

Antinociceptive effects of *Rhus coriaria* L. extract in male rats

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Abstract It is well known that the tendency toward the medicinal plants is increasing in recent years. They have low side-effects and high varieties of efficient components. This study was designed to investigate the analgesic effect of hydro alcoholic leaf extract of *Rhus coriaria* (HRCLE) in a rat model. For this purpose, 42 adult male rats were divided into 7 groups: control, HRCLE (80, 100 and 300 mg/kg, i.p.), morphine (1 mg/kg, i.p.), aspirin (1 mg/kg, i.p.), and HRCLE 300 mg/kg plus naloxone (1 mg/kg, i.p.). The analgesic effects of HRCLE were assessed with writhing, tail flick and formalin tests. The data were compared with control by one-way ANOVA and Tukey post hoc test. All dose levels of HRCLE inhibited the number of contractions induced by acetic acid in the writhing test significantly. None of the dose levels of HRCE have been showed antinociceptive activity in the formalin test except the dose of 100 mg/kg (at chronic phase) and the dose of 300 mg/kg (at chronic– acute phase). In the tail flick model, the highest effect was at the dose of 300 mg/kg of HRCLE ($P < 0.01$). Utilization of naloxone plus extract inhibited the antinociceptive effect of HRCLE. In this study, our findings suggest that analgesic effect for the HRCLE may be mediated via both peripheral and central mechanisms. The presence of flavonoids might be responsible for the antinociceptive activity of this plant.

Keywords Pain · Formaldehyde · *Rhus coriaria* · Medicinal plants · Rat

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Introduction

Pain is a somatic sensation such as touch, pressure and proprioception. Always pain has been a serious challenge in medicine as which has an important protective role in avoiding or treatment of actual or potential tissue damages. Although, nonsteroidal anti-inflammatory or opioid drugs are mostly used to control pain, but these drugs have a many adverse effects and cause gastrointestinal and renal disorders sometimes. Therefore, most people looks for new drugs that have fewer side effects and are cheaper and easily available [1].

There is also increasing evidence that in traditional medicine prescribing medicinal plants to treat a pain and an inflammation is prevalently but the origin and structure of such plants have often remained unknown. Therefore, information about the pharmaceutical effects of these plants can be applied as a logical research approach in order to discover new drugs [2, 3].

It believes that plants belongs to Anacardiaceous family have a variety pharmaceutical effects as an analgesic, anti-inflammatory and antipyretic [4-6]. Sumac with scientific name *Rhus coriaria*, is a perennial plant that contain over 250 individual species of flowering plants belong to Anacardiaceous family [7], its name is derived from the word of sumaga that means red [8]. This plant have a latex stems, simple or compound leaves, small flowers, fruits and dense cluster [9] which grows over the wide in Mediterranean, Iraq and Iran.

Sumac is used in traditional medicine as an anti-bacterial, anti-spasmodic, anti-virus and anti-inflammatory agent and a drug for treatment fever, diarrhea, gastrointestinal diseases and dermatitis [10, 11]. It also has anti-microbial and anti-oxidant effects and these properties have been proven in modern medicine [12-14]. The old claim of traditional medicine for anti-inflammatory properties of sumac due to the strong relationship between inflammatory and pain processes. Previous studies have clearly not showed the analgesic effects of this plant. On this foundation, this study was

designed to investigate antinociceptive effect of hydro alcoholic leaf extract of *Rhus coriaria* using formalin, writhing and tail flick tests in rat model.

Methods

Plant material collection

Some of fresh *Rhus coriaria* leaves were prepared and authenticated by a botanist and then a voucher specimen number of the plant was deposited in the herbarium of the department of biology, faculty of basic sciences, Abu Ali Sina University of Hamadan, Iran. For preparation of hydro alcoholic extract, *Rhus coriaria* leaves were shed dried at room temperature in the shade and were pulverized mechanically using a grinder. One hundred grams of powdered *Rhus coriaria* leaf was placed in one liter of 80 % methanol for 72 hours to extract the required active ingredients. The obtained mixture was placed in a rotary device to remove the solvent and then to dehydrate the substance, it was put in a dish and under a hood for one week. After that, the residual material at the bottom of the container, extract, was dissolved in the appropriate amount of saline (0.9 % saline) to treat rats with different doses.

