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ANTIDIABETIC EFFECT OF HYDROALCHOLIC EXTRACT OF *ALLIUM HIRTIFOLIUM* BOISS. IN ALLOXAN-INDUCED DIABETIC RATS

KAZEMI S[1*], MOSHTAGHIAN SJ[1], SETORKI M[2] AND DOUDI M[3]

1: Department of Biology, University of Isfahan, Isfahan, Iran

2: Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

3: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran

***Corresponding Author: E Mail: so.kazemi8@gmail.com**

ABSTRACT

Allium hirtifolium (Shallot) is one of the medicinal plants used in traditional Iranian medicine as a treatment for diabetes, but little scientific documentation supports its antidiabetic action. This study was designed to investigate the antihyperglycemic effects of ethanolic extract of shallot in diabetic rats. 35 male Wistar rats were divided into 5 groups of 7 each. The groups included normal control, diabetic conrol, diabetic treated with low dose extract (0.1 g/Kg), diabetic treated with high dose extract (1 g/Kg) and diabetic treated with Glibenclamide (0.6 mg/Kg). Diabetic rats with no treatment exhibited an increase significantly in level of fasting blood glucose, cholesterol, triglyceride, LDL and decrease in insulin and HDL level compared to nondiabetic rats. Treatment of diabetic rats with extract and glibenclamide could restore the change of the above parameters to their normal levels. The effects produced by extract were comparable to that of glibenclamide. On the base of histological results, the used herbal medicines had significant effects on increasing the size and number of pancreatic islets in diabetic groups. Also, the administering of extract improved body weight changes in the diabetic rats in experimental period. The observations of this study indicate that phytochemicals present in *Allium hirtifolium* may play a therapeutic role in combating diabetes and diabetes-related complication.

Keywords: Diabetes, Shallot, Alloxan, *Allium hirtifolium*

INTRODUCTION

Diabetes mellitus is a common metabolic disorder, characterized by hyperglycemia and alteration in carbohydrate, protein and lipid metabolism which resulting from perturbation in insulin secretion (type 1) or insulin action (type 2) **[1]**. Chronic hyperglycemia cause complication linked to diabetes, such as retinopathy, nephropathy and neuropathy. The risk for atherosclerotic vascular disease is also increased in persons with diabetes mellitus **[2]**. It is a growing health problem in most countries and its incidence is considered to be high all over the world **[3]**. Insulin and oral hypoglycemic agent are use as standard drug in diabetic patient, but managing diabetes without any side effects is still a subject for challenge between medicinal communities. Plants have long been used for the ethnomedical treatment of diabetes in various systems of medicine and accepted as an alternative for diabetes treatment. In recent years, research on medicinal plants for the management of diabetes has attracted the interest of scientists. The available literature shows that there are more than 400 plant species showing hypoglycemic activity **[4, 5, 6]**. Thought some of these plants have great reputation in the indigenous system of medicine for their antidiabetic activities, many remain to be scientifically established. The edible Allium species are of major economic and dietary importance all over the world. Shallot *(Allium hirtifolium* Boiss.), belonging to the family of Liliaceae, is a native edible plant in Iran. The use of genus Allium plants including shallot, garlic and onion has been widely used as medicine and condiment **[7]**. They are rich in sulphur-containing compounds like allicin and polyphenolic compounds like flavonoids that have perceived benefits to human health **[8]**. There is previous report on the pharmacological effects of shallot such as antiproliferative [9], antioxidant **[10]**, antitrichomonas **[11]**, immunomodulatory effect **[12]** and antibacterial **[13]**. The purpose of this research was to experimentally assess the antidiabetic effect of *A. hirtifollium* in alloxan induced diabetic rats and to compare it with glibenclamide as a reference standard.

