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Antiinflammatory and antioxidant activities of gum mastic

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Abstract. – *Objectives:* *Pistacia lentiscus* has traditionally been used in the treatment of many diseases. Its resin was investigated for its mineral contents, anti-inflammatory and antioxidant activities in rats.

Material and Methods: **Inhibition of carrageenan induced edema was used to evaluate anti-inflammatory activity. Fe^{2+} chelating ability, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) and nitric oxide scavenging activities were used to evaluate antioxidant activities and mineral contents were determined by atomic absorption spectroscopy. Gallic acid content was determined by HPLC.**

Results: **Resin produced statistically significant inhibition of edema at all doses when compared to the control groups. A 100% inhibition of inflammation was observed at 800 mg/kg i.p. Resin exhibit no toxicity up to 3 g/kg body weights i.p. in mice. Weak DPPH and nitric oxide scavenging activities were observed but showed good Fe^{2+} chelating ability (IC_{50}=162 µg ml^{-1}). The amount of elements was decreased in the order: Cu > Fe, Zn > Mn> Ni, Cd. Gallic acid content was 0.1 mg/g resin.**

Conclusions: **These experimental data support the use of *Pistacia lentiscus* resin as an anti-inflammatory and antioxidant agent.**

Key Words:

Antiinflammatory, Carrageenan, DPPH, Gallic acid, *Pistacia lentiscus*.

Introduction

It has been showed that *Pistacia* spp. (Anacardiaceae) possess multiple pharmacological effects such as anti-inflammatory[1], estrogen-like[2] and antiemetic activiy[3]. *Pistacia (P) lentiscus L.* grows in many Mediterranean countries[4]. Many effects such as antimicrobial[5], hypotensive[6], anti-hyperlipidemia[7], gastric and duodenal anti-ulcer[8] has been reported from this plant. The aerial part of *P. lentiscus* L. has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties[9]. Some reports available in the literature, studying the antioxidant properties of extract plant[10,11], as well as the total flavonoids content[12]. *P. lenticus* gum had a great effect in healing of mucosal[8]. Also, anti*Helicobacter pylori* activity has been reported[13]. In this report, anti inflammatory, antioxidant activity and mineral content of *P. lenticus* gum have been studied. Also the amount of gallic acid was determined by HPLC.

Materials and Methods

Chemicals

Ferrozine, Linoleic acid, Trichloroacetic acid (TCA), 1,1-diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide, Carrageenan, Hydrogen peroxide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Gallic acid, Quercetin, Butylated hydroxyanisole (BHA), Ascorbic acid, Sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA and Ferric chloride were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade or purer.

Plant Material

Resin of *P. lentiscus* was collected from local market in Sari, Iran. After identification by Dr. Bahman Eslami a voucher (No. 721) has been deposited in the Faculty of Pharmacy herbarium. The resin was coarsely ground before experiment.

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Animals

All experiments were performed on male Swiss mice (25-30 g) or male Wistar rats (180-220 g) obtained from Institute Pasteur of Iran. Experimental protocols meet the Guidelines of Animal Experimentation approved by the Commission of Ethics in Animal Experimentation of Mazandaran University of Medical Sciences, Sari, Iran.

Anti-inflammatory activity

Carrageenan-induced paw edema and toxicity test carried out according to our published papers[14,15]. Carrageenan (50 µL of 1% suspension) was injected into the sub planar tissue of the right hind paw of each rat. Resin (200-800 mg/kg) or diclofenac sodium (100 mg/kg) were administered *i.p.* to rats 1 hour before carrageenan injection. The volume of edema was measured prior and 3 h after carrageenan injection. The degree of swelling was the ratio of the volume of hind paw before to after carrageenan treatment.

Instrumentation and Analytical Procedures

Resin was ash-dried overnight at 400-420°C in a Vitreosil crucible. Two g of ash (yield = 10%) were dissolved in a 1:3 mixture of hydrochloric and nitric acids[16] diluted to 50 ml with distilled water and used for analysis by means of an atomic absorption spectrometer Perkin Elmer AAS 100 (Wellesley, MA, USA). Three times the standard deviation was used as detection limit.

Determination of Total Phenolic and Flavonoid Contents

Total phenol content was determined by Folin Ciocalteu reagent[17]. A solution of resin (0.5 ml of 1:10 g/ml) or gallic acid was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and then aqueous Na_2CO_3 (4 ml, 1 M) was added. The absorbance of reaction was measured at 760 nm after 2 hrs of incubation at r.t. Results were expressed as gallic acid equivalents. Colorimetric aluminum chloride method was used for flavonoid determination[17]. Resin (0.5 ml of 1:10 g/ml) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The solution remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkins Elmer UV/Visible spectrophotometer (UV-Visible EZ201, Perkin Elmer, Covina, CA, USA). Total flavonoid contents were calculated as quercetin equivalents from a calibration curve.

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used[18]. Different concentrations of resin were added, at an equal volume, to methanol solution of DPPH (100 M). After 15 min at r.t., the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Assay of Nitric Oxide-Scavenging Activity

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline (PBS), was mixed with different concentrations of resin in methanol and incubated at r.t. for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm (UV-Visible EZ201, Perkin Elmer, USA). Quercetin was used as positive control[19,20].

Metal Chelating Activity

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry. The chelating of ferrous ions by extracts was estimated by the method of Dinis et al[21]. Resin (0.2-3.2 mg/ml) was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left standing at r.t. for 10 min. Absorbance of the solution was then read at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as $[(A_0-A_s)/A_s]$ 100, where A_0 was the absorbance of the blank, and A_s was the absorbance of the extract or positive control[20], ethylene diamine tetracetic acid (EDTA).

Table I. Trace elements contents in *P. lentiscus* resin by AAS Analysis (µg/g ash).

Sample	Cr	Cd	Pb	Ni	Mn	Zn	Fe	Cu
P. lentiscus resin	ND	1	ND	1	7	12	12	27.3

Values are averages of three independent measurements having a precision of approx ± 2%. ND: Not detected. Yield of ash was 10%.

Assay of Gallic Acid

A DAD detector Knauer series liquid chromatograph system with a C_{18} reversed phase (D-SS120 Mainz, Germany) was used. Mobile phase was methanol/water/orthophosphoric acid (20: 79.9: 0.1) and the flow rate was 1.0 ml/min. Absorption wave length was selected at 210 nm. The content of gallic acid was calculated on the basis of a calibration curve constructed using authentic reference gallic acid.

Statistical Analysis

Results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test.

Results

Assay of Gallic Acid and Mineral Contents

No quercetine was found but gallic acid content was 0.1 mg/g resin. The amount of elements analyzed in the present work, decreases in the order: Cu > Fe, Zn > Mn> Ni, Cd (Table I).

Total Phenol and Flavonoids Contents

Flavonoid content was 30.52± 1.10 mg/g quercetin equivalent and phenol content was 9.92 ± 0.27 mg g^{-1}.

DPPH Radical-Scavenging Activity

Resin showed a very weak DPPH radical-scavenging activity with only 37% inhibition in 1.6 mg/ml. The IC_{50} values for vitamin C, quercetin and BHA were 1.26 ± 0.11, 1.32 ± 0.07 and 13.49 ± 1.04 µg/ml, respectively.

Nitric Oxide Scavenging Activity

Resin showed very weak NO scavenging activity with only 28% inhibition in 1.6 mg ml^{-1}. IC_{50} for quercetin was 17.01 ± 0.03 µg/ml.

Fe^{2+} Chelating Ability

Resin showed good Fe^{2+} chelating activity (IC_{50} = 162.2 ± 8.7 µg/ml). The effect was dose dependent. EDTA showed very strong activity (IC_{50} = 18 ± 0.4 µg /ml).

Pharmacology

Resin produced statistically significant inhibition of edema induced by carrageenan at all doses when compared to the control groups (Table II). The effect was dose-dependent. The highest activity showed at 800 mg/kg i.p. that inhibited 100% of inflammation. This activity was so higher than diclofenac at 100 mg/kg i.p. (74.4%). At the concentration of 600 mg/kg i.p. the activity was equipotent with diclofenac ($p > 0.05$). Resin exhibits no toxicity up to 3 g/kg body weight when injected i.p. in mice.

Table II. Anti-inflammatory activity of *Pistacia lentiscus* resin on carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg i.p.)	Initial paw thickness (cm)[b]	Paw thichness after 3 hr (cm)[a]	a/b ratio[a]	Inhibition (%)
Solvent	Vehicle	0.31 ± 0.07	0.58 ± 0.05	1.87	0
P. lentiscus resin	200	0.25 ± 0.05	0.43 ± 0.05***	1.72	33.3
	400	0.30 ± 0.06	0.40 ± 0.07***	1.33	62.9
	600	0.35 ± 0.04	0.43 ± 0.02***	1.22	70.3
	800	0.30 ± 0.06	0.30 ± 0.04***	1	100
Diclofenac	100	0.23 ± 0.04	0.30 ± 0.06***	1.30	74.1

[a]A ratio less than 1.5 was considered to be a significant inhibitory effect. Values are means ± SD. (n = 6), ***$p < 0.001$, with respect to control (ANOVA followed by Newman-Keuls multiple comparisons test).

Discussion

The daily requirements of an adult man are as follows (mg/d): 10-15 Fe, 12-15 Zn and 2-3 Cu[18,22]. The knowledge of the chemical form of the elements in plants of economic interest might be crucial because actions can be taken to reduce or minimize the toxic effects of the environment pollutant heavy metals[23]. Resin contained high level of Cu, Fe and Zn. Resin standardization was performed according gallic acid content. It contained 0.1 mg/g resin. Flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. Phenolic compounds are a class of antioxidant agents acting as free radical terminators[17]. Flavonoid content was 30.52 ± 1.10 mg/g quercetin equivalent and phenol content was 9.92 ± 0.27 mg/g. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities[18]. Studies have shown that increasing level of flavonoids in the diet could decrease certain human diseases[17]. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples[19]. Resin showed weak DPPH radical-scavenging activity. Therefore, it could not serve as electron donors for terminating the radical chain reaction. Resin showed weak NO scavenging. In addition to reactive oxygen species, NO is also implicated in inflammation, cancer and other pathological conditions[20]. The plant/plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. In spite of significant anti-inflammatory activity of resin, it showed very weak NO scavenging activity. It suggests other mechanism(s) should be concerned. Chelation therapy reduces iron-related complications in human and thereby improves quality of life and overall survival in some diseases such as Thalassemia major[24]. The transition metal, iron, is capable of generating free radicals from peroxides by Fenton reactions[25]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. The effect was dose dependent. Resin showed good Fe^{2+} chelating activity. Resin produced statistically significant inhibition of edema induced by carrageenan at all doses when compared to the control groups (Table II). The effect was dose-dependent. At 800 mg/kg *i.p.* 100% of inflammation blocked. This activity was higher than diclofenac at 100 mg/kg *i.p.* (74.4%, $p < 0.001$). At 600 mg/kg the activity was equipotent with diclofenac ($p > 0.05$). Resin was safe and did not exhibit any toxicity up to 3 g/kg body weight when injected *i.p.* in mice. The results of present study support the folkloric utilization of *P. lentiscus* resin. Further investigation of individual compounds, to determine the mechanism of anti-inflammatory and antioxidant activities is suggested.

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