

Comparison antinociceptive activity of the aqueous methanolic extracts of *Salvia Hypoleuca* and *Phytolacca americana* in mice

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Abstract. – **OBJECTIVES:** Anti-nociceptive activity of aerial parts of *Phytolacca (P.) americana* and *Salvia Hypoleuca* were investigated, using the hot plate method in mice.

MATERIALS AND METHODS: Antinociceptive activity of aerial parts of *Phytolacca americana* and *Salvia Hypoleuca* were investigated using the hot plate method. In addition, the effect of its aerial parts on morphine dependence was investigated in mice. After induction of dependence by morphine, different concentrations of plants aerial parts extract were injected to treated groups. To assess morphine withdrawal, mice were injected naloxone (5 mg/kg) i.p. on the 5th day. After four consecutive days of morphine injection, withdrawal syndrome was assessed by placing each mouse in a 30 cm high glass box and recording the frequency of escape jumps for 60 minutes.

RESULTS: The aqueous methanolic extract of aerial parts of *P. Americana* and *S. Hypoleuca* produced a statistically significant increase in pain threshold after 30 min of i.p. injection of extract, in comparison with the control groups, at doses of 190 and doses of 500, 1000 and 1500 mg/kg ($p < 0.001$) respectability. The activity was comparable to that of morphine (30 mg/kg i.p., $p > 0.05$). The anti-nociceptive activity of *P. Americana* and *S. Hypoleuca* increased until the 60th min ($p < 0.05$ compared to morphine).

CONCLUSIONS: The results of this study support the extensive use of *S. Hypoleuca* and *P. americana* in Western Asia and America. The LD₅₀ of extracts *S. Hypoleuca* and *P. americana* following a 14 days acute toxicity study were calculated to be about 1800 and 208 mg kg⁻¹ i.p. respectability.

Key Words:

Antinociception; *Salvia Hypoleuca*, *Phytolacca Americana*, Hot plate method, aqueous methanolic extract.

Introduction

Pain is still one of the main health problems of the world's populations¹. Many bioactive substances are involved in the modulation of pain sensation². Eclectic physicians relied upon herbal medicines and natural remedies to treat disease³.

Phytolacca (P.) genus consisting one hundred and fifty species in particular the species of *Phytolacca americana* in Phytolaccaceae family⁴, that dried flowers are available in Eastern North America, northwestern areas of Iran and other parts of the world, they have been used to relieve pain and for the reduction of fever⁵⁻¹⁰. The boiled leaves are used in a popular salad (called grandmother's salad) in the American diet¹¹⁻¹³. It is well known for several medicinal properties, despite its toxicity, especially hepatotoxicity^{14,15}. *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^{16,17}.

Salvia (S.) is an important genus consisting of about 400 species in the *Hypoleuca* family 1. They are several reports that some *Salvia* species has effects on the CNS. *S. haematodes* has CNS-depressant, antinociceptive and anticonvulsant activities^{18,19}. The genus, *Salvia Hypoleuca*, is generally known for its multiple pharmacological effects including analgesic and anti-inflammatory activities^{20,21}, *S. leriifolia* has an effect on morphine dependence and hypoglycaemic effects as well^{22,23}.

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* and *S. Hypoleuca*, using the hot plate method in mice, as well as the determination of its median lethal dose (LD_{50}).

Materials and Methods

Animals

Male albino mice 25-30 g were obtained from a random bred colony, maintained on a special diet in the animal house of Medical Sciences University of Mazandaran. The animals had free access to a standard commercial diet and water *ad libitum* and were kept in rooms maintained at $25\pm 1^\circ\text{C}$ with a 12/12h light/dark cycle.

Drugs

Distilled water and other drug, morphine sulphate (Daru Paksh, I.R. Iran) and plant extracts were injected intraperitoneally in different doses and regimes.

