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Original Article

Antinociceptive study of extracts of *Platanus orientalis* leaves in mice

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Abstract

Platanus orientalis L. (Platanaceae) is a medicinal tree used in Iranian folklore and traditional medicine as a pain and inflammation reliever. In this study, the polyphenolic and total extract of *P. orientalis* leaves were prepared and their antinociceptive effects were studied in mice using the acetic acid-induced writhing, formalin and light tail flick tests. Both hydroalcoholic and polyphenolic extracts of *P. orientalis* at a dose of 2 g/kg significantly (P<0.05) reduced acetic acid-induced abdominal writhes. These extracts were also effective in suppression of formalin-induced pain behavior but they did not show any significant analgesia in light tail flick test. It is concluded that *P. orientalis* extracts have moderate analgesic effect and further studies are needed to elucidate the mechanism and active constituents.

Keywords: *Platanus orientalis*; Platanaceae; Polyphenols; Antinociceptive; Anti-inflammatory

INTRODUCTION

Platanus is a unique living member of Platanaceae family. It is a small genus of trees that known in English as plane trees. The principal use of plane trees are as ornamental trees, especially by roadsides and parks (1,2). The plane trees are widely planted to improve the microclimate (3). The plane leaves commonly known in Iran as "Barge Chenar", have been used in concentrated aromatic liquids, herbal remedies and Iranian traditional medicine to treat several disorders. They are used in Iranian folk and traditional medicines for treating some dermatological, gastrointestinal, rheumatic and inflammatory diseases (4-6). Some Persian scientists and hakims such as Avicenna and Hakim Momen were also familiar with this tree and mentioned its medicinal uses like teeth pain killer and analgesic and antiinflammtory effects for knee pain and inflammation in their books (4,5). The plane leaves contain flavonoids, pentacyclic triterpenoids, tannins and caffeic acid (7-11). Many pharmacological activities such as cytotoxic, cytostatic, astringent, antimicrobial and antiseptic effects have been attributed to the *Platanus* species (7-9, 11). *Platanus orientalis* is the major species of *Platanus* in Iran and is widely distributed in northern and central parts of the country (1,2,11).

The aim of the present study was to evaluate antinociceptive effect of a total extract and a polyphenol-rich extract fractionated from *P. orientalis* leaves, as the major identified species of *Platanus* in Iran, in mice using the formalin, acetic acid-induced writhing and light tail flick tests.

MATERIALS AND METHODS

Plant material and preparation of the extracts

Fresh plane leaves growing in the campus of the Isfahan University of Medical Sciences, Iran were collected in June 2004 at an approximate altitude of 1500 m. The plant identity as *P. orientalis* was confirmed by the herbarium department of School of Biology, Isfahan University, Iran. A voucher specimen of the plant (No. 1746) was deposited in the herbarium of School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran for future evidence.

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For preparation of total hydroalcoholic extract, air-dried and finely powdered leaves (200 g) were soaked by adequate volume of ethanol:water (7:3 v/v) and the extraction was undertaken for 48 h to obtain full extract using maceration method. The extract was then shuddered, filtered and evaporated in a rotary evaporator under reduced pressure until a semisolid and jam nature extract yielded 17.4% w/w (12).

For preparation of polyphenolic extract, same plant materials were weighed out. Extraction of polyphenol compounds was carried out in two steps, first with ethanol: water (9:1 v/v) and second with ethanol:water (1:1 v/v). At each step solvent was added to make slurry with the leaves powder and was left for 24 h. The two extracts were then combined and evaporated. The resultant solution was cleared of low polarity contaminants like fats, xanthophylls and chlorophylls by extraction in a separating funnel with chloroform in several times. This solvent-extracted aqueous layer, containing the bulk of the flavonoids and other phenolics, was then evaporated to dryness under vacuum in an evaporator. Evaporation and solvent removal of extract gave a semisolid syrupy mass yielded 22.5% w/w (13). These extracts were stored in a refrigerator at 3°C.

Animals

Male swiss mice (25-35 g) obtained from the animal house of our school were used. They were housed in polypropylene cages under standard environmental conditions and had free access to pellet diet and tap water. For experimentation six animals were included in each group.

Acetic acid-induced writhing test

This test was carried out according to the method described by Koster et al. (14). Groups of mice (n=6) were orally administered different doses (0.5-2 g/kg) of hydroalcoholic or polyphenolic extracts 1 h prior to an i.p. injection of 1% acetic acid in a volume of 10 ml/kg. Control group received vehicle (10 ml/kg of 1% solution of tween 80). Indomethacin (10 mg/kg, p.o.) was used as the reference drug.

