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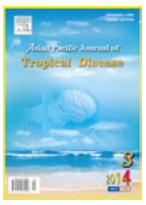

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Antidiabetic activity of aqueous leaf extract of *Atriplex halimus* L. (Chenopodiaceae) in streptozotocin-induced diabetic rats

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PEER REVIEW

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Comments

A. halimus aqueous extract is widely used as a traditional antidiabetic remedy in Algeria. Its rational use is controversial. The present study provides evidence of its main antidiabetic activities.
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ABSTRACT

Objective: To investigate the antidiabetic effect of *A. halimus* leaf in streptozotocin-induced diabetic rats.
Methods: The aqueous extract of the plant leaf was tested for its efficacy in streptozotocin-induced diabetic rats. The extract was evaluated for its acute and short term general toxicity in male mice and for its antihyperglycemic activity using glucose tolerance test in rats. The aqueous extract was subjected to phytochemical screening and determination of total phenolic contents.
Results: The statistical data indicated the significant increase in the body weight and decrease in the blood glucose and hepatic levels. The total protein level was significantly increased when treated with the extract.
Conclusions: These results suggest that the aqueous leaf extract of *A. halimus* has beneficial effects in reducing the elevated blood glucose level and hepatic levels in streptozotocin-induced diabetic rats.

KEYWORDS

Atriplex halimus L., Chenopodiaceae, Aqueous extract, Antidiabetic, Streptozotocin

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the impaired secretion of insulin or insulin insensitivity[1]. Diabetes is associated with a high risk of vascular disease (2 to 4-fold greater risk than individuals without diabetes) and with cardiovascular disease which is the primary cause of death among people with type 1 or type 2 diabetes[2]. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemic and hypolipidemic agents[3]. Of the several indigenous plants used in the local treatment of diabetes mellitus in Tlemcen region, *Atriplex halimus* L. (*A. halimus*) is one of those plants used by tribes to treat diabetes and heart conditions[4,5]. *Atriplex* has always been a popular folk remedy. In the Arab world, *A. halimus* was used to treat chest ailments, as a laxative, to cure stomach pains, for intestinal worms and to regulate gall bladder excretions[4,5]. The Bedouin tribes of the Negev use the plant today for treating muscular pain. Other uses the Bedouin have found for *Atriplex* are to feed sheep and goats, to treat intestinal diseases in animals and to flavor cooking. The leaves are edible but too salty to be taken alone[4,6]. *A. halimus* is also a source of vitamins A, C and D[7] and tannins, flavonoids, saponins, alkaloids and resins,

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and it contains up 10% sodium chloride[6]. *A. halimus* is a xero-halophyte species belonging to Chenopodiaceae widely distributed in non-saline as well as in saline areas, in sub-humid to arid regions of South Europe, East Mediterranean and North Africa, including the Sahara in Algeria[5,8]. This plant is often cultivated as forage because tolerating severe conditions of drought, and it can grow up in very alkaline and saline soils. In addition, it is useful to valorize degraded and marginal areas because it will contribute to the improvement of phytomass in this case[8]. Given these considerations, this study was taken up to investigate the antihyperglycemic activities of the leaf of *A. halimus* in streptozotocin (STZ) induced diabetic rats.

2. Materials and methods

2.1. Plant material

Leaves of *A. halimus* were collected from Beni Ounif located 110 km north-east to the province of Bechar, South Algeria during May 2011. The position of the plant from which the specimens were obtained was determined using the global positioning system. The coordinates were 32°02′49.96″ N, 1°20′19.64″ S, and the altitude was approximately 800 m. Voucher specimen was identified by Pr Nouri Benabadji, Department of Biology, University of Tlemcen, Algeria and deposited in the institutional herbarium. The plants were cut at ground surface and taken to laboratory to obtain aqueous extract from the leaves.

2.2. Preparation of aqueous extract

Fresh leaves were dried at room temperature. Aqueous extract was obtained as follows. In brief, 100 g of dried fresh leaf were extracted by refluxing with distilled water for one hour. Thereafter, it was decanted and filtered with filter paper and followed by centrifugation for 30 min at 5000 r/min. The supernatant was filtered to eliminate any residues. The filtrate was dried in the oven at 40 °C to make a powder yielding 3.2% (w/w). The solid residue was stored in desiccator prior use for subsequent experiment.

2.3. Phytochemical screening and determination of total phenolic contents

Preliminary phytochemical screening of the aqueous extract was determined by employing the methods given in the literature[9]. The concentration of phenolics in plant extracts was determined using spectrophotometric method[10]. Methanolic solution of the extract in the concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic solution of extract, 2.5 mL of 10% Folin-Ciocalteu′s reagent dissolved in water and 2.5 mL 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu′s reagent dissolved in water and 2.5 mL 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λ_{max}=765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg GAE/g).

