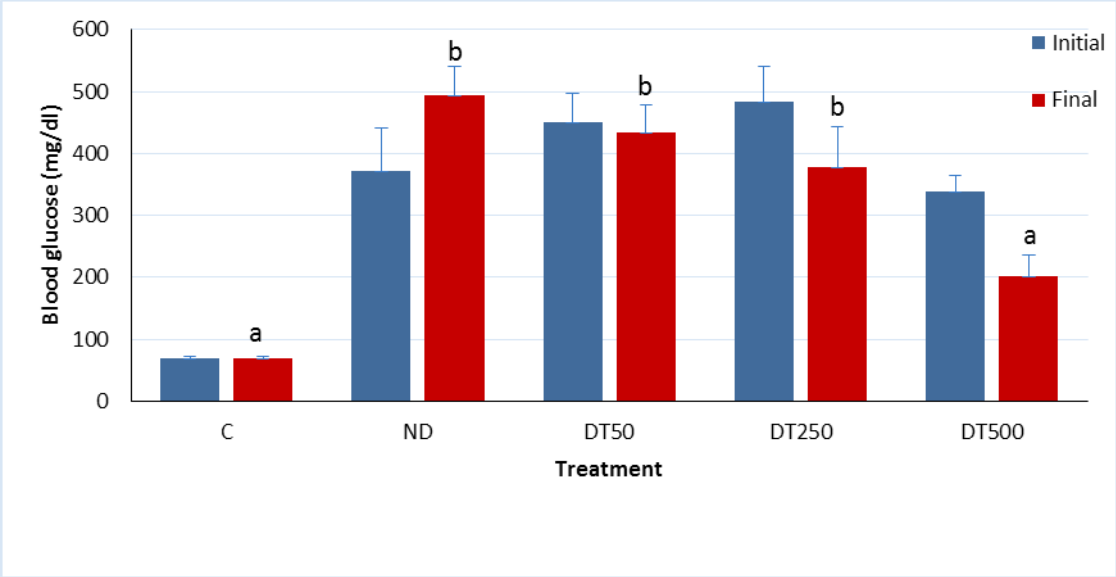

Fig. 1 : Blood glucose levels(mg/dl) in experimental groups.
a: significant difference in comparison with diabetic group(P<0.05).
b: significant difference in comparison with control group(P<0.05).

