



Asian Journal of Plant Sciences

ISSN 1682-3974

science
alert

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Evaluation of Analgesic Effects of Hydroalcoholic Extract of *Marrubium parviflorum* by Formalin Test in Mice

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Abstract: In this research, hydroalcoholic extract obtained from the aerial parts of *Marrubium parviflorum* (Lamiaceae) was subjected to evaluation of analgesic effects using formalin test at the doses of 50, 100 and 200 mg kg⁻¹ in mice. Duration of licking and biting time (min) of the injected paw was recorded at 5 min intervals for 40 min after formalin injection as a pain index. Results of study showed that the dose of 100 mg kg⁻¹ of the extract decreased duration of licking and biting time between 15 and 40 min, but this effect was not statistically significant ($p > 0.05$), while the dose of 200 mg kg⁻¹ of extract showed significant analgesic effects ($p < 0.05$) in the chronic phase (15-40 min) of formalin induced pain that this analgesic effect was equal to morphine. Considering that chronic phase of formalin induced pain is a secondary response to formalin induced inflammation, results of study introduce the *M. parviflorum* as a valuable analgesic herbal medicine that can be used in treatment of inflammatory painful disease. It is possible to assume that phytochemical contents of *M. parviflorum* reduce inflammatory pain by inhibiting the formation of inflammatory mediators such as prostaglandins followed by inhibiting COX-II enzyme.

Key words: *Marrubium parviflorum*, Lamiaceae, analgesic, formalin, pain

INTRODUCTION

The genus *Marrubium* from Labiateae family includes about 40 species all over the world (Rigano *et al.*, 2006). In traditional medicine some of these species are used to treat respiratory conditions, painful and inflammatory diseases and also as cholagogues and sedative agents (Meyre-Silva and Cechinel-Filho, 2010). Various activities such as antimicrobial (Hayet *et al.*, 2007), antioxidant (Weel *et al.*, 1999), anti-hypertensive (Bardai *et al.*, 2001), antidiabetic (Elberry *et al.*, 2011), antispasmodic (Schlemper *et al.*, 1996), hepatoprotective (Ahmed *et al.*, 2010) and analgesic effects (De Souza *et al.*, 1998) have been shown from this plant genus during pharmacological and biological investigations. On the other hand, phytochemical studies have confirmed occurrence of flavonoids (Nawwar *et al.*, 1989), phenylpropanoids (Sahpaz *et al.*, 2002), di- and triterpenes (Karioti *et al.*, 2005; Nicholas, 1958) and volatile oils (Baher Nik *et al.*, 2004) in *Marrubium* species.

Marrubium parviflorum Fisch. and C. A. Mey. is one of the ten species from this genus that grows in Iran (Mozaffarian, 2007). Previous studies have demonstrated presence of high level of phenolic compounds such as flavonoids and phenolic acids in the leaves of *M. parviflorum* and its potent antioxidant activities (Yumrutas and Saygideger, 2010) as well as bicyclogermacrene (26.3%), germacrene D (21.5%) and β -caryophyllene (15.6%) as major constituents of its essential oil (Khanavi *et al.*, 2005). It has been shown that *M. vulgar* L. (white horehound), another member of this genus, has significant analgesic effects in various pharmacological studies (De Souza *et al.*, 1998; Roghani, 2006). Because of close relation between phytochemical contents of the species belong to same genus and occurrences of flavonoids with established analgesic effects in this plant (Rylski *et al.*, 1979; Yumrutas and Saygideger, 2010) we assumed that *M. parviflorum* also may act as an analgesic agent. So, we

put this hypothesis to test by evaluating analgesic effects of hydroalcoholic extract of its aerial parts in mice by using formalin-induced inflammatory pain model.

MATERIAL AND METHODS

Plant material: Aerial parts of *M. parviflorum* were collected from Khalkhal region (Ardabil province, Iran) during its flowering stage in July 2009. This plant species has been identified by herbarium of Institute of Medicinal Plants (Academic Centre for Education, Culture and Research (ACECR), Tehran, Iran.

Extraction: Hydroalcoholic extract was obtained by maceration method from air-dried and grinded aerial parts of *M. parviflorum* (200 g) with 70% mixture of Methanol in water (3×1 L) for 72 h. The obtained extract was concentrated by using rotary evaporator at the maximum temperature of 45°C.

Animals: Thirty six male Swiss albino mice (25-30 g) were purchased from Razi institute (Karaj, Iran). The animals were kept in groups of six in standard polypropylene cages with easily access to water and food. The cages were housed in a room under standard laboratory conditions (24-26°C) with 12:12 h light/dark cycle. All experiments were carried out regarding to guidelines of Tehran University of Medical Sciences Ethics Committee.

Chemicals: Morphine hydrochloride was obtained from Sigma Company (England). Extract and morphine were all dissolved in normal saline, freshly on experimentation days, to prepare appropriate designed concentrations.

Formalin test: Potential analgesic effects of the Hydroalcoholic extract was evaluated using formalin test. Briefly, drug solutions (Hydroalcoholic extract of *M. parviflorum* (50,100 and 200 mg kg⁻¹), morphine (2 mg kg⁻¹) as positive control and normal saline as negative control) were administered intraperitoneally (IP), 15 min before injection of formalin. Formalin (2.5%) was administered subcutaneously (SC) into the dorsal surface of the right hind paw of the mice, then duration of the licking and biting time (min) of the injected paw was recorded at 5 min intervals for 40 min after formalin injection as a pain index. The responses were measured for two distinct phases, the initial 5 min after formalin injection is known as acute or early phase of formalin induced pain and the duration between 15 and 40 min as the chronic or late phase (Hunnskaar and Hole, 1987; Hunnskaar *et al.*, 1985; Rosland *et al.*, 1990; Tjolsen *et al.*, 1992).

Statistics: All data were presented as Mean±SEM. One-way ANOVA (analysis of variance) was applied to data analysis followed by Tukey's t-test. Differences between groups at the level of p<0.05 were considered statistically significant.

RESULTS

According to previous reports, intraperitoneal administration of morphine at the dose of 2 mg kg⁻¹, 15 min before the injection of formalin significantly inhibited both the acute and chronic phases of formalin-induced inflammatory pain (p<0.05) (Fig. 1a-d). Results of study showed that the dose of 200 mg kg⁻¹ from hydroalcoholic extract exerts significant analgesic effect (p<0.05) characterized by decreasing duration of licking and biting time in the chronic or second phase (15-40 min) of formalin induced pain. This analgesic effect was equal to morphine (Fig. 1d). Dose of 100 mg kg⁻¹ of the extract decreased duration of licking and biting time between 15 and 40 min, but this effect was not statistically significant (p>0.05) (Fig. 1c). All of administrated doses of extract have not showed any analgesic effects in the first or acute phase (initial 5 min) of formalin induced pain compared with morphine (Fig. 1).

Discussion: On the basis of previous pharmacological studies, formalin test, by providing two distinct phases of pain, is a useful model to determination of possible mechanisms of analgesic effects (Hunnskaar and Hole, 1987; Hunnskaar *et al.*, 1985; Rosland *et al.*, 1990; Tjolsen *et al.*, 1992). Drugs such as opioids which induced analgesic effects by affecting central nervous system inhibit both acute and chronic phases of formalin induced pain (Shibata *et al.*, 1989). While drugs such as indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs) inhibit chronic or late phase of the pain, only (Zvejniec *et al.*, 2006). In fact, chronic or late phase of formalin induced pain is a secondary response to produced inflammation as a result of subcutaneous formalin injection (Hunnskaar and Hole, 1987). According to the results of this study, analgesic effect of *M. parviflorum* was appeared at the dose of 200 mg kg⁻¹ in the chronic phase of induced pain. It is possible to assume that phytochemical contents of *M. parviflorum* reduce inflammatory pain by inhibiting the formation of inflammatory mediators such as prostaglandins followed by inhibiting COX-II enzyme. Probably, Marrubiin, a furane labdane diterpene, which has been recognized as the main analgesic compound present in *M. vulgare*, could be involved in the analgesic effects of this species (Jesus *et al.*, 2000; Meyre-Silva *et al.*, 2005). Furthermore,

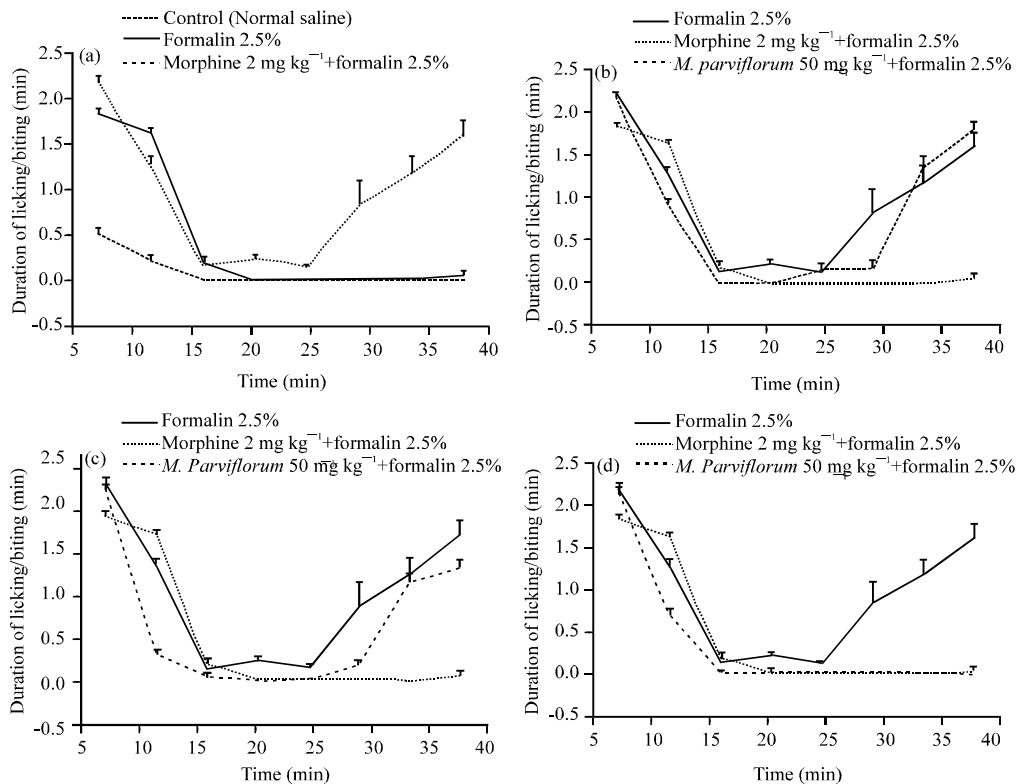


Fig. 1: Effects of hydroalcoholic extract of *Marrubium parviflorum* in different doses and morphine on formalin test. (a): Normal saline as negative control and morphine (2 mg kg^{-1}) as positive control, (b): *M. parviflorum* (50 mg kg^{-1}), (c): *M. parviflorum* (100 mg kg^{-1}) and (d): *M. parviflorum* (200 mg kg^{-1}). Morphine and extracts were administrated IP, 15 min before of the formalin injection. Data are expressed as Mean \pm SEM for six mice. The duration of licking and biting time (min) of the injected paw was recorded at 5 min intervals for 40 min after formalin injection

flavonoids and phenylethanoids compounds that its anti-inflammatory and analgesic activities have been confirmed during previous studies can also be effective in the appearing of this activity (Fu *et al.*, 2008; Kim *et al.*, 2009; Pelzer *et al.*, 1998; Rylski *et al.*, 1979).

CONCLUSION

Results of this study introduce the *M. parviflorum* as a valuable analgesic herbal medicine that can be used in treatment of inflammatory painful disease such as rheumatoid arthritis, inflammatory bowel disease (IBD), etc. Certainly, more studies on the analgesic effects of fractions and isolated compounds of *M. parviflorum* and on its toxicity properties can be useful to evaluating of use of *M. parviflorum* as an analgesic agent.

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