2.4. Induction of diabetes

Diabetes was induced in male Wistar albino rats aged more than 3 months (200-280 g body weight) by intravenous administration of STZ (single dose of 50 mg/kg body weight) dissolved in freshly prepared 0.1 mol/L citrate buffer, pH 4.5[11]. After 10 d rats with marked hyperglycemia (fasting blood glucose≥170 mg/dL) were selected and used for the study. All the animals were allowed for free access to tap water and pellet diet and maintained at room temperature in plastic cages.

2.5. Toxicity evaluation in rat

Acute toxicity study on aqueous extract of *A. halimus* was performed in experimental rats. Graded doses of the aqueous extract of leaf (1 000, 2 500 and 3 000 mg/kg body weight) were administered (*p.o.*) and the animals were observed for 2 weeks following administration. The dosage schedule was fixed as 200 mg/kg body weight/day for each rat for 30 d.

2.6. Experimental design

2.6.1. Effect of long term treatment with aqueous extract A. halimus on glycemic control

The animals were divided into four groups and each group consisted of 5 rats. Group 1: Untreated normal rats; Group 2: Untreated diabetic rats; Group 3: Diabetic rats treated with aqueous extract at 200 mg/kg body weight; Group 4: Normal rats treated with aqueous extract at 200 mg/kg body weight.

Aqueous leaf extract of *A. halimus* was administered to the rats every morning for 30 d by gastric intubation using oral gavage. Blood samples were collected from tail veins before the start of the treatment (t=0) and on 14th, 21th and 30th d of the treatment and fasting blood glucose levels were estimated. Body weights of all the animals were recorded.

2.6.2. Effect of short term treatment with aqueous extract A. halimus on glycemic control

Blood samples were collected from the tail vein at 0, 1, 2 and 3 h after the administration of aqueous extract and blood glucose levels were determined by using glucose oxidase-peroxidase reactive strips. At the end of the experiment, an oral glucose tolerance test was practiced. Animals (four groups) were loaded with glucose (3 g/kg). Blood glucose level was determined at 0, 30, 60 and 120 min after glucose loading.

2.7. Analytical procedures

Estimation of blood glucose was carried out by glucose oxidase-peroxidase method[12].

2.8. Statistical analysis

The results were expressed as mean±SD. Data were analyzed statistically using the student's *t*-test. In all cases, $P<0.05$ was used as the criterion of statistical significance.

3. Results

3.1. Effect of long term treatment with aqueous extract A. halimus on glycemic control

The effect of long term treatment with aqueous leaf extract *A. halimus* on glycemic control is given in Table 1. Aqueous extract exhibited significant antihyperglycemic activity after 30 d of treatment in diabetic rats. It produced 54% ($P<0.001$) decrese in fasting blood glucose levels compared to the initial fasting blood glucose levels prior to the treatment. No significant effect was observed in normoglycemic rats. The body weights of normal, normal treated and diabetic treated rats increased significantly by 45.98 g, 42.16 g and 56.15 g respectively, whereas the body weights of diabetic control group decreased by 23.40 g (Table 1).

Table 1

Effect of long term treatment with aqueous extract *A. halimus* on blood glucose and body weight.

Group	Blood glucose (mg/dL)				
	1st Day	14th Day	21th Day	30th Day	Change in body weights (g)
1	92.75±8.20[a]	83.25±7.30[a]	90.25±6.80[a]	93.04±7.60[a]	+45.98
2	293.66±32.20[b]	266.33±28.20[b]	260.25±34.00[b]	260.33±28.20[b]	+42.16
3	350.25±38.20	232.75±32.20	193.36±24.20	161.12±18.20	−23.40
4	93.50±9.20[a]	90.25±6.60[a]	91.36±5.80[a]	103.10±8.30[a]	+56.15

Values are given as mean±SD from four rats in each group. Values not sharing a common superscript letter differ significantly at $P<0.01$.

3.2. Effect of short term treatment with aqueous extract A. halimus on glycemic control

The effects of aqueous extract of *A. halimus* on the fasting blood glucose levels of diabetic rats are given in Table 2. The fasting blood glucose levels of diabetic untreated rats were significantly higher than those of normal untreated rats (Group 1). When aqueous extract of *A. halimus* at a dosage of 200 mg/kg body weight was tested for their glucose lowering effects, the extract produced the maximum fall of 23% and 41% in the fasting blood glucose levels of diabetic rats after 2 h and 3 h of treatment, respectively.

Table 2

Effects of aqueous extract *A. halimus* on fasting blood glucose levels.

