

Full Length Research Paper

Evaluation of topical antinociceptive effect of *Artemisia absinthium* extract in mice and possible mechanisms

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This study, evaluated the topical antinociceptive effect of dried extract of *Artemisia absinthium* in mice, and some mechanisms underlying these effects were also investigated. Four concentrations (1, 2.5, 4, and 6% W/V) of dried extract of the plant in normal saline were evaluated for topical analgesia in tail flick model test. The mice tail was immersed in normal saline supplemented with different concentrations of extract as well as normal saline as control for 2 min before tail flick test. Atropine (5 mg/kg), metoclopramide (1 mg/kg), ondansetron (0.5 mg/kg) and naloxone (4 mg/kg) were injected intraperitoneally (IP), 20 min before tail immersion in normal saline containing the extract at 4% concentration. Subsequently, maximum possible effect percentage (MPE%) was calculated for each dose. It was found that the plant extract produced antinociceptive effect at (4 and 6% W/V) concentration in tail flick model. Furthermore, analgesic effect of extract at 4% W/V concentration was significantly attenuated by pretreatment with atropine, metoclopramide, ondansetron and naloxone. These results suggest that the extract produced antinociception in tail flick model probably through cholinergic, serotonergic, dopaminergic, and opiodergic system.

Key words: *Artemisia absinthium*, antinociceptive effect analgesia, mechanism of action.

INTRODUCTION

The traditional medicine has used medicinal plants to relieve pain without noticeable side effect. Therefore, it can introduce novel drugs with less complications and cost. Pain is described as a discomforting feeling in a certain part of the body. The obligation of medical science is to maintain human health and ease the pain. Understanding the concept of pain, therefore, is one of the most essential

tools in realizing these goals. Since pain is considered an indicator of disease, all around the world and in all cultures, it is the most common symptom that compels patients to visit doctors (Kruger, 2001). Different classes of drugs have been considered and used for pain relief. Opioids and non-steroidal anti-inflammatory drugs were included. Currently, the side effects of these drugs are

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causing problems in treatment (Katzung et al., 2007). Analgesics, particularly medicinal plants, which have less side effects and addictive characteristics, can gain importance. Localized treatments have many advantages compared to systemic drugs. In limiting the drugs contact to location, central side effects may reduce significantly. As for opioids, this effect can limit side effects such as reduced irritability (sedation), respiratory depression and nausea. Also, for non-steroidal anti inflammatory drugs (NSAID), side effects of the gastro intestinal system reduces (Katzung et al., 2007). One of these medicinal plants is *Artemisia absinthium* (Wormwood). The medical use of this plant dates back to at least Roman times (Lachenmeier, 2010). The plant was used as anti-diarrhea (Zargari, 1989), antihelmintic (Lopes-Lutz et al., 2008; Tariq et al., 2009; Tariku et al., 2011), and antimicrobial (Lopes-Lutz et al., 2008). Anti-proliferative effects on human breast cancer cells were recently studied by Shafi et al. (2012), who showed that absinthium possess potent antioxidant properties, and may be used as a protective agent against disorders associated with oxidative stress (Canadanovic-Brunet et al., 2005; Bora and Sharma, 2011), neuroprotective and may prove to be useful adjunct in the treatment of stroke (Bora and Sharma, 2010).

Its use has been claimed to remedy cure indigestion and relieve gastric pain (European Medicines Agency, 2009). A recent double blind study by Omer et al. (2007) suggested that Wormwood may help patients with Crohn's disease, because of its steroid-sparing effects.

The systemic analgesic and anti inflammatory effects of this drug have been previously evaluated either by Fayyaz et al. (1992) and Sadeghifard and Zareian (2008). This study assessed its topical analgesic effect in mice. The mechanism of action was also discussed. The local name of tested plant is Afsantin.

