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Anti-diabetic effects of *S. aegyptiaca* extract on streptozotocin-nicotinamide induced type 2 diabetes rats

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Objective Type 2 diabetes mellitus is one the most prevalent disorders worldwide which is associated with unhealthy lifestyle and puts great burden on affected individuals and societies. In addition to chemical drugs and treatment options, using natural products including different types of plant extracts have been in use for curing type 2 diabetes for many years. In this research article, we have examined the effects of administrating *Suaeda aegyptiaca* extract on streptozotocin-induced diabetic rats.
Methods Streptozotocin-induced diabetic rats were administrated with 100, 200 and 400 mg/kg of *S. aegyptiaca* extract or metformin for a period of 4 weeks. Blood was taken from animals, and levels of factors including Blood urea, cholesterol, creatinine, HDL, LDL, SGOT, SGPT and triglyceride were evaluated.
Results Using *S. aegyptiaca* extract was associated with dramatic changes in lipid profile. It also decreased serum levels of urea, cholesterol and altered activity of SGOT and SGPT enzymes close to normal in treated rats. In comparison with metformin, *S. aegyptiaca* extract showed more significant results.
Conclusion Results of this study implies that *S. aegyptiaca* extract can be a good candidate for subsequent studies to define a new natural treatment option for type 2 diabetes mellitus.
Keywords *Suaeda aegyptiaca*, streptozotocin-nicotinamide, diabetes

Introduction

Type 2 diabetes mellitus is a complicated metabolic disease which affects energy balance of the cell and consequently the body. Diabetes results in complications like hyperglycaemia and altered lipid metabolism. Among the main and most important complications associated with diabetes are cardiovascular problems.[1] These manifestations are the results of islet β-cells inability to secrete sufficient levels of insulin in response to varying degrees of situation including over nutrition, inactivity, obesity, and insulin resistance.[2]

With 422 million affected individuals diabetes is one of the most prevalent disorders.[3] It affects different organs of the body and due to this complicated nature puts a heavy burden, both financially and emotionally on patients, their families and also on the society and the healthcare system. The estimated worldwide prevalence of diabetes among adults has increased from 108 million in 1980 to 422 million in 2014. This statistics are predicted to rise to around 439 million by 2030.[4] About 90% of cases of diabetes are diagnosed as type 2 diabetes mellitus.[5] Due to the dependency of this disorder on lifestyle, increase of prevalence in developing countries is predicted to be much more than in developed ones with 69% versus 20%.[4] This increase is related to westernization of developing countries. Consuming high energy diet and reduction in daily physical activity are the two determining factors in this westernization, and changes in lifestyle is a great risk factor.[6,7] Statistics show that in developing countries people within the range of working age are affected most, in comparison with those older than 60 years in developed countries.[4]

Diabetes is the result of interaction between genetics and environment. Although the main metabolic defects of type 2 diabetes are present to some degree in most patients, this disorder is highly heterogeneous.[8] Many different susceptibility genes have been identified that interacts with environmental factors in different stages of life.[9]

There are various chemical treatment options available for curing diabetes or decreasing its manifestations. Most of these drugs cause problems including drug resistance (reduction of efficiency), side effects, and even toxicity.[10] Therefore, need for new less harmful drugs is high especially due to high prevalence of this disorder. Most of the plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides which can have anti-diabetic effects.[11,12] Hence, study of plants is a logical approach for finding new treatment options for diabetes. The anti-hyperglycemic effects that results from treatment with plants are often due to their ability to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose.[13]

Suaeda aegyptiaca is a halophile plant which belongs to the family Amaranthaceae and grows in the northern parts of Iran and specifically in Khozestan province.[14] In this article we discuss the effects of administrating different doses of *S. aegyptiaca* extract on diabetic rats and compare it with the results of using metformin on the same animals.

Materials and Methods

Plant Material and Preparation

Fresh leaves of *S. aegyptiaca* were collected in Khozestan province, Iran and confirmed scientifically by the Department of Botany of Ahvaz Jundishapur University of Medical

Sciences, Ahvaz, Iran. Leaves were air dried and then milled using mechanical grinders.

