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

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ARTICLE INFO

Article history:
Received 31 July 2016
Received in revised form 3 October 2016
Accepted 13 October 2016
Available online 26 October 2016

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)

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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.

The percentage of inhibitory activity of sucrase was calculated using the following formula:

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

where $Abs_{control}$ is the absorbance of the control reaction, and Abs_{sample} is the absorbance of the test sample.

2.5.3. Glucose uptake by yeast cells

Yeast cells were prepared according to the method of Cirillo (1962) with slight modifications. Commercial baker's yeast (1 g) was suspended in distilled water (5 ml) and centrifuged repeatedly (3000*g*, 5 min) until the supernatant became clear. A 10% (v/v) suspension was then prepared in distilled water.

Various concentrations of *P. lentiscus* L. extract (3.125, 6.25, 12.5, 25, 50 mg/ml) were added to 1 ml of glucose solution (5 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 μl of yeast suspension, agitated then incubated at 37 °C for 60 min. After incubation, the tubes were centrifuged (2500*g*, 5 min) and glucose was estimated in the supernatant. A control solution containing all reagents except the test sample was prepared.

The percentage of increase in glucose uptake was calculated using the following formula:

$$\% \text{ increase in glucose uptake} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of the control reaction, and Abs_{sample} is the absorbance of the test sample.

2.6. Qualitative analysis by HPLC

2.6.1. Sample preparation

A stock solution of the dry extract was prepared at 20 mg/ml in 80% methanol. This solution was first eluted in Phenomenex Strata CN solid phase extraction cartridges (55 μm, 70A°) then filtered through a 0.22-μm pore size acetate membrane to remove the undesirable contaminants. A sample solution was freshly prepared by diluting the stock solution to 10 mg/ml. The sample was placed in 2-ml amber glass HPLC vials and analyzed by RP-HPLC coupled with DAD.

2.6.2. Standard preparation

Reference compounds for authenticated standards (rutin, quercetin, vanillic acid, kaempferol, epicatechin, caffeic acid, ribovlavin, curcumin, coumaric acid, tannic acid, luteolin, apigenin, gallic acid and chlorogenic acid) were obtained from Sigma-Aldrich, USA. A stock solution of each standard was prepared at 0.5 mg/ml of methanol. The injected standard solution was diluted to 10 μg/ml in 80% acetonitrile.

2.6.3. HPLC analysis

Analysis was carried out on a Thermo Separation system (San Jose, CA) with P4000 pump, SN 4000 system controller, UV 6000 LP detector, 20 μl sample loop and ChromQuest 5.0 software. The separation was achieved on a reversed phase column (Venusil, XBPC18, 5 μm, 100A°, 4.6 × 250 mm; Agela Technologies, USA) with a photodiode array detector (DAD) set at 200 nm to 500 nm. The injection volume was 10 μl for each technical repeat. The analysis was performed with a flow rate of 0.75 ml/min using acetonitrile as solvent A and 20 mM phosphoric acid as solvent B (Table 1). The peaks were simultaneously identified using UV absorbance at 275, 330 and 370 nm. The chromatographic peaks of the analytes were confirmed by comparing their retention time and UV spectra with those of the pure standards.

Table 1
Gradient elution method performed with binary solvent system used as mobile phase.

Time (min)	Acetonitrile (%)	20 mM Phosphoric acid (%)
0	1	99
30	5	95
50	8	92
80	13	87
110	15	85
145	15	85
150	1	99

2.7. Statistical analysis

Data are expressed as mean ± standard deviation of three parallel measurements. The results were subjected to one-way analysis of variance and the significance of differences between sample means was calculated. $P \leq 0.05$ was considered significant.

IC_{50} values were determined by plotting a percent inhibition versus concentration curve for *in vitro* assays, in which the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

3. Results

3.1. Effect of P. lentiscus L. extract on glycemia

Diabetic rats showed a significant increase in glycemia in comparison with normal rats. Oral administration of *P. lentiscus* L. extract induced a significant reduction of glycemia starting at the 7th day of the treatment. In fact, this reduction reached 20% compared to before treatment (day 0) and 36% compared to the control values of the diabetic group on the 14th day. On the other hand, glibenclamid treatment of diabetic rats caused a significant reduction of glycemia on the 14th day of treatment, which reached 34% compared to before treatment and 42% compared to the control values of diabetic group in the corresponding day (Fig. 1).

