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ARTICLE

Antidiabetic effect of *Atriplex halimus* long and short term treatment against streptozotocin induced diabetes in rats

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Resumen

Efecto antidiabético de Atriplex halimus *en tratamiento. corto. largo plazo contra la diabetes inducida por estreptozocina*

El objetivo fue evaluar la actividad antidiabética de hojas de *Atriplex halimus* en ratas diabéticas modelo. Para evaluar se utilizó la glicemia, pérdida de peso, volumen de consumo de agua, parámetros bioquímicos. estudio histológico. Las investigaciones fitoquímicas indican la presencia de flavonoides, taninos, saponinas, mucílagos, glucósidos. proteínas. Los resultados muestran que el tratamiento con extracto acuoso de *A. halimus* presenta reducción significativa de los niveles de glucosa en sangre en ratas de los grupos D100. D200, en comparación con el grupo diabético,. protege. las ratas de complicaciones diabéticas. El estudio histológico del páncreas lo confirman por la mejora en los islotes de Langerhans de rata tratada con este extracto vegetal. *Atriplex halimus* parece ser una planta prometedora para futuros ensayos preclínicos. clínicos en la diabetes de tipo I.

Palabras clave: *Atriplex halimus*; Diabetes: Fitoquímicos; estreptozotocina; Glicemia.

Abstract

The aim of the present study was to evaluate the anti-diabetic activity of *Atriplex halimus* leaves in diabetic model rats. Glycaemia, weight loss, volume of water consumption, biochemical parameters and histological study were used to evaluate the anti-diabetic activity. Phytochemicals investigations indicate the presence of: Flavonoids, tannins, saponins, mucilages, glycosides and proteins. Findings show that the treatment with *A. halimus* aqueous extract presents. significant reduction of blood glucose levels in rats of groups D100 and D200 compared with diabetic group and protect rats from diabetic complications. These results were confirmed by the histological study in pancreas which indicate improvement in Islets of Langerhans of rat treated with this plant extract. *Atriplex halimus* appears to be. promising plant for further preclinical and clinical trials in type 1 diabetes.

Key words: *Atriplex halimus*; Diabetes; Phytochemicals; Streptozotocin; Glycemia.

Introduction

Diabetes is. chronic, metabolic disease characterized by elevated levels of blood glucose which leads to serious damage to the blood vessels, kidneys, eyes and heart. About 422 million people worldwide have diabetes in 2020, the majority living in low-and middle-income countries, and 1.6 million deaths are directly attributed to diabetes each year. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades (WHO 2020). Diabetes prevalence (% of population ages 20 to 79) in Algeria was reported at 6.7% in 2019, according to the World Bank collection of development indicators (World Bank 2020). There are two main types of diabetes mellitus: Insulin-dependent diabetes or type 1 diabetes, pancreatic beta cells produce very little or no insulin because of an autoimmune reaction that destroys them partially or completely, and is known to affect only 10% of the diabetic population. Non-insulin-dependent diabetes or type 2 is a metabolic disorder which is non-insulin dependent, characterized by chronic hyperglycemia. This disease usually occurs in older adults and affects more the obese or overweight people (American Diabetes Association 2020).

Over the past decade phytotherapy has become more important, making an impact on both health and international trade. Return to natural product is essential as it would be less toxic and equally effective (Missoun *et al.* 2018). Algeria has a diverse climate and a large geographical location making it a treasure trove of medicinal plants furthermore the trade of plants is very easy and cheap. In addition, many people are interested in having more autonomy over their medical care in Algeria. Many ethnobotanical studies conducted on the use of medicinal plant in the treatment of diabetes in Algeria. These studies identify 171 plants divided into 58 families, most of these plants were used by diabetic patients and they are not aware of their toxicity, and without any pharmacological experimental studies (Hamza *et al.* 2019).

For this reason we have chosen in this study *Atriplex halimus* L, Sp. Pl. 2: 1052. In traditional medicine, the infusion or decoction of this plant leaves is used as antidiabetic, anti-sterility in female, antilithiasis, antitumor, anti-inflammation and skin rejuvenation in. deep burn. *Atriplex* Genera Plantarum ed.5 is. halophytic shrub belongs to Chenopodiaceae family, are widely distributed in Europe and Northern Africa including the Sahara in Algeria and Morocco. HPLC analysis of ethyl acetate extract indicated that the plant contained flavonol, flavanone and flavone glycosides (Chikhi *et al.* 2014) and the presence of myricetin, quercetin, isorhamnetin glycosides, simple phenolic acids and esters (Clauser *et al.* 2013). However, There are few studies in phytochemical and pharmacological activities of *A. halimus* especially in Algeria.

