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Studying the effect of *Suaeda aegyptiaca* extract in comparison to the metformin on streptozotocin-nicotinamide induced type 2 diabetes rats

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Objective Type 2 diabetes mellitus is a complicated metabolic disease which affects the energy balance of the cell and consequently the body. With 422 million affected individuals, diabetes is one of the most prevalent disorders. Various medicinal properties have been counted for *Suaeda aegyptiaca* in traditional medicine. This study was conducted to comprise the effect of metformin and *S. aegyptiaca* extract on some physiological factors in diabetic rats.
Methods Streptozotocin-induced diabetic rats were administrated with 100, 200 and 400 mg/kg of *S. aegyptiaca* extract or metformin for the period of 4 weeks. Blood was taken from animals and levels of factors including Insulin, FBS, amylase, proteins, albumin, LDH, ALK, CPK, Fe, Na, K, TSH and MDA were evaluated.
Results Our results indicate that with the increasing concentration of *S. aegyptiaca* extract, the FBS and amylase amount of sugar decrease significantly. The mean volume of proteins in the T2 group was significantly higher than other groups and the albumin volume in T1 group was significantly higher than other groups. The CPK volume in the T3 group is approximately same to the negative control group. The rats treated with *S. aegyptiaca* extract had approximately same MDA concentration and which were higher than the metformin group.
Discussion Suaeda aegyptiaca extract can be a suitable candidate for subsequent studies to define a new therapeutic agent for treatment of diabetes.
Keywords *Suaeda aegyptiaca*, streptozotocin-nicotinamide, diabetes

Introduction

Diabetes mellitus type 2 is one of the most common and complex disorders of today's society. More than hundreds of million individuals suffered from diabetes which has caused serious economic and social problems.[1] It has been shown that the type 2 diabetes pathogenesis is associated with various disorders, such as progressive insulin resistance in the liver and peripheral tissues, decreased β cell mass and insulin secretion deficiency.[2] The long-term increase in glucose during diabetes is the main cause of secondary disorders, such as microangiopathy, macroangiopathy and retinopathy.[3] Weakening of antioxidant defense systems, osmotic pressure and metabolic abnormalities are other diabetics type 2 related disorders.[4] These complications damage the physical and physiological functions of various organs and threaten human health. Late complications of the type 2 diabetes, including nephropathy, retinopathy, cardiovascular complications, neuropathy, skin ulcer, hypertension and weight gain have been studied more.[5]

Cardiovascular disease is one of the diabetes complications. Advanced glycation end products (AGEs) through the reactive oxygen species (ROS), oxidized LDL, which cause atherosclerosis and other cardiovascular complications. The most important complications of glycation and formation of AGEs and its association with lipid profiles are enzyme activity reduction, free radical production, and reduction of receptor-ligand affinity. The protein glycation is one of the pathological mechanisms involved in the occurrence of multiple complications by inducing change in shape and function of glycosylated proteins. Glycation of structural and blood proteins are the main result of chronic hyperglycemia due to type 2 diabetes.[6,7]

Suaeda aegyptiaca is a salt-tolerant plant, which is a native plant of Khuzestan province, Iran. Various medicinal properties have been counted for this plant in traditional medicine.[8] This plant is considered as an appetizing because of the presence of the vitamin B group.[9] This plant contains a high volume of potassium, sodium, iron, iodine and other minerals.[18] Blood pressure regulation, diuretic and anti-inflammatory properties are other effect of this plant on human physiology. It is notable that this plant is also a CNS simulator.[10,11]

Although there are various medicines for diabetes, metformin is the most common medication for this disorder and some evidence showed decreased mortality rate;[12] however, this conclusion is questioned.[13] It is notable that metformin should not be used in those with severe kidney or liver problems.[14] Insulin injection can be used as auxiliary treatment or main therapeutic strategy. It is notable that insulin treatment is no need for initial treatment in most patients. When it is used, oral medications can be used for continuing the treatment process. The blood sugar level will be controlled by increasing the dosage. When nightly insulin is insufficient, twice a day insulin may achieve better control.[15]

This study was conducted to considering the importance of diabetic disease in the society and the advantages of *S. aegyptiaca* which is native to Iran, and comprise the effect of metformin and *S. aegyptiaca* extract on some physiological factors in diabetic rats.

