RESEARCH ARTICLE

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SCIENCELeaf Aqueous Extract of *Argania spinosa* Exhibits Antihyperglycemic Effect in Diabetic Rats

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Abstract: *Introduction:* *Argania Spinosa* L. (Sapotaceae) is an endemic species from south-western Morocco. This plant has many traditional uses including its use in the treatment of diabetes.

Objective: The objective of the study was to evaluate the antidiabetic activity of *Argania Spinosa* Leaf Aqueous Extract (A.S.L.A.E).

Methods: The antidiabetic effect of A.S.L.A.E was evaluated in both normal and streptozotocin (STZ)-induced diabetic rats treated at a dose of 20 mg/kg body weight for 15 days. The histopathological changes in the liver were evaluated. In addition, the antioxidant activity of this extract was also studied.

Results: Single oral administration of A.S.L.A.E (20 mg/kg) showed no significant change in blood glucose levels in both normal and STZ induced diabetic rats after 6 hours of administration. Furthermore, in normal rats, repeated oral administration of A.S.L.A.E reduced blood glucose levels. Moreover, blood glucose levels decreased in STZ diabetic rats after fifteen days of treatment. According to the oral glucose tolerance test, the A.S.L.A.E (20 mg/kg) was shown to prevent significantly the increase in blood glucose levels in normal treated rats. Moreover, A.S.L.A.E showed antioxidant activity.

Conclusion: The results show that *Argania spinosa* leaf aqueous extract possesses significant antihyperglycemic activity.

Keywords: Antihyperglycemic effect, antioxidant activity, *Argania spinosa*, diabetes, oral glucose tolerance test, streptozotocin.

1. INTRODUCTION

Traditional herbal medicines have an advantage in the prevention and treatment of diabetes, as they have fewer side effects [1, 2].

The Argan tree, called *Argania Spinosa* (L.) Skeels is an endemic tree of the southwestern region of Morocco, belonging to the Sapotaceae family. Populations of Morocco traditionally use the fruits of *Argania spinosa* (*A. spinosa*) to prepare edible oil [3]. Traditionally, the Argan tree is used in cosmetics for skin and hair care and against acne, gastritis, diarrhea, fever, urticaria and headaches hypercholesterolemia [4-10].

According to a previous ethnobotanical survey which was conducted in Chtouka Ait Baha and Tiznit (Western Anti-Atlas) Morocco, the seed of *A. spinosa* was used in the

traditional medicine by the local population for the treatment of diabetes [11]. Our previous investigation has demonstrated that the fruits' aqueous extract of *A. spinosa* possesses antidiabetic effect [12]. In addition, we demonstrated that Arganimide A extracted from *A. spinosa* had lipid and glucose lowering effect [13]. Nevertheless, no previous pharmacological or clinical study has been carried out to test the anti-diabetic activity of *Argania spinosa* leaf aqueous extract (A.S.L.A.E). Therefore, the present study was designed to evaluate the effect of A.S.L.A.E in normal and diabetic rats. We have also evaluated the histopathological changes induced by this aqueous extract in the liver both in normal and STZ rats. Additionally, an oral glucose tolerance test was realized and the antioxidant potential of the A.S.L.A.E was also demonstrated.

2. MATERIAL AND METHODS

2.1. Plant Material

Leaves of *Argania spinosa* (Sapotaceae) were collected from the Souss region in Agadir (Morocco) in 2016-2017 and air-dried at 40°C. The plant was taxonomically identi-

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fied and a voucher specimen was deposited at the herbarium of the Faculty of Sciences and Techniques, Errachidia.

2.2. Preparation of the Aqueous Extract

Plant material was prepared according to the traditional method used in Morocco (decoction), the dose administered was 20 mg of lyophilized aqueous extract per kg of body weight [12-14].

2.3. Experimental Animals

The antidiabetic activity of the *A.S.L.A.E* was studied in adult male Wistar rats weighing about 190-230 g. The animals were housed under standard environmental conditions and maintained with free access to water and *ad libitum* standard laboratory diet.

2.4. Effect of A.S.L.A.E on Glucose Tolerance Test

Fasted normal rats were randomly assigned to three different groups containing five rats each. The duration of the fast was 12 hours with free access to water.

Group I served as control received only vehicle (distilled water);

Group II received the *A.S.L.A.E* at a dose of 20 mg/kg;

Group III received a reference drug; glibenclamide at a dose of 5 mg/kg;

Glucose 2 g/kg was fed 30 min after the administration of different doses of *A.S.L.A.E* and glibenclamide.

Blood was withdrawn from the tail vein at 0, 30, 60, 90 and 120 min [13]. Moreover, glucose levels were estimated by using a reflective glucometer (ContourTMTS) [14].

2.5. Induction of Diabetes

Diabetes was induced and groups were randomly assigned as it has been previously described [12-15].

