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Cynodon dactylon Rhizome Extract Decreases the Cardiovascular Biomarkers (Cardiac Troponin I And Homocysteine) and Adenosine Deaminase Activity in Streptozotocin-Induced Diabetes Mellitus in Rats

Kaveh Azimzadeh[1] and Farid Digale[2*]

[1] *Elite Club, Urmia Branch, Islamic Azad University, Urmia, Iran*

[2] *Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran*

**Corresponding e-mail: fariddigaleh1@gmail.com*

ABSTRACT

Objective: *The aim of this study was to evaluate the plasma changes of cardiac troponin I level (cTnI), homocysteine (Hcy) and the activity of adenosine deaminase (ADA) in the streptozotocin-induced diabetes mellitus and following administration of pure Cynodon dactylon extract in the diabetic rats. Diabetes Mellitus (DM) is known as a metabolic disease which is followed by different blood and tissue complications.* ***Methods:*** *In this research, the cTnI, Hcy and ADA activity was measured in diabetic group after 3-month administration of Cynodon dactylon extract in three 150, 300 and 450 mg/kg doses.* ***Results:*** *The significant increase($P<0.01$) in cTnl, Hcy and ADA parameters in diabetic (positive control) group was determined in comparison to the control group (negative control) and a significant decrease of those ones ($P<0.01$) in the 450 mg/kg dose of Cynodon dactylon in comparison to the diabetic and control group.* ***Conclusion:*** *In conclusion, firstly heart damage occurs in diabetic rats and secondly the Cynodon dactylon extract tremendously influences the reduction of above-mentioned parameters and usage of dried powder and/or extract of Cynodon dactylon rhizomes in the food of the diabetic cases may play important role in the cardiovascular health and increasing the glucose consumption by cells.*

Keywords: *Cynodon dactylon*, cardiovascular biomarkers, diabetes mellitus, rat

INTRODUCTION

Diabetes Mellitus (DM) is known as a metabolic disease which is identified by the consistent hyperglycaemia and deficiency in the insulin synthesis in animal and human ones [1]. It has involved remarkable morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (heart attack) in patients [2]. Moreover, it causes almost 3.2 million deaths per year because of diabetes mellitus-mediated complications [3]. Now diabetes mellitus impresses more than 230 million people and affected ones is anticipated to attain 350 million by 2025 [4]. DM is considered as one of the most deleterious factors in the heart diseases occurrence and it could be useful to evaluate unknown aspects of herbal medicine such as *Cynodon dactylon* on DM complications (especially cardiovascular).

Myofibrillar proteins such as troponin I, C and T make reaction between actin and myosin via calcium in the heart muscle and skeletal muscles [5]. cardiac troponin I (cTnI) exists exclusively in myocardium and possesses valuable marker in awareness about the cardiomyocyte damage in the animals [6]. Following the increase of cardiomyocyte permeability, cTnI releases into blood and is pointed out as a high-accurate biomarker for diagnosis of the heart muscle damage and positive correlation has been clarified between high serum cTnl and cardiomyocyte damage [7,8]. Generally, several studies have reported the changes of cTnI in diabetes mellitus [9,10].

Adenosine is categorized as a strong essential endogenous inhibitor of the immune system that its suppressive effect has been determined in all cells and its level is regulated by adenosine deaminase (ADA) (EC 3,5,4,4, ADA) [11]. ADA breaks down the adenosine and deoxy adenosine into inosine and deoxy inosine. ADA is known as one of the most essential enzymes in maturation and the differentiation of T-cells and monocyte and also ADA activity in the T-cells is higher than B-cells [12]. It worth mentioning that ADA participates in the mechanisms which are involved in dilation

of the vessels and cell proliferation and its activity increases in diseases caused by stimulation of the immune system like liver cirrhosis, chronic hepatitis, and liver cancer [13,14]. Although the ADA activity is indicatively reported in tuberculosis but it can also increase in other diseases (infectious or non-infectious) like typhoid, sarcoidosis, and chronic lymphoblastic leukaemia [15].

Homocysteine (Hcy) is known as sulfur-containing amino acid which is produced following demethylation of methionine and also has been determined that it participates in endothelial cells damages in laboratory animals and the cardiovascular diseases. Hyperhomocysteinemia takes part in oxidative stress and plays important role in pathologic effects like anaemia. In addition, hyperhomocysteinemia damages the endothelial cells oxidation via multi mechanisms like: auto-oxidation, over-production of reactive oxygen species (ROS) in platelets and producing homocysteine thiolactone [16,17].