Three hundred grams of *S. aegyptiaca* leafs powder was dissolved in 1,200 ml of distilled water and ethanol mixture (70:30) and kept for 72 h at room temperature. The mixture was filtered with Whatman paper and then centrifuged for 20 min with speed of 3,500 rpm. The supernatant was removed and dried at room temperature. Finally, the obtained semisolid mass was freshly used.[15]

Experimental Animals

Thirty six adult male Wistar rats (150–250 g) were purchased from animal house of Ahvaz Jundishapur University of Medical Science (AJUMS) and were kept in cages with standard laboratory conditions (temperature 22 ± 2°C with a 12/12 h light–dark cycle).[16] Rats were allowed *ad libitum* access to normal laboratory diet and tap water. All animals' procedures were in accordance with standards guide for the care and use of laboratory animals, established by the National Research Council of the National Academic.

Induction of Non-insulin Dependent Diabetes Mellitus

For experimental induction of type 2 diabetes mellitus in adult male Wistar rats, after they were fasted overnight, firstly nicotinamide (120 mg/kg body weight for each rat) (Merck, Germany) was dissolved in normal saline and then was administrated intraperitonealy to them. After 15 min the rats received an IP injection of STZ (60 mg/kg BW) (Sigma-Aldrich, USA) dissolved in citrate buffer (pH 4.5). Development of diabetes was confirmed by the assessment of glucose level in blood before and 72 h after STZ injection. Animals with glucose level of more than 126 mg/dl or more were included in the study.[17]

Experimental Design

Animals were randomly divided into six groups (n = 6) and treated daily for 4 weeks as follows: Group I: intact control rats were administered normal saline daily for 4 weeks; group II: diabetic control rats; group III, IV and V: diabetic rats which received *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; group VI: diabetic rats received metformin (100 mg/kg body weight, Sigma-Aldrich, USA) orally for 4 weeks as standard medication. By the end of the experiment, animals were deprived of food overnight (24 h) and after mild anesthesia by ether, blood sample was directly collected from their hearts and were centrifuged at 3,500 rpm for about 15 min to obtain blood serum. Serum samples were refrigerated at −70°C until evaluation of insulin and serum biochemical parameters.[18]

Lipid profiling (including measurements of triglycerides, HDL and LDL) was done using spectrophotometer instrument.

Lipid Profile, SGOT and SGPT Levels Measurement

The activities of pathophysiological enzymes such as serum ALP, SGOT and SGPT, as well as serum.

Triglyceride (TG) total cholesterol (TC), LDL-cholesterol (LDL-c) and HDL-cholesterol (HDL-c) were estimated with Pars Azmoon kits using auto-analyzer. VLDL-cholesterol (VLDL-c) concentration according to Norbert formula, equals one fifth of TG content.[20] Serum leptin concentration was measured by ELISA kit (Labor Diagnostika Nord GmbH, Germany). Inter- and intra-assay coefficients of variation was 5.8 and 4.3% respectively. Low-end sensitivity of this kit was 0.5 ng/ml.

Statistical Analysis

Data was analyzed using SPSS software version 16 by the equation mean ± SEM. One-way ANOVA test and student's t-test were used for statistical analysis followed by significant difference (LSD) test. Values of $P < 0.05$ were considered to be statistically significant.[19]

Results

Effects of S. aegyptiaca Extract on Blood Urea

Results of this study shows that administration of *S. aegyptiaca* extract in 400 mg/kg decreases the amount of blood urea to a comparable level to negative control rats. Administration of *S. aegyptiaca* extract in 100 and 200 mg/kg also decreases the amount of blood urea to a comparable level with metformin.

Effects of S. aegyptiaca Extract on Cholesterol Level

Our findings showed that the level of cholesterol increases in diabetic rats in comparison with control animals (Fig. 1). Using *S. aegyptiaca* extract has reversed this phenomenon and the highest decrease in level of cholesterol as seen in the group of rats that used 400 mg/kg of body weight of the extract ($P < 0.000$). Our results showed that the administration of metformin does not affect levels of cholesterol in the blood samples of rats in comparison with positive control group ($P > 0.615$).