The aim of this study was to evaluate the antidiabetic effect of *A. halimus* leaves collected from Bechar region on Wistar rats. The type 1 diabetes was induced by an antibiotic called streptozotocin. It is particularly toxic to β cells of the islets of Langerhans in mammals; it is therefore indicated in the treatment of insulinomas and used in medical research in the chemical induction of type 1 diabetes in animal model.

Materials and methods

Plant material

The plant material was collected in April 2019 from Bechar region, South-west of Algeria. The samples were identified by Doctor Sekal, FZ, and Voucher Specimen (H.S.02888) of the plant was kept at the Herbarium of Ecology Laboratoiry, University of Es-Senia, Oran, Algeria, for future reference.

Preliminary phytochemical screening

Qualitative Analysis

Chemical tests were carried out on the methanolic and aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Harborne (1998), Evans & Evans (2009), Sofowara (1993) and Ayoola (2008).

Tannins: 5 ml of aqueous extract solution, and. ml of $FeCl_3$ (1%) solution was mixed. In the presence of gallic tannins it develop blue color and green black for catecholic tannins.

Saponins: Their presence is quantitatively determined by the calculation of the foam index. 0.5g of plant material soaked in 10 ml water and was shaken; a foam produced persists for ten minutes

indicate the presence of saponins.

Flavonoids: The plant powder was mixed with 2 ml of HCl, and 1 to 2 drops of NaOH solution was added. It develops a yellow color and it becomes colorless in the presence of dilute acid indicate the presence of flavonoids.

Alkaloids: They were determined by Dragendorff's and Mayer's reagents test using methanolic extract.

Terpenoids: Salkowski test was performed using the methanolic extract.

Glycosides: 0.5 g of plant powder extract was dissolved in 2.0 ml of glacial acetic acid along with one drop of ferric chloride solution and on of 1.0 ml of pure H_2SO_4 was added brown ring develop at the interface indicated the presence of glycosides.

Quinons: 1 g of powder of the plant with 1ml of HCl was mixed after that 5 ml of chloroform was added and left for several hours. The extracts are filtered, diluted ammonia (1/2) then was added to the filtrate. If it is observed that the aqueous phase does not stain it indicates the absence quinons.

Anthraquinons: 0.5 g of plant powder was macerated in water. After filtration, 1 ml of ammonia (10%) was added, the presence of anthraquinons is confirmed by the formation of red rose color.

Coumarins: 10 ml of the extract of the dry powder with diethyl ether was evaporated,. ml of water was added to the extract. The mixture obtained is then divided in two test tubes (one used as. reference). in the second 0.5 ml of NH_4OH (10%) was added. And then observed under UV. The presence of coumarins was indicated by fluorescence in the tube.

Proteins: Proteins were demonstrated by Lowry's method with the Folin Phenol Reagent (Lowry 1951).

Mucilages: 0,5 g of powder was moistened with. mL of ethanol (96%), distilled water was added up to 25 mL, it was shaken vigorously every 10 min for 1 h and then allowed to stand for 3 h. The presence of flaky precipitate indicated the presence of mucilage.

Glycosides: To the alcoholic extract of drug equal volume of water and 0.5 ml of strong lead acetate solution was added, shaked and filtered. Filtrate was extracted with equal volume of chloroform and the chloroform extract was evaporated to dryness. The residue was dissolved in 2 ml of pyridine and sodium nitropruside 2 ml was added followed by addition of NaOH solution to make alkaline. Formation of pink colour in presence of glycosides or aglycon moiety.

Quantitative Analysis

Total phenolic content: It was determined by the Folin reagent method Ciocalteu (FCR) (Mruthunjaya *et al.* 2016). One ml of the plant extract was mixed with FCR (diluted 10 times). After standing for 5 min at 22 ºC, a volume of 750 ml $NaCO_3$ was added to the mixture. The absorbance was measured at 725 nm by spectrophotometer (Shimadzu UV mini 1240). The content of TPCs of each extract was estimated by comparison with the standard curve generated from gallic acid. The results were expressed in gallic acid equivalents (mg GAE/g extract).

Flavonoid content: It was determined using quercetin as. reference compounds. One ml of plant extract in methanol was mixed with 1 ml chloride aluminum. The absorbance was read at 415 nm. The flavonic content is expressed in mg quercetin/g extract (Mruthunjaya *et al.* 2016).

Experimental design

Plant Extract Preparation for in vivo antidiabetic activity

One gram of dried leaves of *A. halimus* was boiled in 100 ml distilled water for 10 min and centrifuge for many times and filtered thereafter, the extract was prepared daily before treatment according to the traditional use of this plant against diabetes.

