



## Original Article

## Study of hypocholesterolemic activity of Algerian *Pistacia lentiscus* leaves extracts *in vivo*



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## ABSTRACT

Plants are a large source of new bioactive molecules with therapeutic potentials. However, only a small amount of worldwide plants have been phytochemically investigated. The aqueous and ethanolic extracts of *Pistacia lentiscus* L., Anacardiaceae, leaves were evaluated for hypocholesterolemic activity *in vivo*. In this study, hypercholesterolemia was induced in animals by feeding them high cholesterol (1%) food. The extracts of *P. lentiscus* were orally administered at a dose of 200 mg/kg body weight along with a high cholesterol diet for thirty successive days. Lipid parameters such as total cholesterol, triacylglyceride, low density lipoprotein, very low density lipoprotein and high density lipoprotein were measured in the plasma. Total phenol and flavonoid contents were also evaluated. Flavonoid content was found to be more present in the ethanolic extract ( $8.218 \pm 0.009$  mg of QE/g) compared to the aqueous extract ( $3.107 \pm 0.014$  mg of QE/g). The administration of *P. lentiscus* extracts produced a significant decrease in total cholesterol, triacylglyceride and low density lipoprotein-cholesterol ( $154.6 \pm 18.10$ ,  $71.2 \pm 4.38$  and  $99.36 \pm 18.77$  mg/dl respectively) in the ethanolic extract, while the aqueous extract showed a significant decrease in total cholesterol and triacylglyceride ( $203.6 \pm 9.18$  and  $97.6 \pm 3.57$  mg/dl respectively). The results of the investigation demonstrated that *P. lentiscus* leaf extract has hypocholesterolemic properties and might be used for the prevention of hypercholesterolemia associated disorders.

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## Introduction

Increases in cholesterol levels (hypercholesterolemia) have become a significant health concern in recent years. Hypercholesterolemia is known to be a risk factor for the development of cardiovascular diseases including atherosclerosis, myocardial infarction and cerebral paralysis (Avci et al., 2006). This dysfunction also enhances free radical generation in various ways (Prasad and Kalra, 1993), as well as the formation of oxygen free radicals, such as superoxide anion radical or peroxy nitrite, which play a significant role in the pathogenesis of many other diseases including, cancer and inflammatory disorders (Das et al., 2000).

Curing hypercholesterolemia without any side effects remains a challenge for modern medicine. Plant-derived products are frequently considered to be less toxic, with few or no side effects, than their synthetic equivalents. Plants play a major role in the introduction of new therapeutic agents, and have received much attention as sources of biologically active substances. Mastic (*Pistacia lentiscus*) is one such example.

*P. lentiscus* is very common in the Mediterranean region. In Algeria, mastic is found in both the Tell region and in forested areas. The aerial portion has traditionally been used as both a stimulant and a diuretic in the treatment of hypertension, coughs, sore throats and stomach aches. *P. lentiscus* leaves contain bioactive molecules such as phenolic compounds (proanthocyanidin tannins and gallic), flavonoid glycosides and anthocyanins.

The aim of this study is hence to study the hypocholesterolemic activity *in vivo* of aqueous and ethanolic extracts of Algerian *P. lentiscus* leaves.

## Materials and methods

## Plant material

*Pistacia lentiscus* L., Anacardiaceae, leaves were collected locally from the Chlef region of Algeria in 2012. Voucher specimens were collected under the registration number FSDB 12.189, at the herbarium of Department of Biology, Faculty of Sciences, University of Hassiba Ben Bouali-Chlef. They were identified by Ms. Medjahed K, a botanist at the Institute of Agronomy, University of Hassiba Ben Bouali-Chlef. After drying in a shadow at room temperature, the plant materials were fragmented.

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### Preparation of aqueous extract

The aqueous extract was prepared by decoction. Fragmented leaves (100 g) were immersed in one liter of water at 100 °C for 15 min. The aqueous extract was filtered through Whatman paper N° 1. The filtrate was concentrated in a rotary vacuum evaporator at 55 °C. The extraction yield was 11.88%.

### Preparation of ethanolic extract

The ethanolic extract was prepared by homogenizing 100 g of fragmented leaves in 900 ml of ethanol solution (50%) for three days, with frequent agitation. The ethanolic extract was filtered through Whatman paper N° 1. The solvent was removed from the sample using a rotary vacuum evaporator at 48 °C (Peixoto et al., 2011). The extraction yield was 15.88%. All of the above dry extracts were stored at 4 °C in sterile glass jars and, sealed for use in further studies.

