Physiology and Pharmacology

Physiol Pharmacol 20 (2016) 189-196 **www.phypha.ir/ppj**

Original Article

Anti-nociceptive effect of *Tanacetum Fisherae* on formalin-induced inflammatory pain in rats

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Abstract

Introduction: The management of pain and inflammation related problem is a real challenge that people face daily. Although several drugs are available for these conditions, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The objective of current study was to investigate the anti-nociceptive effect of *Tanacetum Fisherae* which has been traditionally used for treatment of pain.

Methods: In this experimental study, formalin test was performed with drug (*Tanacetum Fisherae*) or DMSO pretreatment 30 min prior to formalin injection in 40 male Wistar rats. Fifty microliters of 2.5% formalin was injected into the plantar surface of the right hind paw. Immediately after injection, licking and flinching number and paw-shaking responses were observed at 5-min intervals for 1 h. Animals were divided into five experimental groups. There were 8 animals in each group. Each group received vehicle (7% DMSO) or *Tanacetum Fisherae* essential oil (25, 50 or 100 µg) or morphine (5 mg/kg). Two-way and one-way ANOVA were used for data analysis. Differences were considered significant at the level of $P<0.05$ (with 95% confidence interval).

Results: Results showed that *Tanacetum Fisherae* essential oil dose dependently reduced licking and flinching number and also pain score in the late (15-35 min) and recovery phase (35-60 min) of formalin test ($p<0.05$, $p<0.01$, and $p<0.001$). It had no anti-nociceptive effect ($p>0.05$) in early (0-5 min) phase and interphase (5-15 min).

Conclusion: Results demonstrate the effectiveness of Tanacetum Fisherae to mitigate the inflammatory pain.

Keywords:
Pain;
Formalin test;
Tanacetum Fisherae

Received: 16 Apr 2016
Accepted: 14 Aug 2016

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Introduction

Pain is an essential sensation that usually signals tissue injury inflicted by external or internal damaging events (Sawynok, 2003). Inflammation is the body's normal response to injuries or infections (Medzhitov, 2008). It is an intrinsically beneficial event that leads

to the removal of initiating noxious stimuli or offending factors and the restoration of tissue structures and physiological functions (Medzhitov, 2008). Inflammatory responses play key roles in the development and persistence of many pathological pain states (Nathan, 2002).

Despite the availability of a large number of therapeutic options, potential side effects and modest efficacy limit the clinical usefulness of available analgesics agents (Nakamura-Craig and Follenfant, 1995). So an increasing number of studies are being carried out in search of new therapeutics from medicinal plants, especially those with proclaimed popular use as anti-inflammatory and analgesic agents (Gupta et al., 2006).

Recently, many natural medicines derived from medicinal plants were used as effective and safer for relieving inflammation and pain (Stevenson and Hurst, 2007).

The genus Tanacetum L. is belonging to the tribe Anthemideae of Compositae and consists of more than 160 species worldwide (Oberprieler et al., 2006). In Flora Iranica area, this genus is shown by 18 sections and altogether 54 species distributed commonly in Rocky Mountains (Podlech, 1986). Recently, some new species and records from different provinces of Iran have been found (Sonboli et al., 2010; Djavadi, 2008).

Tanacetum Fisherae with the common local name of Mofaroo is a perennial, aromatic and most scented subshrub plant native to Iran, Kerman province. Its strong aromatic odor is due to the presence of volatile oil especially 1,8-cineole. The essential oils composition and biological activities of some Tanacetum species have already been studied (Afsharypour and Jahromy, 2003; Esmaeili et al., 2010).

Tanacetum Fisherae can be distinguished by its pappus structure (entire, unilateral not divided to the base into 3–6 oblong scales), segments of pinnate leaves remote, terete and mucronulate (not approximate and linear), peduncle 3–15 cm long (not 6 cm), and margin color of involucral bracts dark brown (not brownish). *Tanacetum Fisherae* has densely hairy leaves, petiole longer than lamina, peduncle length up to 3–4 cm, diameter of involucre 4–5 mm and achene size 1.8–3.0 mm.

