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Original Article

Analgesic and anti-inflammatory activities of hydro-alcoholic extract of *Lavandula officinalis* in mice: possible involvement of the cyclooxygenase type 1 and 2 enzymes

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ABSTRACT

Lavandula officinalis Chaix, Lamiaceae, extracts can inhibit inflammation and also pain induced by formalin in mice. This study evaluated the effects of *L. officinalis* hydro-alcoholic extract on pain induced by formalin and also cyclooxygenase (COX) type 1 and 2 activity in mice. To evaluate probable analgesic and anti-inflammatory effects of the extract, flowers were prepared by maceration and extraction in alcohol and their analgesic effects were studied in male mice, using formalin and hot plate tests. The effect of intraperitoneal hydro-alcoholic extracts of *L. officinalis* (100, 200, 250, 300, 400 and 800 mg/kg), subcutaneous morphine (10 mg/kg), dexamethasone (10 mg/kg; *i.p.*) and indomethacin (10 mg/kg; *i.p.*) on formalin induced pain were studied. Our results indicated that administration of the extract (100, 200, 250, 300, 400 and 800 mg/kg; *i.p.*) has inhibitory effects on inflammation induced by formalin injection into the animals hind paw. Moreover, this inhibitory effect was equal to the effects of morphine, dexamethasone and indomethacin. The extract in100, 200 and 300 mg/kg; significantly reduced heat-induced pain. The extract also reduced COX activity in dose dependent manner, where the inhibitory effect on COX1 activity was 33% and on COX2 activity was 45%. Here for the first time we show that *L. officinialis* extract can modulate pain and inflammation induced by formalin by inhibition of COX enzymes.

Introduction

Natural products are believed to be an important source of new chemical substances with potential therapeutic application. Several plant species were traditionally used as analgesics. In general, the herbal plant usage in treatment of disease and pain relief is one of the important strategies in medicine. *Lavandula officinialis* Chaix, Lamiaceae, commonly known as "Ostokhoddous", is indigenous to the Arabic, Mediterranean Coasts and Asia Minor (Ghelardini et al., 1999; Akhondzadeh et al., 2003). This plant, from the mint family, has small subdued green leaves, is 30–50 cm height with small purple flowers and has a rather pungent taste. *L. officinialis* is used in traditional and herbal medicine for the treatment of several gastrointestinal, nervous and rheumatic disorders (Leung and Foster, 1996). The chemical composition and pharmacological evaluation of *L. officinialis* has been the subject of several studies over the years. Most of these studies were focused on the extracts, fractions, and essential oils of the aerial parts and flowers of the plant. In pharmacological and biological tests, extracts, fractions, and essential oils of *L. officinialis* are reported to have antispasm and soporific, antitension, antioxidant, CNS-depressant, anticonvulsive, sedative, local anesthetic, antibacterial, and mast cell degranulation inhibitory effects (Ghelardini et al., 1999; Hohmann et al., 1999; Kim and Cho, 1999; Lis-Balchin and Hart, 1999; Shahriary et al., 2005). It is well accepted that *L. officinialis* extract contains linalool, acetate linalool, monotril, cezcoiterpen, luteolin, ursolic acid, coumarin, and umbelliferone (Kakkalou, 1988; Hajhashemi and Ghannadi, 2003; Barocelli et al., 2004). Hajhashemi and Ghannadi (2003) showed that the aquatic, alcoholic, and phenolic extracts of this plant have anti-nociception

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effects in the second phase of formalin test, but only the phenolic and alcoholic extracts had been able to prevent the first phase of the formalin test. Also application of this plant could not prevent the edema evoked by carrageenin administration (Hajhashemi and Ghannadi, 2003). It was proved that inhaling the leaves of the *Lavandula officinalis* could attenuate pain evoked by hotplate test, and stomach graze induced by high dose administration of ethanol and ascetic acid (Barocelli et al., 2004). Therefore, it seems that the components of *L. officinialis* have an important role in the reduction of the inflammatory pain. COX enzyme, responsible for the production of prostaglandins, is the key enzyme in causing inflammatory pain. Over-activity of COX in inflammation can produce peripheral sensitization of primary afferent. Three types of COX (1, 2 and 3) had been identified. Studies have shown that only types 1 and 2 had roles in inflammatory pain (Simmons et al., 2004). Non-steroid anti-inflammatory drugs such as indomethacin could induce their anti-inflammatory and analgesic effects by blocking the action of such enzymes (Payan, 1992). The purpose of the present study was to evaluate the analgesic and anti-inflammatory effect of the hydro-alcoholic extract of *L. officinalis* in mice using formalin and hot plate tests. In addition, the inhibitory effects of the extract on COX 1 and 2 activity was measured *in vitro*.

