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Study of the Potential Impact of Atriplex Halimus L. (*Chenopodiaceae*) on Experimental Animals Infected with Hyperglycemia

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Abstract

The present study aimed to determine the potential effect of Atriplex halimus leaves powder on blood glucose levels in hyperglycemic rats. Thirty adult male albino rats weighing (150±5g) were divided into five groups as follows: Group 1: Negative control group (6 rats) fed on a basal diet, and experimental group (24 rats). The experimental group was infected by alloxan (dose 150 mg per kg) to induce diabetes. Then was divided into four subgroups (6 for each) as follow; positive control group fed on a basal diet, groups (3, 4, 5) diabetic groups were fed on the basal diet, and 2.5, 5, 7.5 % of A. halimus leaves powder respectively for 28 days. Results showed that glucose level in the negative control group was significantly decreased compared with diabetic groups. Blood glucose values were decreased while insulin and BWG were increased with an increase in the percentage of A. halimus leaves powder to the basal diet. The leaves of A. halimus improved the liver and kidney functions; the best and lowest values of creatinine were recorded with groups fed on 7.5% of A. halimus leaves powder compared with the positive control group.

Keywords: *hyperglycemia, liver functions, kidney functions, chromium, diabetes, rats*

Introduction

The World Health Organization[(1)] defined the diabetes as serious, chronic disease that occurs either when the pancreas does not produce enough insulin, or when the body

cannot effectively use the insulin it produces. So, it is an important public health problem. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades. It was the seventh leading cause of death in 2016. It is a major cause of blindness, kidney failure, heart attack, stroke and lower limb amputation[(2)].

Diabetic patients are known to have organ damage, atherosclerotic disease, neuropathy, poor immunity and wound healing and susceptibility to infection, secondary to the many detrimental effects of insulin resistance and hyperglycemia, often leading to adverse postoperative outcomes[(3)]. Nowadays, because of the expensive treatment for diabetes as well as the contraindications that these medications give, people are trying to discover the wonders of alternative herbal-based treatments for diabetes mellitus. According to recent surveys carried out among practitioners of Arabic medicine in the Middle East, *Atriplex halimus* L. is one of the medicinal plants that is strongly recommended as antidiabetic and antioxidant[(4)]. The extracts of *A. halimus* have a very important antioxidant capacity compared to the reference (ascorbic acid) methanol extract of which has the highest percentage in this activity[(5)]. The decoction leaves of *A. halimus* has been used to treat hypertension in Tarfaya province[(6)]. It was mentioned for the treatment of digestive diseases in Ouargla[(7)]. It is used to treat certain diseases and infections in northern Africa[(5)]. Saidi *et al.,*[(8)] showed that the leaves of *A. halimus* have many therapeutic applications including hepatitis, obesity and healing wounds. The leaves are used by Arabic indigenous herbal practitioners to treat Cardiovascular system diseases and Hypercholesterolemia[(9)]. Preventive treatment with an aqueous extract of *A. halimus* maintaining healthy kidney function[(10)].

Dey *et al.,*[(11)] investigated that administration of *A. halimus* leaves powder (3g/day) for type 2 diabetes in humans decreased blood glucose levels. Kadan *et al.,*[(12)] showed that sand rats were fed diets composed of standard laboratory animal chow with or without *A. halimus* leaves lowered their blood glucose levels as well as enhanced insulin secretion.

Brockman,[(13)]said that the leaves of *A. halimus* are edible. It is used in food as a very good source of proteins in the Sahara region of Algeria[(14)]. Chikhi *et al.,*[(15)] showed that *A. halimus* (Mediterranean saltbush) is a good source for vitamins A, C and D. The active principle is of a mineral nature: The tissue chromium of this plant regulates blood sugar by activating the effect of insulin[(16)]. For that, this study aimed to reduce the complications of diabetes mellitus, investigate protective effect of *A. halimus* leaves

powder on hyperglycemia by improve blood sugar control and use the leaves of *A. halimus* as a diet to maintain the level of public health for diabetics.

Material and Methods

Materials

The fresh of *Atriplex halimus* leaves were obtained from Marsa Matrouh, Egypt. in 2018.