In recent years, more attention has been paid on useful effects of herbal plants in alleviation of DM-mediated complications and metabolic disorders. Hence, to our knowledge, evaluation of *Cynodon dactylon* extract effects, as a useful herbal plant, in the streptozotocin-induced diabetes mellitus has not been yet assessed on above-mentioned parameters in rat.

MATERIALS AND METHODS

Preparation of *Cynodon dactylon* Rhizome extract

One kilogram of *Cynodon dactylon* was gathered from the pastures around Urmia city and they were dried and converted to powders and then the powder was combined by ethanol and thereafter was extracted during 18 hours. In the following, the Ethanol (solvent) was removed (recovering process) and the pure extract was dried in oven (60°C). About 200 g pure extract was obtained from one-kilogram rhizome which was daily administered to diabetic rats via stomach gavage for three months in 150, 300 and 450 doses (mg/kg in distilled water).

Methodology and sampling

In present study, fifty male Wistar rats (255 g ± 26 g, 3-4 months) were randomly divided into five groups and were kept in a special place with cages under standard and hygienic situation. Also, the environment temperature and its humidity were assigned 21°C to 25°C and 41%, respectively. After two-weeks adaptation, for induction of DM, streptozotocin solution (Sigma-Aldrich) (60 mg/kg) in sterile distilled water was injected intra-peritoneal for four groups 45 (positive control and three treatment diabetic groups with 150, 300 and 450 mg/kg *Cynodon dactylon* extract). After three months, all rats were anesthetized (with sodium pentobarbital, 50 mg/kg, i.p) and blood sampling carried out via cardiac puncture, transferred to heparinized tubes and centrifuged 6000 rpm for 10 minutes at 4°C to preparation of plasma. cTnI was determined (RA1000) by ELISA method (Cobas kit, Elisa). The ADA activity was assessed using electrochemiluminecsence technique (ECL) (Elecsys, Roche, 2010). Insulin measurement took place using ELISA method (Crystal Chem, USA) and finally Hcy was measured by Spectrophotometric method (Spekoll 1500) (Parsazmoon company, Tehran, Iran).

Data statistical analysis

Statistical analysis was accomplished in all analyses. The Mean ± SD and the determination of variation between the data results were carried out with student's t-test through SAS v9.1 (SAS Institute Inc., Cary, NC, USA). The significance level was specified at ($P<0.01$).

RESULTS

The changes of the parameters are denoted in Table 1. Remarkable increase ($P<0.01$) in cTnI, ADA and Hcy in diabetic groups in comparison to the healthy ones and significant decrease of all parameters ($P<0.01$) were observed in the diabetic groups which were administered different doses of the pure extract of *Cynodon dactylon* (especially 450 mg/kg) in comparison to the healthy ones.

Table 1 Alterations of plasma cTnI, Hcy, ADA, Glucose, Insulin and Zn^{2+}, among different Groups

Parameters	Control negative group	Control positive group	Diabetic group received 150 mg/kg	Diabetic group received 300 mg/kg	Diabetic group received 450 mg/kg
cTnI, pg/ml	28.39 ± 2.33	139.86 ± 12.28†	109.64 ± 13.85	87.41 ± 7.59	32.16 ± 3.75
Hcy, mg/dl	4.02 ± 0.74	12.19 ± 1.23†	9.46 ± 1.53	7.63 ± 1.86	5.72 ± 1.49
ADA, U/L	16.29 ± 2.18	46.51 ± 2.54†	25.32 ± 2.61	19.84 ± 3.92	15.68 ± 2.82
Glucose, mg/dl	121 ± 9.48	342 ± 9.18†	251.44 ± 9.81	193.21 ± 5.87	118.86 ± 5.99
Insulin, µg/L	0.61 ± 0.03	0.04 ± 0.002†	0.11 ± 0.007	0.29 ± 0.01	0.52 ± 0.04
$Zn^{2+,}$ µg/dl	169.91 ± 7.69	52.85 ± 4.61†	47.11 ± 3.89	58.13 ± 5.12	43.68 ± 5.67
Note: Data are expressed as mean ± standard deviation. † Significantly different from the control group ($P<0.01$).					

DISCUSSION

In this study, high ADA activity was observed in diabetic group and also decrease of its activity was detected in the diabetic groups with *Cynodon dactylon* extract especially in the 450 mg/kg dose. Adenosine plays different roles such as, platelets aggregation, regulation of blood flow and also one of most important effects of adenosine is known as facilitator of glucose entrance into the cell [18,19]. Also, adenosine is considered as one of the strong anti-inflammatory molecule which modulates the leukocytes function in inflammation process. Furthermore, adenosine inhibits neutrophil-mediated endothelial damage and modulates the interaction of endothelial cells with leukocytes [20].