Plant Material

Aerial parts (flowered browse) of *P. Americana* were collected from Mazandaran (a northern state in Iran) in April 2009, and *S. limbata* were collected from Tehran and were identified and confirmed by Dr. Gohari at the Drugs plants Sciences research center, School of Pharmacy, Medical Sciences University of Tehran. A voucher specimen (NO.783) have been deposited in Tehran School of Pharmacy Herbarium. Aerial parts of plants were dried at room temperature and coarsely ground before extraction. One hundred g of the powdered samples (*P. Americana* and *S. Hypoleuca*,) were extracted at room temperature by percolation with methanol/ water (80:20, 400 mL \times 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 extracts were 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. An aqueous methanolic extract of the aerial parts of *S. Hy-*

poleuca, was given at a doses of 500, 1000 and 1500 mg/kg i.p. to the animals, as a multiple dose. Anti-nociceptive activity was assessed by measuring the hot plate latency to heat, as described by Leimbach and Eddy²⁴. 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 Byahatti²⁵. Mice were placed in a thermostatically controlled hot plate apparatus (Tahgheh Gostran Teb, Mashhad, khorasan Razavi, IR) maintained at $52 \pm 0.5^\circ\text{C}$ 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 method²⁶. 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 of *P. americana*) and (100, 200, 400, 800, 1600, 2000 mg kg⁻¹ of *S. Hypoleuca*), 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.

Statistical Analysis

Statistical analysis was performed using the SPSS software for Windows (Ver. 10, SPSS Inc., Chicago, IL, USA). Data were analyzed by one-way analysis of variance (ANOVA) and presented as mean \pm sem. Student-Newman-Keuls test was used for statistical analysis and $p < 0.05$ was considered to be significant.

Results

Aqueous methanolic extract of the aerial parts (flowered browse at 190 mg kg⁻¹) of *P. Americana* and (flowered browse at 1000 mg kg⁻¹) of *S. limbata* produced a statistically significant in-

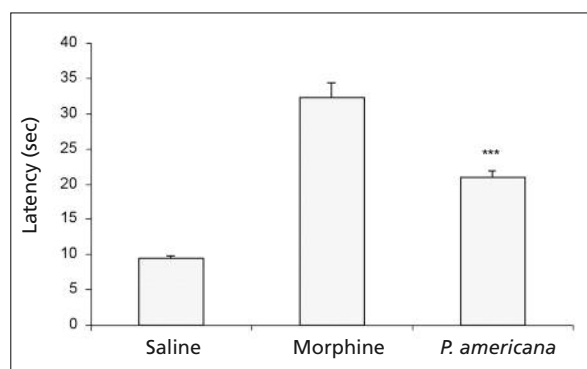


Figure 1. Anti-nociceptive activity of aqueous methanolic extract of *P. americana* aerial parts after 30 min. Values are presented as mean \pm SEM (n = 7), *** p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

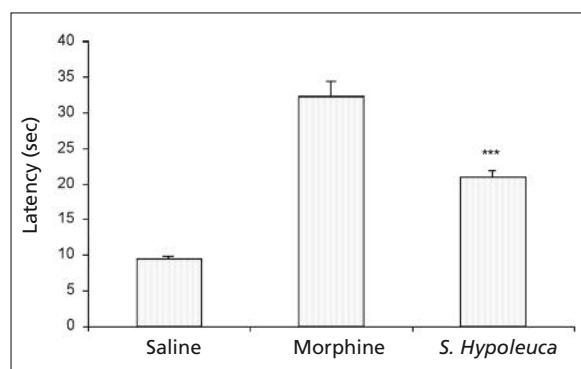


Figure 3. Anti-nociceptive activity of aqueous methanolic extract of *S. Hypoleuca*, aerial parts after 30 min. Values are presented as mean \pm SEM (n = 7), *** p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

crease in the pain threshold, after 30 min, in comparison with the control (Figures 1 and 3). The effect or activity was rather low special for *S. Hypoleuca*; 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 extracts increased until the 60th min. The p -value was greater than 0.05, compared to morphine (Figures 2 and 4).

