Evaluation of the Hypoglycemic Effect and Antioxidant Activity of Methanol Extract of Ampelodesma mauritanica Roots

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Abstract

In the present study, the hypoglycemic effect and antioxidant activity of the methanol extract of Ampelodesma mauritanica roots are studied. In the first part of this study, we evaluated the levels of total phenolic and the total flavonoid content in Ampelodesma mauritanica roots and we determine its antioxidant capacity. In the second part, the methanol root extract was tested on normal glucose-fed hyperglycemic mice to evaluate its efficacy as a hypoglycemic agent. The methanol extract administered intraperitoneally to mice significantly reduced blood glucose level. This acute effect presents a comparable glucose lowering effect with the oral hypoglycemic drug, glibenclamide, and this methanol root extract could be a valuable source of hypoglycemic compounds.

Keywords: Ampelodesma mauritanica; ABTS; DPPH; antioxidant; antihyperglycemic.

1. Introduction

Ampelodesma mauritanica T., Durand & Schinz (Vernacular name Diss), family Poaceae, is a plant native of northern Africa and southern Europe, is perennial and luxuriant, growing spontaneously in wild state around the Mediterranean basin. The phytochemical screening revealed the presence of flavonoids, saponins, cardenolides and tannins [1,2]. It is observed that in some regions of Algeria, Ampelodesma mauritanica has been used for reducing the blood glucose levels in diabetics.

A growing demand for natural active compounds has been observed in the past few years. There is a growing interest in herbal remedies, which are apparently effective, produce minimal or no side effects, and are of relatively low cost as compared to oral synthetic hypoglycemic agents [3-6]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research.

Diabetes and its complications is becoming the third leading cause of death, after cancer and cardiovascular diseases and according to an estimation of the World Health Organization (WHO), more than 220 million people worldwide have diabetes and the number of diabetes deaths will double between 2005 and 2030 [7]. Diabetes also known as the “silent killer”, is a metabolic disorder characterized by elevated blood glucose concentration and caused by an insufficiency of insulin secretion and/or insulin action [8-10], and has serious and fatal complications affecting the retina, kidney, skin, nervous system, erectile dysfunction and limb amputation [11-16]. Many serious side effects of insulin therapy and oral hypoglycemic agents necessitated the search for newer effective and safer class of compounds to overcome diabetic problems.

At the present time, much attention is being paid to antioxidant substances because they play a fundamental role in the human life. Antioxidants in biological systems have diverse functions as potential health protecting action and the intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and diseases associated with aging and the antidiabetic activity of several plants may be attributed to its antioxidant activity [17,18]. So, finding new natural sources of antioxidant compounds with free radical scavenging molecules could prevent or reduce diabetic complications and we think that antioxidant action may be an important property of plant medicines associated with diabetes.

Then, the objectives of this study are to determine the content of total phenols, total flavonoids and total antioxidant potential of a methanol extract of Ampelodesma mauritanica roots by using vitamin C equivalent antioxidant capacity (VCEAC) tests, and finally an evaluation of hypoglycemic effect is carried out.
2. Methods

**Plant material**: *Ampelodesma mauritanica* roots were collected from El Kala National Park in the north east of Algeria [19] during April 2008. The plant was identified by Professor M.C. Younos, Ethnobotany and Pharmacology Laboratory, Paul Verlaine-Metz University, France. The roots were air dried in shade at room temperature, ground on mill to obtain coarsely powder and stored in a glass flask to protect it from humidity and light.

**Chemicals**: Folin-Ciocalteu’s phenol reagent, aluminum chloride, catechin, gallic acid and vitamin C were purchased from Acros Organics. Potassium persulfate, sodium carbonate, sodium nitrite, hydrochloric acid, sodium hydroxide, sodium sulfate anhydrous, methanol, glibenclamide, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) diammonium salt (ABTS) were obtained from Sigma and Roth (France). The chemicals used were all of analytical grade.