Aerial parts of *Tanacetum Fisherae* were used as a traditional herbal medicine for the management of gastrointestinal (GI) disorders also as a flavoring agent for many types of food products in Kerman (Rajaei and Mohamadi, 2012).

In view of the importance of medicinal plants as a potential source of cheaper, safer and effective treatment of diseases, and the traditional use of *Tanacetum Fisherae* in various disorders, and because 1,8-cineole has anti-inflammatory and analgesic effects (Santos and Rao, 2000) in the present study, we sought to investigate the analgesic effect of *Tanacetum Fisherae* by using formalin test in rats. Therefore the main question was study the Anti-nociceptive effect of *Tanacetum Fisherae* on formalin-induced pain in rats. We hypothesized that *Tanacetum Fisherae* could alleviate inflammatory pain.

Materials and methods

Preparation of *Tanacetum Fisherae* essential oil

Tanacetum Fisherae essential oil was obtained from Dr. Sonboli's Lab. Pure *Tanacetum Fisherae* was obtained from Kerman, Iran where the plant grows naturally. The aerial parts of *Tanacetum Fisherae* were collected at full flowering stage, from the Hezar Mountain, at an altitude of 3800 m, Kerman, Iran. A voucher specimen (MPH-1418) was deposited in the Medicinal Plants Research Institute Herbarium (MPH), Shahid Beheshti University, Tehran, Iran. Air-dried aerial parts (100g) of *Tanacetum Fisherae* were subjected to hydrodistillation for 3.5h, using a Clevenger-type apparatus and analyzed by Gas chromatography (GC) and Gas chromatography–mass spectrometry (GC-MS, Rajaei et al., 2011).

The distilled oil was dried over anhydrous sodium sulfate and stored in sealed vial at 4 °C until bioassay and GC-MS analyses. GC-MS analysis was carried out on a Thermoquest-Finnigan. Trace GC-MS equipped with a fused silica capillary DB-1column (60 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C /min, then held at 250 °C for 10 min; transfer line temperature, 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; split ratio, 1/50. The quadruple mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 µA (Rajaei et al., 2011).

DMSO was used as the vehicle for the essential oil. It is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water (Pope and Oliver, 1966).

Experimental animals

In this experimental study, forty adult male Wistar rats (weighing 250-300 g) of approximately the same age were used. They were grouped into 5 groups of 8 animals. Rats were held in standard condition with access to food and water ad libitum. They were held in cages made of Plexiglass (at most 4 rats were kept in each cage at each time) and in controlled temperature (22±2 °C) and in a 12-12 hour light-dark cycles. In order to avoid the effect of circadian rhythms, all experiments were performed at 9:00 a.m. to 11:00 a.m. All animal care and experimental procedures were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals used for Experimental Purposes, and the protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

The rats were used because their genetic, biological and behavior characteristics closely resemble those of humans, and many symptoms of human conditions can be replicated in rats. We studied on male rats because of female's cyclic hormones fluctuation. This hormones fluctuation might influence our experiment.

Formalin test

The formalin test was carried out as described by Santos and Calixto (1997), in five experimental groups: Group I, control group treated with 7% dimethyl sulfoxide (DMSO) intraperitoneally (i.p.); Group II, animals treated with *Tanacetum Fisherae* (25 µg, i.p.); Group III, animals treated with *Tanacetum Fisherae* (50 µg, i.p.); Group IV, animals treated with *Tanacetum Fisherae* (100 µg, i.p.); Group V, animals treated with Morphine (5 mg/kg, i.p.).

The drug was administered i.p. because of the ease of administration compared with other methods and similar bioavailability to intravenous (iv) injection and also to avoid drug degradation by gastric acid (Turner et al., 2011).