Animal experiments and drug administration

Forty-two adult male Wistar rats (200–250 g) were purchased from Pasteur's institute of Iran. The animals were housed three to four per cage and kept at a controlled temperature of 23 ± 1 °C under a light/dark cycle of 12:12 h with food and tap water available ad libitum. All experiments were conducted between 10:00 and 16:00. All rats were treated humanely and were conducted in concordance with the IASP guidelines on the use of laboratory animals [15]. The animals were randomly divided into seven equal groups ($n = 6$ rats per group): control, HRCLE (80, 100, and 300 mg/kg, i.p.), morphine (1 mg/kg, i.p.), aspirin (1 mg/kg, i.p.) and 300 mg/kg of HRCLE plus naloxone (1 mg/kg i.p.). Sulfate morphine, naloxone and aspirin were purchased from Darou Pakhsh (Iran), and acetic acid and formalin from Merck Inc (Germany).

Tests of pain

Writhing test: On the experiment day, 30 minutes before running the experiments, the animals were sent into a standard experiment glass box to get used to the conditions. The HRCLE was solved in sterile physiologic serum and injected intraperitoneally in doses of 80, 100, and 300 mg /kg. After 15 minutes, acetic acid on the scale of 1 mg/kg of the body weight with the density of

6 % was injected and immediately after the intraperitoneal injection of the acetic acid, the number of abdominal contractions was counted for 30 minutes (both legs stretched). It is also necessary to mention that each animal was used only once [16]. In the control group, after intraperitoneal injection of saline, the writhing test was run. It is worth mentioning, based on CPCSEA report in 2004 which was stated that laboratory animals used for the experimentation should be properly used and pain and sufferings inflicted in animals should be avoided or minimized if avoidance is not possible so, we proceeded on the basis that experimental procedures that cause pain or sufferings in human beings will also cause similar pain or sufferings in animals be minimize [17].

Tail Flick Test: The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat. It was first described by D'Amour and Smith in 1941 [18]. Most commonly, a light beam is focused on the animal's tail and a timer starts. When the animal flicks its tail, the timer stops and the recorded time (latency) is a measure of the pain threshold. For the tail flick test, we used a tail flick analgesimeter apparatus made in Borj Sanat Iran Company. Animals were separately put in a restrainer and 30 minutes after ingestion, the baseline reaction time was measured by focusing a light beam on the distal one-third portion of the animal's tail. Each 15 minutes interval, the reaction time was recorded until 2 hours. However a 15 seconds cut off time was used for preventing tissue damage. Percent of maximum possible antinociceptive effect was calculated for each time.

Formalin test: In this experiment, suggested model of Dubuisson and Dennis, was used in order to evaluate the chronic pain. One hour before the test, the animals were sent into the special box of formalin test in order to get used to the experiment condition. The box was made of Plexiglas in the dimensions of $30 \times 30 \times 30$ cm. Positioned in 45° , a mirror was inserted below the box and in front of the observer to observe the animal's behaviors more clearly. Thirty minutes after the intraperitoneal injection of the drugs, there was a subcutaneous injection of 50 μ l of 2.5 % formalin solution into the sub plantar surface of the left hind paw; then the animal was sent to the test special box again. The animal's behavior was observed and labeled for 60 minutes as following: once every 15 seconds, the motor response to pain was rated and recorded on a scale of 0, 1, 2, and 3. The numbers indicate the following reactions: number 0, the animal moves with complete balance and its weight distributed equally on both feet; number 1, the animal could not tolerate its body weight on the being-injected foot or take care of that foot; number 2, the animal raised the painful claw and has no contact with