Experimental animals

A total of 30 adult normal male albino rats (Sprague Dawley strain) weighting 150±5 g were obtained from the Medical Insects Research Institute, Doki, Giza, Egypt.

The chemical kits

Alloxan used for induction for diabetic in rats, obtained from pharmaceutical Company, Cairo, Egypt, All analysis kits from Bio-diagnostic, Co., Giza, Egypt.

Methods

Preparation of Atriplex halimus leaves powder

The fresh leaves of *A. halimus* were washed then dried at 50ºC for 6 hour using vacuum oven. The leaves were ground by an electrical grinder (Moulinex) to pass through 1.6 mm sieve then packed in dark polyethylene bags and stored in a freezer until used according to AOAC [(17)].

Chemical composition of Atriplex halimus leaves

Moisture, fat, protein, fiber and ash contents were determined in *A. halimus* leaves according to AOAC [(18)]. The carbohydrate was calculated by difference.

Induction of diabetic rats

Diabetes was induced by single intraperitoneal administration of alloxan monohydrate in rats (injection of 150 mg/kg body weight) according to the method described by Desai and Bhide,[(19)].

Experimental Design

The work was carried out at the Faculty of Home Economics, Menoufia University, 30 male albino rats were housed in cages in arrows maintained at 25±2ºC and kept under normal condition, Rats were fed on a standard diet according to AIN[(20)] for 7 days as an adaptation period. The rats were distributed into 5 groups (6 rats each) according to the following: group (1): Negative control still fed on standard diet; group (2): Positive control (rats with diabetes) fed on standard diet only; group (3): Diabetic rats were treated with dried *Atriplex halimus* leaves powder in a dose of 2.5%; group (4): Diabetic rats

were treated with dried *A. halimus* leaves powder in a dose of 5%; group (5): Diabetic rats were treated with dried *A. halimus* leaves powder in a dose of 7.5%.

Blood sampling

Blood samples were collected after 12 hours fasting at the end of the experiment (4 weeks). Using the retro-orbital method by means of a micro capillary glass tubes. Blood samples were collected into a dry clean centrifugal tube and left to clot in a water bath (37°C) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum a part of was subjected to glucose determination and the reminder was carefully aspirated and transferred into clear quit fit plastic tubes and kept frozen at (-20°C) until analysis.

Relative body weight (BWG%), feed intake (FI) and feed efficiency ratio (FER)

Feeding and growth performance were carried out by determination of body weight gain (BWG**%**) and feed efficiency ratio (FER) according to Chapman *et al.,*[21] using the following formulas:

$$BWG\% = \frac{(Final\ weight - Initial\ weight)}{Initial\ weight} \times 100$$

$$FER = \frac{Gain\ in\ body\ (g)}{Food\ intake\ (g)}$$

Biochemical analysis

Blood glucose and serum insulin

Serum glucose and serum insulin were estimated according to Astoor and King,[22] and Wilson and Miles,[23] respectively.

Liver and kidney functions

Serum aspartate and alanine amino transferase (AST and ALT) were measured according to the methods described by Kachmar and Moss,[24]& Bergmeyer and Harder,[25]respectively. Determination of serum creatinine, urea and uric acid [26,27,28] respectively.

Statistical Analysis

Results were expressed as the mean and SD**.** Statistical analysis was performed by using computer program, Statistical Package for Social Science and compared with each other using the suitable tests [29].

Results

Data in table (1) showed the chemical composition of dry leaves of *Atriplex halimus* powder (g/100g). The results indicated that *A. halimus* leaves content 33.56% carbohydrate, 22.89% moisture, 3.28% fat, 11.5% protein, 14.19% ash and 14.58 % fiber respectively.

Table (1): Chemical composition of dried leaves of Atriplex halimus L. powder

Parameter	*Atriplex halimus*
Moisture (g/100 g)	22.89
Carbohydrates (g/100 g)	33.56
Fat (g/100 ml)	3.28
Protein (g/100 g)	11.5
Ash (g/100 g)	14.19
Fiber (g/100 g)	14.58

The data in table (2) indicated that the mean value of feed intake, body weight gain and FER for normal and diabetic rats. It is clear that the feed intake value showed non-significant in group supplement with 2.5% of *A. halimus* leaves powder compared with positive group. While 5 and 7.5% *A. halimus* leaves powder recorded significant ($p \leq 0.05$) increase compared with positive group. On the other hand, body weight gain recorded significant ($p \leq 0.05$) increased in group which supplement with 5% *A. halimus* leaves powder. While group fed on 7.5% *A. halimus* leaves powder recorded high significant increasing at ($p \leq 0.01$).