Discussion

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 pain²⁷. The pain relief compo-

sition is prepared from roots of the *Phytolacca* family and in particular the species *Phytolacca americana*⁴. *P. americana* contains aromatase inhibitors and has antioxidant properties²⁷. A number of anti-inflammatory components have also been reported in *P. americana*^{5,10,28}. Among the constituents, oleanolic acid appears to be the most significant, with its anti-inflammatory and prostaglandin synthesis inhibitory properties²⁷. Our findings support the extensive use of this plant in America⁷⁻⁹. It is possible that the same components could lead to anti-nociceptive activity in our extract. This needs to be justified in future investigations. Based on our data, *P. americana* could be candidated as an analgesic agent. Although, the mechanism of plant action to increase anti-nociceptive activity in mice is unclear.

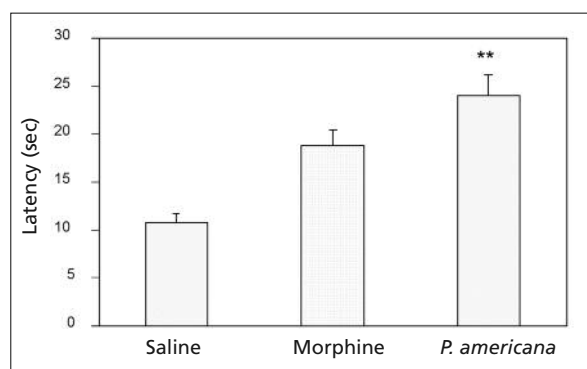


Figure 2. Anti-nociceptive activity of aqueous methanolic extract of *P. americana* aerial parts after 60 min. Values are presented as mean \pm SEM (n = 7), *** p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

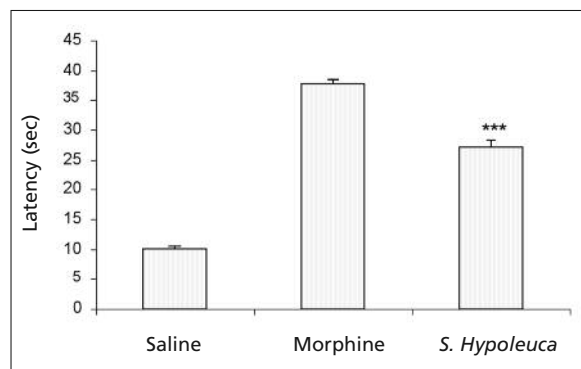


Figure 4. Anti-nociceptive activity of aqueous methanolic extract of *S. limbata* aerial parts after 60 min. Values are presented as mean \pm SEM (n = 7), *** p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

Phytolaccatoxin and the related triterpene saponins, believed to be the primary toxic constituents, are present in the berry juice and other plant parts²⁹⁻³¹. Several 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 discarded²⁹⁻³¹. The lethal dose 50% (LD₅₀) is most frequently used to characterize the response of animals, such as rats and mice, as a general indicator of an agent acute toxicity test³². Based on our data, the LD₅₀ values, after the 14 days acute toxicity study was calculated to be 204 mg kg⁻¹ i.p. the extract was of partial toxicity.

The present results indicate that the aqueous extract of *S. Hypoleuca*, has central antinociceptive activity, because it showed a significant antinociceptive effect in the hot-plate test and also its effect was inhibited by naloxone, a specific antagonist of opioid receptors. ***Antinociceptive and/or anti-inflammatory activities have been reported for some *Salvia genera* such as *S. hemaematodes*, *S. aethiopsis*^{22,33}, *S. leriifolia* leaf³⁴ and other genera³⁵. This study and other research on aerial parts of *S. limbata* also confirm that *Salvia genera* are good candidates for anti-inflammatory and analgesic uses of this plant in western Asia. With regard to the LD₅₀ value and in comparison with a toxicity classification³⁶, the extract was of low toxicity.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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