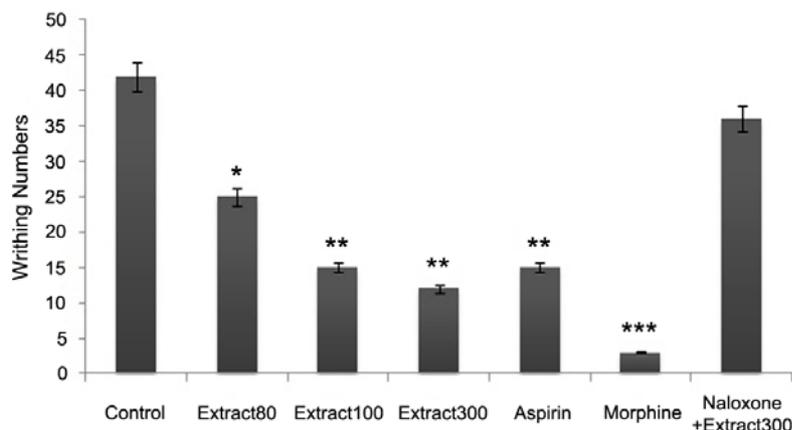


Fig. 1 Effect of different doses of hydro alcoholic extract of *Rhus coriaria* on acetic acid induced writhing in rats. As compared with control: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (n=6, mean ± S.E.M)

the box floor; and number 3, the animal licks the painful claw, chewed or moved severely. The average of first 5-minute grades was considered as phase 1 (acute phase) and the average of minutes 15 to 60 was considered as the phase 2 (chronic phase) [19].

Lethal dose (LD50)

The acute toxicity was determined by the previous laboratory model [20]. Various doses of the extract were injected separately and intraperitoneally to the male rats. The number of deaths of the animals was counted within the next 72 hours and the LD50 of the plant extract was determined.

Data analysis

All data were expressed as mean ± S.E.M. For analysis of data, one-way ANOVA followed by Tukey’s post hoc test was used and a calculated $P < 0.05$ was considered statistically significant.

Results

Writhing test: Statistical analysis of this study revealed that injection doses of 80, 100 and 300 mg/kg of the beneficial effect of HRCLE caused a significant reduction in comparison with the control group (with $P < 0.05$ and $P < 0.01$, respectively). As it was shown in Fig. 1, there was a very significant reduction in morphine and aspirin groups in comparison with control group ($P < 0.001$ and $P < 0.01$, respectively).

Tail flick test: According to Fig. 2, group of the HRCLE 300 mg/kg showed a very significant increase of tail flick latency when compared to the control group ($P < 0.01$). Injection of morphine and aspirin were increased tail flick latency as well as ($P < 0.001$ and $P < 0.01$, respectively).

Regarding formalin test, injection of 300 mg/kg of HRCLE was strongly decreased pain score in both acute and chronic phase in comparison with control group ($P < 0.01$), while injection dose of 100 mg/kg of HRCLE led to the significant decrease of the pain score in chronic phase ($P < 0.05$). Although, injection dose of 80 mg/kg of HRCLE was shown no significant change in pain score. Groups of aspirin and morphine were also significantly decreased pain score when compared to the

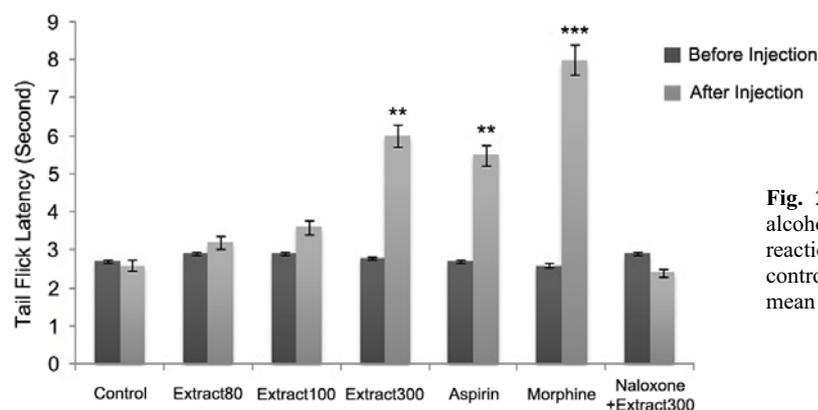


Fig. 2 Effect of different doses of hydro alcoholic extract of *Rhus coriaria* on the reaction time in rats. As compared with control: ** $P < 0.01$ and *** $P < 0.001$ (n = 6, mean ± S.E.M.).

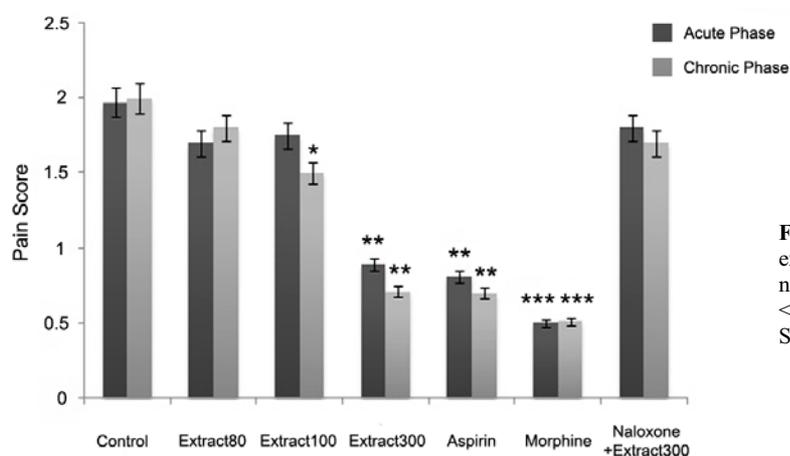


Fig. 3 Effect of different doses of hydro alcoholic extract of *Rhus coriaria* on formalin induced nociception in rats. As compared with control: * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ ($n = 6$, mean \pm S.E.M.).

control group ($P < 0.001$; Fig. 3). At the all of tests of pain, the treatment of naloxane plus HRCLE (300 mg/kg) inhibited the antinociceptive effect of HRCLE. There was also no significant change in weight of animals in each group. Lethal dose of the plant intraperitoneally was 5100 mg/kg.

Discussion

The proper management of pain has been identified as a primary indicator of quality assurance. Pain and substance abuse co-occur frequently, and each can make the other more difficult to treat [21]. Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin is widely used in the treatment of pain, but often of these cause gastrointestinal injury [22]. The present research indicates antinociceptive effect of hydro alcoholic extract of *Rhus coriaria* leaf (HRCLE) on an animal model. Standard tests of writhing, tail flick and formalin were used in order to investigate the antinociceptive effects of HRCLE.

One of the most important tests is writhing test which usually used to screen possible antinociceptive mixtures. In this test acetic acid is a chemical stimulation which is extensively used to evaluate peripheral antinociceptive activity [23]. The HRCLE prevented abdominal constriction caused by acetic acid therefore, it is imagined that its alleviative effects are supported by the environmental mechanisms. Intraperitoneal injection of acetic acid can cause the acute inflammation of the peritoneum [24]. In this model, it seems that peripheral antinociceptive effects of HRCLE are indirectly due to internal mediators such as bradykinin, serotonin, histamine, substance P, and prostaglandin. It is justified that all of these mediators are associated with the stimulation of peripheral nociceptive neurons [23].

The tail flick assay or tail flick test uses a high-

intensity beam of light aimed at a rodent's tail to detect nociception [18]. In this study, injection of the extract high dose decreased pain. Experimental tests of the tail flick testing method showed that the temperature of the skin of the tail plays a major role in the critical temperature, i.e., the temperature at which the tail flicks in response to pain. Since tail flick test performed to evaluate the spinal reflexes and the central analgesic pathways [25] therefore, it seems that the antinociceptive effect of the extract involves a central nervous component which may be elicited from several defined areas in the CNS.