Formalin test

This test was carried out according to Hunskaar and Hole (15). Briefly, 1 h after oral administration of vehicle or above-mentioned doses of the hydroalcoholic or polyphenolic extracts or 30 min after i.p. injection of morphine (10 mg/kg), 20 µl of 2.5% formalin v/v in 0.9% saline was injected into the subplantar space of the right hind paw and the duration of paw licking was determined 0-5 min (first phase) and 20-30 min (second phase) after formalin injection.

Light tail flick test

The analgesic activity of the extracts was also determined by the tail-flick method (16) using a tail flick apparatus (Pooya-armaghan, Iran). Briefly, each animal was placed in a restrainer 2 min before treatment, and baseline reaction time was measured by focusing a beam of light on the distal one-third portion of the animals' tail. Extracts, vehicle or morphine was orally administered immediately after this step and the post drug reaction time was measured at 15 min intervals up to 2 h. A 12 s cut-off time was used in order to prevent tissue damage. The MPE% (percent of maximum possible analgesic effect) was calculated for each time interval according to the following formula.

MPE%= [(test latency – control latency)] / [(cut-off time – control latency)] × 100

Statistical analysis

The results are presented as mean ± S.E.M. and statistically analyzed by One-way ANOVA followed by the Duncan test. $P<0.05$ was considered significant.

RESULTS

As it is seen in Table 1. both hydroalcoholic and polyphenolic extracts of *P. orientalis* at a dose of 2 g/kg significantly ($P<0.05$) reduced acetic acid-induced abdominal writhes. Indomethacin, as a reference drug (10 mg/kg), produced 72% inhibition of abdominal writhes and its effect was significant ($P<0.001$).

In acute phase of formalin test hydroalcoholic extract at doses of 0.5, 1 and 2 g/kg inhibited formalin-induced pain by 40%,

Table 1. Effect of hydroalcoholic and polyphenolic extracts of *Platanus orientalis* leaves on acetic acid-induced writhing in mice (n=6). * $P<0.05$; *** $P< 0.001$ compared with control group. POHE, *Platanus orientalis* Hydroalcoholic extract; POPE, *Platanus orientalis* Polyphenolic Extract.

Treatment	Dose (g/kg, p.o.)	Number of writhes (Mean ± S.E.M)	Percent inhibition
Control	0.00	51.7 ± 2.5	-
POHE	0.50	40.0 ± 2.8	23
	1.00	38.5 ± 5.1	26
	2.00	30.2 ± 3.1*	42
POPE	0.50	41.2 ± 5.0	20
	1.00	40.0 ± 5.1	23
	2.00	23.8 ± 3.0*	54
Indomethacin	0.01	14.7 ± 2.1***	72

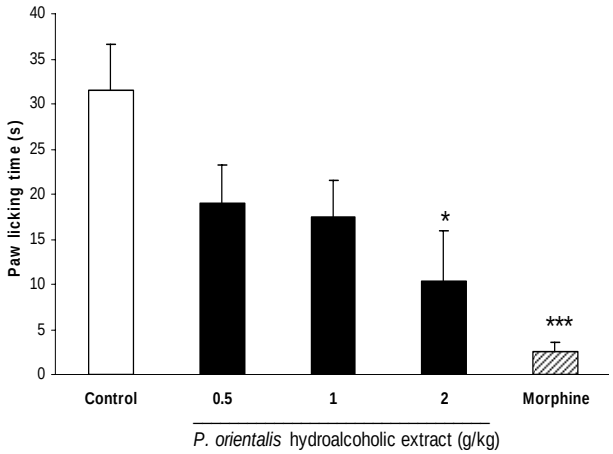

Fig. 1. The antinociceptive activity of *P. orientalis* hydroalcoholic extract on paw licking during acute phase of formalin test. Vehicle and different doses of the extract were orally administered 1 h prior to subplantar injection of formalin and the time spent for licking was measured during a 0-5 min. period starting after formalin injection. Morphine (10 mg/kg, i.p.) was used as reference drug. Data are mean ± S.E.M. of 6 animals in each group. * $P<0.05$; *** $P<0.001$ significantly different from control group.

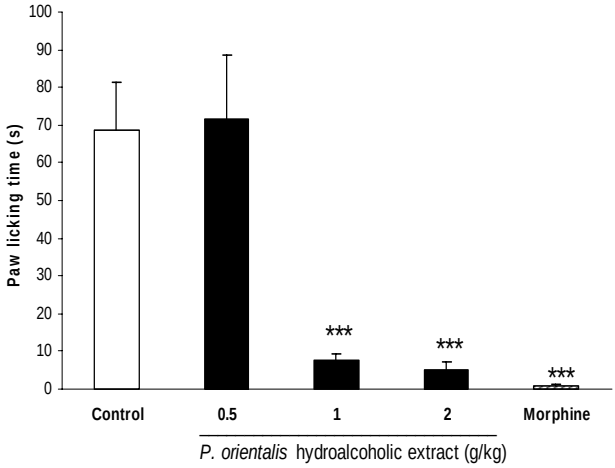

Fig. 2. The antinociceptive activity of *P. orientalis* hydroalcoholic extract on paw licking during chronic phase of formalin test. Vehicle and different doses of the extract were orally administered 1 h prior to subplantar injection of formalin and the time spent for licking was measured during a 20-30 min. period starting after formalin injection. Morphine (10 mg/kg, i.p.) was used as reference drug. Data are mean ± S.E.M. of 6 animals in each group. *** $P<0.001$ significantly different from control group.

44% and 67%, respectively and this effect was only significant ($P<0.05$) at the dose of 2 g/kg. Morphine as an standard analgesic drug significantly ($P<0.001$) reduced pain behavior by 92% (Fig.1). In chronic phase, hydro-alcoholic extract (1 and 2 g/kg) and morphine caused significant ($P<0.001$) inhibition in formalin-induced licking behavior (Fig. 2). The effect of polyphenolic extract of *P. orientalis* on acute and chronic phases of formalin test has been depicted in Fig. 3 and Fig. 4. As it is observed polyphenolic extract at doses of 1 and 2 g/kg significantly ($P<0.05$) inhibited paw licking behavior of mice in both phases of formalin test and the dose of 0.5 g/kg of this extract was ineffective.

In light tail flick test only morphine, as the reference drug, showed analgesic activity and both extracts failed to produce any analgesic effect (Fig. 5).

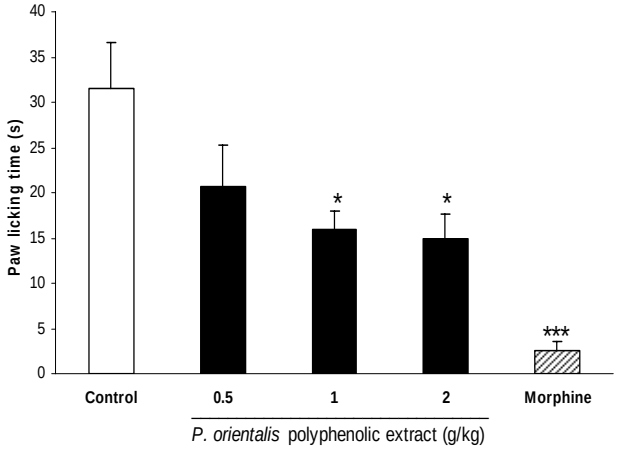

Fig. 3. The antinociceptive activity of *P. orientalis* polyphenolic extract on paw licking during acute phase of formalin test. Vehicle and different doses of the extract were orally administered 1 h prior to subplantar injection of formalin and the time spent for licking was measured during a 0-5 min. period starting after formalin injection. Morphine (10 mg/kg, i.p.) was used as reference drug. Data are mean ± S.E.M. of 6 animals in each group. * P<0.05; *** P<0.001 significantly different from control group.

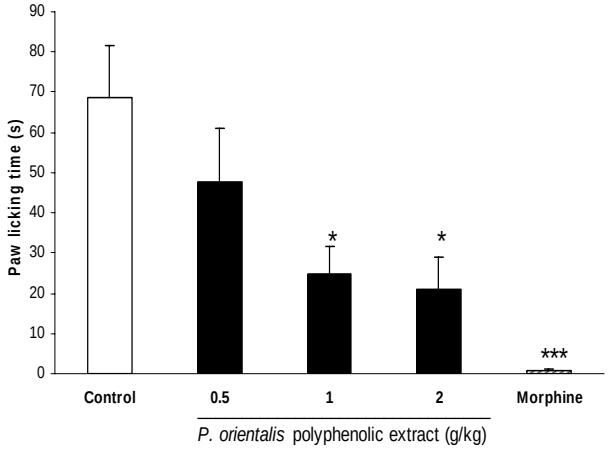

Fig. 4. The antinociceptive activity of *P. orientalis* polyphenolic extract on paw licking during chronic phase of formalin test. Vehicle and different doses of the extract were orally administered 1 h prior to subplantar injection of formalin and the time spent for licking was measured during a 20-30 min. period starting after formalin injection. Morphine (10 mg/kg, i.p.) was used as reference drug. Data are mean ± S.E.M. of 6 animals in each group. * P<0.05; *** P<0.001 significantly different from control group.