MATERIAL AND METHOD

Preparation of Extract and Quantitative Analysis of allicin

The bulbs of *A. hirtifollium* were purchased from Medicinal Plants Research Division, Isfahan's Department of Natural Resources (Isfahan, Iran), in May 2008 and a voucher specimen No.1583 kept in the herbarium of the Pharmacy Faculty of Isfahan University of Medical Sciences.

Dried and powdered bulb (100 g) of the plant were extracted in two steps, firstly with %90 ethanol and secondly with %50 ethanol for 48h. The two extract were combined and concentrated in a rotaring evaporator, consequently was decanted with chloroform, At last evaporated in the oven under temperature about of 50̊ C and sterile condition **[12]**. This method 51.9 g extract was obtained from 100 g powder.

Animals

Male wistar rats weighing 180-220 g were purchased from Ahvaz University of Medical Sciences (Jondishapoor), Ahvaz, Iran. The animals were housed in clean cages and maintained in Animal House of Department of Biology of the Isfahan University, Isfahan, Iran, in well ventilated room under conditions of temperature and natural light and dark cycle. The Ethics Committee of the Isfahan Cardiovascular Research Center approved the present study. All animals had free access to standard pallet diet and tap water.

Induction of Diabetes

Diabetes was induced by intraperitoneally injection of freshly solution of alloxan monohydrate (Sigma, Germany) in sterile normal saline. The dose of alloxan used was 120 mg/kg BW as a single dose [14, 15]. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia [16]. After 72 hours of alloxan injection, fasting blood glucose was measured by Glucometer [17, 18]. The diabetic rats with blood glucose level over 130 mg/dl were used for the experiments [19].

Experimental Design and Treatment Schedule

In the present study, a total of 35 rats were randomly divided into 5 groups of 7 rats each.

Group 1: Normal control rats

Group 2: Diabetic control rats

Group 3: Diabetic rats were supplemented with 0.1 g/kg BW of shallot extract

Group 4: Diabetic rats were supplemented with 1 g/kg BW of shallot extract

Group 5: Diabetic rats were received 0.6 mg/kg BW of reference drug, glibenclamide

Shallot extract and glibenclamide suspended in normal saline and administered to animals daily using a single intraperitoneally injection for 4 weeks.

Collection of Blood and Biochemical Analysis

After 4 week of treatment, the animals were fasted overnight and blood samples were collected, then serum was separated by centrifugation at 3000 rpm for 15 min, and then utilized for biochemical assay **[19]**.

The concentration of glucose determined with diagnostics kits from Biosystemes. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured using biochemical test kits (Parsazmun Co., Karaj), with the colorimetric method [20]. Insulin was measured using the Monobind (Lake Forest, CA) insulin microplate enzyme-linked immunosorbent assay test.

Histopathological Study

After the animals were sacrificed, the pancreas tissue were removed and washed with normal saline, put in %10 formalin solutions. The tissue samples were dehydrated in ascending grades of ethanol. The specimens were cleared in xylene, embedded in paraffin wax, sectioned at 5 micron thickness, stained with Hematoxylen and Eosin and then studied under microscopy **[15]**.

Statistical Analysis

Analysis of data performed using the SPSS statistical software with one way ANOVA. The differences among means were tested for significance using LSD post hoc test. The criterion for statistical significance was $p< 0.05$. Data are presented as means with their standard deviation.

RESULTS

In **Table 1,** results of biochemical tests for glucose, insulin, lipids, lipoproteins, CRP is mentioned. The results of weight changes and histological findings are presented in Table 2 and 3, respectively.

A: Comparison of the Groups in Glucose and Insulin Level

In diabetic control group, serum glucose levels compared with normal controls showed a significant increase. Consumption of high and low dose shallot extract (0.1 and 1 g/kg) in diabetic rats causes a significant decrease in glucose levels compared to diabetic control, like taking glibenclamide (p <0/05) **(Table 1)**.

In the diabetic group, insulin levels decreased compared with normal controls.

Consumption of high dose shallot extract with glibenclamide increased rate of this factor in comparison with diabetic control, but these changes were not significant.