Feed efficiency ratio recorded significant ($p \leq 0.05$) increased in groups which supplement diet with 2.5 and 5% *A. halimus* leaves powder. Whereas group fed on 7.5% *A. halimus* leaves powder recorded high significant increasing at ($p \leq 0.01$).

Table (2): Effect of Atriplex halimus leaves powder on relative body weight, feed intake and feed efficiency ratio of diabetic rats

Parameters / Groups	FI (g) Mean ± SD	BWG (%) Mean ± SD	FER Mean ± SD
Control –ve	15.43±1.18**	16.37±1.56**	0.073±0.01***
Control +ve	11.64±1.28	5.29±0.17	0.023±0.03
2.5% *A. halimus* leaves powder	12.78±1.62	6.04±0.67	0.038±0.01*
5 % *A. halimus* leaves powder	13.21±0.11*	8.87±1.13*	0.051±0.02*
7.5% *A. halimus* leaves powder	13.62±0.71*	10.92±1.86**	0.058±0.02**

$^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$

The data in table (3) demonstrated that, the serum glucose is high in positive control and low in the negative control. Groups which supplemented with 2.5% *A. halimus* leaves powder showed non-significant change in insulin compared with positive group. But serum glucose was significantly decreased at ($p \leq 0.05$). While groups fed on 5 and 7.5% of *A. halimus* leaves powder recorded the highest significant ($p < 0.01$) decrease in serum glucose. Whereas insulin had significant increased at ($p \leq 0.05$) in groups which supplement diet with 5 and 7.5% of *A. halimus* leaves powder compared with positive control.

Table (3): Effect of Atriplex halimus leaves powder on serum glucose and insulin (mg/dl) of diabetic rats

Groups	Blood Glucose Levels Mean ± SD	Insulin Mean ± SD
Control –ve	106.52±3.04***	11.63±1.66**
Control +ve	202.58±5.61	6.05±1.01
2.5% *A. halimus* leaves powder	163.54±3.35*	7.26±1.83
5 % *A. halimus* leaves powder	141.24±7.45**	9.01±1.22*
7.5% *A. halimus* leaves powder	122.63±2.37**	9.32±0.91*

$^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$

Data in table (4) illustrate the mean value of AST and ALT for normal and diabetic rats. Levels of Aspartate amino transferase (AST) in groups which supplement diet with 2.5 and 5% *A. halimus* leaves powder had significant decreased ($p \leq 0.05$) compared with positive group, the highest significant decreased (at $p \leq 0.01$) in 7.5% *A. halimus* leaves powder.

The value of Alanine amino transferase (ALT) was significant decreased ($p \leq 0.05$) in groups fed on 2.5 and 5% of *A. halimus* leaves powder compared with positive group, the highest significant decreased (at $p \leq 0.01$) in 7.5% *A. halimus* leaves powder.

Levels of creatinine was no significant in group fed on 2.5% of *A. halimus* leaves powder, but urea and uric acid had significant decreased at ($p \leq 0.05$) compared with positive group. Whereas group fed on 5% of *A. halimus* leaves powder had significant decreased at ($p \leq 0.05$) in urea, uric acid and creatinine compared with positive group. On the other side group fed on 7.5% of *A. halimus* leaves powder recorded the highest significant decreased (at $p \leq 0.01$) in urea, uric acid and creatinine compared with positive group.