Acute toxicity study

Acute toxicity study was assessed in rats by using an acute oral toxic class method of Organization of Economic Co-operation and Development guidelines (OECD/OCDE 2002). This test consists in administering gradual doses (300 mg, 400 mg, 1000 mg, 5000 mg kg BW) to the animals and observed for any manifestation of toxicity, increase in locomotors activity, salivation, convulsion, coma and death. These observations are made regularly up to 24 hours.

Induction of diabetic in rat

Diabetes was induced in rats by single intra peritoneal (IP) injection of streptozotocin (STZ, Sigma chemical, Aldrich) at. dose 80 mg/kg BW freshly dissolved in 0.1 M cold citrate buffer of pH 4.5 (Mostafavinia *et al.* 2016). 48 hours later blood samples were collected and blood glucose

levels were determined by the ACCU-CHEK Performa glucometer (USA) to confirm the development of diabetes. Those rats which showed hyperglycemia (blood glucose levels>300 mg/dl) were used in experiment.

In vivo anti-diabetic activity

Fifty healthy adults females Wistar rats weighing (148-160). were obtained from Algerian Pasteur Institute. Animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature at 25±5 °C with 12:12 light: dark cycles) and water *ad libitum*. Food was provided by dry pellets. Animals were randomly divided into five groups of ten rats each. Experimental work was carried out at UMAB in accordance with the Algerian Legislation (Law Number 12-235/2012) inherent to the protection of animals designed to experimental and other scientific purposes as well as with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA). This study was done from June, July until August. After induction of diabetic in rats. Treatment with the aqueous extract of *A. halimus* orally, daily in the morning starts seven days after injecting streptozotocin.

Rats were divided into five groups of ten rats:

- Group C: Control group received orally physiological NaCl-solution .
- Group NT: Normal rats treated with aqueous extract of *A. halimus* at dose 200 mg/kg BW.
- Group D: Untreated diabetic rats received physiological NaCl-solution.
- Group D100: Diabetic rats treated with aqueous extract of *A. halimus* at dose 100 mg/kg BW.
- Group D200: diabetic rats treated with aqueous extract of *A. halimus* at dose 200 mg/kg BW.

Evaluation of anti-diabetic activity

The antidabetic effect of aqueous extract of *A. halimus* leaves in rats were determined by measuring fasting plasma glucose levels and body weights weekly. Water consumption was determined daily (ml). Serum triglycerides, total cholesterol, and liver enzymes (serum glutamic oxaloacetate transaminase (SGOT) and serum glutamic pyruvate-transaminase (SGPT), alkaline phosphatase (PAL). were determined on day 14 and 42 post extract administration using semi-automatic biochemistry analyser (BA88A). The animals were divided in two groups, group 14 sacrificed by decapitation in day fourteen and group 42 sacrificed by decapitation after forty two days.

Histopathological examination

Two rats were randomly selected from each group for histopathological investigations on days 14 and 42. Tissue samples were taken, pancreas were fixed in 10% formalin for 48 h, incorporated into paraffin and sectioned to obtain paraffin sections 4 µm thick using a slide microtome. The tissue sections obtained were collected on glass slides, dewaxed in xylene, hydrated in descending series of ethyl alcohol, stained with hematoxylin and eosin (H&E) stains, dehydrated in ascending series of ethyl alcohol, eliminated in two changes of xylene, assembled with DPX (Bancroft & Gamble 2008) then examined with optical microscope.

Statistical analysis

For numerical outcomes, one-way analysis of variance (ANOVA) with test. comparisons of two variances were performed using XLSTAT (2020.5.1.1065) and all graphs were made by utilizing Microsoft office 2007 software. The results were expressed as an average. SEM. The values of $p<0.05$; $p<0.01$; $p<0.001$ were considered significant (*), very significant (**) and highly significant (***) respectively.

Results

Phytochemical screening

The phytochemical screening of *A. halimus* leaves extracts has shown that this herb contains: Flavonoids, tannins, saponins, mucilages, glycosides and proteins, whereas the absence of alkaloids, quinons, coumarins, terpenoids and combined anthraquinones (Table 1).

Type of compounds	Test name or reagent	
Alkaloids	Mayer- & Dragendorff's reagent	-
Flavonoids	Shinoda test	+
Quinones	NAOH + Extract	-
Tannins	Ferric chloride test	+
Saponins	Froth test	+
Coumarins	Fluorescence test	-
Triterpenoids	Salkowski's test	-
Proteins	Trichloroacetic acid test	+++
Glycosides	Legal test	+++
Combined anthraquinones	Borntrager's test	+
Mucilages	Extract + Alcohol	+++

-Absence, +Presence in low concentration, ++Presence, +++Presence in high concentration

Tabla 1. Resultados del cribado fitoquímico de *Atriplex halimus*.