ADA catalyzes adenosine to inosine and causes decreasing of adenosine concentration and enhancement of ADA activity decreases the level of adenosine and subsequently declines the entrance of glucose into the cell and the result of high activity of ADA is hyperglycaemia [21]. ADA is noticed as an enzyme which is needed for proliferation, maturing and differentiation of the lymphocytes (especially T cells) [22] and also elevation of ADA activity takes place after the inflammation diseases which occur along with activation and proliferation of T cells. Hence, the serum level of ADA is considered as a marker of activation of T cells [23]. As a result, high activity of ADA in diabetic rats demonstrates higher T cells activity.

One of the other probable reasons of high activity of ADA is known to be the oxidative stress. Oxidative stress is accompanied with increase of free radicals and lipid peroxidation along with decreasing anti-oxidant enzymes and the role of oxidative stress in elevation of T cells and ADA activity has been reported in several studies [24]. So, the increase of ADA activity in diabetic rats can be related to the DM-induced oxidative stress.

In this study, ADA activity decreased during administration of *Cynodon dactylon* extract with different doses in diabetic rats. ADA is an important enzyme in insulin bioavailability [25,26] and Rutkiewicz, et al. [27] have reported the reduction in ADA activity after injection of insulin in rats. Bopp, et al. [28] have demonstrated the effects of water extract of *Syzgium cumini* fruit in decreasing and even inhibition of the ADA activity in hyperglycaemic patients and since ADA is pointed out as one of the most important factors in hyperglycaemia, so the *Cynodon dactylon* extract is effective in decreasing the blood sugar and its normalization. There is no study about the effects of *Cynodon dactylon* on the biochemical parameters like glucose and ADA in the diabetic rats but Karthik and Ravikumav [29] have determined the effects of *Cynodon dactylon* in decreasing the blood sugar in rats DM and the same results were reported by Ramya, et al. [30] in alloxan-induced diabetes mellitus in rat.

Since high ADA activity impresses on hyperglycaemia and also effects of the *Syzgium cumini* (as herbal plant) has been reported in decreasing of ADA activity and even its inhibition, so it is possible that the *Cynodon dactylon* extract participates in ADA decreasing processes in diabetic rats with the same *Syzgium cumini* mechanisms and/or there are unknown mechanisms which decreases the ADA activity. It is worth mentioning that, best dose of *Cynodon dactylon* extract in decreasing the ADA activity and even normalizing of glucose level was 450 mg/kg and in this dose the ADA activity reaches the normal level of healthy rats.

One of the other probable factors in decreasing of ADA which could be considered along with other mechanisms, is physiologically decreasing of ADA activity by cell. Since, DM is offered as one of the important factors in occurrence of endothelial cells damage and the protective role of adenosine in alleviation of damaged endothelial cells has been reported in several studies. Hence, it is possible that for alleviating of cell damage, high concentration of adenosine

is needed and the cell for reaching to this target, decreases the ADA activity. Furthermore, due to positive effect of adenosine in facilitation of glucose entrance into the cells, as a result, the cell decreases ADA activity. In addition, significant decline of zinc was determined during administration of *Cynodon dactylon* extract with different doses in diabetic rats in comparison of control group (negative group) and positive ones (diabetic group). The relation between zinc and the immunity of cell (T-cell) has been demonstrated and its shortage causes the severe decrease of the immune system performance of immune cells (T-lymphocyte) [31]. In addition, zinc cation is known as essential element of ADA and is placed in its active site and participates in catalyzing mechanism [32]. So, there is a close relationship between zinc and the ADA activity. Hence, one of the other probable reasons of ADA activity decreasing in extract receiving group could be due to hypozincaemia.