But, consumption of low dose shallot extract causes a significant increase of insulin comparison to diabetic control (p <0/05) **(Table 1)**.

B: Comparison of the Groups in Cholesterol and Triglycerides Level

The results of this research indicate that triglycerides and cholesterol levels, in diabetic control group compared to normal controls increases significantly.

Diabetic rats treated with the extract (both high dose and low dose) significantly reduced cholesterol and triglyceride levels compared to diabetic control (p <0/05) **(Table 1)**.

As **Table 1** implies, effect of glibenclamide on blood cholesterol lowering is not enough (as much as extract shallot) and therefore no significant difference seen in comparison with diabetic control group.

C: Comparison of the Groups in Lipoprotein Level

Consumption of extract in diabetic rats, has been reduced significantly serum LDL levels in diabetic rats.

The results indicate that HDL lowering in diabetic control group compared to the normal control group was not significant, but the diabetic rats treated with glibenclamide is able to increase rate of HDL significantly compared to diabetic control. While, increasing this factor in treated with extract group is not significant (p <0/05) **(Table 1)**.

D: Comparison of the Groups in CRP

Survey results showed that this factor is increased in diabetic control group compared to other groups, treated with the extract (both high dose and low dose) are able to decrease rate CRP significantly. Effect of glibenclamide-treated group compared to the diabetic control is not significant (p <0/05) **(Table 1)**.

E: Weight Changes Results

Table 2 illustrated the effect of hydroalcolic extract and glibenclamide on body weight changes in the diabetic rats in experimental period. Result showed that after 2 and 4 weeks of treatment, only diabetic control group decreased significantly in body weight when compared to week 0 (p <0/05).

F: Results of Histology

Histomorphologic study has shown that the average size and number of islets Langerhans is different in experimental groups. The size and number of islets in the diabetic group compared with the normal control has been decreased significantly, but in shallot extract and glibenclamide groups to be close to the level of normal controls. High and low doses of the extract increased the average size of islet compared with diabetic control (p <0/05) **(Table 3, Figure 1)**.

DISCUSSION

The results of this study showed that treatment of diabetic rats with shallot extract for 4 weeks reduced glucose, cholesterol, triglycerides, LDL, CRP levels compared to diabetic control group.

Also, this herb increases blood HDL levels compared to diabetic control, but this has not significantly increased. This plant could increase blood insulin levels, but, this effect is significant in groups treated with the extract lower doses.

A number of researches have reported the beneficial effects of Allium genus, including onion and garlic on diabetes, but no reports exist about the effectiveness of shallot. For example, Similar to our results, Thomson et al is reported effects of hypoglycemic *Allium sativum* in diabetic rats with streptozotocin **[21]**.

Also, Eidi et al stated that the methanol extract of *Allium porrum* may reduce serum glucose in diabetic rats with streptozotocin **[22]**. Loss of body weight is a characteristic condition in diabetes, owing to defect in glucose metabolism and excessive breakdown of tissue protein. Administration of extract for 4 weeks to diabetic rats improved body weight. This could be due to a better control of the hyperglycemic state in diabetic rats.

Histologic changes were seen in the mean size and number of islets in the diabetic group compared to the control group. As the average size of islands in the diabetic group was significantly lower than in normal control that obtained in other studies **[15, 23]**.

The results obtained in this study, the average size of pancreatic islets increased in groups treated with the extract compared to diabetic control. This implies shallot extract on the healing and regeneration of pancreatic tissue. Following this operation, the number of B cells increased and more insulin is secreted. As a result, some adverse effects are corrected in diabetic and insulin deficiency. Antioxidant compounds of plant can cause regeneration and reconstruction of damaged cells and tissue. Antioxidants effect on signal transduction, increasing of mRNA levels and an increase in cell division. Studies have shown that treatment of rat pancreatic islets with quersetin will increase the number of islands. This is due to increased DNA replication in cells of pancreatic islets **[24]**. Research Jelodar et al shows that consumption of Allium causes a significant effect in increasing the volume density of pancreatic islets volume density of, beta cells and alpha cells is compared to the number of beta cells **[25]**. Inhibiting of histological changes in shallot extract group provides the immediate cause for the improvement and prevention of complications of diabetes by this plant.