Table 1. Results of phytochemical screening of *Atriplex halimus*.

The results show that the total polyphenols compounds in *A. halimus* leaves was higher in methanolic extract (5,24±0,07mg GAE/g of extract) as well as flavonoids with (1,266±0,06mg EQ/g of extract. than in aqueous extract (Table 2).

	Flavonoids (mg Quercetin equivalent/g of extract)	**Total phenolic compound** (mg Gallic acid equivalent/g of extract)
Methanolic Extract	1,266 ±0,06	5,24 ±0,07
Aqueous extract	0,848 ±0,01	1,092 ±0,08

Tabla 2. Flavonoides y contenido total de compuestos fenólicos de *Atriplex halimus*.

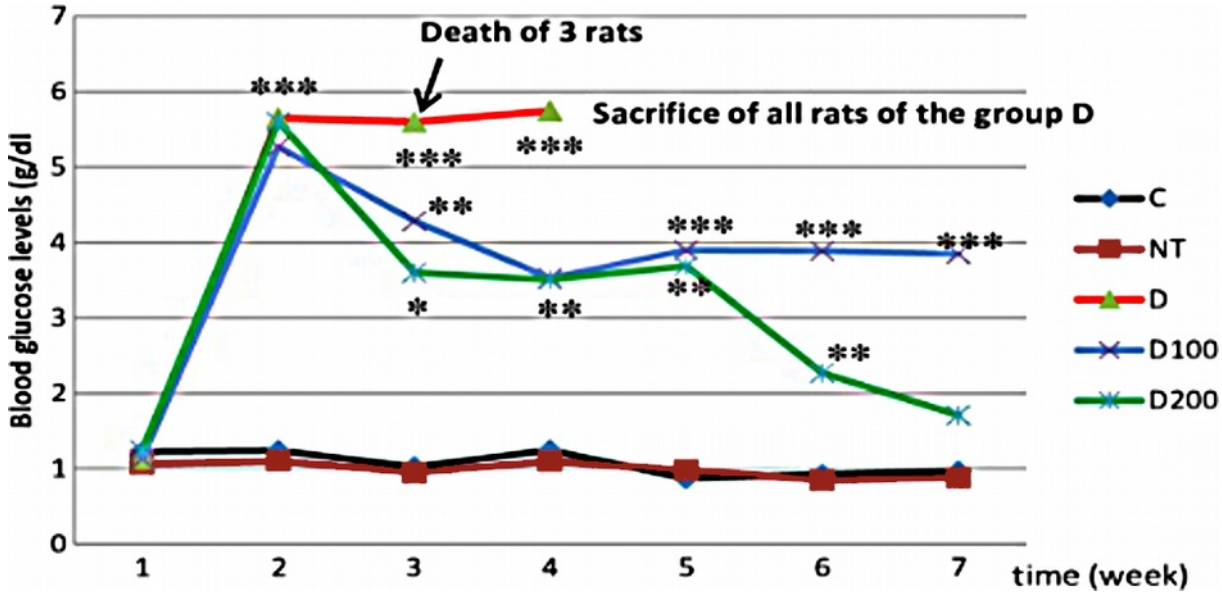
Table 2. Flavonoids and Total phenolic compound contents of *Atriplex halimus*.

In vivo anti-diabetic activity

Oral administration of the aqueous extract of *A. halimus* at doses of (300 mg, 400 mg,1000 mg, 5000 mg)/kg BW to rats did not induces any signs of acute toxicity during the 48 h of observation.

Results indicate that streptozotocin administration increased significantly ($p<0.001$) the blood glucose levels in groups (D, D100, D200) one week post IP injection (Fig. 1) compared to control. All clinical manifestations were observed in rats: Hyperglycemia, fatigue and weakness, a rapid weight loss, increased urination, rapid breathing and high volume of water consumption. Two weeks post injection of streptozotocin, three rats from the diabetes group were died due to complications caused by hyperglycemia. The seven rats left were dramatically weak for this reason all rats of this group were sacrificed after 21 days post injection of streptozotocin. Results in (Fig. 2) revealed highly significant weight loss in diabetic rats compared to control, however weight of rat was improved after 42 days of treatment with *A. halimus* extract at doses 200 and 100 mg/kg. Rats treated with *A. halimus* consume less water than diabetes rats in the first fifteen days of treatment, this difference was significant at the end of the experiment between control. Normal treated and rats treated with *A. halimus* 200 and 100 mg/kg (Fig. 3). Rats continue consuming water may be due to the salinity of aqueous extract of *A. halimus*.

