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Short Communication

Anti-nociceptive Activity of Aqueous-methanolic Extract of *Phytolacca americana* Growing in Iran

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Abstract

Abstract Anti-nociceptive activity of aerial parts of *Phytolacca americana* were investigated, using the hot plate method in mice. Results of the present study showed that the aqueous methanolic extract of aerial parts of *P. americana* produced a statistically significant increase in pain threshold after 30 min, in comparison with the control, at adose of 190 mg kg^{-1} ($P < 0.001$). The activity was comparable to that of morphine (30 mg kg^{-1} i.p., $P > 0.05$). The anti-nociceptive activity of *P. americana* increased until the 60th min ($P < 0.05$ compared to morphine). The results of this study support the extensive use of *P. americana* in Western Asia and America. The LD_{50} of extract following a 14 days acute toxicity study was calculated to be 208 mg kg^{-1} i.p.

Keywords: Antinociception; *Phytolacca americana*; Hot plate method; Aqueous extract; Morphine.

Introduction

Pain is still one of the main health problems of the world's populations (1). Many bioactive substances are involved in the modulation of pain sensation (2). Eclectic physicians relied upon herbal medicines and natural remedies to treat disease (3). The pain relief composition is prepared from roots of the Burdock plants family and in particular from at least one of the species, such as *Arctium lappa*, or as *Arctium minus* in combination with roots of the Phytolaccaceae family and in particular the species *Phytolacca americana* (4). Pokeweed, *P. americana*, is a perennial plant native to North America. The boiled leaves are used in a popular salad (called grandmother's salad) in the American diet (5-7). *P. americana* grows widely in northwestern parts of Iran, mainly in the coastal areas and forest lands (8, 9). It is well known for several medicinal properties, despite its toxicity, especially hepatotoxicity (10, 11).

P. americana has been most commonly used as laxative. It has been shown to possess pain relieving, anti-inflammatory, anti-rheumatism and anti-arthritic activities. Also, it is suitable for the treatment of various skin diseases (12, 13). Nowadays, pokeweed is still used cautiously by some herbalists to treat the above-mentioned conditions. There are a few reports on its anti-viral, anti-cancer, anti-fungal

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and immunostimulant activities (14, 15). One hundred and fifty species of *Phytolacca* dried flowers are available in Eastern North America, northwestern areas of Iran and other parts of the world, they have been used to relive pain and for the reduction of fever (16-21). They are used alone or as a mixture.

The purpose of this study was the evaluation of anti-nociceptive activity of an aqueous methanolic extract of the aerial parts (flowered browse) of *P. americana* growing in Iran, using the hot plate method, as well as the determination of its median lethal dose (LD_{50}).

Experimental

Morphine was purchased from the American Peptide Company (Sunnyvale, CA, USA). The experimental protocol used in this study was carried out in accordance with the ethics committee of the Mazandaran University of Medical Sciences.

Animals

Male Swiss albino mice weighing between 25-30 g were used for these studies. They were housed in groups of five, under standard light (7.00 to 19.00) and temperature (22 ± 1°C), with food and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. Experiments were performed during the day (08:00-16:00 h). Each animal was used only once.

Preparation of the plant extract

Aerial parts (flowered browse) of *Phytolacca americana* were collected from Mazandaran (a northern state in Iran) in April 2006, identified and confirmed by Dr. Saeidnia at the Department of Pharmacognosy, School of Pharmacy, Mazandaran University of Medical Sciences. Aerial parts were dried at room temperature and coarsely ground before extraction. One hundred grams of the powdered sample was extracted at room temperature by percolation with methanol/ water (80:20, 400 mL × 3 times). The resulting extract was concentrated over a rotary vacuum evaporator, until a solid extract sample was obtained. The resulting crude extract was freeze-dried. The extract was prepared in phosphate buffer (pH 7.4) and tween 80 (4:1) for pharmacological studies.

Hot plate method

Morphine was injected intraperitoneally (i.p.) to mice, as a single dose of 30 mg kg^{-1} (as a positive control). Solvent was injected to the negative control group (10 mL kg^{-1}, i.p.). An aqueous methanolic extract of the aerial parts of *P. americana* was given at a dose of 190 mg kg^{-1} i.p. to the animals, as a single dose. Anti-nociceptive activity was assessed by measuring the hot plate latency to heat, as described by Leimbach and Eddy (22). A minimum of three trials was recorded for each animal and toxicity studies carried out in mice, according to the method stated by Reddy and Byahatti (23). Mice were placed in a thermostatically controlled hot plate apparatus (Harvard, UK), maintained at 52 ± 0.5 °C and the reaction time (time elapsed between placing the mouse on the hot plate and appearance of signs of acute discomfort) for licking or kicking of the fore-or hind paws was recorded using a stop watch. The controlled reaction time in was recorded before the start of experiment. Mice, which did not show any reaction after 15 sec, were discarded. Reaction time (in sec) before and at 0, 30 and 60th min after administration of the drugs was recorded. A cut-off time of 45 sec was imposed to avoid tissue damage.