Effects of S. aegyptiaca Extract on Creatinine

In this study by induction of diabetes in rats using STZ, level of creatinine in the blood samples of affected rats shows a slight decrease in comparison with healthy animals, although this difference was not statistically significant ($P > 0.677$).

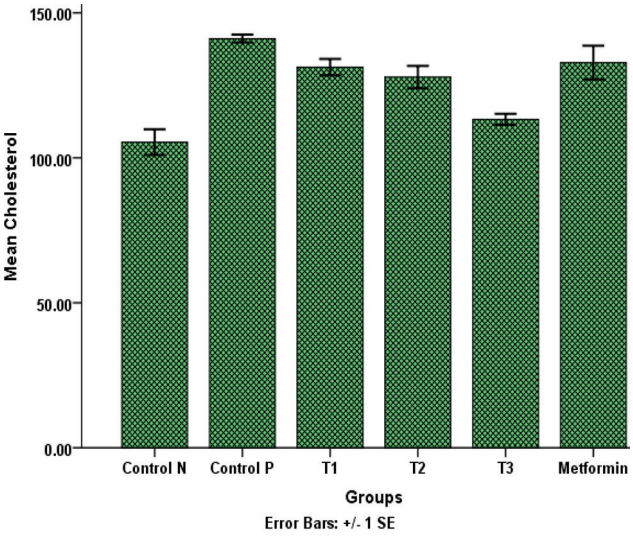

Fig 1. **Variations in cholesterol level in different studied groups. Control N: Positive Control. Control P: Negative Control. T1: *S. aegyptiaca* extract (100 mg/kg). T2: *S. aegyptiaca* extract (200 mg/kg). T3: *S. aegyptiaca* extract (400 mg/kg). Metformin: Metformin (100 mg/kg).**

Same difference was observable in changes of the levels of creatinine between control group, the group that has administered *S. aegyptiaca* extract and the group that has used metformin ($P < 0.933$, 0.945, 0.100 and 990 for *S. aegyptiaca* extract 100, 200, 400 mg/kg and metformin respectively).

Effects of S. aegyptiaca Extract on HDL and LDL Levels

Results of this study showed increase in levels of LDL and decrease in levels of HDL in diabetic rats in comparison with healthy animals. In case of LDL, administration of different doses of *S. aegyptiaca* extract was associated in attenuation of effects of diabetic condition in affected rats. This decrease in levels of LDL using *S. aegyptiaca* extract was higher than the effect that administration of metformin causes. In case of HDL, using *S. aegyptiaca* extract has resulted in increase in level of this substance in a comparable level to healthy animals ($P < 0.001$). In this case using metformin did not significantly changed the level of HDL in blood samples of studied animals.

Effects of S. aegyptiaca Extract on SGOT and SGPT

As demonstrated in Table 1, administration of STZ increased levels of SGOT and SGPT enzymes in serum of animals. For SGOT, using *S. aegyptiaca* extract was associated with increase in levels of the enzyme in the serum of treated animals ($P < 0.050$, 0.006 and 0.001 for 100, 200 and 400 mg/kg of *S. aegyptiaca* extract respectively). In comparison with administration of metformin, *S. aegyptiaca* extract (especially with 400 mg/kg dose) was much more effective in lowering levels of SGOT in the blood ($P < 0.037$). For SGPT, our results showed a similar trend for all four drugs, meaning that all of them have similar effect on decreasing levels of the enzyme in the serum samples of studied animals (Table 1).

Effects of S. aegyptiaca Extract on Triglyceride

Administration of STZ increased the level of triglyceride in diabetic rats in comparison to healthy animals. This increase has been meaningfully lowered by the administration of 100, 200 and 400 mg/kg of *S. aegyptiaca* extract ($P < 0.000$ for all three doses). In case of using metformin the decrease in amount of triglyceride was lesser in comparison with *S. aegyptiaca* extract concentration. Based on our results, using metformin does not make any significant difference in levels of triglyceride between affected rats and healthy animals.