### Phytochemicals screening of the leaves extracts

#### Determination of total flavonoid content

The total flavonoid content in the extracts was determined spectrophotometrically using an aluminum chloride method involving the formation of a flavonoid–aluminum complex at 420 nm (Zhou et al., 2005). The concentration of the total flavonoid content was calculated by comparison with the absorbance of different concentrations of quercetin (QE), and the result was expressed as milligrams of QE equivalents per gram of plant powder. Samples were prepared in triplicate for each analysis, and the mean value of absorbance was obtained.

#### Assay of hypocholesterolemic activity in vivo

##### Animals

Twenty-five male Swiss albino mice weighing between 25 and 30 g were used in the present study. They were housed in polypropylene cages with controlled levels of temperature (24 ± 2 °C) and light (a 12 h light, 12 h dark photoperiod), and fed with standard laboratory pellets. Food and water were given ad libitum.

The protocol employed was approved by the laboratory of toxicology and pharmacology (Antibiotic group – Medea, Algeria), following the recommendations of the European Pharmacopoeia 8.0 under reference number 215/2013.

#### Experimental design

Hypercholesterolemia was induced in the mice via administering of food containing high cholesterol (1%) for thirty days. All mice had free access to food and water for the duration of the experiment. The test group received 200 mg/kg body weight plant extracts at 10 ml/kg every morning, administered orally. The mice were divided into five groups of five animals each as follows:

Group 1: Received normal diet (control group).

Group 2: Fed with a high cholesterol diet and received 0.5 ml of sterile water, administered orally.

Group 3: Fed with a high cholesterol diet and received aqueous extract of *P. lentiscus*, 200 mg/kg body weight for 30 days, administered orally.

Group 4: Fed with a high cholesterol diet and received ethanolic extract of *P. lentiscus*, 200 mg/kg body weight for 30 days, administered orally.

Group 5: Fed with a high cholesterol diet and received reference drug Atorvastatin, 10 mg/kg body weight (Khera and Bhatia, 2012) for thirty days, administered orally.

At the end of the experiment, blood samples were collected from the mice in all groups in vials without anti-coagulant. Biochemical parameters measured in the study were TC (total cholesterol), TG (triacylglyceride), LDL (low density lipoprotein), HDL (high density lipoprotein) and VLDL (very low density lipoprotein), using assay kits (Beckman Coulter, Ref: 467825, 445850, 969706, and 650207 – USA.).

#### Statistical analysis

The experimental results were expressed as mean SEM (standard error of the mean). Data were assessed by ANOVA. Tukey's test was then applied using XL Stat Pro 7.5 software. A *p* value of <0.05 was considered to be statistically significant.

## Results

### Flavonoid content

Flavonoid levels in the aqueous and ethanolic extracts of *P. lentiscus* leaves were 3.107 ± 0.014 and 8.218 ± 0.009 mg of QE/g respectively. Ebrahimzadeh et al. (2008) tested the gum of *P. lentiscus*. They found the flavonoid levels to be 30.52 ± 1.10 mg of QE/g. A flavonoid content of 38.7 ± 0.02 mg of QE/g was found by Cherbal et al. (2012) for the methanol extract of *P. lentiscus* leaves.

### Hypocholesterolemic activity in vivo

Table 1 shows the values of serum lipid profile in the normal, hypercholesterolemic, control and extract treated groups.

Cholesterol and triacylglyceride are important building blocks in the structure of biological membranes. They are also used in the biosynthesis of steroid hormones. However, high cholesterol concentration in the blood increases the risk of developing atherosclerosis and related cardiovascular diseases.

In the present study, mice fed with a high cholesterol diet had higher (*p* < 0.05) levels of total cholesterol in the serum (253 ± 31.60 mg/dl) than mice fed with a normal diet (116 ± 0.89 mg/dl).

The treatment of group 3 with aqueous extract of *P. lentiscus* at 200 mg/kg body weight, significantly decreased TC (203.6 ± 9.18 mg/dl) and TG (97.6 ± 3.57 mg/dl) compared to the hypercholesterolemic control (group 2). However, there was no significant decrease in LDL-cholesterol.

In the cholesterol-fed mice (group 4), the treatment with 200 mg/kg body weight of *P. lentiscus* ethanolic extract also significantly decreased TC (154.6 ± 18.10 mg/dl), TG (71.2 ± 4.38 mg/dl) and LDL-cholesterol (99.36 ± 18.77 mg/dl).

TC, TG and LDL-cholesterol also showed a significant decrease as compared to the high cholesterol-fed mice receiving 10 mg/kg body weight doses of atorvastatin (group 5).