Recently, oxidative stress has been as one of the mechanisms governing on diabetes mellitus that influence the metabolism of carbohydrates, lipids and proteins. In people with diabetes, some types of changes have nature of oxidative, or are associated with increased oxidative stress **[26]**. Some of sugary compounds **[27]** and hypoxia-induced hyperglycemia false **[28]** may be an imbalance of oxidants and redox status within cells **[29]**.

Antioxidants are compounds that protected different combinations and cell membranes against oxidation. The mechanism of action of these compounds, free radicals collecting, transferring the electrons to these oxidants and disable them **[30, 31]**. Effect of antidiabetic shallot probably is due to antioxidant compounds.

Several reports suggest that the hypoglycemic effects of Allium mainly depending on the active sulfur compounds, although, their flavonoids and other compounds may also be effective **[22]**. Compounds such as allicin and allyl propyl disulfide exert effects of hypoglycemic by increasing pancreatic secretion of insulin and also the release of more insulin **[32]**.

Allium is rich in antioxidant compounds such as saponin and phenolic compounds.Eidi et al reported that hypoglycemic effects *Allium porrum* due to the presence of saponin and flavonoids found in this plant **[22]**. Compounds flavonoids such as quercetin have hypoglycemic activity in diabetic rats **[33]**.

Our results are consistent with the results of the work Liu *et al*. These researchers were studied effect of garlic oil and its diallyltrisulfide compounds on serum insulin level in diabetic rats with streptozotocin **[34]**. Yield evaluated on the hyperinsulinemia can be expressed as follows:

Compounds of antioxidant effects on pancreatic beta-cell repair and causes increasing cell number. Several studies have shown that in diabetic rats treated with antioxidants significantly increased the number of beta cells **[15]**.

Our results on parameters of lipid are similar to the results of the work Kumari&Augusti. These researchers are studied effect of S-methyl cysteinsulfoxide (a combination sulfide) isolated from onion (*Allium cepa*) on serum lipids and lipoproteins in hypercholesterolemic rats **[35]**.

Function of these plants on lipid parameters can be attributed to the following reasons:

Sulfur material in Allium, causes oxidation of SH groups in lipid synthesis enzymes and thus lipid synthesis are reduced or prevented. Also, these compounds cause oxidation of NADPH to NADP. Since, in stages of synthesis lipid, hydrogen, is supplied by NADPH, this action causes inhibition of lipid synthesis **[36]**.

Based on experiments of Slowing et al, saponins of Allium, through inhibition cholesterol absorption from the intestinal lumen (without changes in HDL-cholesterol) can reduce cholesterol. Regulations of cholesterol synthesis take places in the beginning and in the course hydroxy methyl glutaryl – COA reductase **[37]**. This reaction catalyzes conversion hydroxy methyl glutaryl – COA to mevalonate, which the site of cholesterol-lowering drugs. Since, cholesterol participates in biosynthesis lipoprotein and there is a maximum amount of cholesterol is in LDL, thus, subsequent reduction of cholesterol, LDL decreases.

CRP increases as an important indicator of the inflammatory process and directly implicated in the atherogenic process. Since diabetes is a major independent risk factor for atherosclerosis, thus, increases it in type 1 and type 2 diabetes **[38]**. Goyal *et al.*, showed that the induction of diabetes in rats, causes increasing this factor **[39]**.

