South African Journal of Botany 108 (2017) 157–162

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antidiabetic and hypolipidemic activities of Algerian *Pistachia lentiscus* L. leaves extract in alloxan-induced diabetic rats

A. Cherbal [a,b,*], M. Kebieche [b], E.m. Yilmaz [c], Z. Aydoğmuş [c], L. Benzaouia [d], M. Benguessoum [d], M. Benkedidah [d], K. Madani [a]

[a] Laboratoire de Biomathématique, Biophysique, Biochimie, et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algérie
[b] Laboratoire de Biologie Moléculaire et Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université Mohammed Seddik Benyahia, 18000 Jijel, Algérie
[c] Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34116 Beyazıt, Istanbul, Turkey
[d] Département de Biologie Moléculaire et Cellulaire. Faculté des Sciences de la Nature et de la Vie, Université Mohammed Seddik Benyahia, 18000 Jijel, Algérie

ARTICLE INFO

Article history:
Received 31 July 2016
Received in revised form 3 October 2016
Accepted 13 October 2016
Available online xxxx

Edited by J Van Staden

Keywords:
Diabetes
Pistachia lentiscus L.
Antidiabetic
α-Amylase
Sucrase
Phenolic compounds

ABSTRACT

Aim: The current study was designed to investigate the antidiabetic and hypolipidemic properties of the 80% methanolic leaves extract of *Pistachia lentiscus* L., a medicinal plant commonly used in Algerian folk medicine for the treatment of diabetes.
Methods: We evaluated the effects of *P. lentiscus* L. leaves extract on blood glucose, cholesterol, triglycerides and insulin levels in alloxan-induced diabetic rats. The effects of the extract on α-amylase, sucrase and glucose uptake by yeast cells *in vitro* were also evaluated. For qualitative determination of biologically active compounds, RP-HPLC analysis of the extract was carried out.
Results: P. lentiscus L. extract exhibited a significant decrease in blood glucose as well as cholesterol and triglyceride levels and caused a significant increase in plasma insulin. In addition, it significantly increased glucose uptake in yeast cells and inhibited α-amylase and sucrase activities.
Conclusion: Based on its strong antidiabetic activity, *P. lentiscus* L. extract appears to be a potential herb for the treatment of diabetes and can be further explored for isolating the active component(s).

1. Introduction

Diabetes mellitus (DM) is an endocrine metabolic disorder characterized by hyperglycemia and glucosuria, with disturbances of carbohydrate, fat and protein metabolism resulting from deficiency of insulin secretion, insulin action or both (WHO, 1999). It is one of the three known major killers that cause deterioration in human health after cancer and cardio-cerebral vascular diseases, and its incidence has been gradually rising over the years (Li-xia et al., 2011). At present, many synthetic hypoglycemic drugs are available. However, these agents can cause serious side effects and they are not suitable for use during pregnancy (Larner, 1985).

Plants have formed the basis of traditional medicine that has been in existence for thousands of years. In fact, more than 800 plants are reported to be used as traditional remedies for the treatment of diabetes (Alarcon-Aguilara et al., 1998). Plant drugs are frequently considered to be less toxic and have fewer side effects and relatively low costs than synthetic ones (Gupta et al., 2005).

Pistachia lentiscus L. (mastic), an evergreen shrub or tree from the family Anacardiaceae, is largely distributed throughout the Mediterranean regions (Zohary, 1952). It has a long tradition in folk medicine dating from the times of the ancient Greeks (Palevitch and Yaniv, 2000). It has been generally used internationally as traditional medicine for its several therapeutic properties such as its antifungal, antibacterial, antioxidant and antiproliferative effects (Kordali et al., 2003; Balan et al., 2007; Cherbal et al., 2012). In Algeria, the leaves of *P. lentiscus* L. were used to purify water and increase the time of conservation of dry figs, sun-dried tomatoes, fish and meat products (Djenane et al., 2011) as well as for treating various diseases such as asthma, ulcer, diarrhea, inflammation, eczema, throat infections and diabetes (Ali-Shtayeh et al., 1998; Bakkali et al., 2008).

Based on the above background and since *P. lentiscus* L. possesses no earlier reports related to its antidiabetic property, the objective of this research was to investigate the effect of a hydro-methanolic extract of leaves of *P. lentiscus* L. on hyperglycemia in alloxan-induced diabetic rats, and to contribute to the elucidation of some possible mechanisms of action by evaluating its effect on blood insulin level and on some carbohydrate hydrolyzing enzymes (α-amylase and sucrase) as well as on glucose uptake by yeast cells. Additionally, as hyperlipidemia is one of the disorders caused by diabetes, the blood levels of total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL)

* Corresponding author at: Laboratoire de Biologie Cellulaire et Moléculaire. Faculté des Sciences de la Nature et de la Vie, Université de Jijel, 18000 Jijel, Algérie.
E-mail address: phytopharmaco@yahoo.fr (A. Cherbal).

http://dx.doi.org/10.1016/j.sajb.2016.10.024
0254-6299/

and triglycerides (TG) were also determined in normal and diabetic rats. In order to determine the active compounds in *P. lentiscus* L. leaves, a RP-HPLC analysis of the extract was carried out.