In the recent years, the vast attention has been paid on the effects of herbal plants on cardiovascular function. One of the medical plants which are proposed as a cardiovascular tonic and improving the operation of them in traditional medicine of Iran is *Cynodon dactylon*. The vasodilatory effect of *Cynodon dactylon* has been made clear in dilation of coronary arteries. In DM, due to overproduction of free radicals, cell damage occurs and one of vital cells that can affect with free radicals is known cardiomyocytes. Following cardiomyocyte damages, the cardiac troponin I (cTnI), which is considered the main high sensitive biomarker in diagnosing the cardiomyocytes damage in comparison of the lactate dehydrogenase (LDH) and creatine kinase (CK) significantly increase and release into blood. In this study, significant decrease of cTnI in the *Cynodon dactylon* treated groups (especially 450 mg/kg dose) were determined in comparison of the diabetic group (positive control) and the control ones (negative group). There is no study in terms of *Cynodon dactylon* effects on cTnI alterations in diabetic rats. But Atabek, et al., and Silvestrini, et al. [33,34] showed elevation of cTnI in diabetic ketoacidosis patients and in dogs with leishmaniosis which is in accordance with our study. The occurred heart damage in this study can be due to DM-mediated anaemia. Following DM occurrence, anaemia and tissue hypoxia are spotted the essential specifications of DM [35] and releasing of cardiac troponins like serum cTnI may happen in a response to the lack of oxygen caused by ischemia which can damage myocardium in diabetic groups. Few studies have been performed in respect of herbal medicine impacts on cTnI decrease, but can refer to Saravanan, et al. [36] who have reported the positive effect of the *Amaranthus viridis* Linn. plant extract in decreasing of serum cTnI in ischemic rats and attributed to its protective effect on the myocardium function which is in accordance with present study. In this study following the use of *Cynodon dactylon* rhizomes especially at 450 mg/kg dose in diabetic rats, significant decrease of serum cTnI was observed. In traditional medicine the very positive effect of *Cynodon dactylon* on cardiovascular health has been reported [37]. The useful effects of the *Cynodon dactylon* in decreasing of myocardium hypertrophy, improving the heart performance, positive ionotropic specification, and the increase of the contraction power of the heart muscle has been identified. Also, dose-dependent role of *Cynodon dactylon* extract has been reported in significant increase of the left ventricle contraction power in rats during elevation of *Cynodon dactylon* dose. Garjani, et al. [38] reported that the *Cynodon dactylon* rhizome are rich of sugars, flavonoids, steroids, saponins and estrols. Redout, et al. [38] determined that free radicals play important role in progression of left ventricle hypertrophy and becoming of it to the congestive heart failure. Since, the *Cynodon dactylon* is full of flavonoids, so, one of the important causes that cardiac damage was not detected in the *Cynodon dactylon* treated groups (because of the significant decrease in cTnI and Hcy), can be attributed to beneficial effects of flavonoids in removing the free radicals.

One of the other major components of *Cynodon dactylon* is steroidal saponins. Saponins are the main glycosides derived from the *Cynodon dactylon* which can be in both forms, steroidal saponins and triterpenoid saponins. In recent years, interesting biological functions of steroidal saponins have been clarified and one of their well-known important biological roles are referred as cardiac protective effect, anti-bacterial, anti-inflammation, and cholesterol reduction effect [39,40]. Since, the extract of *Cynodon dactylon* contains steroidal saponins so it can highly improve the myocardium function in the *Cynodon dactylon* extract treated rats.

Many studies have been carried out about the serum changes of Hcy in diabetic cases and the animal models and all of them have clarified the Hcy is known as a cardiovascular risk factor. In line with this, Nehler [41] has pointed that Hcy is an independent cardiovascular risk factor. In this study, significant increase of Hcy in diabetic group and significant decrease in the *Cynodon dactylon* treated diabetic rats were determined in comparison of the diabetic group (positive control) and control ones (negative control) and even Hcy concentration decreased by increasing the *Cynodon dactylon* dose. There is not any study in respect of Hcy alterations in diabetic rats but the increase of

the Hcy has been reported in streptozotocin-induced diabetes mellitus in rabbit [42]. Also, Azimzadeh [43] reported the increase of Hcy in cattle leptospirosis which is in accordance with this study. It is possible that due to vitamin B12 and acid folic deficiency in the diabetic group, (following anorexia and/or malabsorption of the vitamins), Hcy has not been converted to methionine and finally has elevated in the diabetic ones. It is hypothesized that flavonoid components of *Cynodon dactylon* extract participates in decreasing of Hcy and converts it to methionine. Meanwhile, it is possible that *Cynodon dactylon* extract includes high source of acid folic which decreases Hcy.

It is concluded that cardiovascular damage occurs in the diabetic rats which needs more attention in this issue. On the other word, *Cynodon dactylon* especially in 450 mg/kg dose plays effective role in preventing the heart damage. Also, following administration of *Cynodon dactylon* extract, decline of ADA activity firstly can be attributed the extract-mediated immune system suppress (especially T-cells) and secondly may be associated with the high demand of adenosine concentration for facilitation the entrance of glucose into the cell.

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