The report about the effect of consumption extract Allium on serum concentrations of CRP in diabetic rats and in normal rats not found. It is probable that the anti-inflammatory effect of this plant is related to its antioxidant compounds such as flavonoids. Lean et al stated that quercetin which is the class flavenols, protect against free radicals and inhibit oxidizes and lypoxygenase **[40]**.

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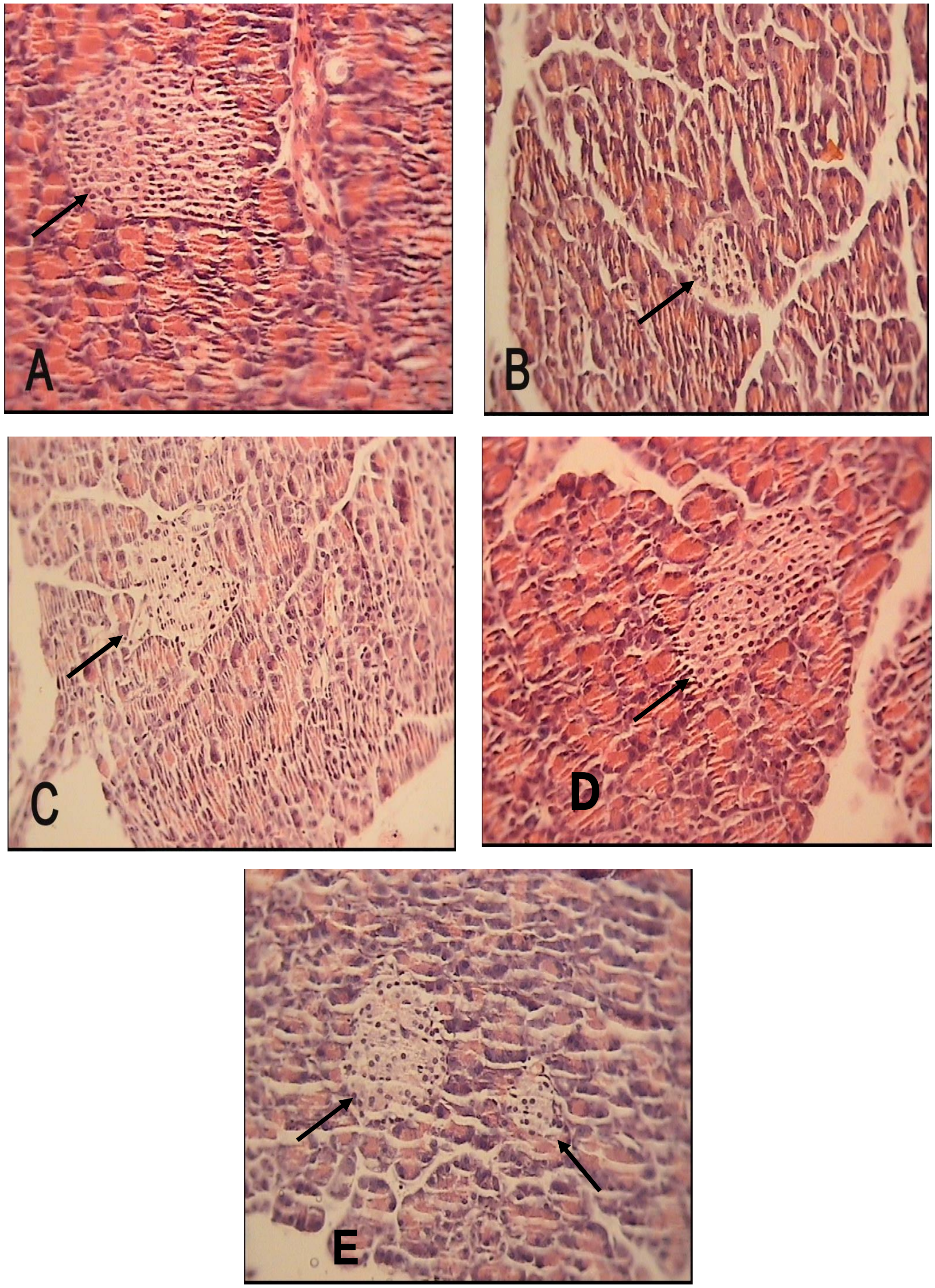