Animals were habituated for 20 min to an individual Plexiglas observation chamber (55 cm long×30 cm wide×26 cm high) before testing. A mirror was placed behind the chamber, which provided a clear view of the rat's paw movement. Formalin (2.5%, 50 µL) was injected subcutaneously via a 29-gauge needle into the plantar surface of the right hindpaw while the rat was manually restrained. Immediately following the injection, rats were returned to the observation chamber and individuals who were not familiar with the experimental conditions recorded the number of licking and flinches by the animal. Flinching was defined as a rapid and brief withdrawal or flexing of the injected paw. *Tanacetum Fisherae* essential oil diluted with DMSO was injected 30 min before formalin injection. The ability of the test compound to inhibit formalin-induced licking and flinches was measured as the number of liking and flinching incidences in a 5-min period for 60 min. Responses were determined for four characteristic time periods: 0-5 min (phase I, an initial acute state) and 5-15 (interphase), 15-35 min (phase II, chronic state), and 35-60 min (recovery phase) after formalin administration.

The response produced by formalin is biphasic: the first phase, usually within the first 5 minutes after formalin injection, this response is neurally mediated; then there is an interphase of about 10 minutes characterized by inhibitory pain mechanisms and the second phase (15–30 minutes). This response follows the release of inflammatory mediators (Hunskaar and Hole, 1987).

Briefly, the pain scoring measurements were as follows: 0 = normal weight bearing on the injected paw; 1 = limping during locomotion or resting the paw lightly on the floor; 2 = elevation of the injected paw; and 3 = licking or biting of the injected paw (Damaj et al., 1999). Recording of nociceptive behaviors began immediately following formalin injections (time 0) and was continued for 60 minutes. The first 5 minutes was considered as early phase and minutes 15 to 35 were considered as the late phase of formalin test. After 35 min, the recovery period was considered and pain related behaviors were evaluated till 60 min. The different behavioral parameters including flexing and licking were monitored as total counts per 5 minutes or total duration in seconds per 5 minutes, respectively (Damaj et al., 1999).

Pain related behaviors were recorded by individuals who were not familiar with the experimental conditions for avoiding the experimenter bias.