Group	Blood glucose (mg/dL)			
	0 h	1 h	2 h	3 h
1	81.75±6.30	80.50±5.30	94.50±7.20	89.50±4.10
2	263.36±25.30	253.10±21.30	259.56±26.30	243.33±22.30
3	364.25±21.10	316.25±30.30*	280.11±28.30*	214.75±21.30*
4	73.25±5.30	87.25±4.20	97.50±9.25	92.75±8.50

*$P<0.0001$ compared with the initial level of blood glucose (0 h) in the respective group.

3.3. Effect of aqueous extract of A. halimus on oral glucose tolerance in normal rats

Oral glucose tolerance test in diabetic and normal rats showed an increase in blood glucose level for 60 min after glucose administration (due to glucose load), but just after that there was a decrease in the fasting blood glucose levels of Groups 2 and 3 when compared to Groups 1 and 4. The results are depicted in Figure 1.

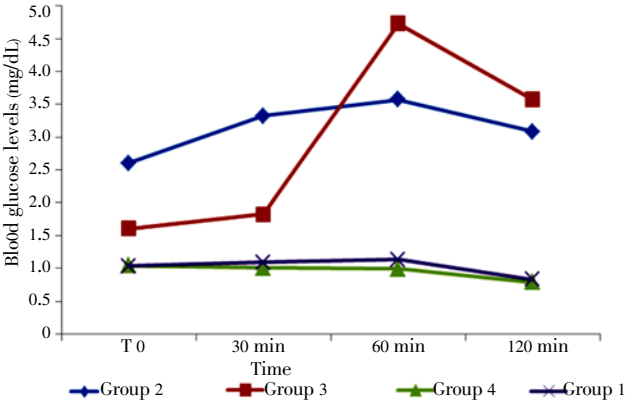

Figure 1. Effect of aqueous extract on oral glucose tolerance in normal and diabetic rats.

3.4. Total phenolic contents and identification of phytocomponents

Qualitative analysis performed in the aqueous extract of *A. halimus* demonstrated the presence of phytocomponents like tannins, flavonoids, saponins and alkaloids. The content of total phenolic was 12.47 mg GAE/g.

4. Discussion

The present manuscript discussed about the antidiabetic effects of the aqueous leaf extract of *A. halimus* on normal and STZ-induced-diabetic rats. During the short term study the aqueous extract produced significant antihyperglycemic activity at a dosage of 200 mg/kg body weight in diabetic treated rats. Acute toxicity studies revealed the non-toxic nature of the aqueous extract of *A. halimus*. There was no lethality or any toxic reactions found with the selected dose until the end of the study period. The results of the study have shown that the aqueous extract of leaf at a dose of 200 mg/kg body weight has a marked antihyperglycemic activity by improvement of the glucose tolerance test in normoglycemic rats and by lowering the blood glucose levels of 54% in STZ-induced-diabetic rats. Induction of diabetes by STZ leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins[3,13]. The gain in body weight was observed both in normal treated and diabetic treated groups. The possible mechanism of antidiabetic action of aqueous extract may be by increasing the pancreatic secretion of insulin from the existing beta cells, by its release from the bound form. Qualitative analysis performed in the aqueous extract of *A. halimus* demonstrated the presence of tannins and flavonoids. Over 150 plants extracts and some of their active principles including flavonoids are

known to be used for the treatment of diabetes[14,15]. Several phenolic compounds and flavonoids possess marked anti-diabetic activity[16]. Possibly the insulin-like activity of these bioactive compounds inherent in aqueous extract *A. halimus* is responsible for the antihyperglycemic effects.

In conclusion, this study has undoubtedly provided scientific confirmation and evidence for the safe use of the leaf of *A. halimus* by traditional healers in the treatment of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Diabetes, a metabolic disorder, has reached epidemic proportions worldwide. Although several drug treatments currently are available, researchers recognize the need for new agents for the prevention and treatment of diabetes. Plants play a major role in the discovery of new therapeutic agents and have received much attention as sources of biologically active substances.

Research frontiers

The data obtained from the present experiments are in agreement with its use in traditional medicine.

Related reports

The results showed that, in diabetic rats treated with the aqueous extract there is an significant increase in the body weight and decrease in the blood glucose and levels hepatic.

Innovations & breakthroughs

There is no work for antidiabetic activity. The present report serves as the first hand information on antihyperglycemic activity of this plant.

Applications

Diabetes is associated with long-term complications that affect almost every part of the body. Some oral hypoglycemic agents are also employed in this regard, such as renal damage and neurological disturbances. There are great deals of evidences to suggest that the use of carefully chosen herbal remedies and dietary supplements (as *A. halimus*) to prevent and control the diabetes.

Peer review

A. halimus aqueous extract is widely used as a traditional antidiabetic remedy in Algeria. Its rational use is controversial. The present study provides evidence of its main antidiabetic activities.

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