Materials and Methods

Plant material and preparation

Fresh leaves of *S. aegyptiaca* were collected in Khuzestan province, Iran and confirmed scientifically by Department of Botany of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Leaves were air dried and then milled using mechanical grinders.

About 1200 ml of distilled water (70%) and ethanol (30%) mixture was used for dissolving 300 g of the *S. aegyptiaca* leaves powder and kept at room temperature for 74 h. Whatman paper was used for filtering the mixture. The mixture then centrifuged for 20 min with speed of 3500 rpm. The supernatant was dried at room temperature to obtained semisolid mass.[16]

Experimental animals

A total of 36 adult male Wistar rats with 150–250 g weight were prepared and kept in cages with standard laboratory conditions (temperature 22 ± 2°C with a 12/12 h light–dark cycle). Rats will have allowed natural diet and fresh water. Standards guide for the care and use of laboratory animals, established by the National Research Council of the national academic was used for all animals.

Induction of non-insulin dependent diabetes mellitus

Type 2 diabetes mellitus was induced in overnight fasted adult male Wistar rats. Intraperitoneal injection of nicotinamide (120 mg/kg body weight for each rat) (Merck, Germany) has been done at the first step. The streptozotocin (STZ) (60 mg/kg BW) (Sigma Aldrich, USA) dissolved in citrate buffer (pH 4.5) was injected intraperitoneally after 15 min. The glucose level in blood before and 72 h after STZ injection was evaluated to confirm development of diabetes. Animals with glucose levels of more than 126 mg/dl or more were included in the study.[17]

Experimental design

Rats were divided into six equal groups ($n = 6$). The treatment period of each group was 6 weeks. The first group considered as a negative control group (N). The rats of the group N received normal saline daily. The positive control group (P) contained six diabetic rats. The groups III–V contained diabetic rats which treated by *S. aegyptiaca* leaves hydro-alcoholic extract orally by gastric tube in doses of 100, 200 and 400 mg/kg body weight, respectively, daily for 4 weeks. The last group (group Met) received metformin (0.25 mg/kg bodyweight, Sigma Aldrich, USA) orally for 4 weeks as standard medication. After 4 weeks, all rats experienced 24 h food deprivation. After mild anesthesia by ether, blood sample was directly collected from the heart of each rat. Blood samples were centrifuged at 3500 rpm for about 15 min. The serum samples were refrigerated at −70°C until evaluation of various parameters.[18]

Urine albumin analysis

The protein and urinary albumin levels were evaluated by rat's urine, which was collected for 24 h every 4 weeks. Western blot analysis of the urine with affinity purified rabbit anti-rat albumin antibody (Immunology Consultants Laboratory, Newberg, OR, USA) was used for determination of urinary albumin extraction.

Determination of serum insulin

Serum insulin levels were determined from the blood samples obtained at the end of the 4-week study period by Insulin Rat ELISA Kit (Thermo Fisher Scientific, USA).

Determination of blood glucose

The blood sample of tail veins was used to determine non-fasting, morning blood glucose by glucometer (Bayer Elite XL, Bayer, Pittsburgh, PA, USA).

Determination of minerals

Serum iron was measured by atomic absorption spectroscopy (AAS). Sodium and Potassium were also measured by photoelectric flame photometer or atomic absorption spectroscopy.

MDA concentration

The MDA concentration was measured using a TBARS kit with chemical colorimetric method and based on the kit instructions. In this method, a pink complex was formed by the MDA reaction with thiobarbituric acid (TBA) in an acidic medium and a temperature of 90°C. The optical absorption was measured by using a spectrophotometer at 532 nm. The standard OD curve based on Tetra-toxin-propane dilutions were prepared for MDA concentration measurement.

Statistical analysis

Data were analyzed using SPSS software version 16. One-way ANOVA test and Student's *t*-test were used for statistical analysis. Values of $P < 0.05$ were considered to be statistically significant.

Results

The volume of various factors related to the blood glucose was statistically analyzed. The results of this experiment have been shown in Table 1. As shown in this table, the volume of all insulin, FBS and amylase have statistically significant difference between groups. The highest and lowest volume of insulin were seen in the control N and control P groups, respectively. Based on this result, the highest and lowest FBS were vice versa. On the other hand, the T3 group had the lowest rate for amylase.