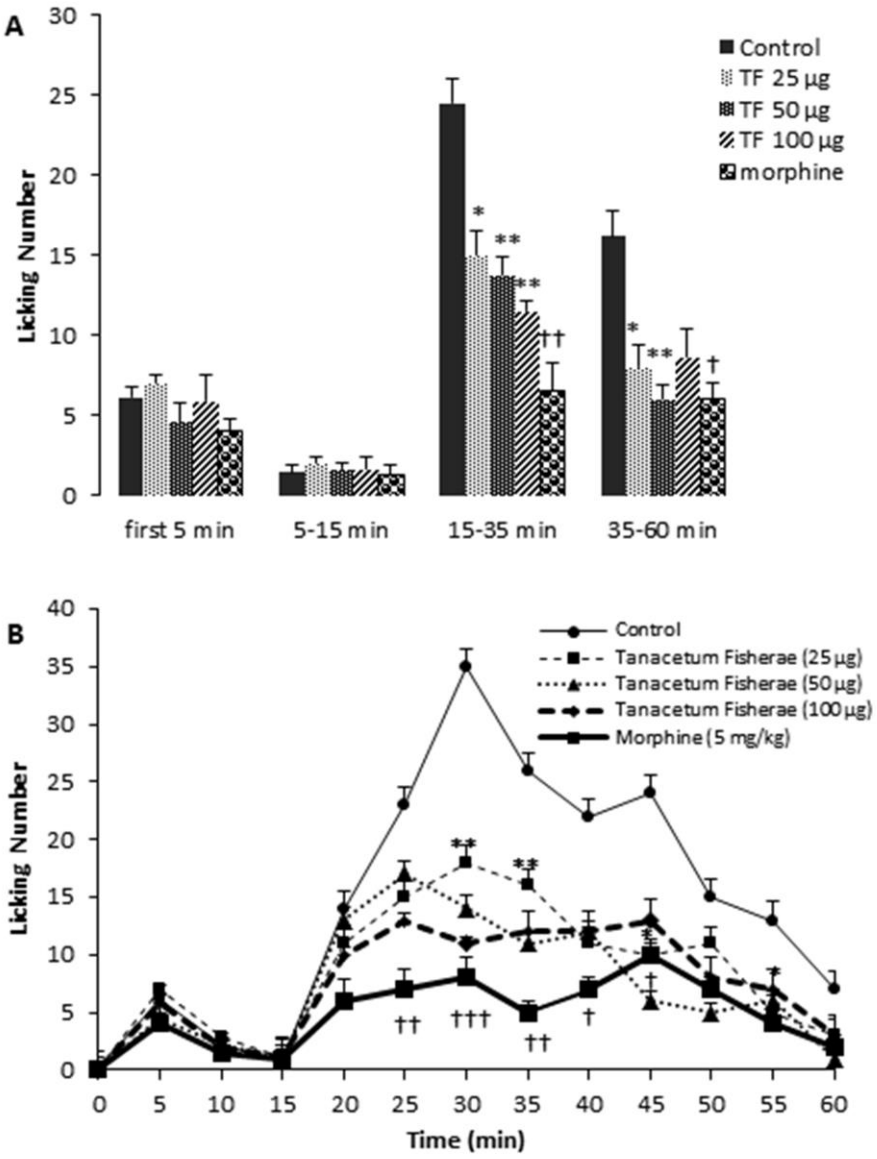

Fig.1. Effect of *Tanacetum Fisherae* on formalin induced licking response in rats. Animals were pretreated with *Tanacetum Fisherae* (25, 50, and 100 µg) or morphine (5 mg/kg) 30 min prior to formalin (2.5%v/v, 50 µl). Bar charts represent the mean of licking number in each phase: phase 1 (1–5 min), interphase (5–15 min), phase 2 (15–35 min), and recovery phase (35-60 min, A). Number of lickings was counted for 60 min with bin of 5 min interval (B), after formalin injection. Data are expressed as mean ± S.E.M. of 8 rats per group.*, † P<0.05; **, †† P<0.01 and, ††† P<0.001 vs. Control, two-way ANOVA followed by Bonferroni's posttest.

Statistical analysis

Data were presented as mean±SEM and processed by commercially available software GraphPad Prism® 6.0. Two-way and One-way ANOVA followed by post hoc analysis (Bonferroni test and Tukey's or Dunnett's test respectively), were used. Differences were considered significant at the level of P<0.05 (95% confidence interval).

Results

The results obtained revealed that there were no significant differences (ANOVA, p>0.05) in formalin induced pain related behavior at any time intervals between the intact (n=8) and vehicle (n=8) groups. Hence, in all experimental animals, the pain scores in formalin tests were compared to respective control group.