**Extract preparation**: Roots were extracted in cold methanol (100 %) by percolation for 48 h. The green organic phase was filtered through Whatmann paper N°1, concentrated under reduced pressure and freeze dried.

2.1. Dosage of phenolic compounds and antioxidant activity

2.1.1. Determination of total phenols

Total phenolic contents were evaluated with Folin-Ciocalteu’s phenol reagent [20] using the spectrophotometric analyses (Cary 50 Scan UV-Visible apparatus). Briefly, an aliquot (1 ml) of standard solutions of gallic acid at different concentrations or appropriately diluted extracts was added to a 25 ml volumetric flask containing 9 ml of ddH2O. A reagent blank using ddH2O was prepared. One milliliter of Folin-Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na2CO3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with ddH2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100g dry weight (dw). Samples were analyzed in three replications.

2.1.2. Determination of total flavonoids

Total flavonoid content was measured according to a colorimetric assay [21]. A 1 ml aliquot of standard solutions of catechin at different concentrations or appropriately diluted samples was added to a 10 ml volumetric flask containing 4 ml ddH2O. At zero time, 0.3 ml 5% NaNO2 was added to the flask. After 5 min, 0.3 ml 10% AlCl3 was added. At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to volume (10 ml) with ddH2O and mixed thoroughly. Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. Total flavonoid content was expressed as mg catechin equivalents (CE)/100g dry weight (dw). Samples were analyzed in three replications.

2.1.3. Antioxidant activities

Two tests have been used to determine the total antioxidant capacity, the ABTS and the DPPH tests [20,22].

2.1.3.1. ABTS radical scavenging test

Total antioxidant activity was determined by scavenging blue–green ABTS radicals and was expressed as mg vitamin C equivalent (VCE) per g dry weight. ABTS radical cation (ABTS+) was generated according to the experiment using an improved method [23]. It is produced by reacting of ABTS solution (7 mM in water) with 2.5 mM potassium persulfate (final concentration) for 16 h at ambient temperature in the dark (stock solution). Then the ABTS+ stock solution was diluted with methanol to an absorbance of 0.7±0.2 at 734 nm. A quantity of 1 mg of the extract was dissolved in 5 ml of 70% aqueous methanol. A quantity of 50 µl of extract solution, was added to 2.0 ml of diluted ABTS+ solution (A = 0.7 ±0.2). The decrease of absorbance was measured after 5 min of incubation at room temperature in the dark and plotted as function of concentration of antioxidants. All determinations were carried out in triplicate on each occasion and at each separate concentration of the standard. Methanol and L-ascorbic acid were used as negative and positive control, respectively. All radical stock solutions were prepared fresh daily.

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2.1.3.2. DPPH radical scavenging activity

The antioxidant activity of plant extract was estimated using a slight modification of the DPPH radical scavenging protocol reported by Chen et al. [24]. Two and nine tenths mL of 100 mM DPPH solution in methanol was mixed with 0.1 mL of plant extract. The reaction mixture was incubated in the dark for 30 min and thereafter the optical density was monitored at 517 nm against the blank. Vitamin C equivalent antioxidant capacity (VCEAC) was calculated by using ascorbic acid as a reference compound to prepare the standard curve and was expressed as mg/100 g of dry matter of VCEAC. For the control, 2.9 mL of DPPH solution in methanol (100 mM) was mixed with 0.1 mL of methanol. The radical solution was prepared daily.

2.1.4. Hypoglycemic effect

**Animals:** Swiss albino mice (male, 8–10 weeks old) and weighing 30-40 g at the receipt from the breeder company (Charles River, France), were used. The animals were kept in quarantine during 1 week for acclimatization, housed with a 12 h light: 12 h dark schedule with free access to water and food (SDS Dietex-France) and maintained at a constant temperature (22 ± 2°C) and a relative humidity of 55 ± 10%. All animal procedures were carried out in accordance with the European Community Council Directive of 24th November 1986 (86/609/EEC).