The concentration of various proteins was also analyzed in this study (Fig. 1). Based on the results, the mean volume of proteins in the T2 group was significantly higher than other groups (Fig. 1A). On the other hand, the albumin volume in T1 group was significantly higher than other groups (Fig. 1B). Due to the fact that LDH is a marker of common injuries and disease such as heart failure, it was measured in this study. Our results indicate that the T3 group had the lowest rate of this enzyme among diabetic rats (Fig. 1C). The metformin group had the lowest rate of ALK among diabetic rats (Fig. 1D). The creatine kinase (CPK) level will deregulate in rich tissues in accidence with various disorders, such as myocardial infarction, rhabdomyolysis, muscular dystrophy, autoimmune myositides and acute kidney injury. Our results showed that the CPK volume in the T3 group is approximately same to the negative control group (Fig. 1D).

The differences of some elements between studied groups were also investigated in this study. Based on the available results of Table 2, the Fe concentration had a significant difference between groups and the T1 group had the highest volume in comparison with the other groups. It is notable that there were no statistical differences between groups for Na and K concentration.

The results of this study about TSH concentration showed that the negative control group had the lowest volume of TSH. Although the *S. aegyptiaca* extract concentration did not have any significant effect on TSH concentration, but *S. aegyptiaca* extract significantly decreased TSH concentration in comparison to the metformin group (Fig. 2A). The MDA is a marker for oxidative stress and its highest and lowest concentration

Table 1. **The statistical analysis of the blood glucose-related factors**

Variable	Group	Min	Glucose relate Max	Mean	SD	*P*-value
Insulin	Control N	13.00	18.00	15.4286	1.90238	0.000
	Control P	4.00	7.00	5.2857	1.11270	
	T1	8.00	12.00	9.7143	1.38013	
	T2	8.00	15.00	10.8571	2.26779	
	T3	11.00	16.00	12.8571	1.95180	
	Met	9.00	14.00	12.1429	1.57359	
FBS	Control N	130.00	160.00	144.0000	10.13246	0.000
	Control P	312.00	720.00	563.0000	126.01984	
	T1	130.00	380.00	254.1429	88.37313	
	T2	201.00	380.00	276.2857	56.94065	
	T3	155.00	180.00	166.1429	10.12305	
	Met	150.00	171.00	163.4286	7.72134	
Amylase	Control N	19.00	29.00	23.7143	3.63842	0.015
	Control P	28.00	33.00	30.2857	1.60357	
	T1	26.00	34.00	29.4286	2.87849	
	T2	23.00	32.00	29.1429	3.02372	
	T3	25.00	32.00	28.7143	2.05866	
	Met	24.00	34.00	29.8571	3.02372	

Table 2. **Statistical analysis for blood elements**

Variable	Group	Min	Max	Mean	SD	*P*-value
Fe	Control N	186.00	298.00	228.2857	40.52042	0.004
	Control P	115.00	210.00	144.1429	30.73233	
	T1	198.00	380.00	245.1429	61.89892	
	T2	106.00	206.00	168.4286	36.96781	
	T3	140.00	350.00	237.2857	71.73729	
	Met	131.00	281.00	212.2857	53.64921	
Na	Control N	133.00	149.00	143.0000	5.50757	0.342
	Control P	137.00	148.00	142.5714	3.59894	
	T1	139.00	147.00	142.8571	2.91139	
	T2	135.00	150.00	142.8571	6.20292	
	T3	129.00	157.00	143.8571	10.00714	
	Met	143.00	159.00	148.4286	5.15937	
K	Control N	4.30	8.20	6.1857	1.24824	0.214
	Control P	4.90	7.30	5.6429	0.78498	
	T1	4.70	8.40	5.8143	1.32467	
	T2	3.90	5.80	4.9000	0.81240	
	T3	5.20	8.90	6.1000	1.29872	
	Met	4.40	6.60	5.2286	0.70407	