In this set of experiments, we evaluated the effect of

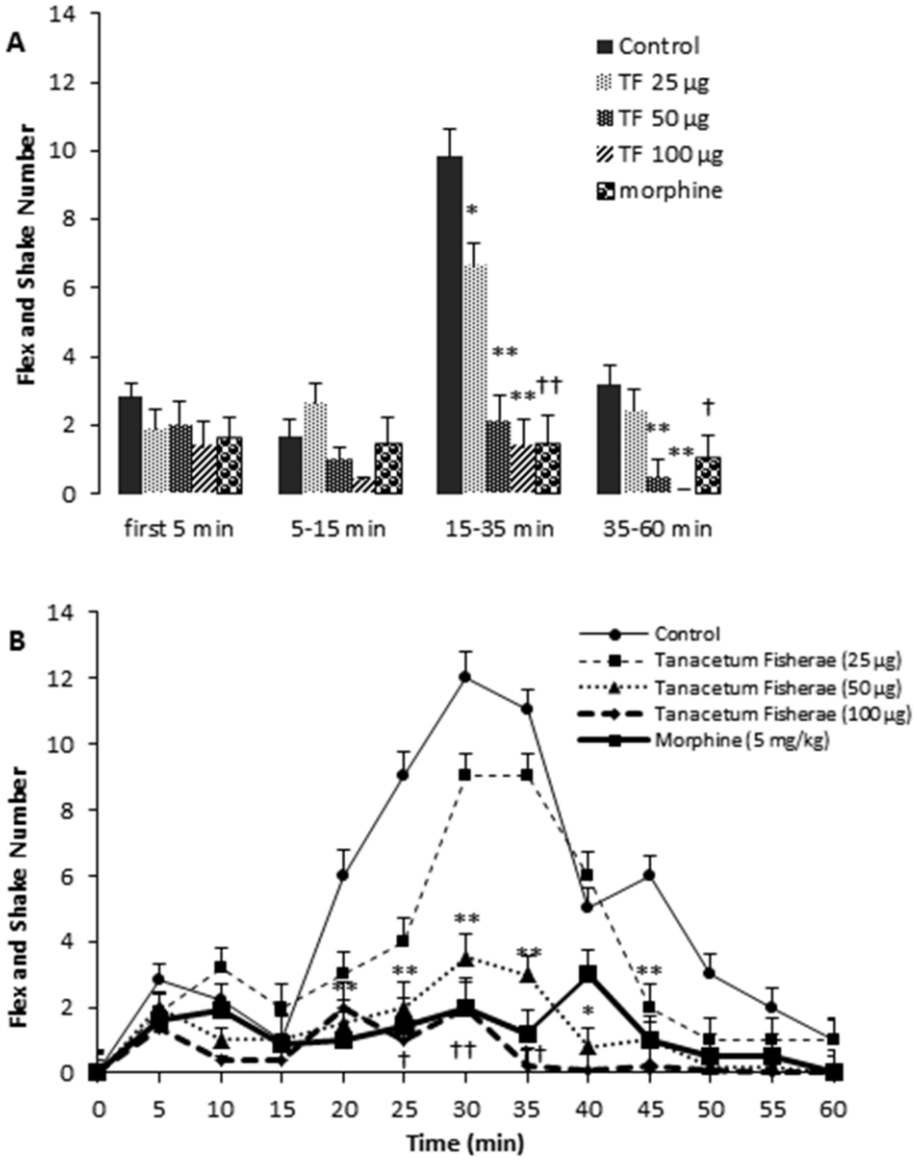

Fig.2. Effect of *Tanacetum Fisherae* on formalin induced flinching response in rats. Animals were pretreated with *Tanacetum Fisherae* (25, 50, and 100 µg) or morphine (5 mg/kg) 30 min prior to formalin (2.5%v/v, 50 µl). Bar charts represent the mean of licking number in each phase: phase 1 (1–5 min), interphase (5–15 min), phase 2 (15–35 min), and recovery phase (35-60 min, A) Number of flinches was counted for 60 min with bin of 5 min interval (B), after formalin injection. Data are expressed as mean ± S.E.M. of 8 rats per group.*, † P<0.05; **, †† P<0.01 vs. Control, two-way ANOVA followed by Bonferroni's posttest.

different doses of essential oil of *Tanacetum Fisherae* (25, 50, 100 µg; n = 8-10 rats in each group) on pain related behaviors including licking and flinching number and pain score during a 60 min period after formalin injection.

As shown in Fig. 1A and B, the administration of different doses of essential oil significantly reduced licking number in phase 2 (ANOVA, p<0.05, p<0.01). *Tanacetum Fisherae* did not have antinociceptive effect in phase 1 or interphase (p>0.05). Treatment with *Tanacetum Fisherae* also reduced licking number during recovery time (p<0.05). Pretreatment with morphine also reduced licking number (ANOVA, p<0.05, p<0.01). There was no significant difference between licking number in *Tanacetum Fisherae* and morphine pretreated rats (p>0.05).

Tanacetum Fisherae inhibited formalin induced flinching response in late phase (Phase 2) as compared to the vehicle. It showed no effect on the early phase, which is considered as a nociceptive phase (Fig. 2A and B). Flinching number reduced in late phase in animals treated with *Tanacetum Fisherae* essential oil and morphine (ANOVA, p<0.05, p<0.01).