Figure 1: Pancreatic Islets in the Normal Control (A), Diabetic Control (B), Diabetic Rats Treated With Glibenclamide (C), Diabetic Rats Treated With Shallot Extract (0.1 g/kg)(D), Diabetic Rats Treated With Shallot Extract (1 g/kg) (E) (40 X)

Table 1: Effect of Hydroalcholic Extract of *A. hirtifolium* on Different Biochemical Parameter Levels in this Study

Parameter	Normal control	Diabetic control	Diabetic+Shallot Extract (0.1 g/kg)	Diabetic+Shallot Extract (1 g/kg)	Diabetic+Glibenclamide (0.6 mg/kg)
Glucose mg/dl)	69.71±6.21*	246±30.33†	110.6±16.88*	111.71±7.29*†	105.5±8.61*
Insulin (µU/ml)	2.06±0.49	1.04±0.22	3.35±0.96*	1.7±0.06	2.12±0.49
Cholesterol (mg/dl)	78.85±4.1*	107.4±5.51†	72.8±8.62*	88.14±4.52*	94.83±9.4
Triglycerides (mg/dl)	58.42±9.73*	103.6±22.89†	43.4±8.99*	48.16±3.57*	41.83±8.24*
LDL (mg/dl)	18.66±1.05*	24.6±1.12†	17.5±1.32*	17.16±1.22*	22.66±2.24
HDL (mg/dl)	40.57±1.6	39.8±3.56	39.5±2.8	45.71±1.83	51.66±4.2*†
CRP (mg/dl)	39.71±2.45*	52.4±1.53†	40.8±2.81*	42±2.07*	50.33±1.42†

NOTE: Results were Expressed as Mean ± SEM (n=7); * Significance Difference Vs Diabetic Control ($p < 0.05$); † Significance Difference Vs Normal Control ($p < 0.05$)

Table 2: Effect of Hydroalcholic Extract of *A hirtifolium* on Body Weight in this Study

Groups		Body weight (g)	
	0 week	2 week	4 week
Normal control	218.66±8.92‡£	233.66±12.44*	237±15.28*
Diabetic control	241.2±12.9‡£	188.4±11.1†	176.2±6.09†
Diabetic + extract (0.1 g/kg BW)	227.42±6.73‡	201.85±7.38†	203.66±12.51
Diabetic + extract (1 g/kg BW)	236±13.71	226±11.5*	232.85±13.87*
Diabetic+Glibenclamide (0.6 mg/kg)	228.16±8.93‡£	204±11.47	210±12.03

NOTE: Results were expressed as Mean ± SEM (n=7); * Significance difference Vs diabetic control ($p < 0.05$); † Significance difference Vs normal control ($p < 0.05$).; ‡ Significance difference Vs week 0 and week 2 ($p < 0.05$); £ Significance difference Vs week 0 and week 4 ($p < 0.05$)

Table 3: Effect of hydroalcholic Extract of *A. hirtifolium* on Size and Number of Langerhans Islets in This Study

Parameter	Normal control	Diabetic control	Diabetic+Shallot Extract (0.1 g/kg)	Diabetic+Shallot Extract (1 g/kg)	Diabetic+Glibenclamide (0.6 mg/kg)
Size of islets (micron)	1.3±0.14*	0.5±0.02†	0.99±0.13*	0.99±0.18*	0.91±0.12
Number of islets	6±0.54*	2.6±0.23†	4.6±0.74	4±0.4†	4±0.68†

NOTE: Results Were Expressed as Mean ± SEM (n=7); * Significance Difference Vs Diabetic Control ($p < 0.05$); † Significance Difference Vs Normal Control ($p < 0.05$)