3.2. Effect of P. lentiscus L. extract on plasma insulin level

Fig. 2 presents the effect of two weeks of treatment with *P. lentiscus* L. hydro-methanolic extract on plasma insulin level in alloxan-induced diabetic rats. Diabetic control rats showed a highly significant decrease (66%) in plasma insulin level with reference to normal control rats. On the other side, diabetic rats treated with *P. lentiscus* L. extract or glibenclamid exhibited a highly significant increase of plasma insulin level which reached 94% and 96%, respectively, compared to diabetic

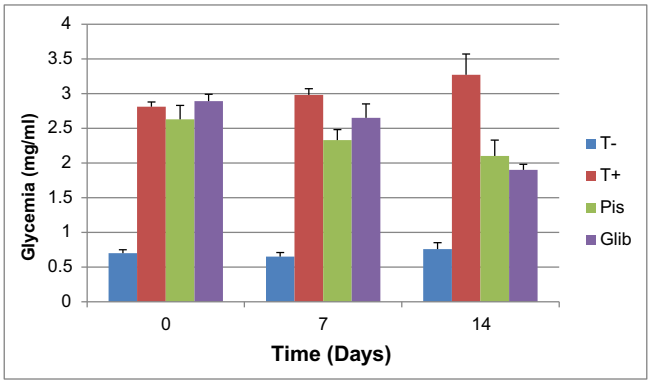

Fig. 1. Effect of two weeks of treatment with hydro-methanolic leaves extract of *Pistachia lentiscus* L. (300 mg/kg) on glycemia in diabetic rats.

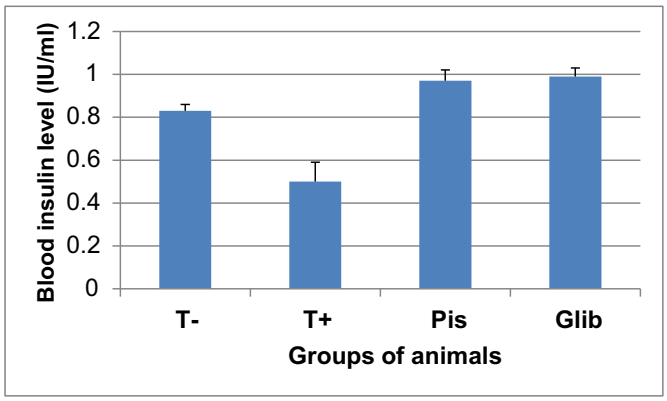

Fig. 2. Effect of two weeks of treatment with hydro-methanolic leaves extract of *Pistachia lentiscus* L. (300 mg/kg) on plasma insulin level in diabetic rats.

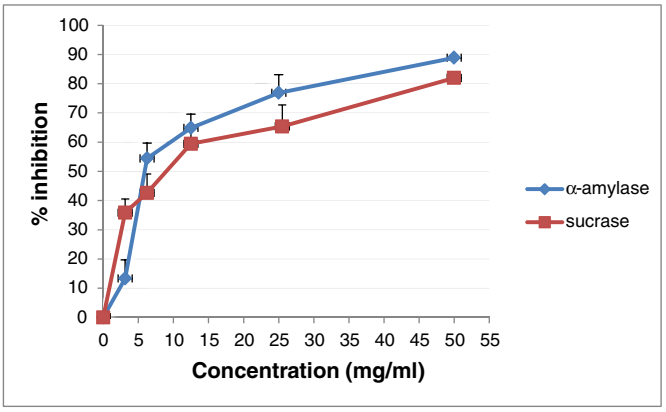

Fig. 4. Effect of hydro-methanolic leaves extract of *Pistachia lentiscus* L. (3125–50 mg/ml) on α-amylase and sucrase activities.

control values, and 17% and 19%, respectively, compared to normal control values.

3.3. Effect of P. lentiscus L. extract on serum lipid profile

Serum total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (HDL) and triglyceride (TG) levels of the experimental groups of animals are shown in Fig. 3. There was a highly significant increase in the levels of total cholesterol, triglycerides and LDL in the untreated diabetic rats compared to normal rats. The treatment with *P. lentiscus* L. extract or glibenclamid significantly decreased and brought back the levels of serum lipids to normal values. No significant changes in HDL serum levels could be detected.