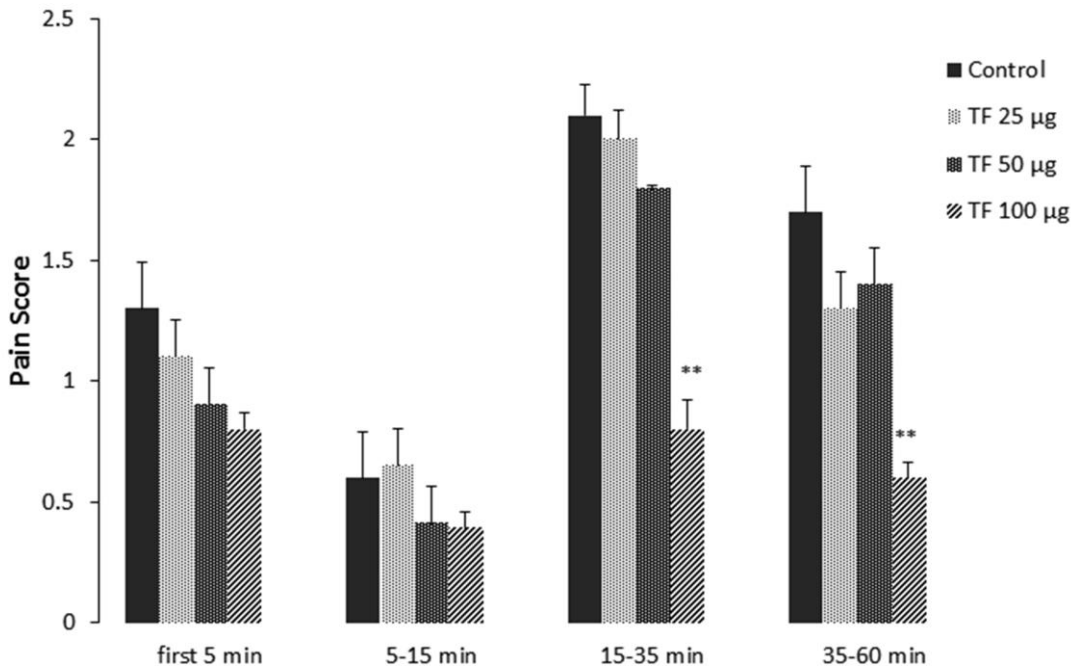

Fig.3. Effect of *Tanacetum Fisherae* on pain score. The columns represent the mean of nociceptive score in each phase: phase 1 (minutes 1–5), interphase (minutes 5–15), phase 2 (minutes 15–35) and recovery phase (minutes 35–60). Data are expressed as mean ± S.E.M. of 8 rats per group, ** P<0.01. one-way ANOVA followed by Tukey's test.

The anti-nociceptive effect of *Tanacetum Fisherae* was not significantly different from morphine effect (p>0.05). These results demonstrate the effectiveness of *Tanacetum Fisherae* mitigation of inflammatory pain. *Tanacetum Fisherae* with high dose (100 µg) also reduced pain score in late phase and recovery phase (p<0.01) but had no effect on pain score during first phase and interphase (Fig. 3). Effects of *Tanacetum Fisherae* were compared with morphine as a known analgesic substance. Its anti-nociceptive effect was as 72 percent as of morphine potency when different formalin induced behavior was considered.

Discussion

In the present study, we have shown that *Tanacetum Fisherae* in different doses modulates pain responses during late phases of formalin test. The anti-nociceptive effect of *Tanacetum Fisherae* is as 72 percent potent as morphine.

Over one-third of the world's people suffer from continuous pain (Loeser et al., 2001). Reliving of pain is probably one of the most common and yet most difficult problem in medical practice. Although many analgesics and anti-inflammatory agents have been developed, but there is considerable opportunity for conceptual innovation.

There is an increasing attention over herbal extracts due to their potentially positive action against certain diseases including cancer, diabetes, atherosclerosis, and coronary heart diseases (Kumar and Pandey 2013). Several species of the genus Tanacetum are traditionally used in a variety of health conditions including pain, inflammation, respiratory and gastrointestinal diseases (Bukhari et al., 2007; Rajaei and Mohammadi, 2012). The results of the present study demonstrated significant anti-nociceptive effects of *Tanacetum Fisherae* essential oil in the second phase of the formalin test at different doses.