2.6. Determination of Parameters

Blood glucose levels were determined by the glucose oxidase method using a reflective glucometer (ContourTMTS) from Bayer Diabetes Care.

2.7. Histopathological Changes in the Liver, Morphometric Analysis

Histopathological and morphometric analysis of the liver followed the protocol used in our previous study [12-16].

2.8. Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) Radical Scavenging Activity

The free radical scavenging activity of *A.S.L.A.E* was analysed as it has been described previously [12-17].

2.9. Statistical Analysis

Data were expressed as mean $\pm$ S.E.M. Statistical differences among the means studied were assessed by two-way ANOVA followed by Bonferroni multiple comparisons test

with GraphPad Prism 6 software. Differences were considered to be significant when $p < 0.05$.

3. RESULTS

3.1. Single Oral Administration

In normal and diabetic rats, a single administration of *A.S.L.A.E* had no effect on reducing blood glucose levels. On the other side, the glibenclamide revealed a significant decrease ($p < 0.0001$) in both normal and STZ induced diabetic rats after four and six hours after administration (Fig. 1).

3.2. Repeated Oral Administration

In normal rats, a significant reduction in blood glucose levels was observed after 15 days of *A.S.L.A.E* oral administration ($p < 0.01$). In STZ rats, a significant reduction in blood glucose levels was observed at the second day of oral treatment ($p < 0.0001$) with *A.S.L.A.E* (20 mg/kg) and the decrease in blood glucose levels became more significant at the fifteenth day of treatment ($p < 0.0001$). On the other hand, the normal and the diabetic rats treated with glibenclamide showed a significant diminution in blood glucose levels at the end of treatment ($p < 0.0001$) (Fig. 2).

3.3. Body Weight

In normal and STZ rats, treated with *A.S.L.A.E* for 15 days, no significant change in body weight was observed. The same result was also observed in glibenclamide-treated group (Fig. 3).

3.4. Effect of A.S.L.A.E on Glucose Tolerance

A.S.L.A.E has prohibited the increase in blood glucose levels significantly 30 min ($p < 0.0001$), 60 min ($p < 0.05$) and 120 min ($p < 0.05$) after glucose administration when compared to the control group (Fig. 4). Glibenclamide treatment has prohibited the increase in blood glucose levels significantly 30 min ($p < 0.01$), 60 min ($p < 0.0001$), 90 min ($p < 0.0001$) and 120 min ($p < 0.0001$) after the administration of 2 g/kg of glucose.

3.5. Histopathological Changes in the Liver and Morphometric Analysis

The results show that the diameter of the core of diabetic rats treated with *A.S.L.A.E* (3.42 ± 0.20) was larger than the diameter of the core of control diabetic rats (3.13 ± 0.16) (Table 1). In addition, the number of hepatocytes counted in an area of $40000 \mu\text{m}^2$ in diabetic rats treated with *A.S.L.A.E* was equal to 25 hepatocytes. Fig. (5) illustrates the histopathological changes in the liver of diabetic rats fifteen days after oral administration of *A.S.L.A.E* (20 mg/kg) or glibenclamide (5 mg/kg).

In diabetic untreated rats (Fig. 5B), as compared to diabetic treated rats with *A.S.L.A.E* (Fig. 5C) or glibenclamide (Fig. 5D), the hepatocytes were observed to be disorganized with noticeable hepatocellular damages along with the disordered liver architecture. Sinusoids were enlarged with the wall of veins thickened (Fig. 5B). After 15 days of treatment by *A.S.L.A.E* or glibenclamide, STZ diabetic rats showed

Fig. (1). Plasma glucose levels over 6 h after a single oral administration of A.S.L.A.E (20 mg/kg) in normal (**A**) and diabetic rats (**B**). Data are expressed as means $\pm$ S.E.M. $n=6$ rats per group. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ and **** $p<0.0001$ when compared to baseline values.

Table 1. Morphometric analysis of hepatocytes of diabetic rats treated with A.S.L.A.E (20 mg/kg) and the controls groups. Values are expressed as means $\pm$ SEM. No significant when compared to control diabetic.

Groups	Diameter of the Core (μm)	Number of Hepatocytes
Control normal	3.23 ± 0.15	29
Control diabetic	3.13 ± 0.16	24
Diabetic rats treated with A.S.L.A.E	3.42 ± 0.20	25
Diabetic rats treated with glibenclamide	3.32 ± 0.26	25

Fig. (2). Plasma glucose levels over once daily repeated oral administration of A.S.L.A.E (20 mg/kg) for 15 days in normal (A) and diabetic rats (B). Data are expressed as means $\pm$ S.E.M. $n = 6$ rats per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ and **** $p < 0.0001$ when compared to baseline values.

more progressive changes, improvement of liver architecture and lack of central hemorrhagic necrosis. The results showed that the rat liver histopathology of A.S.L.A.E was almost similar to the glibenclamide-treated group (Fig. 5C and Fig. 5D).