METHODOLOGY

Plant and preparation of plant extract

A. absinthium L. is a member of the Asteraceae or Compositae family. The plants were planted and grown in Avicenna Medicinal Plants Research Station of Hamadan Agricultural and Natural Resources Research Center, Hamadan, Iran. The plant recognition and identification was carried out by herbarium of the research center. The leaves and inflorescence of the plants were harvested in two plant growth stages including: before opening flowers in May and last stage of flower maturity in late August. The selected parts of these plants were then dried in shade at temperatures between 21 and 30°C for 15 to 30 days, after which these parts of plants were chopped and ground. 100 g of the plant was mixed with 120 ml of 8% acetic acid for two weeks and the extract was obtained through pressing and grinding of the plant using stainless steel press. The extract was concentrated at room temperature. Different concentrations (1, 2.5, 4, and 6%) of the extract were prepared in normal saline.

Drugs

The drugs used were as follows: morphine, naloxone (T.D., Iran), metoclopramide (Tehran Chemie, Iran), ondansetron (EXIR, Iran), and atropine (Darou Pakhsh, Iran). All drugs were freshly dissolved in 0.9% saline for intraperitoneal (IP) injection. Morphine (10 mg/ml) was used topically.

Animals

Male Albino N-MR (Institute of the Razi, Tehran) weighing 25 to 32 g was used in the experiments. All experiments were performed in accordance with institutional animal use guidelines. The animals were housed in standard stainless steel cages in a controlled room temperature (22±2°C) with a 12/12 h light/dark cycle. Mice were divided into 9 groups. Each group contains 8 animals.

Nociceptive assay

Antinociception was assessed using the tail flick apparatus. The tail withdrawal latency (s) was measured before administration of any drug or vehicle. Normal response latencies were usually between 2.5 and 3.0 s. An 8-s cut-off was used to prevent tissue damage. The response was tested at 10, 20, 30, 60, 90 and 120 minutes after drug administration, respectively. All drugs were injected (IP, 0.1 ml/10 g) 20 min before extract administration. After drug injection (Naloxone 1 to 5 mg/kg, ondansetron 0.5 mg/kg, atropine 5 mg/kg, metoclopramide 1 mg/kg), 3 cm of tail was put in the extract as treatment group or normal saline as control group for 2 min. Antinociception was quantified as the percentage of maximum possible effect (%MPE) using the method of Keil and Delander (2011).

The following formula was used to calculate %MPE:

$$\text{MPE (\%)} = 100 \times [(\text{test} - \text{control latency}) / (\text{cutoff} - \text{control latency})]$$

Statistic analysis

Statistical analysis was carried out using Statistical package for Social Sciences (SPSS) for Windows, version 16. The parametric test Mann-Whitney U and Kruskal-Wallis tests were used to compare different groups with control and different groups with each other, respectively. Trend analysis test was used for comparison between times in different groups.

RESULTS

Antinociceptive effect of *A. absinthium* was studied at different concentrations. It was found that at 1 and 2.5 concentrations, the extract did not show any analgesic effect until 4 and 6% concentrations. The difference of effects were significant between 4.6% concentration and lower doses and control ($P < 0.05$) (Figure 1). There is a significant linear trend for mean of MPE% to increase across the different time in both groups ($P < 0.05$) (Figure 1). Topical analgesic effect of morphine (10 mg/ml) was compared with absinthium 4 and 6%. There was no significant difference between them ($P > 0.05$) (Figure 1).

Analgesic effect of 4% concentration of absinthium was reduced after injection of naloxone, metoclopramide,

