Glucose Lowering Activity of the Aqueous Extract of Warionia saharae in Normal and Diabetic Rats

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Abstract: Background: Warionia saharae is a medicinal plant used in the Morocco for the treatment of diabetes mellitus.

Objective: The aim of the study was to assess the effect of the aqueous extract of this plant on blood glucose levels in diabetic rats.

Method: The current study was carried out to investigate the effect of a single dose and daily oral administration for 15 days of the Aerial Part Aqueous Extract (A.P.A.E) of Warionia saharae (W. saharae) at a dose of 5 mg/kg body weight on blood glucose levels and the histopathological changes in liver and pancreas both in normal and streptozotocin-induced diabetic rats (STZ). Also, the effect of this extract to improve glucose tolerance in normal rats was demonstrated.

Results: Single oral administration of W. saharae A.P.A.E reduced blood glucose levels 6 h after administration in normal rats and in STZ-induced diabetic rats. Furthermore, blood glucose levels were decreased in normal and STZ-induced diabetic rats after 15 days of treatment. The aqueous extract of W. saharae was shown to improve the increase on blood glucose levels in normal treated rats significantly (p< 0.001) 90 min after glucose administration as compared to the control groups.

Conclusion: In conclusion, the results show that APAE of W. saharae possesses significant antihyperglycemic and hypoglycemic activity.

Keywords: Warionia saharae, streptozotocin, diabetes, aqueous extract, glucose tolerance, antihyperglycemic activity.

1. INTRODUCTION

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world and there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [1]. In Morocco, several medicinal plants are used in traditional medicine for the treatment of diabetes [2-4].

Warionia Benth. & Coss. is a monotypic genus of Asteraceae, endemic to the northwestern edge of the African Sahara desert. The species Warionia saharae Benth. & Coss., known by the vernacular name of “afessas”, may be found in several localities on dry shale in northwestern Africa, in Morocco and Algeria [5]. This species grows on slopes of the High Atlas, Anti-Atlas and Saharian Atlas, in the coast of western Morocco, and in desert areas on basic and siliceous rocks, from 0 to 1300 m [6]. The plant is known to possess varied medicinal properties. The crude extracts of the plants showed antibacterial and cytotoxic activities against a cancer cell line (KB cells) [7]. In addition, the local women anoint themselves with the perfume of the leaves and believe that the supernatural powers attributed to the plant make them more seductive [8].

Phytochemical studies reported by Amezouar et al. revealed that ethanolic extract of Moroccan Warionia saharae Benth. & Coss. contains phenolic compounds and flavonoids [9]. On the other hand, the chemical composition of the hexane extract of W. saharae was studied by Essaqui et al. which demonstrated that the Moroccan W. saharae extract is characterized by the higher percentage of oxygenated components particularly the hexadecanoic acid [10].

According to previous ethnobotanical survey realized in Tata province, Morocco, showed that W. saharae is used by the local population as a hypoglycaemic agent and as a remedy against rheumatoid arthritis, rheumatism, gastroenteritis, colds, jaundice and cardiac ailments [11], but no previous pharmacological or clinical study was carried out to test the hypoglycaemic activity of this plant. Since the hypoglycaemic...
effect of *W. saharae* has never been experimentally confirmed, thus, the present study was carried out to investigate the effect of the aqueous extract of *W. saharae* on blood glucose levels in normal and STZ-induced diabetic rats after single and repeated oral administration and amelioration of sensibility of glucose tolerance in normal rats. Finally, the histopathological changes in liver and pancreas of rats treated with the aqueous extract of *W. saharae* were investigated.

2. MATERIAL AND METHODS

2.1. Plant Material

Specimens of *Warionia saharae* (*Asteraceae*) were collected from the Tafilalet region (semi-arid area) of Morocco in March–April 2015 and air-dried at 40°C. A voucher specimen was deposited at the herbarium of the Faculty of Sciences and Techniques, Errachidia (Voucher specimen No. MEWS 15).

2.2. Preparation of the Aqueous Extract

Plant material was prepared according to the traditional method used in Morocco (decoction): 1 g of powdered aerial parts, mixed with 100 ml distilled water, was boiled for 10 min and then cooled for 15 min [12]. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. Finally, the filtration of the extract was lyophilized. The dose administered was 5 mg of lyophilized aqueous extract per kg of body weight [12].