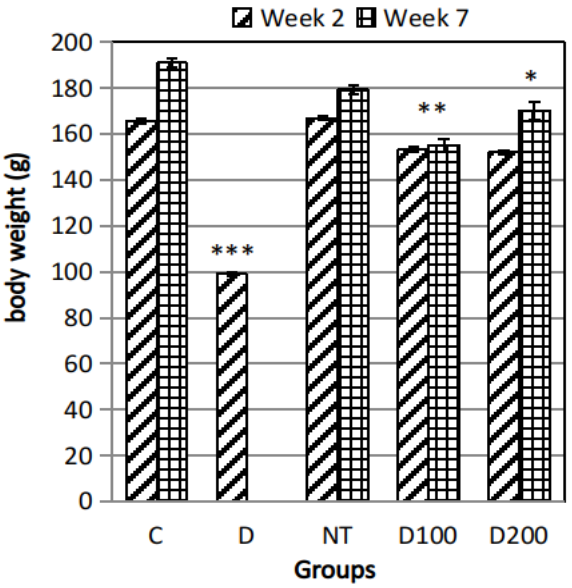
Treatment with the aqueous extract of *A. halimus* (200 mg/kg BW) induces highly significant decrease in glucose levels ($p<0.001$) at the end of experiment (Fig. 1). This anti-hyperglycemia was dose dependent and was observed after long treatment (seven weeks) compared to short term (two



* p <0.05, ** p <0.01, *** p <0.001

Figura 1. Efecto del extracto acuoso de *Atriplex halimus* sobre los niveles de glucosa en sangre contra la diabetes inducida por estreptozotocina en ratas y ratas normales. C: control; NT: ratas tratadas normales; D: ratas diabéticas no tratadas; D100: ratas diabéticas tratadas con extracto acuoso a dosis de 100 mg/kg de peso corporal. D200: ratas diabéticas tratadas con extracto acuoso a dosis de 200 mg/kg de peso corporal. Media ± DE, n=10 animales por grupo.

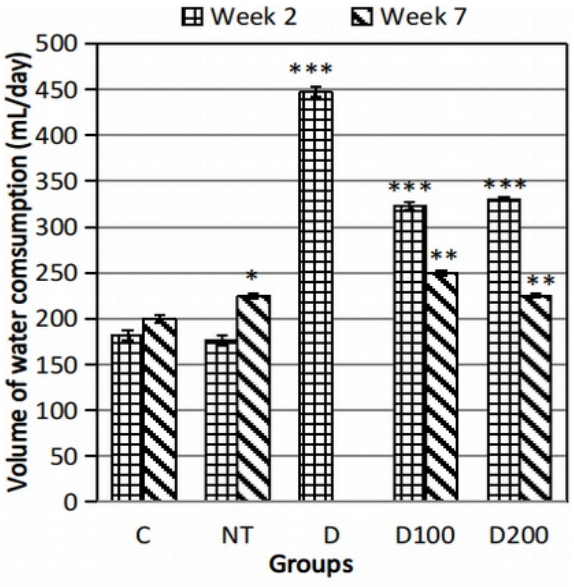
Figure 1. Effect of aqueous extract of *Atriplex halimus* on blood glucose levels against streptozotocin induced diabetes in rats and normal rat. C: Control; NT: Normal treated rats; D: Untreated diabetic rats; D100: Diabetics rats treated with aqueous extract at dose 100 mg/kg BW; D200: diabetic rats treated with aqueous extract at dose 200 mg/kg BW. Mean±SD, n=10 animals per group.



* p <0.05, ** p <0.01, *** p <0.001

Figura 2. Efecto de *Atriplex halimus* sobre el peso del animal (g), en la semanas 2 y 7. C: control; NT: ratas tratadas normales; D: ratas diabéticas no tratadas; D100: ratas diabéticas tratadas con extracto acuoso en dosis 100 mg/kg de peso corporal; D200: ratas diabéticas tratadas con extracto acuoso a dosis de 200 mg/kg de peso corporal. Media ± DE, n=10 animales por grupo.

Figure 2. *Atriplex halimus* effect on weight of animal (g), at weeks 2 and 7. C: Control; NT: Normal treated rats, D: Untreated diabetic rats; D100: Diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 100 mg/kg BW; D200: diabetic rat treated with aqueous extract at dose 200 mg/kg BW. Mean±SD, n=10 animals per group.



* p <0.05, ** p <0.01, *** p <0.001

Figura 3. Efecto de *Atriplex halimus* sobre el consumo promedio de volumen de agua (ml / día), en la semanas 2 y 7. C: control; NT: ratas tratadas normales; D: ratas diabéticas no tratadas; D100: ratas diabéticas tratadas con extracto acuoso en dosis 100 mg/kg de peso corporal; D200: ratas diabéticas tratadas con extracto acuoso a dosis de 200 mg/kg de peso corporal. Media ± DE, n=10 animales por grupo.