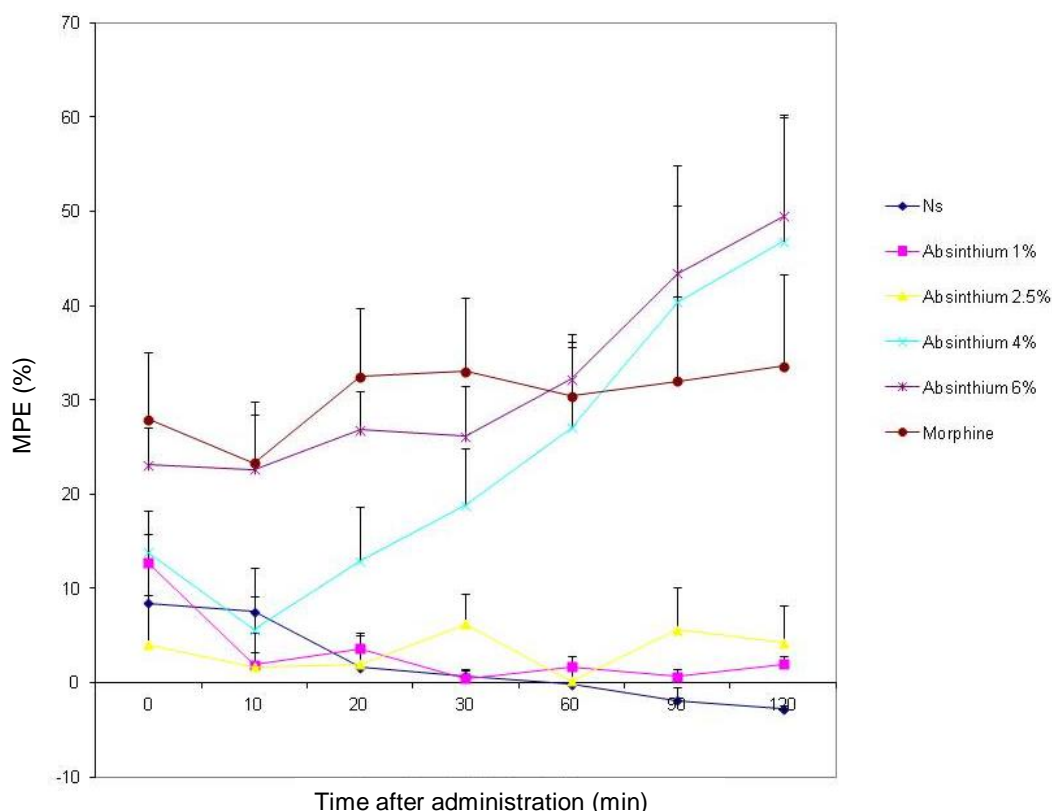


Figure 1. Comparison of different concentration of the extract with morphine 10 mg/ml and normal saline in mice by tail flick test. MPE: Maximum possible effect.

ondansetron and atropine ($P < 0.05$) (Figure 2). Naloxone inhibited analgesic effect of herbal extract at the 60, 90, and 120 min and metoclopramide at the same time with naloxone (Figure 2). Ondansetron had inhibitory effect at 30, 60, 90, and 120 min and atropine at the 90 and 120 min (Figure 2).

DISCUSSION

To evaluate the topical antinociceptive effect of *A. absinthium* extract on mice, different concentrations of the plant were prepared and tested. The plant is already known to remedy indigestion and lessen gastric pain (European Medicines Agency, 2009). The systemic analgesic effects of this extract have been previously evaluated by Fayyaz et al. (1992). The tail-flick response is believed to be a spinally mediated reflex (Chapman et al., 1985). Moreover, Grumbach (1966) has shown that the effectiveness of analgesic agents in tail-flick pain model is highly correlated with relief of human pain.

In this study, the extract showed antinociceptive effect at 4.6% concentration ($P < 0.05$), but did not show antinociceptive effect in lower doses. Thus, antinociceptive effect of the extract was dose dependent. The non-

significant effect between morphine (10 mg/ml) and the extract shows that it has antinociceptive effect as well as morphine. Fayyaz et al. (1992) had compared the systemic effect of absinthium extract with acetylsalicylic acid. In spite of rapid onset of analgesic action, the plant extract, even at high dose, showed less potent analgesia (both in terms of intensity and duration) when compared with acetylsalicylic acid. In this study absinthium extract showed similar effect (both in terms of onset and intensity) of topical analgesia when compared with morphine ($P > 0.05$). The anti-inflammatory effect of absinthium extract was assayed (Fayyaz et al., 1992). In the other study, it resulted that this extract may inhibit inflammatory cytokines such as TNF- α and NF kappa B (Krebs et al., 2010).

