Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Ethnopharmacology 105 (2006) 235–240
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of pistachio, *Pistacia vera* L.

I. Orhan [a], E. Küpeli [a], M. Aslan [a], M. Kartal [b], E. Yesilada [a,*]

[a] *Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara, Turkey*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Tandogan 06100, Ankara, Turkey*

Received 29 July 2005; received in revised form 25 October 2005; accepted 31 October 2005
Available online 6 December 2005

Abstract

The ethanolic and aqueous extracts prepared from different parts of *Pistacia vera* L. (Anacardiaceae) as well as its oleoresin were evaluated for their in vivo anti-inflammatory and antinociceptive activities. Among the extracts screened, only the oleoresin was shown to possess a marked anti-inflammatory activity against carrageenan-induced hind paw edema model in mice without inducing any gastric damage at both 250 and 500 mg/kg doses whereas the rest of the extracts were totally inactive. While the oleoresin was found to display significant antinociceptive activity at 500 mg/kg dose, the ethanolic and aqueous extracts belonging to fruit, leaf, branch and peduncle of *Pistacia vera* did not exhibit any noticeable antinociception in *p*-benzoquinone-induced abdominal contractions in mice. Fractionation of the oleoresin indicated the *n*-hexane fraction to be active, which further led to recognition of some monoterpenes, mainly α-pinene (77.5%) by capillary gas chromatography–mass spectrometry (GC–MS) as well as the oleoresin itself. α-Pinene was also assessed for its antinociceptive and anti-inflammatory activities in the same manner and exerted a moderate anti-inflammatory effect at 500 mg/kg dose.

Keywords: Anti-inflammatory activity; Antinociceptive activity; *Pistacia vera*; Anacardiaceae; Pistachio; Oleoresin; GC–MS; α-Pinene

1. Introduction

Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti-inflammatory agents exert their effect through a spectrum of different modes of action (Samuelsson et al., 1978). However, chronic inflammatory diseases are still one of the major health problems worldwide and non-steroid anti-inflammatory drugs (NSAIDs) are the most prescribed drugs for treatment of inflammatory diseases. Although the NSAIDs provide the patients with symptomatic relief, they do not modify the pathogenesis of inflammation and do not reduce the disabling bone and cartilage damage (Ford-Hutchinson et al., 1981). Therefore, it has become a must to search for new initiatives in the treatment of chronic inflammation.

On the other hand, plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases. The nuts of *Pistacia vera* L. (Anacardiaceae), commonly referred to pistachio, is a quite popular flavoring foodstuff and snack in Turkey and in the world. The oleoresin of *Pistacia vera*, a widely-distributed plant throughout the south-east region of Anatolia, has been used to treat asthma by chewing as folk remedy in this part of Turkey (personal note) as well as against stomachache (by chewing the gum) and hemorrhoids (externally) (Baytop, 1999). On this purpose, we aimed to evaluate in vivo anti-inflammatory and antinociceptive potentials of the aqueous and ethanol extracts obtained from various parts of the plant along with its oleoresin in order to validate medicinal utilization of the plant in mice using carrageenan-induced hind paw edema model for anti-inflammatory activity as well as *p*-benzoquinone induced abdominal contractions for antinociceptive activity.

* Corresponding author. Tel.: +90 216 578 00 00x3021; fax: +90 216 5780068.
E-mail addresses: iorhan@gazi.edu.tr (I. Orhan), yesilada@yeditepe.edu.tr (E. Yesilada).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.10.023

2. Experimental

2.1. Plant material

Plant material was collected from Korucak village, Nizip, Gaziantep (Turkey) in July 2004. Authenticated voucher specimen (GUE 2381) was preserved in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

2.2. Preparation of plant extracts

The plant parts used in extraction were classified as fruits, leaves, branches and peduncles, while the oleoresin occurring naturally on trunk of the plant was exuded. Each of the above cited plant part was dried under shade and powdered to a fine grade by using a laboratory scale mill. Ten grams of the each plant part was weighed accurately and, except for the oleoresin, two kinds of the extract were prepared with 96% ethanol and water, separately, at room temperature macerating for two times (×200 ml). Following the filtration, the ethanol and aqueous extracts were evaporated to dryness in vacuo to give crude extracts (percent yields for the ethanolic extracts; fruit 34.87%, leaf 27.9%, branch 8.7%, peduncle 16%; and for the aqueous extracts; fruit 24.1%, leaf 21.7%, branch 22.5%, peduncle 9.4%).

2.3. Fractionation of the oleoresin

The oleoresin (75 g) was firstly extracted with 300 ml of ethanol (90%) by sonication. Following, the solution was extracted with *n*-hexane (300 ml, Merck Co.) in a separatory funnel. The *n*-hexane phase was collected and evaporated under vacuum until dryness affording the "*n*-hexane fraction" (yield 59.7 g). A precipitate was formed by the dilution of the remaining ethanolic phase with distilled water and was obtained through filtration "Oleoresin-precipitate fraction" (yield 4.8 g) and remaining hydroalcoholic phase was evaporated to dryness under reduced pressure "hydroalcoholic fraction" (yield 4.9 g). Each fraction of the oleoresin was submitted to the bioactivity assays in doses estimated from the yields in the 500 mg of oleoresin, i.e., 429.7, 34.9 and 35.4 mg/kg for *n*-hexane fraction, oleoresin-precipitate fraction, and hydroalcoholic fraction, respectively. In order to obtain comparable results fractions were also administered in 40, 400 and 400 mg/kg doses, respectively.

2.4. Methyl esterification of n-hexane fraction and the oleoresin of Pistacia vera

n-Hexane fraction of the oleoresin and the oleoresin itself were independently saponified with 0.5 N methanolic NaOH solution by heating on a steam bath until fat globules entered the solution, an approximately 5 min step and boiled for 2 min. After cooling, saturated NaCl solution was added to each solution. The mixtures were transferred to a separatory funnel individually and each extracted with 30 ml of petroleum ether and converted to their methyl ester forms with 20 ml of boron trifluoride-methanol complex reagent (20%, Merck Co.) (Morrison and Smith, 1964).

2.5. GC–MS analysis of the n-hexane fraction and oleoresin

Chromatographic analysis was carried out on Agilent 6890N Network GC system combined with Agilent 5973 Network Mass Selective Detector (GC–MS). The capillary column used was an Agilent 19091N-136 (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 μm). Helium was used as carrier gas at a flow rate of 0.8 ml/min with 1 μl injection volume. Samples were analyzed with the column held initially 60 °C for 1 min after injection with 10 min hold time, then increased to 220 °C with 4 °C/min heating ramp and kept at 220 °C for 10 min. Then final temperature was increased to 240 °C with 1 °C/min heating ramp. The injection was performed in splitless mode. Detector and injector temperatures were 280 and 250 °C, respectively. Run time was 80 min. MS scan range was (*m/z*) 35–450 atomic mass units (a.m.u.) under electron impact (EI) ionization (70 eV).

2.6. Pharmacological procedures

2.6.1. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals left for 2 days for acclimatization to animal room conditions were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day prior to the experiment, but was allowed free access of water. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals.

2.6.2. Preparation of test samples for bioassays

All extracts were administered in both 250 and 500 mg/kg doses after suspending in 1% Tween 80 in distilled H_2O. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (100 and 200 mg/kg) in 1% Tween 80 was used as reference drug.

2.6.3. Antinociceptive activity

2.6.3.1. p-Benzoquinone-induced abdominal constriction test in mice. In accordance with the method of Okun et al. (1963) 60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 ml/10 g body weight of 2.5% (v/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the fifth minute after the PBQ injection. The data represent average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls.