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RESEARCH ARTICLE



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ANTINOCICEPTIVE ACTIVITY OF THE AQUEOUS METHANOLIC EXTRACT OF WITHANIA COAGULANS IN MICE

Abstract

Objectives: The present experiments were undertaken to study the protective effect of withania coagulans(Wc)(family: Solanaceae) for antinociceptive activity by hot plate test in mice.

Material and Methods: A methanol/ water (80:20) extract of roots parts of withania coagulans was investigated for antinociceptive activity by hot plate test in mice.

Results: Results of the present study showed that the aqueous methanolic extract of roots parts of withania coagulans produced a statistically significant increase in pain threshold after 30 min of i.p. injection of extract, in comparison with the control groups, at adose of 50 and doses of 100 and 200 mg/kg(P<0.001) respectibility. The activity was comparable to that of morphine (30 mg/kg i.p., p>0.05). The anti-nociceptive activity of withania coagulans increased until the 60th min (P<0.05 compared to morphine).

Conclusions: The results of this study support the extensive use of withania coagulans in Western Asia and America. The LD50 of extract following a 14 days acute toxicity study were calculated to be about 400 mg kg-1 i.p.

Keywords: Antinociception, withania coagulans, Hot plate method, aqueous methanolic extract

INTRODUCTION

Many bioactive substances are involved in the modulation and relief of pain sensation(1).Eclectic physicians relied upon herbal medicins and natural remedies to treat disease². Withania coagulans Dunal belongs to family Solanaceae. It is distributed in the East of the Mediterranean region and extend to South Asia(2). Different parts of this plant have been reported to possess a variety of biological activities. Its berries are used for milk coagulation. The fruits are reported to be sedative, emetic and diuretic. Fruit has been shown to exert hepatoprotective, anti-inflammatory activity and hypoglycemic activity(3,4). They are useful indyspepsia, flatulent colic and other intestinal infections. They are employed for the treatment of asthma, biliousness and stranguary. The berries are used as a blood purifier. It is well known in the indigenous system of medicine for the treatment of ulcers, rheumatism, dropsy, consumption and sensile debility(5). Antifungal and antibacterial properties have also been demonstrated in extract of the whole plant and leaves (6,7). The purpose of this study was the evaluation of antinociceptive activity of an aqueous methanolic extract of the roots parts Withania coagulans, using the hot plate method in mice, as well as the determination of its median lethal dose (LD50).

Materials and methods Animals

Male albino mice 25-30 g were obtained from a random bred colony, maintained on a specialdiet 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 ± 1 °C with a 12/12h light/dark cycle.



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Drugs

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

Plant material

Root parts of Withania coagulans were collected from From Tehran and were identified and confirmed by Dr. Gohari at the Drugs plants Science sresearch center, School of pharmacy, Medical Sciences University of Tehran. A voucher specimen (0506-15) have been deposited in Tehran School of Pharmacy Herbarium. Root parts of plants were dried at room temperature and coarsely ground before extraction . One hundred grams of the powdered sample (W.coagulans) were extracted at room temperature by percolation with methanol/ water (80:20, 400 mL×3 times). The resulting extract was concentrated over a rotary vacuum vaporator, 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 apositive control). Solvent was injected to the negative control group (10 mL kg-1, i.p.). Anaqueous methanolic extract of the root parts of W. coagulans was given at a doses of 50,100and1200 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 Eddy(8). 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(9). Mice were placed in a thermostatically controlled hot plate apparatus (Harvard, UK), maintained at $52 \pm 0.5^{\circ}$ 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 (LD50)

Extract was dissolved in phosphate buffer (pH 7.4) and Tween 80 (4:1)(2) and was given as a single dose to mice intra peritoneally. Acute toxicity assays were conducted based on our recently published method(10). 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 W. coagulans), 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, 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

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Results

Results of the present study showed that the aqueous methanolic extract of the root parts (200 mg kg-1) of W. coagulans 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 high special for W. coagulans , 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 (Figure 2).



Figure 1.Anti-nociceptive activity of methanolic extract of W. coagulans root parts after 30 min. Values are presented as Mean \pm SD (n = 7), ***p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test)



Figure 2. Antinociceptive activity of aqueous methanol extract of W. coagulans root parts after 60 min. Values are presented as Mean \pm SD (n = 7), ***p < 0.001 with respect to control (ANOVA followed by Newman-Keuls multiple comparison test).

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DISCUSSION

Administration of aqueous extract of fruits of Withania coagulans Dunal significantly lowered the blood sugar, serum cholesterol, serum LPO, and hepatic LPO levels at the highest concentration of 1 g/kg; po in streptozotocin induced diabetic rats. In normal rats as well, the blood sugar levels were significantly decreased following treatment with the above drug⁷. Withania coagulans also exhibited free radical scavenging activity in an in vitro system(11). Fruit and root of Withania coaggulans are used in treating burns and infectious wounds, arthritis, inflammation and rheumatism(12).

Recently we have shown the high inhibition of morphine dependence in chloroform, Alcoholic and Ethyl acetate extracts of roots of Withania coaggulans. Alcoholic extract of roots of Withania coaggulans was safe and showed a high protective effect. The next portions, chloroform and Ethyl acetate extract were moderate protective effect on the development of dependence to morphine in mice. Two new withanolides, epoxywithanolide I and 17 β -hydroxywithanolide K have been isolated from the whole plant of Withania coaggulance and their structures were elucidated by spectroscopic techniques to be active against a number of potentially pathogenic fungi(4). The identification of Polyphenols as biologically active compounds has been previously reported(6,13).

The protective effect on the development of dependence to morphine and inflammation and pain relief composition are prepared from roots of the Withania family and in particular the species Withania coagulance(14,15).

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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