Formalin test is known as an appropriate method for producing and quantifying the chemical pain in the rat model. In this test, pain intensity is determined based on some objective behavioral categories and the observations are converted to numerical values ((Dubisson and Dennis, 1997). Formalin test first described by Dubisson and Dennis (Dubisson and Dennis, 1997) has been represented as the most predictive of acute pain (Le Bars et al., 2001) and a valid model of clinical pain (Costa-Lotufo et al., 2004; Vissers et al., 2003). The formalin test, is a well-characterized method in preclinical screening of analgesics (Vissers et al., 2003; Abbott, 1988). Intradermal injections of formalin into the rat paw

resulted in a biphasic nociceptive response evidenced by flinching, licking or biting of the injected paw as reported earlier (Dubisson and Dennis, 1997; Wheeler-Aceto et al., 1990). An analgesic drug would tend to reduce the incidence of flinching, licking or biting of the injected paw (Courteix et al., 1998). It is suggested that the first phase of the formalin response is due to the direct stimulation of nociceptors by formalin, sensitive to central analgesics (Chau, 1989; Le Bars et al., 2001; Szolcsanyi et al., 2004) whereas the second phase involves inflammatory components with the release of different pain mediating substances that possibly activate small afferents (Le Bars et al., 2001; Malmberg and Yaksh, 1992; Yashpal and Coderre, 1998).

We used formalin induced pain model for evaluating antinociceptive effect of *Tanacetum Fisherae* in rats. Our data demonstrated that *Tanacetum Fisherae* has potent antinociceptive effects in second phase of formalin induced pain based on the results of this study, it can be concluded that *Tanacetum Fisherae* essential oil possess analgesic and anti-inflammatory properties. These findings justify the use of the plant in traditional medicine in the management of pain and inflammation related diseases.

Injection of this essence before formalin injection greatly influenced pain behavior. In this study three doses (25, 50, 100 µg) were used. The analgesic effect was small at low dose (25 µg) but by increasing dose to 50, 100 µg, analgesic effects of *Tanacetum Fisherae* were greatly enhanced. It seems that *Tanacetum Fisherae* essential oil has compounds with analgesic effects especially by decreasing the inflammation. The lower dose than 25 µg and higher dose than 100 µg were also studied. The lower dose had no significant effect and the effect of higher dose was not different from 100 µg.

Chemicals in Tanacetum may reduce the body's production of substances that initiate and prolong inflammation. They may prevent platelets from releasing inflammatory substances and also may reduce the body's production of prostaglandins. The presence of various compounds in Tanacetum including caffeic acid, ferulic acid, luteolin, apigenin, rutin and also 1,8-Cineole has been shown (Esmaeili et al., 2010). Caffeic acid is found in the plant leaves and it is reported that this compound has anti-inflammatory activity (Da Cunha et al., 2004; Norata et al., 2007) and might be considered for antinociceptive effect of *Tanacetum Fisherae*. Eleven components representing 99.9% of the total oil were identified. 1,8-Cineole (79.9%) was characterized as the principal compound. It has been shown that1,8-cineole has anti-inflammatory and analgesic effects (Santos and Rao, 2000). So the effect of *Tanacetum Fisherae* to reduce pain related behavior may be attributed to this compound. Further studies are needed to determine the exact effective compound of the *Tanacetum Fisherae* essential oil and determine the effective dose and possible side effects.

Conclusion

Based on the obtained result it can be concluded that *Tanacetum Fisherae* essential oil has anti-inflammatory effects and reduces pain responses dose dependently during late phases of formalin test.

Acknowledgments

This article has been extracted from the thesis written by Mr. Fathi (Registered Number: 356), medical student of Shahid Beheshti University of Medical Sciences. The Authors would like to thank Dr. Gila Behzadi for her scientific comments.

Conflict of interest

The authors have no conflict of interest to declare.

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