Table (4): Effect of Atriplex halimus leaves powder on AST, ALT, urea, creatinine and uric acid of diabetic rats

Parameters / Groups	AST (U/L) Mean ± SD	ALT (U/L) Mean ± SD	Urea (mg/dl) Mean ± SD	Creatinine (mg/dl) Mean ± SD	Uric acid (mg/dl) Mean ± SD
Control –ve	29.34±2.16***	34.55±2.03**	35.35±3.21**	0.66±0.02***	1.44±0.01***
Control +ve	52.28±1.38	53.56±1.11	51.28±6.84	1.28±0.13	2.69±0.16
2.5% *A. halimus* leaves powder	41.80±1.09*	45.97±3.64*	46.83±2.12*	1.16±0.17	2.28±0.11*
5% *A. halimus* leaves powder	38.01±3.46*	42.49±2.42*	43.86±3.28*	0.96±0.45*	2.06±0.27*
7.5% *A. halimus* leaves powder	33.74±2.85**	38.21±1.23**	40.38±4.41**	0.86±0.22**	1.82±0.18**

$^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$

Discussion

In this study, the Chemical composition of dried leaves of *Atriplex halimus* powder (g/100 g). contained 14.19 ash, 14.58 fiber and 11.5 % protein respectively. Selmi *et al.,* [30] reported that *A. halimus* attained high value in crude protein (20.40%), higher levels of ash (28.03%) and crude fiber (14.8%).

Haddad *et al.,* ([31] suggested that several in vitro studies and studies on alloxan-induced diabetic rats suggest that *A. halimus* leaf ash containing trace amounts of chromium potentiates the effect of insulin on glucose uptake. *A. halimus* is a nutritious plant, rich in dietary fiber (cellulose), with its fiber-rich content, it facilitates digestion[16]. Furthermore, chromium-magnesium salts contained in *A. halimus* evidenced as an insulin cofactor to facilitate glucose entry into muscle and fat cells[32].Proteins were detected in moderately high levels on the leaves of *A. halimus* and were associated with high digestibility[33]**.**

The present study discussed about the antidiabetic effects of *A. halimus* leaves powder on normal and alloxan-induced-diabetic at a dose of (150 mg per kg) rats during 28 days. *A. halimus* leaves powder produced the highest significant ($p<0.01$) antihyperglycemic activity at 5 and 7.5% in diabetic treated rats, Whereas insulin had significant increased at ($p\leq0.05$) compared with positive control. This finding was agreement with Chikhi *et al.,* [15] reported that administration of aqueous extract of *A. halimus* leaves (200mg.kg^{-}

^{1}b.wt.) on STZ-induced rat model for 30 days. It produced significant antihyperglycemic activity up to 54% ($P<0.001$) reduction in fasting blood glucose levels compared to the initial fasting blood glucose levels prior to the treatment due to rich in flavonoids, Tannins, Saponins, Alkaloids and Resins. The action of this drug can be attributed to the insulin-like behavior in the regulation of glucose levels. So, *A. halimus* is one of Plants which stimulates the β-cells in the pancreas to release more insulin. These results agreement with Ouazeta *et al.,*(34) evaluated the activities: Hypoglycemic and antihyperglycemic of the methanolic extract of the leaves of *A. halimus* on an animal model and demonstrated a significant hypoglycemic activity of the extract especially in rats that were temporarily hyperglycemia caused by a glucose solution at a dose of 250mg/kg, and normoglycemic rats show that the methanolic extract of *A. halimus* possesses good hypoglycemic and antihyperglycemic potential. Also, Harlev *et al.,*(35) said that alloxan-diabetic albino rats showed a significant hypoglycemic effect when fed with the green leaves of *A. halimus* with oral, but with no decrease in appetite. The possible mechanism of antidiabetic action of *A. halimus* leaves may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form.

In this study, alloxan exposure at a dose (150 mg per kg) rats resulted in decreased body weight, and group fed on 7.5% *A. halimus* leaves powder recorded high significant increasing at ($p\leq0.01$). On the other hand, Chikhi *et al.,*(15) reported that the aqueous extract of *A. halimus* at a concentration of 200 mg.kg^{-1}b.wt. showed a significant increase in BWG of diabetic treated rats.

The finding of this study indicates that alloxan-induced diabetic in the rat at a dose (150 mg per kg) resulted in a significant increase serum liver enzymes (ALT and AST) and kidney functions (serum urea, uric acid and creatinine levels) compared with normal rats. Moirangthem *et al.,* (36) reported that the increased levels of urea and creatinine are considered as the marker of impaired kidney functions.