2.3. Experimental Animals

The hypoglycaemic activity of the aqueous extract of *W. saharae* was studied in adult male Wistar rats weighing about 100-145 g. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h/12 h light/dark cycle) and maintained with free access to water and ad libitum standard laboratory diet.

2.4. Oral Glucose Tolerance Test (OGTT)

Fasted rats were divided into two groups of six rats each. Group I served as a control and received distilled water. Groups II received an aqueous extract of *W. saharae*, at the oral dose of 20 mg/kg as a fine aqueous suspension. After 30 min of extract administration, the rats of all groups were orally treated with 3 g/kg of glucose. Blood glucose levels were measured immediately by glucose oxidase method using a reflective glucometer (Contour™ TS) just prior to glucose administration and at 30 and 90 min after glucose loading [12].

2.5. Induction of Diabetes

After an overnight fast, streptozotocin (STZ; Sigma, St. Louis, MO) at a dose of 65 mg/kg dissolved in 0.1M fresh cold citrate buffer, pH 4.5, was injected intraperitoneally to rats [11]. Stable hyperglycaemia was confirmed after 18h of STZ injection. Only rats with post-absorptive blood glucose levels more than 17 mmol/L (> 300 mg/dl) were used in this study. In the percentage of rat, mortality due to streptozotocin injection was 20% [11].

Normal and diabetic rats were randomly assigned to three different groups containing six rats each. One control group received distilled water, a second treated group received the aqueous extract of *W. saharae* at a dose of 5 mg/kg (5 mg of lyophilized aqueous extract of *W. saharae* per kilogram of body weight) and the third group received a reference drug (Glibenclamide at a dose of 5 mg/kg). The dose of 5 mg/kg was used according to the Moroccan traditional use. For single oral administration, distilled water (control), glibenclamide or the aqueous extract were administered and blood glucose levels were monitored for 6 h. For repeated oral administration, rats were treated once daily for 15 days and blood glucose levels were followed during this period. The rats (n = 6 in each group) were treated orally [12].

All experiments were performed in fasted rats. All animal experimental procedures have been performed according to guidelines of the ethical committee (Faculty of Sciences and Techniques Errachidia, Morocco) [11].

2.6. Determination of Parameters

Blood samples from rats were collected from the retro-orbital sinus under ether anesthesia. Blood glucose levels were determined by a reflective glucometer (Contour™ TS) from Bayer Diabetes Care [11, 13].

2.7. The Relative Organs Weight

After 15 days of treatment with the aqueous extract of *W. saharae*, all the rats are sacrificed under sodium pentobarbital anesthesia before the measure of their body weight [11]. Then, their organs (liver, kidney, pancreas and Brown Adipose Tissue) were removed and weighed. The Relative Organ Weight (ROW) of each organ was then calculated according to the following equation:

\[
RWO = \frac{\text{Absolute organs weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100
\]

2.8. Histopathological Changes in the Liver and pancreas

At the end of the experiment, the organs (pancreas and liver) of the animals were isolated for histopathological observation on optical microscopy. The isolated organs (pancreas and liver), washed with a phosphate buffer solution, then fixed at 10% natural buffered formaldehyde (formalin), dehydrated by passing through a graded series of alcohol, cleared in toluene to remove the alcohol or other agents in which both alcohol and paraffin are miscible, and embedded in paraffin blocks and 5 μm sections were prepared using a semi-automated rotatory microtome (MICROM GmbH, HM 310, Ser. Nr. 6146, Germany) [11]. Hematoxylin and eosin were used for staining [14]. Finally, Histopathological changes were examined under phase contrast microscope (Olympus B x 41, Japan) and images were captured at a magnification of 40× using a computer-assisted image analyzer (Nikon H600L, Nikon DS camera control Unit DS-U2, Version 4.4) [11].

2.9. Statistical Analysis

Data were expressed as mean ± 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 [11].
3. RESULTS

3.1. Single Oral Administration

Fig. (1) shows the change in plasma glucose levels after a single oral administration of *W. saharae* aerial part aqueous extract in normal and STZ-induced diabetic rats. In normal rats, a single administration of *W. saharae* A.P.A.E (5 mg/kg) caused a significant reduction in blood glucose levels until the sixth hour after administration (p< 0.05). However, in diabetic rats, we observed a significant reduction in blood glucose levels until the fourth hour after the single administration (p< 0.01) and a very significant decrease (p< 0.001) after the sixth hour of administration. Significant reduction in blood glucose levels was observed in glibenclamide treated normal rats. Additionally, glibenclamide in diabetic rats caused a significant reduction in blood glucose levels after six hours of treatment (p< 0.05). The blood glucose levels of untreated diabetic and normal rats were unchanged during the six-hour of treatment.