The median lethal dose (LD_{50})

Extract was dissolved in phosphate buffer (pH 7.4) and Tween 80 (4:1) (2) and was given as a single dose to mice intraperitoneally. Acute toxicity assays were conducted based on our recently published method (24). Briefly, doses in the tested dose-interval were progressively increased such that each dose was 50% higher than the previous one (0, 12.5, 25, 50, 100, 200, 400, 800 mg kg^{-1}), until the dose lethal to half of the test population had been attained. The animals were observed during a 14 days study period and deaths were recorded.

Analyses of the data

Statistical analysis was performed using the SPSS software for Windows (Ver.10, SPSS Inc.,

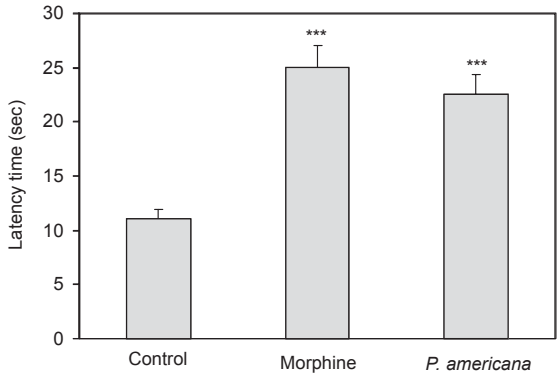

Figure 1. Anti-nociceptive activity of aqueous methanolic extract of *P. americana* aerial parts after 60 min. Values are presented as mean ± SEM (n = 7), ***P < 0.001 with respect to control (ANOVA followed by Newman–Keuls multiple comparison test).

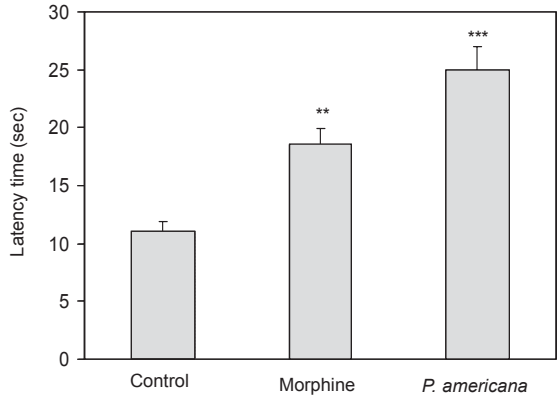

Figure 2. Anti-nociceptive activity of aqueous methanolic extract of *P. americana* aerial parts after 60 min. Values are presented as mean ± SEM (n = 7), **P < 0.05 and ***P < 0.001 with respect to control (ANOVA followed by Newman–Keuls multiple comparison test).

Chicago, USA). Data were analyzed by one-way analysis of variance (ANOVA) and presented as mean±SEM. Student-Newman-Keuls test was used for statistical analysis and $P < 0.05$ was considered to be significant.

Results and Discussion

Results of the present study showed that the aqueous methanolic extract of the aerial parts (flowered browse at 190 mg kg^{-1}) of *P. americana* produced a statistically significant increase in the pain threshold, after 30 min, in comparison with the control (Figure 1). The effect or activity was rather low, however enough for treatment and blocking the pain. This activity was comparable to that of morphine (30 mg kg^{-1} i.p., $P > 0.05$). The anti-nociceptive activity of extract increased until the 60th min. The P-value was greater than 0.05, compared to morphine (Figure 2). Poke root is an herbal medicine used to treat inflammation (swelling) of the mouth, throat, nose, and breast. It is also used to treat skin infections and stop pain (25). The pain relief composition is prepared from roots of the *Phytolacca* family and in particular the species *Phytolacca americana* (4). On other hand, the anti-cancer effects appear to work primarily based upon anti-tumor and anti-inflammatory properties, along with immuno-stimulant functions (14, 15). Furthermore, it contains aromatase inhibitors and has antioxidant properties (25). A number of anti-inflammatory components have also been reported in *P. americana* (15, 16, 21). Among the constituents, oleanolic acid appears to be the most significant, with it's anti-inflammatory and prostaglandin synthesis inhibitory properties (25). The results of this study support the extensive use of this plant in western Asia and America (18-20). It is possible that the same components could lead to anti-nociceptive activity in our extract. This needs to be justified in future studies. Based on our results, *P. americana* could be candidated as an analgesic agent. Although, the mechanism of plant action to increase anti-nociceptive activity in mice is unclear. On the other hand, phytolaccatoxin and the related triterpene saponins, believed to be the primary toxic constituents, are present in the berry juice and other plant parts (26-28). Other toxic constituents have also been identified, including the alkaloids phytolaccine and phytolaccotoxin, as well as a glycoprotein and histamines. When pokeweed is used as food, the water in which it is boiled, must be discarded (26-28). The lethal dose 50% (LD_{50}) is most frequently used to characterize the response of animals, such as rats and mice, as a general indicator of an agent acute toxicity test (29). Based on our data, the LD_{50} values, after the 14 days acute toxicity study was calculated

to be 208 mg kg^{-1} i.p.

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