2. Materials and methods

2.1. Plant material and preparation of the extract

Leaves of *P. lentiscus* L. were collected from the Elmzayer district (Jijel, Algeria) in the month of April (2013) and the material was identified and authenticated by the Department of Environment and Agronomy Sciences of the University of Jijel. Fresh intact leaves of *P. lentiscus* L. were shade-dried and ground in an electric grinder into a fine powder. About 100 g of this powder was submitted to extraction with 1000 ml of methanol (80%) for 72 h. After extraction, the solvent was filtered, centrifuged, de-fatted by hexane and then evaporated by Rotavapor (Heidolph, LABOROT 4003) at 40 °C to obtain solid residue (Yu et al., 2005; Nack and Shahidi, 2006).

2.2. Experimental animals

Male Wistar rats weighing about 150 to 200 g, obtained from Pasteur Institute of Algiers, were used. They were housed in cages at an ambient temperature of 25 °C–27 °C, with a 12 h light and dark cycle and free access to standard rat chow and tap water. Before starting the experiment, the animals were acclimatized to the laboratory environment for a period of one week. All the experimental procedures were conducted in accordance with the ethical guidelines for the care and use of laboratory animals.

2.3. Induction of diabetes mellitus

Experimental diabetes was induced by a single intra-peritoneal injection of alloxan prepared freshly at a dose of 150 mg/kg bw. After four hours of alloxan injection, tap water was replaced with a 5% glucose solution for 24 h, in order to overcome fatal hypoglycemia caused by alloxan as a consequence of β cell's destruction and high release of insulin (Stanely et al., 2004). One week after alloxan injection, the blood glucose level of the overnight fasted animals was tested with the help of a glucometer for evidence of a diabetic state. The animals that exhibited fasting glucose levels higher than 180 mg/dl were considered as diabetic rats and included in the study (Manickam and Periyasamy, 2013).

2.4. In vivo antidiabetic assays

2.4.1. Treatment protocol

Twenty rats were randomly divided into four groups (five rats in each group):

Group 1: normal untreated rats given distilled water (vehicle)
Group 2: diabetic untreated rats given distilled water
Group 3: diabetic rats given *P. lentiscus* L. extract (PLE) at a dose of 300 mg/kg bw
Group 4: diabetic rats given glibenclamid at a dose of 2.5 mg/kg bw

Distilled water and the drug preparations were fed orally by gastric intubation to rats of respective groups using a force-feeding needle, once daily for two weeks.

2.4.2. Collection of blood samples

All four groups of rats were sacrificed on the last day of the treatment after overnight fasting, blood samples were withdrawn through the retro-orbital plexus using capillary tubes and plasma was separated immediately by centrifugation (3500 rpm for 10 min) for evaluation of biochemical parameters relevant to diabetes (glycemia, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and insulin).

2.4.3. Determination of biochemical parameters

Biochemical parameters namely fasting blood glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) levels in blood plasma were measured using special kits (Abbott Laboratories, USA) which utilized the colorimetric method in an autoanalyzer (Architect c system, Abbott, USA).

Plasma insulin levels were estimated by enzyme-linked immunosorbent (ELISA) method, using the commercially available kit obtained from Abbott Laboratories, USA.

2.5. In vitro antidiabetic assays

2.5.1. α-Amylase activity

The effect of various concentrations of *P. lentiscus* L. extract (3.125, 6.25, 12.5, 25, 50 mg/ml) on α-amylase activity was studied according to the method of Ou et al. (2001) with minor modifications. Each of the samples were mixed by stirring with 25 μl of potato starch (4%) in a beaker, 100 μl of porcine pancreatic α-amylase were added to the starch solution, stirred vigorously and incubated at 37 °C for 60 min. After the incubation period, NaOH (0.1 M) was added to inhibit α-amylase activity. The mixture was centrifuged for 15 min (3000*g*) and glucose content in the supernatant was determined by glucose oxidase peroxidase method. An untreated enzyme solution (containing all reagents except the test sample) was used as the control. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. Acarbose was used as positive control. All experiments were carried out in triplicate.

The percentage of inhibitory activity of α-amylase was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control reaction, and $\text{Abs}_{\text{sample}}$ is the absorbance of the test sample.

2.5.2. Sucrase activity

Brush border membranes prepared from rat intestine according to the method of Dahlqvist (1962) were used as the source of rat intestinal sucrase. Overnight fasted rats were sacrificed and their small intestines were immediately excised and washed with ice-cold phosphate buffer (pH 6, 0.1 M). The brush border was carefully removed and homogenized with phosphate buffer (1:5 w/v) in cold condition. The homogenate was then centrifuged for 15 min (10,000*g*, 4 °C) and the supernatant was used as a source of sucrase.

The effect of various concentrations of *P. lentiscus* L. extract (3.125, 6.25, 12.5, 25, 50 mg/ml) on sucrase activity was assayed according to the procedure of Honda and Hara (1993) with slight modifications. The enzyme solution (10 μl) and the sample (10 μl of the extract solution) were incubated together with 180 μl phosphate buffer (pH 6) for 10 min at 37 °C. The enzyme reaction was started by adding 100 μl sucrose solution (60 mM). After 30 min, the reaction was stopped by adding 200 μl of 3,5-dinitrosalysilic acid and treating the mixture in a boiling water bath for 5 min. The absorbance of the solution was read at 540 nm. An untreated enzyme solution (containing all reagents except the test sample) was used as the control. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with buffer. Acarbose was used as positive control. All experiments were carried out in triplicate.