3.4. Effect of P. lentiscus L. extract on α-amylase and sucrase activities

The effect of various concentrations of *P. lentiscus* L. hydro-methanolic leaves extract on α-amylase and sucrase activities is presented in Fig. 4. *P. lentiscus* L. extract potentially inhibited α-amylase and sucrase activities in a dose-dependent manner. This high inhibitory activity was expressed in terms of IC_{50} value. The IC_{50} of α-amylase was 5.81 mg/ml and that of sucrase was 9.32 mg/ml. A lower IC_{50} value indicates higher inhibition, which means that *P. lentiscus* L. extract have higher inhibitory activity on α-amylase than on sucrase.

3.5. Effect of P. lentiscus L. extract on glucose uptake by yeast cells

The effect of hydro-methanolic leaves extract of *P. lentiscus* L. on glucose transport across yeast cell membrane was studied in an *in vitro* system comprising yeast cells suspended in a glucose solution at 5 mM in the presence/absence of the extract. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The presence of *P. lentiscus* L. extract in the medium resulted in a dose-dependent increase in glucose uptake in yeast cells (EC_{50} = 3.6 mg/kg). This increase was directly proportional to the extract concentration and reached 92.85% at 50 mg/ml (Fig. 5).

3.6. HPLC results

Reversed phase HPLC was used to separate individual chemical constituents of methanolic extract of *P. lentiscus* L. leaves. The chromatographic profile was compared with the retention times of reference standards used (Fig. 6). In reversed phase conditions, more polar compounds are eluted from the column faster than less or nonpolar compounds. Therefore, peaks at the beginning of chromatogram indicate more polar compounds whereas peaks at the end indicate less polar ones. The chromatographic profile revealed that the extract had high content of both polar and less polar molecules and showed the peaks corresponding to gallic acid (4.65% from total peak area, retention time 15.797 min), caffeic acid (10.21% from total peak area, 50.110 min), vanillic acid (3.59% from total peak area, 58.247 min), and traces of

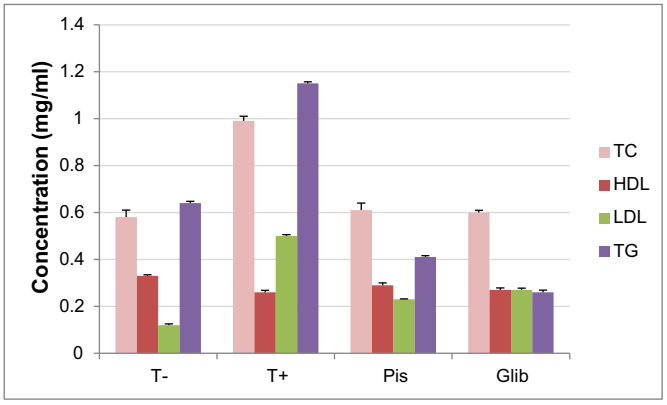

Fig. 3. Effect of two weeks of treatment with hydro-methanolic leaves extract of *Pistachia lentiscus* L. (300 mg/kg) on total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TG) in diabetic rats.

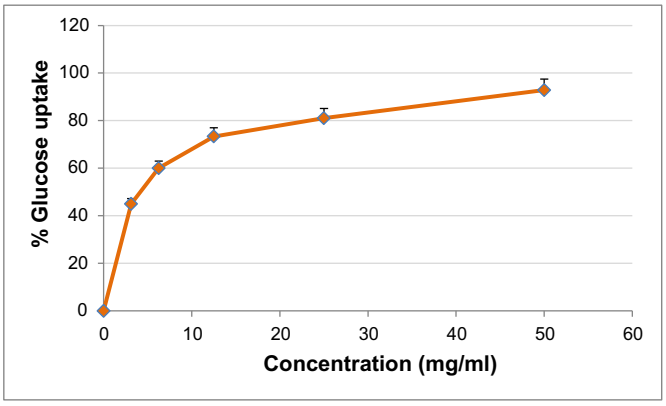

Fig. 5. Effect of hydro-methanolic leaves extract of *Pistachia lentiscus* L. (3125–50 mg/ml) on glucose uptake by yeast cells.