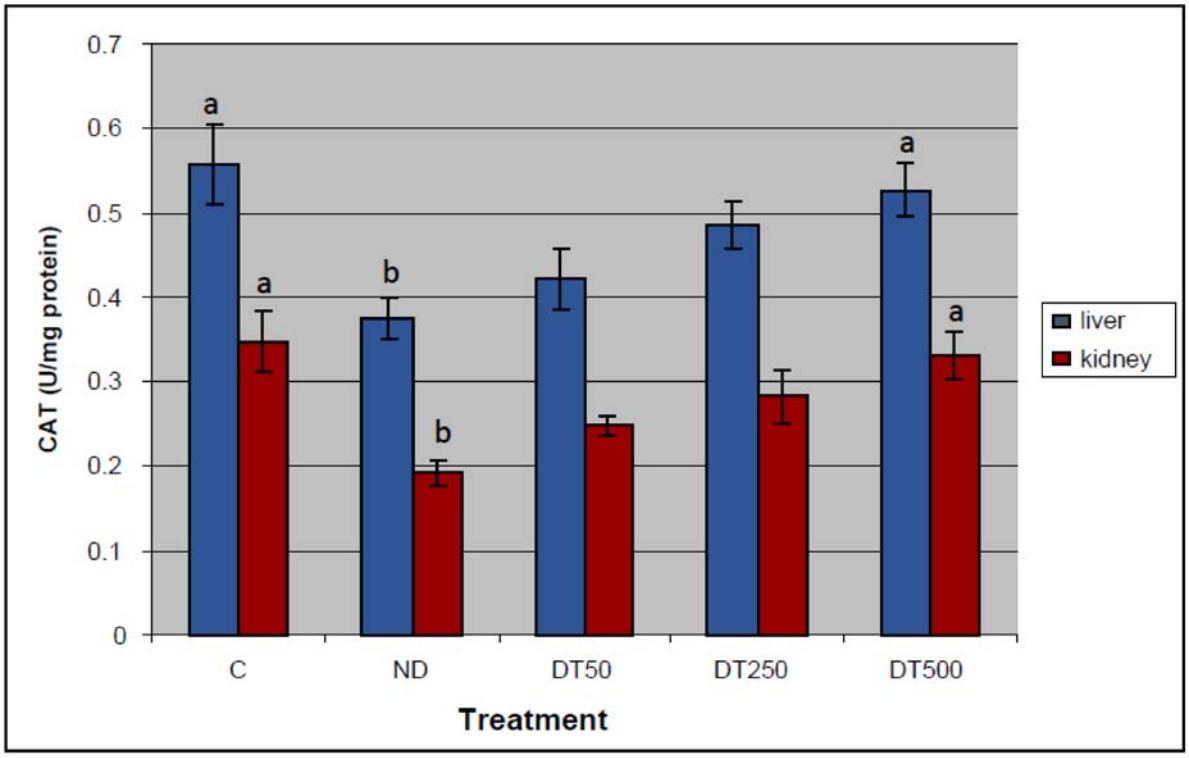

Fig. 2 : CAT activity(u/mg protein) of the kidney and Liver in experimental groups.
a: significant difference in comparison with diabetic group(P<0.05).
b: significant difference in comparison with control group(P<0.05).

the GLUT2 glucose transporter. STZ generates reactive oxygen species (ROS) in a cyclic redox reaction such as superoxide radicals, hydrogen peroxide and, in a final iron-catalyzed reaction step,

hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defense capacity (15). Based on ancient Persians traditional books, use of herbal medicine has a positive effect on the treatment of different diseases especially in diabetes mellitus (5). Numbers of plants which have this effect are: barberry, cynodon dactylon, tarragon, sumac, cinnamon, some tea specious and onion. Investigation shows these plants contain antioxidant agents (16).

Phytochemical investigation of Cynodon dactylon reveals the presences of flavonoids and sterols (17, 18). Flavonoids are products of plant metabolism that have free radical scavenging properties are effective antioxidants. This utility capable herbal plant to protect tissues against free oxygen radicals and lipid peroxidation (19). It is well known that certain flavonoids exhibit hypoglycemic activity and are also known for their ability of beta cell regeneration of pancreas (20). Stars have also shown to decrease blood sugar in experimental animal models (21). Thus, the significant antidiabetic effect of aqueous extract of Cynodon dactylon may be due to the presence of more than one antihyperglycemic principle and their synergistic properties.

Oxidative stress has been involved in the pathogenesis of chronic diabetes mellitus. Oxidative stress was characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems (22). MDA is one of the final products of polyunsaturated fatty acid peroxidation in the cells, increase in free radicals causes overproduction of MDA (23). In the present study, estimation the liver and kidney tissue contents of MDA which is formed as a result of lipid peroxidation were done. MDA levels in diabetic animals treated with extract were found to be lower than those in non-treated diabetic group. These results provided evidence for the free radical-scavenging properties of cynodon dactylon.

Seven et al (2004) reported a significant increase in the MDA level of the livers of diabetic rats (24). Fervid et al. Have found that supplementation with Antioxidant decreases the MDA level in diabetic patients (25). The flavonoids present in the aqueous extract of Cynodon dactylon might be responsible for its marked antioxidant efficacy at the tissue level in STZ-induced diabetic rats.

In diabetes mellitus, hyperglycemia can simply inactivate antioxidant enzymes such as SOD, CAT and GPX by fluctuating these proteins and induces oxidative stress which in turn causes

Lipid peroxidation (26, 27). In the enzymatic antioxidant defense system, CAT is one of the important enzymes and elimination of H_2O_2. The observed decrease in CAT activity in diabetic rats could result from inactivation by H_2O_2 or by glycosylation of the enzyme (28).

Liver and kidney are essential tissues where important complications of diabetes mellitus occur. It was shown that the severity of diabetic complications in tissues is related to the damage in their oxidative-antioxidative systems (29). In the present study, treatment with extract of C. Dactylon caused a significant increase in CAT activity in treating diabetic animals when compared with that in non-treated diabetic animals. The result was in collaboration with Rai (2010) who reported that the CAT activity was also markedly decreased following ROS formation and aqueous extracts of Rhus coriaria fruit prevented both ROS formation and oxidative stress (30).

The significant recovery in the CAT activities in liver and kidney tissues because of treatment of diabetic rats with the C. Dactylon aqueous extract reflects the antioxidant potential of this herbal preparation. There may be a possibility that the aqueous extract of C. Dactylon might have contributed in preventing glycation and inactivation of this enzyme (10). Phytochemical studies also indicate that the total phenolic content is 20 mg/g in the C. dactylon extract powder. In the case of medicinal plants, it has been reported that there is a positive correlation between the total phenolic content and antioxidant activity (31). In other related studies, the role of flavonoids in reducing oxidative stress associated with diabetes as well as in the regulation of plasma glucose concentration has been reported (32). Thus, the presence of phenolics and flavonoids in the aqueous

extract of C. dactylon is attributed to its significant antioxidant activity in the diabetic rats.

In conclusion, it suggested that Cynodon dactylon administration would be beneficial in the treatment of diabetes as an antioxidant and a free radical scavenger in controlling glucose levels and MDA levels, CAT activities of the kidney and liver.

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