**Preparation of the test samples and collection of blood samples:** All samples were suspended in solution of Tween 80 (0.05%). Glibenclamide (3 mg/kg; body weight) was used as a hypoglycemic model drug. Control mice received only the vehicle (0.05% Tween 80) in the same volume (0.5 ml of vehicle/100 g, body weight) by the same route. Blood samples were collected from the tip of tail at the defined time patterns. Blood glucose levels (mg/dl) were estimated by enzymatic glucose oxidase method using a commercial glucometer. Percentage variation of glycemia for each group was calculated with respect to initial (0 h) level according to:

\[
\text{% variation of glycemia} = \left( \frac{G_t - G_i}{G_i} \right) \times 100
\]

where \(G_i\) is initial glycemia value and \(G_t\) is the glycemia value at different times after sample administration.

**Toxicity evaluation in mice:** To study sub-acute (short term) toxicity, four groups of mice were used and were orally fed with the methanol extract of *Ampelodesma mauritanica* roots in increasing dose levels of 100, 250, 500, 1000 mg/kg body weight. The animals were observed continuously for 2 h for behavioral, neurological and autonomic profiles and for any lethality or death for the next 72 h.

**Assessment of hypoglycemic activity in normal healthy mice:** The animals fasted overnight and were divided into four groups of 6 animals each. Control mice (group I) were given vehicle (0.05% Tween 80) only, while groups II, III, IV, V and received the methanol extract of *Ampelodesma mauritanica* roots (suspended in 0.05% Tween 80) orally at doses 150, 200, 300, and 400 mg/kg, respectively. The sixth group received 3 mg/kg of glibenclamide, used as a reference standard drug. Blood glucose levels were estimated before and after 1, 2, 4 and 6 h of extract administration.

**Assessment of anti-hyperglycemic activity in normal mice:** Oral Glucose Tolerance Test (OGTT). After overnight fasting, the mice were divided into three groups each of twelve animals. Group I was kept as control which received vehicle (1 ml of 2% Tween 80); group II used as reference received reference drug and group III received extract (300 mg/kg). After 30 min later, glucose (10 g/kg) was administered orally to all mice. Blood samples were collected before the administration of the glucose and at 30, 60, and 120 min after administration [25–27].

2.2. Statistical analysis

All experimental data were expressed as mean ± standard deviation. Statistical analysis was done using one-way analysis of variance (ANOVA). For all statistical comparisons, the level of significance was set at \(P < 0.05\). All statistical analyses were carried out using the Statview™ 4.5 statistical package (Abacus Concepts, Inc).

3. Results and Discussion
3.1. Determination of total phenols, total flavonoids and total antioxidant capacity (VCEAC assay)

Figure 1 shows that the concentrations of total phenols and flavonoids in *A. mauritanica* roots were respectively 129.8±9.25 mg GAE and 74.3±3.87 mg CE per 100 g dry weight, respectively. It shows also the total antioxidant capacity expressed as vitamin C equivalent antioxidant capacity (VCEAC). The values of this capacity were 248.1±11.87 mg and 205.1±8.74 mg VCE per 100g dry weight obtained respectively by ABTS and DPPH tests.

![Figure 1: Total phenol and flavonoid content in root extract of *A. mauritanica* and quantification of its total antioxidant activity by VCEAC assay.](image)

**Toxicity evaluation in mice:** No deaths or hazardous signs were recorded during treatment or the observation period (24 and 72 h) in either control or treated groups of mice with extract (100, 250, 500 and 1000 mg/kg).