Antinociception exerted by *A. absinthium* extract in tail flick test appears to depend upon opioidergic neurotransmission, since the effect of these compounds was partially antagonized by the opioid receptor antagonist naloxone. The antinociceptive effects elicited by the extract were antagonized by atropine. This antagonism indicates a muscarinic receptor-mediated interaction in the antinociceptive activity induced by herbal extract and suggests a very important involvement of muscarinic cholinergic mechanisms in the expression of the antinociceptive

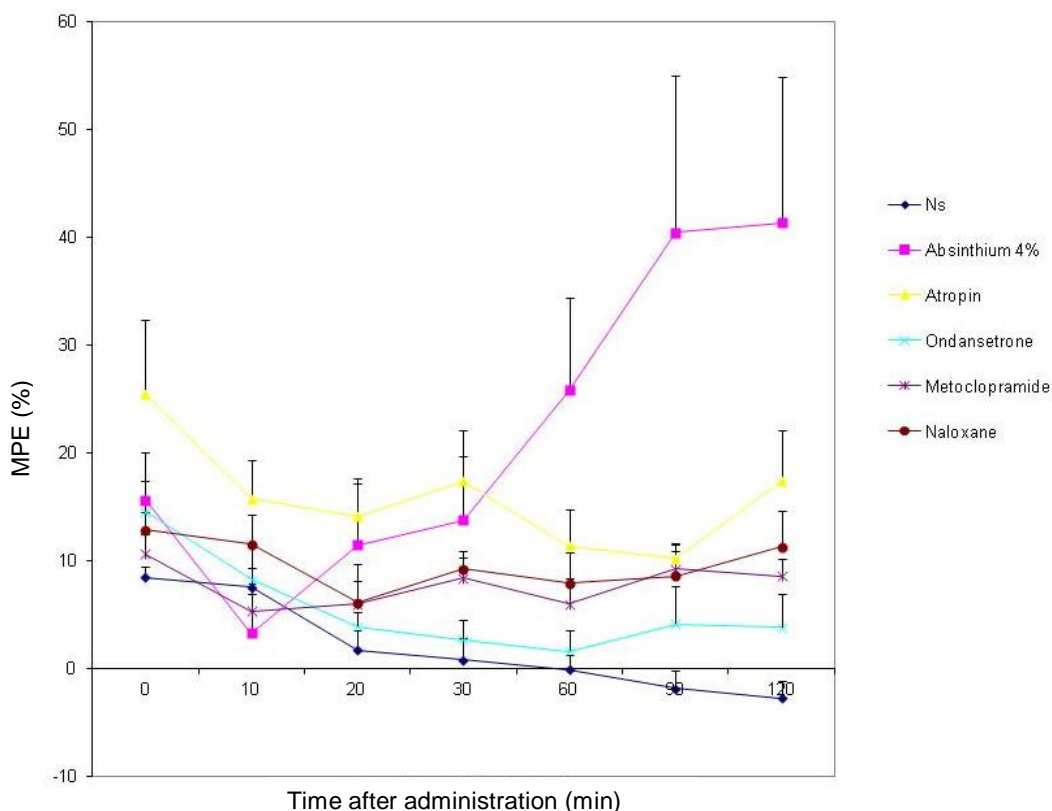


Figure 2. Comparison of absinthium 4% analgesia effect before and after pretreatment with drugs (naloxone 1 to 5 mg/kg, ondansetron 0.5 mg/kg, atropine 5 mg/kg and metoclopramide 1 mg/kg) by IP injection 20 min before the extract administration in mice. MPE: Maximum possible effect.

effect.

Several reports support a role for acetylcholine (ACh) in the inhibition and modulation of the nociceptive information transmission (Eisenach, 1999). Studies have shown the involvement of the dopaminergic system in mechanisms of antinociception. Of note, dopamine receptors agonists were described as facilitating analgesic response (Michael-Titus et al., 1990; Suaudeau and Costentin, 1995).

The present results show that the antinociceptive action of *A. absinthium* extract was attenuated by metoclopramide. Pivotal studies have shown a spinal analgesic action of 5-HT released from brainstem structures (Yaksh and Tyce, 1979; Yaksh and Wilson, 1979). In this study analgesic effect of the extract was inhibited by ondansetron, a serotonin antagonist.

Conclusion

A. absinthium extract has topical analgesic effect. Pretreatment with naloxone 1 to 5 mg/kg, ondansetron 0.5 mg/kg, atropine 5 mg/kg and metoclopramide 1 mg/kg reduced analgesic effect of the extract ($P < 0.05$). It

can result that these pathways may have important role in analgesic effect of *A. absinthium* extract.

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