Discussion

With estimated prevalence of 6.4% in 2010, diabetes mellitus is one of the disorders that puts a heavy burden not only on affected individuals and their families, but also on the health-care system and society. Therefore, finding new ways for curing this disorder is of immense importance. In this study, we examined the effects of using *S. aegyptiaca* extract as a substance for reversing effects of diabetes in rats that have been under administration of STZ for induction of diabetes.

Suaeda egyptiaca belongs to halophytes, a group of plants comprising about 1% of the flora of the world.[14] Our findings indicate drastic changes in levels of triglycerides, blood urea, LDL, HDL and cholesterol. Our results also implied on the positive effects of *S. aegyptiaca* extract on the levels of SGOT and SGPT enzymes in the studied diabetic rats. There are numerous studies that examined effects of using plant extracts on diabetic rats. Eidi and colleagues observed similar results by administrating garlic (*Allium sativum* L.) on streptozotocin-induced diabetic rats. In their study oral administrations of the garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, SGOT and SGPT levels, while increased serum insulin in diabetic rats but not in normal rats ($P < 0.05$).[20]

In another study, Ahangarpour and colleagues evaluated ant diabetic effects of *Dorema aucheri* hydroalcoholic leave extract. They found that *D. aucheri* has highly significant blood glucose lowering effect. It also has a dramatic effect on serum lipid profiles, insulin and leptin levels. Similar to our study, they found that SGPT and SGOT levels will dramatically change using different dosages of *D. aucheri* in streptozotocin-induced diabetic rats.[21]

Patel and colleagues have used Dihar, a polyherbal formulation consisting of extracts from eight different herbs to measure anti-hyperglycemic, anti-hyperlipidemic and antioxidant activities of it. Results of this study demonstrated that administration of Dihar for streptozotocin-induced diabetic rats significantly lowers the serum creatinine and urea levels in studied animals.[22]

Table 1. **Effect of *S. aegyptiaca* extract on enzymatic profile of rats**

		Mean	Std. deviation	Std. error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Lower bound		
SGOT	Control N	40.1429	1.57359	0.59476	38.6875	41.5982	38.00	42.00
(AST)	Control P	62.4286	1.98806	0.75142	60.5899	64.2672	59.00	65.00
	T1	59.2857	1.25357	0.47380	58.1264	60.4451	58.00	61.00
	T2	56.1429	5.17779	1.95702	51.3542	60.9315	50.00	63.00
	T3	54.4286	5.28700	1.99830	49.5389	59.3182	48.00	62.00
	Metformin	60.4286	1.51186	0.57143	59.0303	61.8268	59.00	63.00
SGPT	Control N	56.4286	4.92805	1.86263	51.8709	60.9863	49.00	62.00
(ALT)	Control P	87.2857	7.82548	2.95775	80.0484	94.5231	77.00	98.00
	T1	85.4286	4.68534	1.77089	81.0954	89.7618	81.00	95.00
	T2	84.8571	2.91139	1.10040	82.1646	87.5497	82.00	89.00
	T3	83.5714	4.64963	1.75739	79.2712	87.8716	75.00	88.00
	Metformin	83.4286	3.95209	1.49375	79.7735	87.0836	78.00	90.00

All in all we showed that the administration of different doses of *S. aegyptiaca* extract on streptozotocin-induced diabetic rats is associated with changes of different factors in serum of the animals. This difference was particularly emphasized for creatinine, blood urea, HDL, LDL and also SGOT and SGPT enzymes. Moreover, results of the present study showed for the first time that *S. aegyptiaca* extract has comparable effects with metformin in reducing complications of diabetes in diabetic rats. Therefore, subsequent studies can be particularly helpful in providing more details about mode of action of this substance which consequently might result in introduction of an effective and natural treatment option for diabetes.

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, and 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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