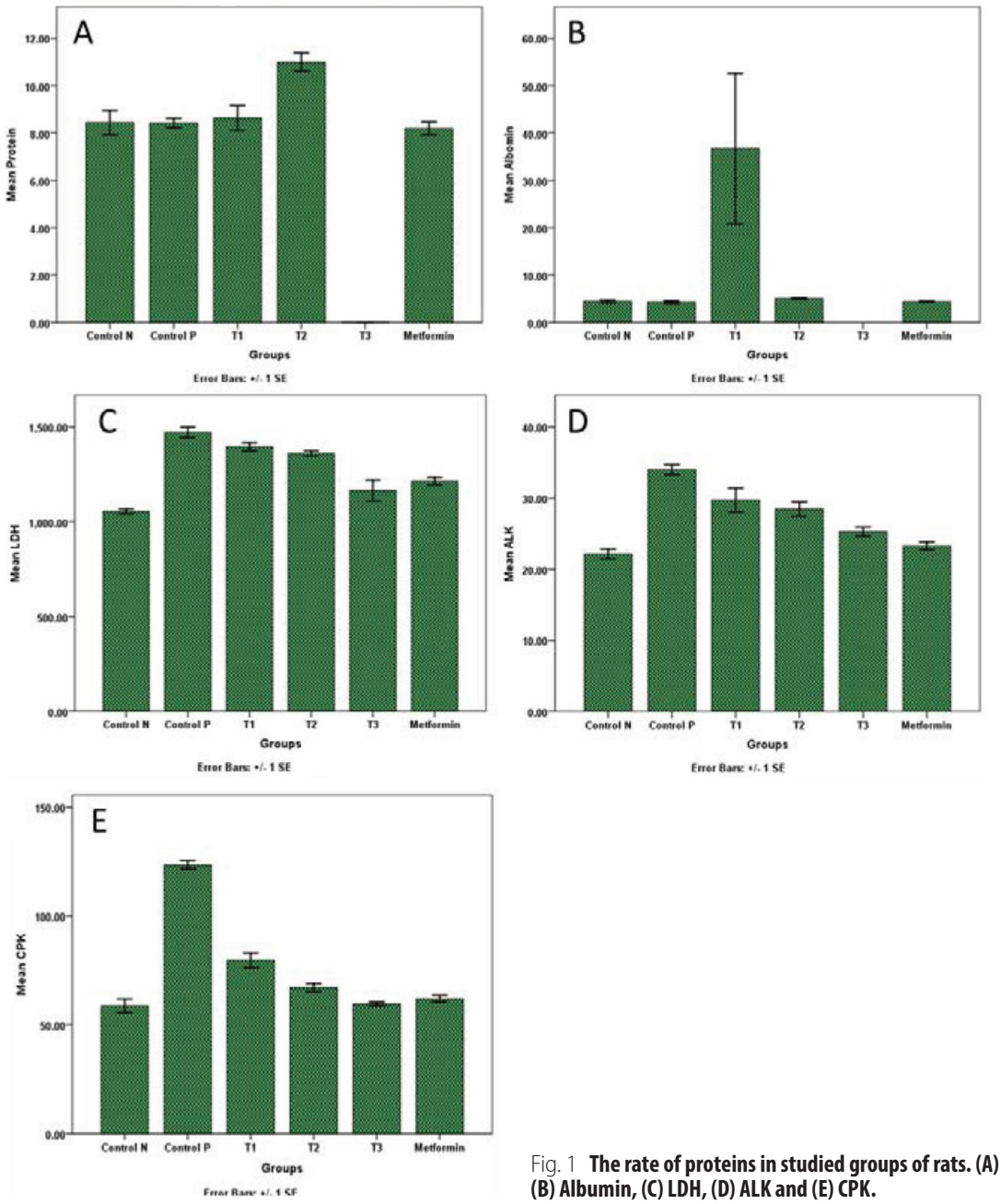

Fig. 1 **The rate of proteins in studied groups of rats. (A) Total protein, (B) Albumin, (C) LDH, (D) ALK and (E) CPK.**