Materials and methods

Animals

Male NMRI mice with mean weight of 25–30 g were purchased from Pasteur Institute (Tehran, Iran). The animals were exposed to 12 h of daylight and were fed standard rat food and tap water (environment temperature, 23 ± 2 °C). In each group, six mice were studied. This study was conducted according to the standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Sciences Committee on the Use and Care of Animals, 87/381, July 25, 2009). The experiments were done in the light period (10 am–4 pm).

Preparation of plant extract

Dried flowers and head branches of *Lavandula officinalis* Chaix, Lamiaceae, were harvested in summer 2012 from the botanical farm of the Faculty of Medicine of Baqiyatallah (a.s.) University. The flowers were sent to the laboratory of Pharmaceutical College of Shahid Beheshti Medical University, assessed by M. Kamalinejad, and given the voucher number code of 408. Plant flowers and head branches were powdered and the extract was prepared using 100 g of the powder and 100% ethylic alcohol by maceration. The extract was separated and filtered by Whatman filter papers. The prepared extract was concentrated by vacuum evaporation and then was dried in low temperatures. The extract was kept in a capped bottle at 4 °C in a refrigerator for future use.

Estimation of plant extracts effective dose 50%

For this propose, the effect of different doses of the extract on formalin-induced pain were evaluated and the dose-response curve was drawn. The R^2 was calculated for the curve and was chosen as ED_{50}%. The doses used in this study are according to the ED_{50}% which was 185 mg/kg. The doses of the extract were chosen as ED ± 2 SD and then were corrected.

Formalin test

Formalin test was done according to the modified method of Dubuisson and Dennis, (1977). Each animal was placed inside a Plexiglas box with the dimensions of 30 cm × 30 cm × 30 cm (Length × width × height) after injection of formalin in plantar part of right foot. The position of the foot and the way animals responded to formalin (20 μl; 2%) injection were evaluated by observers and scored on a scale of 0–3 depending on the condition of the animal's foot. No pain and normal movement of the animal was scored zero. If the animal put its foot on the floor of the box but avoided putting its body weight on the injected foot (claudicating), it was scored 1. Score 2 was given to the animals that avoided putting their injected foot on the floor of box. Score 3 was documented when the rat bit or licked the injected foot, which was taken to be an indication pain. Each animal was injected with one of these drugs 30 min before the injection of formalin: *Lavandula* hydro-alcoholic extract, morphine, dexamethasone and indomethacin.

Determining the degree of inflammation

For determining the degree of inflammation induced by formalin (Ferreidoni et al., 2000; Ahmadiani et al., 2000), each animal's left foot was considered as the control foot, in which saline was injected. Animal's right and left feet were separately placed in a container that contained mercury and the exact weight of the foot was determined as follows. By calculating the weight change of mercury due to immersion of the left foot (control) and the right foot (test), foot weight changes were determined after formalin injection. This weight change was deduced from the volume change through dividing it by 13.6 (density of mercury). The anti-inflammatory effect of the chemicals was assessed in 5, 10, 20, 25 and 30 min intervals.

Assay of the effects of L. officinalis *extract on COX activity*

As mentioned in the previous section, interstitial fluid from the formalin-injected paw was collected using a fine needle (gauge 30) and was added to the ELISA kit which was poured in three wells by COX type 1 or 2 enzymes. After 30 min of incubation at 37 °C, the enzyme product was measured by an ELISA reader at 870 nm.

Hot-plate test

The hot-plate test was performed according to the method described by King et al. (2001). The animals were placed individually on a hot plate (Pars AZMA Co., Isfahan, Iran), maintained at 46 ± 1 °C and the time between the placement of the animal on the hot plate and the occurrence of either the licking of the hind paws, shaking or jumping off from the surface was recorded as the response latency. A cut-off time of 30 s was considered if the animal did not show any reaction to the painful stimulus.

Chemicals

The following drugs were used in this study: Morphine sulfate (Temad Co., Tehran, Iran), indomethacin hydrochloride (Sigma, USA) and dexamethasone (Sina Darou, Iran). Drugs were dissolved in saline and injected intraperitoneally to the animals in volumes of 10 ml/kg except for morphine which was given subcutaneously. The extract of *L. officinalis* was dissolved in physiologic saline and then injected intraperitoneally. In this study, morphine was used as a standard analgesic drug, indomethacin as a non-steroid anti-inflammatory drug and dexamethasone, as an anti-inflammatory drug were used to provide positive witnesses to compare the anti-inflammatory and analgesic function of the extract.

Experimental design

Experimental animals received subcutaneous morphine (10 mg/kg), or dexamethasone (10 mg/kg; *i.p.*), indomethacin