**Normal mice:** Table 1 gives the levels of blood glucose in the control and experimental groups of mice where a dose-response study was carried out. Indeed, different doses of the roots extract were orally administered and falls in plasma glucose levels were observed. Administration of the extract at a dose of 100 mg/kg brought about fall in fasting plasma glucose from 111.8±5.3 to 104.5±3.9 (6.5% fall) at 6 h. Maximum reduction in blood glucose level was seen at a dose of 250 mg/kg (25.3% fall at 6 h). However, further increase in the dose (300 and 500 mg/kg) did not improve the hypoglycemic response (23.7% and 23.2% fall at 6 h). Glibenclamide showed maximum reduction (29.9%) after 6 h.

**Table 1:** Effect of the *A. mauritanica* methanol root extract on blood glucose levels in normal mice (mean±SEM).

<table>
<thead>
<tr>
<th>Treatment (mg/kg/body wt)</th>
<th>Blood glucose (mg/dl)</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>112.1±3.2</td>
<td>111.8±2.6</td>
<td>109.9±4.9</td>
<td>108.1±6.1</td>
<td>108.1±3.1</td>
</tr>
<tr>
<td>Extract 100</td>
<td></td>
<td>111.8±5.3</td>
<td>112.9±2.2</td>
<td>109.3±6.1</td>
<td>103.9±3.5</td>
<td>104.5±3.9</td>
</tr>
<tr>
<td>Extract 250</td>
<td></td>
<td>111.5±5.6</td>
<td>108.3±6.3</td>
<td>105.8±3.8</td>
<td>91.3±2.8</td>
<td>83.8±3.7</td>
</tr>
<tr>
<td>Extract 350</td>
<td></td>
<td>112.9±4.2</td>
<td>111.1±5.4</td>
<td>106.1±6.8</td>
<td>99.2±4.1</td>
<td>86.1±6.3</td>
</tr>
<tr>
<td>Extract 500</td>
<td></td>
<td>111.4±6.1</td>
<td>109.8±5.2</td>
<td>105.2±4.9</td>
<td>101.7±3.9</td>
<td>85.5±5.2</td>
</tr>
<tr>
<td>Reference</td>
<td></td>
<td>110.1±6.2</td>
<td>108.1±5.2</td>
<td>103.9±3.7</td>
<td>84.2±2.5</td>
<td>77.2±3.3</td>
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</table>

Values are expressed as mean±SEM, n=12, P<0.05 when compared with control.

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Glucose induced hyperglycemic mice: The mean blood glucose levels of fasted hyperglycemic animals at 30, 60 and 120 min after oral administration of methanolic extract, Glibenclamide and vehicle are shown in Table 2. The methanolic extract of Ampelodesma mauritanica roots (250 mg/ kg, p.o.) and Glibenclamide (10 mg/kg, p.o.) significantly depressed the peak of blood glucose level at 30 min after glucose loading but reference drug (Glibenclamide) showed more potent activity than the extract and oral administration of vehicle (10 ml/kg) did not change significantly the level of blood glucose level.

Table 2: Effect of the A. mauritanica methanol root extract on oral glucose (10 g/kg) tolerance test in normal control mice (mean±SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fasting</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>109.6±4.2</td>
<td>310.2±6.4</td>
<td>196.6±4.2</td>
<td>259.1±4.1</td>
</tr>
<tr>
<td>Extract 250</td>
<td>110.5±3.4</td>
<td>199.9±6.8</td>
<td>181.6±3.2</td>
<td>127.1±3.5</td>
</tr>
<tr>
<td>Reference</td>
<td>112.1±5.1</td>
<td>126.1±4.5</td>
<td>137.4±5.2</td>
<td>116.4±3.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM, n=6, P<0.05 when compared with control.

4. Conclusion
The present study confirms the antihyperglycemic effect of A. mauritanica extract in normal glycemic mice by lowering of blood glucose and strong antioxidantive properties in vitro were shown. Chemical and pharmacological investigations should be carried out to evaluate the hypoglycemic activity in diabetic animals. In conclusion, the extract of the roots of this plant should be studied further and studies are in progress to isolate and identify active compounds in the extracts responsible for the hypoglycemic effect and antioxidant activity.

References