Figure 3. *Atriplex halimus* effect on average of water volume consumption (mL/day), at weeks 2 and 7. C: Control; NT: Normal treated rats, D: Untreated diabetic rats; D100: Diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 100 mg/kg BW; D200: diabetic rat treated with aqueous extract at dose 200 mg/kg BW. Mean±SD, n=10 animals per group.

weeks) treatment protocol.

We noticed that the dose of 100 mg/kg BW induced significant decrease in blood glucose levels. Aqueous extract of *A. halimus* induce. slight reduction of glucose levels in normal rats compared to control.

Streptozotocin cause slight decrease in cholesterol level and triglycerides but was not significant, however administration of aqueous extract of *A. halimus* cause a significant decrease ($p<0.05$) in cholesterol and triglycerides in group D100 and D200 and highly significant decrease in normal treated group two weeks after starting treatment (Table 3). Seven weeks after starting treatment triglycerides increase significantly in group D100, we noticed also an increase of triglycerides in group D200 but was not significant (Table 4).

Streptozotocin injection caused a highly significant increase in blood enzymes levels in group D ($p<0.001$) compared to control group which suggested liver cells damage. The administration of aqueous extract of *A. halimus* reduce slightly the levels of these enzymes. The STGP level in group D200 was close to control at the end of experiment (Tables 3, 4).

Histological study

The histological study in the pancreas tissues of control rats (C) and normal rats treated with *A. halimus* (NT) showed normal structure of Islets of Langerhans at 400x in day 14 and day 42, however streptozotocin induced severe degenerative and necrotic changes in diabetic rat after 21 days of induction of diabetes, all rats of this group were scarified after 21days.

Diabetic rats treated with aqueous extract of *A. halimus* showing marked improvement of Islets of Langerhans at the dose of 100 mg/kg BW and dose 200 mg/kg BW (Fig. 4).

Discussion

Our results of phytochemical screening from leaves collected from Bechar (Southwest of Algeria) are in the same order with the results of

Group	Cholesterol (g/L)	Triglycerides (g/L)	STGO (U/L)	STGP(U/L)	PAL(U/L)
C	1,017±0,120	1,450±0,026	31±2,000	29,333± 4,041	187,667±9,074
NT	1,493±0,567 NS	0,313±0,025***	32±3,606 NS	31,000± 4,000NS	191,667±11,015NS
D	1,077±0,576 NS	1,000±0,030 NS	80 ±10,817***	70,000±11,136***	456,3±60,451***
D100	0,583±0,015 *	0,643±0,031*	69,333±2,082 **	60,667± 2,517**	369,667± 4,163**
D200	0,720±0,036 *	0,450±0,026**	50,667±2,309**	49,000±2,646 **	357,6±38,527**

C: Control; NT: Normal treated rats; D: Untreated diabetic rats; D100: Diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 100 mg/kg BW; D200: diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 200 mg/kg BW.

Results are expressed as the mean ± SD (n=10 animals per group). *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the Control group (C). NS: non-significant.

Tabla 3. Efecto de *Atriplex halimus* sobre los triglicéridos séricos, el colesterol total y las enzimas hepáticas de ratas diabéticas y normales dos semanas después de iniciar el tratamiento. SGOT: transaminasa glutámico-oxalacética sérica; SGPT: glutamato-piruvato transaminasa sérica; PAL: fosfatasa alcalina.

Table 3. *Atriplex halimus* effect on Serum triglycerides, total cholesterol, and liver enzymes of diabetic and normal rats two weeks after starting treatment. SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamate-pyruvate transaminase; PAL: alkaline phosphatase.

Group	Cholesterol (g/L)	Triglycerides (g/L)	STGO (U/L)	STGP(U/L)	PAL(U/L)
C	1,153±0,182	1,157± 0,240	29±1	27,667± 1,528	193,33± 6,807
NT	1,053±0,182 NS	1,057± 0,240 NS	27± 3,464NS	26,667± 1,528NS	186,66± 12,503NS
D100	0,637±0,090*	2,280± 0,171*	65±4,583**	51±3,606**	379±9,165***
D200	0,557±0,038*	1,933± 0,700NS	58,667± 3,215**	34±4,163NS	381,66± 12,423***

C: Control; NT: Normal treated rats; D100: Diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 100 mg/kg BW; D200: diabetic rat treated with aqueous extract of *Atriplex halimus* at dose 200 mg/kg BW.