In this study, diabetic rats fed with 7.5% of *A. halimus* leaves powder treatment significantly ($p<0.01$) decreased the raised serum urea and creatinine, this means maintained healthy kidney function, and significantly restored the liver enzymatic activity compared with positive control. On the other hand, Azamel,(37) reported that the serum urea was significantly lowered in two groups of growing lambs fed on *A. halimus ad*. Libitum plus 50% and 75% barley in comparison with control group "lambs fed on

Berseem hay plus 100% barley". Also, Khaoula and Djahra,[38] showed that the treatment of rats by *A. halimus* had significantly decreased ($p \leq 0.01$) in (ALT) when compared with benzene group, and restored the liver enzymatic activity.

Possibly the insulin-like activity of these bioactive compounds inherent in the leaves of *A. halimus* is responsible for the antihyperglycemic effects, However, our study indicated that *A. halimus* has a dramatically preventive effect on alloxan-induced diabetic. There was no lethality or any toxic reactions found with the selected dosages until the end of the study period.

Conclusion:

These results concluded that the tested leaves of *Atriplex halimus* contained several important compounds such as fiber have therapeutic effects in reducing the elevated blood glucose level on diabetic rats. Feeding with7.5% of *A. halimus* leaves powder was effective in decrease blood glucose levels followed by group which treated with5% of *A. halimus* leaves powder. Therefore, the data recommended that the leaves of *A. halimus* selected in a moderate amount to be included in daily diets especially in salads or using it as herbal tea.

Conflict of interest

The authors declare that they have no conflict of interest concerning the publication of this article. This article is extracted from a Master's thesis submitted to the Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt.

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دراســـة التأثير المحتمل لأوراق نبات القطف على حيوانات التجارب المصـــابة بارتفاع جلوكوز الدم

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قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر

الملخص العربي:

تهدف الدراسة الحالية لمعرفة التأثير المحتمل لأوراق نبات القطف المطحون على مستوى جلوكوزالدم في الفئران المصـابة بإرتفاع سـكر الدم. أجريت الدراسـة على ثلاثين من ذكور الفئران البيضـاء البالغة تتراوح أوزانهم بين (150±5جم) وتم تقسيمهم إلى 5 مجموعات كل منها 6 فئران كما يلي: المجموعة الأولي ضـابطة سـالبة وتتغذي علي الوجبة القياسـية، باقي المجموعات (24 فأر) تم حقنها بالألوكسـان بنسـبة (150 ملجم/كج من وزن الجسـم) لإحداث مرض السكري حيث المجمـــــوعة الثانية ضابطة موجبة تتغذي علي الوجبة القياسية والمجموعة الثالثة والرابعة والخامسـة مصـابة بالسـكري وقد تم إضـافة أوراق نبات القطف المطحون بنسـبة 2.5،5،7.5٪ علي التوالي إلى الغذاء الأسـاسـي واسـتغرقت التجربة 28 يوم. وأظهرت النتائج أن مسـتوى الجلوكوزفي المجموعة الضـابطة السـالبة إنخفض بشـكل واضـح مقارنة بمجموعة السـكري وانخفض قيم السـكر في الدم، بينما زيادة الأنسولين، BWG بزيادة نسبة أوراق نبات القطف المطحون المضـاف للغذاء الأسـاسـي. أوراق القطف حسـنت وظائف الكبد والكلي، أفضـــل وأقل قيمة للكرياتينين ســـجلت مع المجموعة التي تم تغذيتها على 7.5٪ من أوراق القطف المطحون مقارنة بالمجمـــوعة الضابطة الموجبة. في الختام، تشير الدراسة الحالية إلى أن نسبة 7.5٪ من أوراق القطف المطحون المضـاف للغذاء حسـنت في مسـتوى جلوكوزالدم، وترجع بعض هذه التأثيرات إلى أملاح الكروميوم والمغنيسيوم الموجودة بأوراق القطف حيث تعد كعامل مسـاعد للأنسـولين لتسـهيل دخول الجلوكوز إلى خلايا العضلات والدهون.

الكلمات الكاشـــفة: ارتفاع ســـكر الدم، وظائف الكبد والكلي، الكروميوم، معدل الزيادة فى وزن الجســـم، فئران التجارب.