Among the several models of persistent nociception, formalin test has well been established as a valid model for screening of anti-inflammatory and antinociceptive agents that act through central pain route from peripheral pain [26]. Intraplantar injection of formalin evokes signs of nociception such as flinching and licking of the injected paw early (phase 1), and subsequently a quiescent period was characterized by fewer pain behaviors and late-hyperalgesic components (phase 2) that last for approximately 1 hour. The early phase or neurogenic nociception results direct activation of peripheral nociceptors whereas the late phase due to inflammatory nociception that reflect induction of a spinal state of facilitation, central sensitization, development of inflammation and enlargement of receptive fields and also the concurrent presence of low level input from both large and small afferents [27]. The results showed that HRCLE have an inhibitory effect over the pain. The HRCLE under investigation in this study showed antinociceptive activity in both phases of formaldehyde-induced pain in rat. It was found that its decreasing effect is more potent in the chronic phase than the acute phase. Inhibition of the chronic phase of the formalin test by HRCLE can be a result of inflammation, so that part of the antinociceptive effect seems to be mediated by releasing compounds like prostaglandins F 2α and E 2 in some amounts sensitized by central nociceptive neurons [28].

To evaluate opioid system interference with antinociceptive effect of this extract, we were used one of the antagonist drugs in opioid system i.e., naloxane, which prevents the activation of opioid receptors [29]. The results indicate that naloxone attenuates the antinociceptive effect of HRCLE. Therefore, it seems that the effect of HRCLE in pain relief is due to the opioid receptors.

However, the analgesic activity of HRCLE is mediated by opioid receptors and may produce the dependence effect similar to opioids therefore, the further studies and suitable methods need to be established for the identification the dependence effects.

Biologic or therapeutic activity of herbs has a close relationship with their chemical combinations [30]. It has been known that *Rhus coriaria* contains several compounds such as organic acids, phenolic acids and derivatives, hydrolysable derivatives, anthocyanins and derivatives, flavonoids and isoflavonoid derivatives, terpenoid derivatives and other compounds [10, 31, 32] which possess antioxidant, anti-inflammation and antinociception properties [33]. The active ingredients or major bioactive components of *Rhus coriaria* which produce morphine-like antinociceptive effect are the flavonoids and isoflavonoid derivatives. A total of 61 flavonoid derivatives were detected and characterised in sumac. Apigenin-7-*O*-(6"-*O*-galloyl)- β -D-glucopyranoside which corresponds to apigenin in structure. This compound was reported as an active compound in Euphoria. In the same manner, dihydrotamarixetin galloyl-hexoside was tentatively proposed as this effect [31, 32]. As far as we know, there is no alkaloids compound with structures similar to morphine in this plant [10, 30, 34].

Previous studies have been shown that inhibition of N-methyl-D-aspartate receptor cut intracellular calcium down. Consequently, the synthesizer enzyme of calcium-related nitric oxide and phospholipase A₂ decreases too and with the reduction of nitric oxide and prostaglandins, especially the prostaglandin E₂ and F_{2 α} reveals its antinociceptive effects [35]. Many flavonoids and tannins are able to chelate free radicals such as hydroxyl radicals and subsequently dismutate ROS. In this regard, there are reports that tannins have roles in creating antinociceptive and anti-inflammatory effects [36]. Therefore, another part of antinociceptive effect of this extract is due to tannins inside the plant.

Conclusions

The results of the present study suggest that the antinociceptive effect of this extract may be due to their content of flavonoids. In this study reduction in writhing, increase in tail flick and inhibition of both phases of the formalin test approved antinociceptive effect of *Rhus coriaria*.

Finally, we conclude that extract possess analgesic effect that is probably due to inhibition of prostaglandin synthesis and inhibition of the central and peripheral nervous system. So this extract could potentially be used to control the painful disease.

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Conflict of interest The authors declare that they have no conflict of interest.

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