3.2. Repeated Oral Administration

Fig. (2) represents the change in blood glucose levels in normal and STZ rats during 15 days of daily oral administration. In normal rats, a significant reduction in blood glucose levels was observed (p< 0.0001) after 15 days of *W. saharae* oral administration. In STZ rats, highly significant reduction in blood glucose levels (p< 0.0001) was observed since the second day of oral treatment (p< 0.0001) with *W. saharae* A.P.A.E (5 mg/kg) and the decrease in blood glucose levels was maintained during 15 days of treatment (p< 0.0001). In addition, the diabetic rats treated with glibenclamide caused a significant (p< 0.0001) reduction in blood glucose levels from the 2nd to the 15th day of treatment.

3.3. Effect of *W. saharae* Aerial Parts Aqueous Extract on Glucose Tolerance

Fig. (3) reports the effect of *W. saharae* A.P.A.E (5 mg/kg) on glucose tolerance. The aqueous *W. saharae* extracts as compared to the control group have prevented the increase in blood glucose levels significantly (p< 0.0001) 30 min after glucose administration and the maximum glucose tolerance was observed at the 90th min (p< 0.001) after administration of 3 g/kg of glucose.
3.4. Body Weight

Fig. (4) depicts the body weight variation in normal and STZ-induced diabetic rats after 15 days of administration of *W. saharae* A.P.A.E (5 mg/kg). However, in normal and STZ-induced diabetic rats, treatment with *W. saharae* A.P.A.E had no significant change in body weight, along the study period. The same result was also observed in glibenclamide-treated groups after 15 days of treatment.

![Graph showing body weight change](image)

Fig. (4). Body weight change after repeated oral administration of an aqueous *W. saharae* extract (5 mg/kg) for 15 days in normal (A) and diabetic rats (B). Data are expressed as mean ± S.E.M., n = 6.

3.5. The Relative Organs Weight

Table 1 shows the relative weights of some organs (g/100 g of body weight) (R.O.W) in normal untreated rats, normal treated rats with *W. saharae* (5 mg/kg), diabetic untreated rats, and diabetic treated groups with *W. saharae* (5 mg/kg) after 15 days of treatment.

![Table showing relative organs weight](image)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Liver</th>
<th>Kidney</th>
<th>Pancreas</th>
<th>Brown Adipose Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal untreated rats</td>
<td>3.74±0.62</td>
<td>0.69±0.03</td>
<td>0.51±0.04</td>
<td>0.17±0.06</td>
</tr>
<tr>
<td>Normal treated rats with <em>W. saharae</em></td>
<td>4.65±1.12</td>
<td>0.8±0.10</td>
<td>0.54±0.10</td>
<td>0.19±0.05</td>
</tr>
<tr>
<td>Diabetic untreated rats</td>
<td>4.87±0.08</td>
<td>0.92±0.01</td>
<td>0.39±0.23</td>
<td>0.25±0.05</td>
</tr>
<tr>
<td>Diabetic treated rats with <em>W. saharae</em></td>
<td>3.47±0.99*</td>
<td>0.98±0.16</td>
<td>0.65±0.12</td>
<td>0.06±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SEM; n=6. (*) p< 0.05; when compared to control (normal or diabetic untreated rats).

We observed no statistically significant differences in relative organs weight (liver, kidney, pancreas and brown adipose tissue) between the normal treated rats with *W. saharae* when compared to the relative organs weight of normal untreated rats. The same result was observed in the R.O.W of diabetic treated rats by *W. saharae* when compared with the R.O.W of diabetic untreated rats. Except for the R.O.W of the liver of diabetic treated rats with *W. saharae* which showed a significant decrease (p< 0.05) when compared with the R.O.W of diabetic untreated rats.

3.6. Effect of *W. saharae* Aqueous Extract on Histopathological Changes in the Liver

Fig. (5) illustrates histopathological changes in the liver of diabetic rats fifteen days after oral administration of *W. saharae* A.P.A.E (5 mg/kg) or glibenclamide (5 mg/kg) treatment.