*P. lentiscus* leaf extracts showed a non-significant increase of HDL-cholesterol level in comparison to the hypercholesterolemic control (group 2).

## Discussion

From the results obtained, it is apparent that phenolic and flavonoid contents differ according to the plant and solvent used during the extraction. Many factors can affect the content of phenolic compounds. Recent studies have shown that extrinsic factors

**Table 1**Effect of aqueous and ethanolic extract of *P. lentiscus* on serum lipid profile of mice fed with high cholesterol diet after 30 days.

Groups	Dose (mg/kg)	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control group		116 ± 0.89	146.4 ± 10.89	48.2 ± 6.40	38.52 ± 8.13	29.28 ± 2.17
Hypercholesterolaemic group		253 ± 31.60 <sup>#</sup>	200 ± 12.32 <sup>#</sup>	31.6 ± 1.74 <sup>#</sup>	160 ± 31.60 <sup>#</sup>	40 ± 2.46 <sup>#</sup>
Aq <i>P. lentiscus</i>	200	203.6 ± 9.18 <sup>*</sup>	97.6 ± 3.57 <sup>***</sup>	38.2 ± 1.48	145.88 ± 9.76	19.52 ± 0.71 <sup>**</sup>
Et <i>P. lentiscus</i>	200	154.6 ± 18.10 <sup>***</sup>	71.2 ± 4.38 <sup>***</sup>	41 ± 1.87	99.36 ± 18.77 <sup>*</sup>	14.24 ± 0.87 <sup>**</sup>
Atorvastatin	10	130.6 ± 8.14 <sup>***</sup>	121.8 ± 42.65 <sup>***</sup>	62.8 ± 3.49 <sup>***</sup>	43.44 ± 9.35 <sup>***</sup>	24.36 ± 8.53

Aq: aqueous extract.

Et: ethanolic extract.

<sup>\*</sup>  $p < 0.05$  significant from hypercholesterolaemic control.<sup>\*\*</sup>  $p < 0.01$  significant from hypercholesterolaemic control.<sup>\*\*\*</sup>  $p < 0.001$  significant from hypercholesterolaemic control.<sup>#</sup>  $p < 0.05$  significant from control animals.

(such as geographic and climatic factors), genetic factors, degrees of maturation of the plant, and storage time all have a strong influence on the content of phenolic compounds (Falleh et al., 2008).

High levels of cholesterol in the diet have been shown to elevate total cholesterol, and may increase the risk of cardiovascular complications.

Regarding the cholesterol-lowering effect of *P. lentiscus* leaf extracts in mice fed with a high-cholesterol diet, the *P. lentiscus* extracts showed hypocholesterolemic properties. This may be due to either the individual or the synergistic action of the phenolic components. A possible mechanism here may be the hydrolysis control of certain lipoproteins and their selective uptake and metabolism by different tissues.

Flavonoids may enhance the lecithin acyl transferase (LCAT) by increasing their activity, which regulates blood lipids (Yoo et al., 2008). LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and its transfer back to VLDL and LDL, which are later returned in liver cells (Dobiášová and Frohlich, 1999).

Flavonoids might represent another beneficial group of naturally occurring hypolipidemic compounds (Cook and Samman, 1996), and they appear to have intensive biological properties that promote human health and help reduce the risk of disease. Flavonoids act as antioxidants, protect LDL cholesterol from oxidation, inhibit platelet aggregation, and act as anti-inflammatory and anti-tumor agents (Cook and Samman, 1996; Manach et al., 1996).

In addition to direct oxidant scavenging, flavonoids may inhibit the enzymes involved in generating pro-oxidant molecules. For example, flavonoids have been shown to inhibit the generation or release of free radicals derived from lipoxygenase (LOX). It has been suggested that LOX is involved in the early events of atherosclerosis by inducing plasma LDL oxidation in the subendothelial space of the arterial wall (Huang et al., 1997).

In conclusion, the present study clearly demonstrates that *P. lentiscus* leaf extract is rich in phenolic compounds. Furthermore, in terms of hypercholesterolemic activity, *P. lentiscus* extracts significantly decrease TC levels in plasma. Future studies are proposed in order to confirm these results.

#### Authors' contributions

MC did the analysis, interpretation and acquisition of the data and drafted the paper. RA designed the study, supervised the laboratory work and contributed to critical reading of the manuscript.

Both the authors have read the final manuscript and approved for submission.

#### Conflicts of interest

The authors declare no conflicts of interest.

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