Results are expressed as the mean ± SD (n=10 animals per group). *p<0.05, **p<0.01, ***p<0.001 were considered significant when compared with the Control group (C). NS: non-significant.

Tabla 4. Efecto de *Atriplex halimus* sobre los triglicéridos séricos, el colesterol total y las enzimas hepáticas de ratas diabéticas y normales siete semanas después de iniciar el tratamiento. SGOT: transaminasa glutámico-oxalacética sérica; SGPT: glutamato-piruvato transaminasa sérica; PAL: fosfatasa alcalina.

Table 4. *Atriplex halimus* effect on Serum triglycerides, total cholesterol, and liver enzymes of diabetic and normal rats seven weeks after starting treatment. SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamate-pyruvate transaminase; PAL: alkaline phosphatase.

(Ounaissia *et al.* 2020) and (Gattouche *et al.* 2020) with *A. halimus* collected from Ouargla desert region in southern and Biskra respectively in Algeria. However, Gattouche *et al.* (2020) found alkaloids in aerial part of the plant. Our findings agree with previous reports of Benhammou *et al.* (2009) with *A. halimus* from Bechar region collected in 2008 which found a very low quantity of polyphenols and flavonoids in leaves. Oueld kadour *et al.* (2019) found. high concentration of polyphenols and flavonoids in *A. halimus* from north of Algeria (Mostaganem). These differences in the contents of total polyphenols, flavonoids and the absence or presence of phytochemicals in different regions can be explain by experimental conditions, climatic and environmental area, genetic heritage and period and time of harvest (Allaoui *et al.* 2016, Atmani *et al.* 2009).

The antidiabetic activity and mechanism of action of *A. halimus* still unknown. The possible mechanism of antidiabetic activity of aqueous extract of this plant is its improvement of islets of Langerhans which are clear in histological tissues in rat treated with *A. halimus* in group D100 and D200 (Fig. 4), or by increasing the pancreatic secretion of insulin from the existing beta cells. This insulin like activity may be due also to phytoconstituents (Missoun *et al.* 2018). Several studies reported antidibetic activity of *Atriplex* species from desert region (Chikhi *et al.* 2014, Slama *et al.* 2020, Oueld kadour *et al.* 2019).

The chemical composition analysis by several authors (Goyal & Kaur 2019, Emam, 2010) showed the presence of secondary metabolites (flavonoids, alkaloids, glycosides, sponosides and tannins) in *A. halimus*. It was demonstrated by several studies that phenolic compounds and flavonoids possess marked anti-diabetic activity (Kumari *et al.* 2012, Suba *et al.* 2004, Chikhi *et al.* 2014), minerals (P, K, Mg, Fe, Cu, Mn, Se, Ca, Na,Cr). These minerals, especially the chromium and magnesium salts, from tissues of this plant regulate blood sugar by activating the effect of insulin (Rashad *et al.* 2021). The presence of amino acids (aspartic acid, threonine, serine, glu-