3.6. DPPH (1-1-diphenyl 2-picryl Hydrazyl) Radical Scavenging Activity

The different concentrations of the A.S.L.A.E (31.25, 62.5, 125, 250 and 500 $\mu\text{g/ml}$) showed antioxidant activities in a dose-dependent manner (23.26%, 33.64%, 40.60%, 60.33% and 67.60% inhibition, respectively) on the DPPH

radical scavenging assay. On the other hand, the synthetic antioxidant BHT gave the following values: 49.17%, 52.89%, 59.67%, 68.66% and 82.87% inhibition (Fig. 6). Linear regression analysis was used to calculate IC₅₀ values. A.S.L.A.E revealed inhibitory concentrations of 50% of free radicals (IC₅₀) of 248.27 $\mu\text{g/ml}$. In contrast, the synthetic antioxidant butylhydroxytoluene (BHT) showed an IC₅₀ equal to 13.65 $\mu\text{g/ml}$.

4. DISCUSSION

In the present study, the results showed that A.S.L.A.E (20 mg/kg) induced a significant decrease in blood glucose

Fig. (3). Body weight change after once daily repeated oral administration of A.S.L.A.E (20 mg/kg) for 15 days in normal (A) and diabetic rats (B). Data are expressed as mean $\pm$ S.E.M. $n=6$ rats per group. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ and **** $p<0.0001$ when compared to baseline values (the start of treatment).

Fig. (4). Effect of A.S.L.A.E (20 mg/kg) on oral glucose tolerance diabetic rats. Values are means $\pm$ SEM; $n=5$. (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$; (****) $p<0.0001$ vs. control.

Fig. (5). Effect of orally administered A.S.L.A.E on liver histology. Representative images of the liver in normal rats (A), STZ-induced diabetic rats (B), diabetic rats treated with 20 mg/kg/day A.S.L.A.E (C) and diabetic rats treated with glibenclamide (5 mg/kg/day) (D) Images were taken under 40× magnification.

H = Hepatocytes, CV = Central Vein, S = Sinusoid, N= Necrosis.

Fig. (6). DPPH radical scavenging activity of A.S.L.A.E.

levels both in normal and STZ induced diabetic rats after fifteen days of treatment. Our results are in agreement with our previous study which demonstrated a strong antihyperglycemic effect on diabetic rats of *Argania spinosa* fruits aqueous extract [12, 18]. Concerning body weight, no signif-

icant reduction was observed in normal and diabetic rats treated with A.S.L.A.E after 15 days of treatment. Streptozotocin as an antibiotic agent has been widely used for inducing type I diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic beta cells [19]. STZ

injection also led to increased oxidative stress apart from inducing liver damage. In STZ induced diabetic rats, A.S.L.A.E was found to provide significant protection from all the serious effects of STZ after 15 days of treatment. Concerning the oral glucose tolerance test, conducted on normal rats A.S.L.A.E prevented the increase in blood glucose levels significantly after glucose administration (2 g/kg). The results suggest that increased levels of glucose tolerance may be due to increased secretion of insulin, inhibition of α -amylase and α -glucosidase. Otherwise, the ability of A.S.L.A.E to lower the blood glucose levels in the oral glucose tolerance test suggests that rats treated with this extract had an increased glucose uptake [16, 20, 21]. Additionally, A.S.L.A.E revealed an antioxidant capacity. On the other hand, polyphenols from the leaves of *A. spinosa* belong to the flavonoid (17%) and catechin tannin (14%) family [22, 23]. Furthermore, flavonoid extract *A. spinosa* has been demonstrated to protect the skin against UV-light [24]. Consequently, the properties of the flavonoids were further studied leading to the discovery of the anti-acne properties of the flavonoid extract and inhibitory properties of their matrix metalloprotease (MMP) [25-29]. MMP constitutes a group of more than twenty enzymes responsible for the degradation of the extracellular matrix. Collagenases and elastases are well known as enzymes involved in the skin-aging process. This may be attributed to the important role of flavonoids as antioxidants. Thus, it is possible that these active compounds are responsible for the antidiabetic activity observed in A.S.L.A.E.

CONCLUSION

The study shows that A.S.L.A.E exerts antidiabetic and antioxidant activity in STZ-induced diabetic rats. More investigations are needed, such as to isolate the active(s) principle(s) of this plant and to clarify its mechanism of action, in addition to toxicological studies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study on animal was approved by the local committee FSTE/2015. Faculty of Sciences and Techniques Errachidia, Morocco.

HUMAN AND ANIMAL RIGHTS

No human were used in this research. All animal research procedures were followed in accordance with the standards set forth in the eighth edition of Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences, The National Academies Press, Washington, D.C.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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