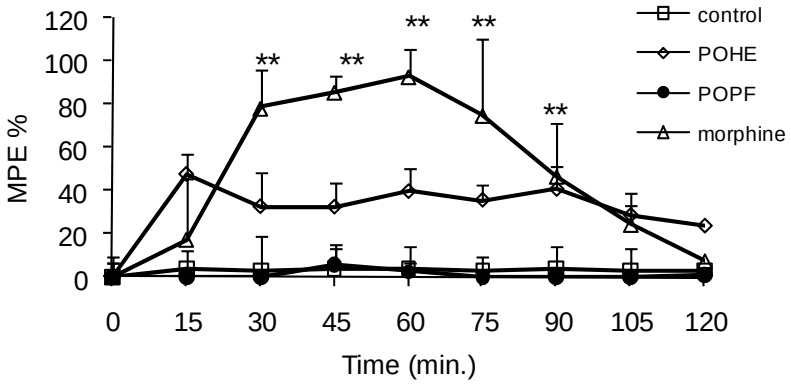

Fig. 5. The antinociceptive activity of *P. orientalis* extracts in light tail flick test. Vehicle and extracts (2 g/kg) were orally administered 1 h prior to test and reaction time of mice was measured at 15 min intervals until 2 h and MPE% (percent of maximal possible antinociceptive effect) was calculated for each time and compared. Morphine (10 mg/kg, i.p.) was used as reference drug. Data are mean ± S.E.M. of 6 animals in each group. ** P<0.01 significantly different from control group. POHE, *Platanus orientalis* Hydroalcoholic extract; POPE, *Platanus orientalis* Polyphenolic Extract.

DISCUSSION

In this study both hydroalcoholic and polyphenolic extracts of *P. orientalis* showed moderate analgesic effect in acetic acid and formalin models but not in light tail flick test. It has been shown that centrally acting analgesics e.g. opioids reveal their analgesic activity in thermal tests such as hot plate and light tail flick (16) and our results indicating the lack of such alkaloids in the extracts. In acetic acid test, while indomethacin as the reference drug inhibited abdominal constrictions by 72%, hydroalcoholic and

polyphenolic extracts of the plant at the highest doses applied, showed 42% and 54% inhibition, respectively. Although acetic acid test is a non specific test for the assessment of analgesic activity, it has been used in a numerous studies as a preliminary screening test (16).

In the formalin test, formalin could induce a typical biphasic pain response as shown in previous work (15). Pain in the early phase is predominantly caused by activation of C-fibers, while in the late phase a combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord are involved (17). Both extracts particularly suppressed pain response of second phase of formalin test and therefore they are promising agents for relieving pain with inflammatory origin. Comparing the effects of the same doses of the extracts in second phase of formalin test show that hydroalcoholic extract has more potent activity than polyphenolic extract and this difference may be due to different chemical constituents of the extracts. *Platanus orientalis* contains various tannins and polyphenols, including flavonoids such as quercetin, kaempferol and their glycosides (7,8,11). It has been reported that these flavonoids have potent anti-inflammatory and analgesic actions on inflammation and pain (18-20). Depending on the chemical substitutions on the flavone-skeleton, flavonoids can play a modulating, biphasic and regulatory action on inflammation and immunity. Flavonoids exert their properties both as plant extracts and as purified aglycone molecules (18). Also caffeic acid is found in the plant leaves and it has been reported that this compound has anti-inflammatory activity (21-22). It seems these natural compounds in the polyphenolic and total extracts of the plant leaves have been partly associated with our pharmacological findings, although it is not clear whether these plant constituents are the only contributing components of this extract.

CONCLUSION

In conclusion, the pharmacological activities of the plant leaves showed moderate antinociceptive effects and further pharmacological and biological studies are needed to elucidate the mechanisms of its action.

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