![Histopathological images](image)

Fig. (5). Effect of orally administered *W. saharae* A.P.A.E on liver histology. Representative images of the liver in normal (A), STZ-induced diabetic rats (B), diabetic rats treated with 5 mg/kg/day *W. saharae* A.P.A.E (C) and diabetic rats treated with glibenclamide 5 mg/kg/day (D). Images were taken under 40× magnification. Three repetitions are represented by image. H = Hepatocytes, CV = Central Vein, S = Sinusoid.
In diabetic untreated rats (Fig. 5B), as compared to diabetic treated rats with *W. saharae* A.P.A.E (Fig. 5C) or glibenclamide (Fig. 5D), the hepatocytes were disorganized with noticeable hepatocellular injuries and disordered liver architecture was observed. Sinusoids were enlarged with the wall of veins thickened (Fig. 5B). However, these same changes are to be improved by *W. saharae* A.P.A.E or glibenclamide treatment at least by some beneficial effects like improved liver architecture, lack of central hemorrhagic necrosis and mild sinusoid hyperemia (Fig. 5C).

3.7. Effect of *W. saharae* A.P.A.E on Histopathological Changes of the Pancreas

Fig. (6) shows histopathological changes in the pancreas of rats treated with *W. saharae* A.P.A.E (5 mg/kg) after 15 days of treatment. The microphotography of pancreas in STZ-untreated rats (Fig. 6B) as compared to STZ treated rats with *W. saharae* aqueous extract (Fig. 6C) shows a remarkable reduction at the number of islets of Langerhans, and damaged or reduced the size of pancreatic cells. Thus, *W. saharae* aqueous extracts exposed regular distributions with apparently normal architectures of pancreatic islets. However, no pathological changes were observed both in the pancreas of normal untreated rats (Fig. 6A) and normal rats (Fig. 6D) treated with aqueous extract of the plant.

**Fig. (6).** Effect of *W. saharae* A.P.A.E on pancreas histology. Representative images of pancreas in normal (A), STZ-induced diabetic rats (B), diabetic rats treated with 5 mg/kg/day *W. saharae* A.P.A.E (C) and normal rats treated with 5 mg/kg/day *W. saharae* A.P.A.E (D). Images were taken under 40× magnification. Three repetitions are represented by image. i.L: islets of Langerhans.

4. DISCUSSION

According to previous ethnomedical survey, conducted in the Tata Province in the South-eastern region of Morocco, *W. saharae* is used by the local population as a hypoglycaemic agent, a remedy against rheumatoid arthritis, rheumatism, gastroenteritis, colds, jaundice and cardiac ailments [12], but no previous pharmacological or clinical study has been carried out to test the hypoglycaemic activity of this plant. Therefore, the present study was designed to confirm the hypoglycaemic effect of *W. saharae* A.P.A.E in normal and streptozotocin-induced diabetic rats.

During the present investigation, streptozotocin was used to induce diabetes in rats and their serum glucose levels were found to be significantly elevated as compared to normal rats. Streptozotocin is a remarkable substance with regard to its specificity for the beta cell and has been extensively used in the induction of insulin-dependent diabetes mellitus in experimental animals [15].

Our results showed that *W. saharae* A.P.A.E (5 mg/kg) decreased significantly the blood glucose levels in normal and streptozotocin-induced diabetic rats, as compared to the initial values of glucose levels. In normal rats, a single administration of the A.P.A.E (5 mg/kg), caused a significant reduction in blood glucose levels (p< 0.01) after 6 hours of treatment and lowered blood glucose levels after 15 days of treatment (p< 0.0001). The hypoglycaemic effect of A.P.A.E of *W. saharae* in normal rats was perhaps dependent on insulin released from beta cells in the pancreas. A similar mechanism was proposed to explain the hypoglycaemic effect of *Lythrum salicaria* which may increase circulating insulin level in normoglycaemic rats. Another mechanism which can lead to the same effect may be the enhancement of glycogen content in the liver [16]. This hypothesis is supported by the results of the present study showing that the relative organs weight of liver of normal treated rats (4.65±1.12) is higher than the R.O.W of the liver in normal untreated rats (3.74±0.62). Indeed, the change of liver weight induced by A.P.A.E of *W. saharae* should be explained by the increase of liver glucose content. In addition, the R.O.W of brown adipose tissue was higher in diabetic treated rats. This fact should be explained by a possible lipolytic action of the A.P.A.E of *W. saharae* in brown adipose tissue.

On the other hand, the oral glucose tolerance test in normal rats supports the potential effect of *W. saharae* to improve glucose tolerance revealed previously by the test of single and repeated oral administration of the extract. In fact, the dose of *W. saharae* A.P.A.E (20 mg/kg) was sufficient to decline significantly (p< 0.001) the glucose blood levels compared to control groups after 30 and 90 min of the glucose’s load. The results suggest that increased levels of glucose tolerance may possibly be due to increased secretion of insulin. Otherwise, the ability of *W. saharae* A.P.A.E to lower the blood glucose level in oral glucose tolerance test suggests that the rats treated with the extract have improved glucose utilization capacity [17].

However, in STZ-induced diabetic rats, orally administered A.P.A.E was able to lower blood glucose significantly (p< 0.0001) after sixth hours of treatment and better glucose homeostasis by the repeated oral administration at low dose used (5 mg/kg). Furthermore, repeated oral administration of
the same extract exhibited a very significant reduction in blood glucose levels (p< 0.0001) until the second day of treatment and was sufficient to normalize the blood glucose levels after 15 days of treatment in severely diabetic rats with fasting glycaemia greater than 16 mmol/L. These effects might have been due to the increased release of insulin from remnant β-cells and/or regenerated β-cells [18, 19], restored insulin sensitivity [20], interference on absorption of dietary carbohydrates as well as disaccharides in small intestine or facilitate utilization of glucose by peripheral tissues mediated by GLUT-4, an insulin-dependent glucose transporter [21, 22]. Moreover, it is well known that diabetes increase levels of oxidative stress in liver of diabetic rats and W. saharae may help to reduce this effect likely via scavenging the free radicals that are highly elevated in diabetes, which was consistent with other finding [23].

Concerning the histopathological studies of the sections of pancreas and liver, this study showed a disturbance in morphological features for STZ-induced diabetic rats. Islets of Langerhans containing β-cells were restored to nearly normal in STZ-induced diabetic treated rats after 15 days of therapy. Hepatic injuries were decreased in liver of STZ-induced diabetic rats treated with W. saharae A.P.A.E when compared to the control. In fact, streptozotocin is known to induce characteristic degenerative changes which include shrinkage of the beta cell, a decrease or complete loss of cytoplasmic granules and nuclear pyknosis [24]. With time, these changes become more marked; the nucleus undergoes karyolysis; vacuolization and disintegration of cytoplasm occurs and finally, the cell boundaries disappear, leading to the formation of a mass of cellular debris [25]. Many studies have shown that streptozotocin has effects on the liver, Salih et al. demonstrated that STZ-induced diabetes affects significantly the biochemical function of the liver and causes disturbances in the activity of liver enzymes [26].

From the phytochemical analysis, it was found that the major constituents of the extract were mono- and sesquiterpenes, flavonoids, triterpenoids, sterols, alkaloids, coumarins, essential oils and tannins [27]. The chemical composition of the Moroccan W. saharae extract was studied by Essaqui et al., and it has been characterized by higher percentage of oxygenated component like hexadecanoic acid (17.8%), ethenolxy-1-octadecane (9.5%), tridecene (7.3%), eicosene-9 (6.7%), octadecanoic acid (6.7%), (E)-2-decenol (6.7%), eicosene-3 (5.1%) and eicosane (4.5%) [10].

More than 150 plants extracts and some of their active principles including flavonoids are known to be used for the treatment of diabetes [28-33]. Additionally, phytochemical studies reported by Amezuor et al. revealed that Moroccan W. saharae ethanol extract contains phenolic compounds and flavonoids [9]. The hypoglycaemic effect of W. saharae may be partly due to phenolic compounds especially flavonoids present in the plant. And at least one or some of the other miscellaneous compounds of the plant may have also contributed to the hypoglycaemic effect of the aerial parts of aqueous extract of W. saharae.

CONCLUSION

In conclusion, the results obtained in this study have shown that the aerial parts aqueous extract of W. saharae (5 mg/kg) possess a potent blood glucose lowering effect in normal and streptozotocin-induced diabetic rats. This finding provides a quantitative basis to explain the traditional folkloric use of W. saharae as a hypoglycaemic agent in Moroccan population. More investigations are needed, such as isolation of the active(s) principle(s) of this plant and clarification of its mechanism of action in addition to toxicological studies.

ETHICS APPROVAL AND CONSENT TO PARTICIPEATE

The study is according to the ethical committee (Faculty of Sciences and Techniques Errachidia, Morocco).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. The studies involving animals were conducted as per FSTE/2015 guidelines.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

This work was supported by the CNRST under grant N° PPR/2015/35.

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