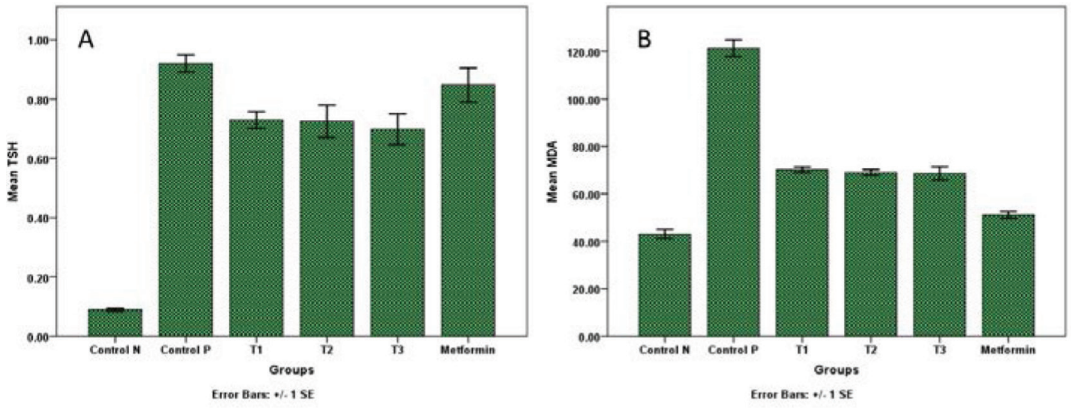

Fig. 2 **TSH (A) and MDA (B) concentration in treated rats.**

were observed in the positive and negative control groups, respectively (Fig. 2B). The rats treated with *S. aegyptiaca* extract had approximately same MDA concentration and which were higher than the metformin group (Fig. 2B).

Discussion

Due to the various complications associated with type 2 diabetes is typical, this disorder considered as a chronic disease

associated with a 10-year-shorter life expectancy.[19] As an example, the risk of cardiovascular disease will increase (2–4 times) in these patients.[19] Non-traumatic blindness and kidney failure are the main disorders caused by this type of diabetes in the developed world.[20] Other complications include acanthosis nigricans, sexual dysfunction and frequent infections.[21]

Suaeda aegyptiaca is a salt-tolerant plant in the family Amaranthaceae that grow naturally in salt-affected areas of Iran. It is notable that various medicinal usages have been counted for this plant.[8,11]

The medical effect of various plants extracts has been studied on diabetic rats. Adhikari et al. (2015) reported that curcumin has a property of protecting the islets β cells, decrease the insulin resistance and decrease the oxidative stress.[22] Our results also indicated that *S. aegyptiaca* extract decrease MDA volume, as an oxidative stress marker, in diabetic rats in comparison to the diabetic rats of the group P.

Sellamuthu et al. (2008) investigated the effect of the *Salacia chinensis* extract on various biochemical parameters of diabetic rats.[23] Their results showed that using *S. chinensis* extract can decrease FBS in diabetic rats. Same to our results, the FBS volume of healthy rats and treated diabetic rats are approximately similar. *S. aegyptiaca* extract decrease the LDH volume significantly in comparison to the diabetic rats. Sellamuthu et al. also showed same results by using *S. chinensis* extract.

The garlic (*Allium sativum* L.) extract decrease the FBS, triglycerides and total cholesterol volume in diabetic rats.[24] Our experiment also showed similar results in term of FBS by using *S. aegyptiaca*. Although Eidi et al. did not show any significant effect by using various concentration of *S. chinensis* extract on insulin volume, but our results indicated that the insulin volume will decrease by increasing *S. aegyptiaca* extract concentration.

Ahangarpour et al. (2014) evaluated the effect of *Dorema aucheri* hydroalcoholic leave extract on FBS and insulin level in streptozotocin-nicotinamide induced type 2 diabetes in male rats.[16] In this study, they comprised the effect of *D. aucheri* extract with Glibenclamide. They showed that rats treated by both *D. aucheri* and GLB have approximately same FBS level. It is notable that our results also showed similar effect on FBS level by metformin and *S. aegyptiaca* treatment. On the other hand, they showed insulin level will decrease more by GLB treatment in comparison to *D. aucheri* extract, but our results showed that *S. aegyptiaca* extract decrease insulin level more than metformin treatment.

Conclusion

In this study, we examined anti-diabetic effects of *S. aegyptiaca* extract on streptozotocin-induced diabetic rats. Our data showed that S. *aegyptiaca* extract in different concentrations has a positive effect on diabetic profile of rats. Our data also showed that this extract has comparable, in some cases better, anti-diabetic effects with a routinely used drug metformin. Therefore, *S. aegyptiaca* extract can be a suitable candidate for subsequent studies to define a new therapeutic agent for treatment of diabetes.

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