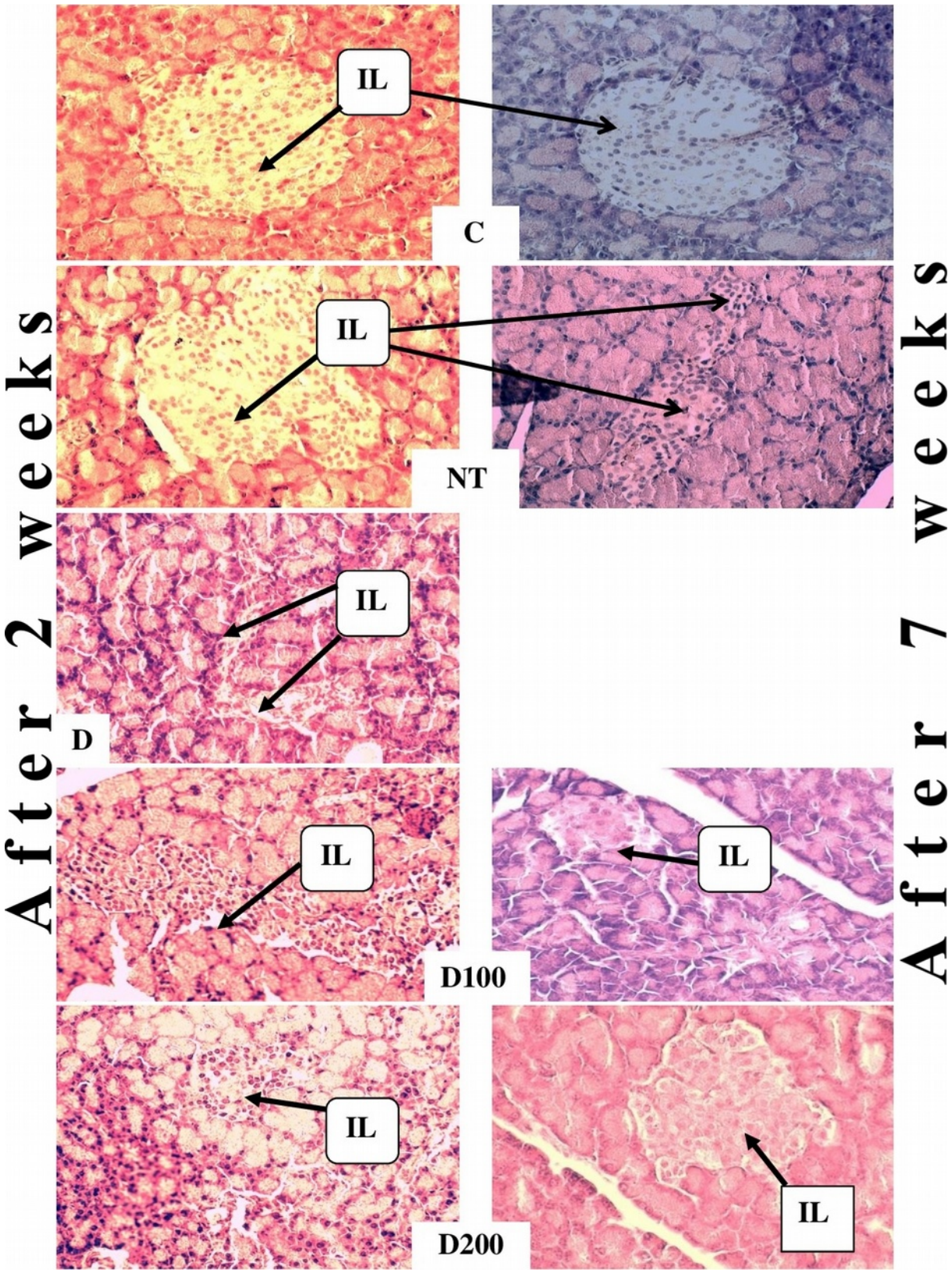


Figura 4. Efecto protector de *Atriplex halimus* contra la diabetes inducida por estreptozotocina en tejidos del páncreas de ratas. C: control; NT: ratas tratadas normales; D: ratas diabéticas no tratadas; D100: ratas diabéticas tratadas con extracto acuoso a dosis de 100 mg/kg de peso corporal. D200: ratas diabéticas tratadas con extracto acuoso a dosis de 200 mg/kg de peso corporal. IL: Islotes de Langerhans (X40).

Figure 4 . Protective effect of *Atriplex halimus* against streptozotocin induced diabetes in pancreas tissues of rats. C: Control; NT: Normal treated rats; D: Untreated diabetic rats; D100: Diabetic rats treated with aqueous extract at dose 100 mg/kg BW; D200: diabetic rats treated with aqueous extract at dose 200 mg/kg BW. IL: Islets of Langerhans (X40).

tamic acid, proline, methionine, isoleucine, leucine, tyrosine, histidine and arginine) and insulin-like proteins was reported in plant materials (Marella 2018) and fatty acid (stearic acid, palitolic acid, myristic acid palitoleic, acid linolenic acid, pelargonic acid).

Cholesterol level is usually elevated in diabetic patient, this suggested that aqueous extract of *A. halimus* can protect diabetic patient from coronary heart disease caused by increased cholesterol. Serum glutamic oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) and alkaline phosphatase (PAL) are well-known enzymes used as biomarkers to predict possible toxicity to the liver since the elevation of these enzymes indicate liver injury (McGill 2016, Wolf *et al.* 1972). The findings of this study demonstrate that *A. halimus* exhibit liver enzymes lowering activity in diabetic rats. These results are in agreement with Rashad *et al.* (2021), who reported that *A. halimus* leaves powder had significant decreased in liver enzymes in diabetic rats. Fall in enzymes activities was observed after long treatment (7 weeks) compared to short term treatment protocol.

No death in rats or any toxic signs was observed with the selected doses until the end of the study period.

Conclusion

Based on our findings from this study ,it can be concluded that the aqueous extract of the leaves of *A. halimus* collected from Bechar south-west of Algeria was effective against hyperglycemia caused by streptozotocin and decrease significantly glucose levels and protect rats from diabetes complications. The phytochemical investigation showed that the leaves of this plant are rich in the term of secondary metabolites. The synergistic effect of these metabolites or one of these phytoconstituents could be responsible for the anti-diabetic activity. Other researches are taking place in our laboratory to isolate and identified the active compound responsible for this pharmacological activity.

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