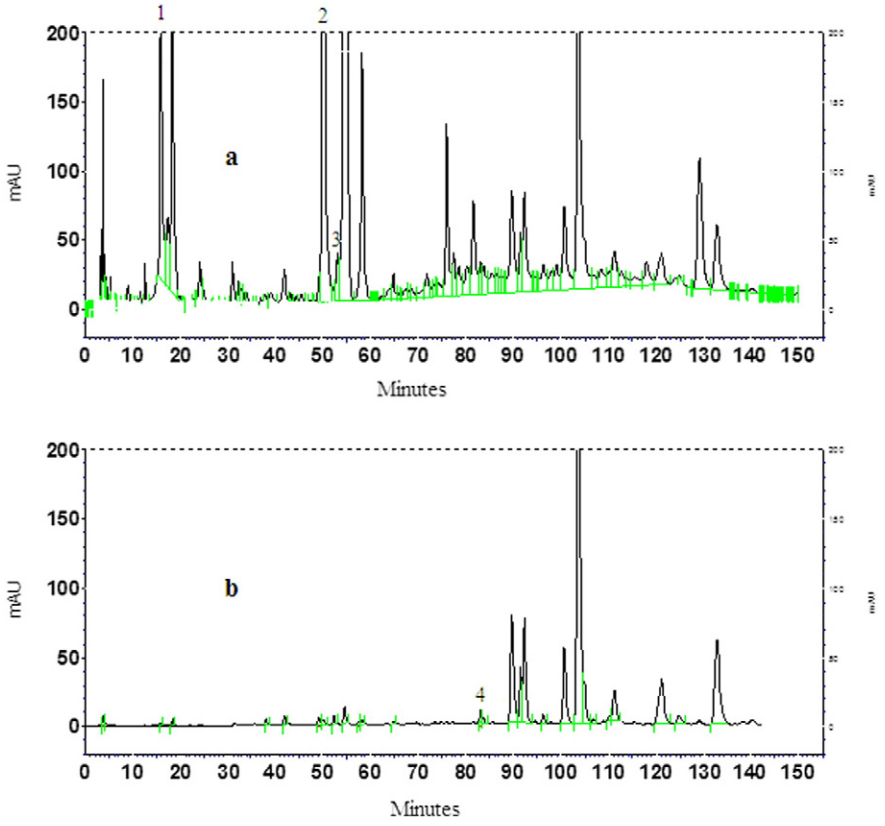

Fig. 6. HPLC chromatogram of *Pistachia lentiscus L.* extract. (a) Detected at 275 nm: (1) gallic acid, (2) caffeic acid, (3) vanillic acid. (b) Detected at 330 nm: (4) coumaric acid.

tannic acid, riboflavin and coumaric acid (0.04%, 62.438 min; 0.15%, 73.532 min and 0.62%, 83.137 min, respectively).

4. Discussion

Diabetes mellitus is probably the fastest-growing metabolic disease in the world, which increases the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes. In Algeria, *P. lentiscus* L. leaves are widely used in folk medicine by diabetic patients to attenuate hyperglycemia caused by diabetes mellitus. Therefore, we have investigated the effect of hydro-methanolic extracts of *P. lentiscus* L. leaves on glycemic control and serum lipid profile in alloxan-induced diabetic rats, and on the activity of two carbohydrate hydrolyzing enzymes, namely α-amylase and sucrase, as well as on glucose transport across yeast cell membrane.

In the present study, the administration of alloxan, as expected, resulted in a significant hyperglycemia, hypoinsulinemia, increased cholesterol, LDL and triglycerides. Alloxan, a cytotoxic glucose analog, causes a state of insulin-dependent diabetes through its ability to induce reactive oxygen species (ROS) formation, leading to a selective necrosis of the pancreatic beta cells (Lenzen, 2008).

After two weeks of treatment of diabetic rats with 300 mg/kg of *P. lentiscus* L. extract, the levels of blood glucose were significantly reduced to 36% compared with diabetic control rats. Untreated diabetic rats on the other hand suffered from persistent alteration in blood glucose. Complementing our findings, previous studies reported that *P. lentiscus* L. extract possesses a potent antioxidant activity due to its high content of phenolic compounds (Kivçak and Akay, 2005; Cherbal et al., 2012). Thus, the obtained results lead us to suggest that the hypoglycemic effect of *P. lentiscus* L. extract may in part be mediated by its high antioxidant potential.

In addition, the present data indicate that *P. lentiscus* L. extract significantly increased plasma insulin levels in treated diabetic rats compared with diabetic control rats. This effect could be due to natural substances present in the plant extract, which stimulates insulin secretion or protects the intact functional β-cells from further deterioration, so that they remain active and continue producing insulin.

Our study reveals that caffeic acid is one of the active components found in *P. lentiscus* L. extract using HPLC. It has been reported that caffeic acid preserves beta cell function and exerts anti-degenerative effect on pancreatic islets in mice. It has also been found that caffeic acid enhances hepatic glucose utilization by increasing glucokinase activity, which catalyzes phosphorylation of glucose to glucose-6-phosphate, decreasing the hepatic release of glucose. A significant increase in glucokinase mRNA expression and a decrease in glucose-6-phosphatase gene expression have been noticed in animal treated with caffeic acid (Iynedjian et al., 1995; Jung et al., 2006). Caffeic acid has been found to enhance the GLUT4 protein expression in adipose tissue. This adipose membrane glucose transporter mediates insulin-stimulated glucose uptake in adipocytes, thus preventing insulin resistance which is the earliest defect in type 2 diabetes (Jung et al., 2006; Huang and Shen, 2012). Caffeic acid has also been reported to inhibit alpha-amylase and alpha-glucosidase activities in the gastrointestinal tract. Inhibitors of pancreatic alpha-amylase delay carbohydrate digestion, lowering the postprandial glycaemia (Oboh et al., 2014).

Our results indicate the presence of gallic acid in the extract of *P. lentiscus* L. Many studies have demonstrated that phenolic compounds, included gallic acid, play a role in the prevention of several chronic diseases such as diabetes (Willcox et al., 2004). According to the study of Al-Salih (2010), the treatment of alloxan-induced diabetic rabbits with gallic acid led to decreased levels of blood glucose. Generally, the antidiabetic action of gallic acid may be due to the enhancement of insulin receptor sensitivity (Huang et al., 2005).

Vanillic acid is also present in the extract of *P. lentiscus* L. extract, according to the results obtained in our survey. Chang et al. (2015) highlighted the protective effect of vanillic acid against hyperglycemia and hyperlipidemia via alleviating hepatic insulin resistance and

inflammation in high-fat diet-fed rats. However, the mechanism underlying such effects was still unidentified.

Diabetes is associated with profound alterations in serum cholesterol, triglycerides and lipoprotein profile which increase the risk of coronary heart disease (Meghrani et al., 2004) Continuous administration of *P. lentiscus* L. extract for two weeks resulted in a significant improvement of serum lipid profile in diabetic rats. It is well known that glycemia is the major determinant of plasma VLDL and triglycerides (Lemhadri et al., 2006), so the strong hypolipidemic effect of *P. lentiscus* L. could also be mediated by the improvement of glycemia.

The results reveal that *P. lentiscus* L. was effective inhibitor of α-amylase and sucrase. Alpha amylase and sucrase are the enzymes involved in the metabolism of carbohydrates. Alpha amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, and sucrase converts sucrose into glucose and fructose. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia. Inhibition of alpha amylase and sucrase results in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemia (David and Bell, 2004).

The inhibition of carbohydrate hydrolyzing enzymes by medicinal plants might be attributed to several possible factors such as encapsulation of starch by the fibers present in the sample thus reducing accessibility of starch to the enzyme or direct adsorption of the enzyme on fibers that may hold inhibitors leading to decreased enzyme activity (Ou et al., 2001).

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as an *in vitro* method for hypoglycemic effect of various compounds and medicinal plants (Ahmed et al., 2009). In the present study, it was observed that *P. lentiscus* L. increased glucose uptake by yeast cells in a dose-dependent manner until saturation of the yeast cells.

5. Conclusion

The present study shows an interesting antidiabetic and antihyperlipidemic properties of *P. lentiscus* L. leaves extract in alloxan-induced diabetic rats. It is concluded that the hypoglycemic action of *P. lentiscus* L. may be mediated by more than one biological mechanism including improvement of insulin secretion and alterations in lipid profile, as well as inhibition of carbohydrate hydrolyzing enzymes and enhancement of glucose transport across the cell membrane. Therefore, the traditional use of this plant as an antidiabetic agent is justified and experimentally verified. However, more studies are required to identify all the active compounds present in *P. lentiscus* L. and their precise mechanisms of action as well as their possible toxicological effects.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to University Mohammed Seddik Benyahia-Jijel and University Aberrahman Mira-Bejaia for the financial support of this work.

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