In vivo analgesic and anti-inflammatory effects of the essential oil from *Tanacetum balsamita* L.

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Tanacetum balsamita locally called Shahesparam is an aromatic plant that grows widely in Azerbaijan Province, Iran. Due to the widespread use of *T. balsamita* as a pain killer and relief of inflammatory based disorders in Iranian folk medicine and considering the high content of essential oil in *T. balsamita* aerial parts, we were prompted to investigate the anti-nociceptive and anti-inflammatory properties of *T. balsamita* essential oil (TBEO) for the first time. The carrageenan-induced Paw Edema was used for inflammation evaluation in rat, and hot-plate method was used for pain assessment in mice. Different doses of TBEO were administered. Morphine and Mefenamic acid were used as the standard drugs in anti-nociceptive and anti-inflammatory evaluation tests, respectively. TBEO (100 mg/kg) showed significantly anti-nociceptive activity in hot-plate test. The anti-inflammatory activity of TBEO was found to be more than mefenamic acid. The studied oil was analyzed by GC and GC–MS. The major component of the oil was characterized as carvone (39.8%) which might be responsible for the observed activities. The results suggested that TBEO possessed biologically active constituents that had significant analgesic and anti-inflammatory effects which support the ethno-medicinal claims of the plant application in the management of pain and inflammation.

Keywords: *Tanacetum balsamita.* Essential oil. Analgesic. Antiinflammatory.

INTRODUCTION

Nowadays in spite of great progress in pharmaceutical sciences and synthesis of many drugs, pain control is still a problem. Opioid drugs have high analgesic properties but dependency precedes this pain reduction after a while. Non steroid anti-nociceptive and anti-inflammatory drugs, regardless of good efficacy, are also associated with different side effects including gastro intestinal problems. Hence, traditional medicine and herbal therapy have always been under consideration especially in countries with ancient history like Iran.

The genus *Tanacetum* (Compositae) consists of 26 species in Iran one of which is *T. balsamita* L. (Mozaffarian, 1996). *T. balsamita* locally called Shahesparam is an aromatic plant that grows widely in Azerbaijan Province, Iran. It has been used in Iranian folk medicine as a pain killer, tranquilizer and cardiac tonic

(Nickavar, Amin, Mehregan, 2010). Previous studies reported that the *Tanacetum* sp. oil possessed digestive, diuretic, antitussive, analgesic/anti-inflammatory and antimicrobial effects (Lotfipour *et al.*, 2008; Yousefzadi *et al.*, 2009).

Also, *T. parthenium* extract has been reported to be an effective remedy for the prophylactic treatment of migraine, as it inhibits platelet aggregation, histamine release from mast cells (Johnson *et al.*, 1985), and the production of prostaglandins, thromboxanes and leukotriens (Hayes, Foreman, 1987).

The *Tanacetum* sp. oil are used in Iranian traditional medicine for health problems such as inflammation, infection and urinary tract disorders (Lotfipour, Nazemiyeh *et al.* 2008; Yousefzadi, Ebrahimi *et al.* 2009). In addition to traditional medicinal usage, Shahesparam can also be used commercially in the food industry. In Iran, a tea prepared from *T. sonboli* is used to treat intestinal disorder, such as diarrhea. In addition, some of the medicinal *Tanacetum* species such as *T. microphyllum* and *T. larvatum* are useful for treating various inflammatory

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disorders (Abad, Bermejo, Villar, 2003) and protecting against indomethacin-induced ulcerogenesis, respectively (Petrovic *et al.,* 2003).

Due to the widespread use of *T. balsamita* in Iranian and other traditional medicines for the relief of pain and inflammation and considering the high content of essential oil in *T. balsamita* aerial parts, we were prompted to evaluate the analgesic and anti-inflammatory activities of the essential oil and investigate the pharmacological basis for its folklore as an anti-inflammatory and pain killer agent. The present study explores the central analgesic and acute anti-inflammatory properties. The essential oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in order to identify the potentially responsible compounds for the observed properties. To the best of our knowledge, this is the first attempt to study the ethno-pharmacological properties of *T. balsamita* essential oil (TBEO) in a comprehensive manner.

MATERIAL AND METHODS

Drugs, chemicals and plant material

TBEO, anesthetic ether, morphine sulfate, mefenamic acid and carrageenan were purchased from Giah Essence Phytopharm Co., Iran, PubChem CID: 3283, Temad Pharmaceutical Co. (Iran), Temad Pharmaceutical Co. (Iran) and Sigma (U.S.A), respectively.

Powdered morphine was dissolved in saline. The solutions were prepared immediately before use and injected intraperitoneally in a volume of 10 mL/kg.

Analysis of TBEO

Analysis of TBEO was performed on a HP-6890 gas chromatography (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, temperature programmed as follows: 60-240 °C at 4 °C/min. The carrier gas N_2 was at a flow rate of 2.0 mL/min; the temperatures of injector port and detector were 250 and 300 °C, respectively. Sample was injected by splitting and the split ratio was 1:10. The analysis of GC/MS was performed on a Hewlett-Packard 6890/5972 system with a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness. The operating conditions were the same as described above but the carrier gas was He. Mass spectra were taken at 70 eV. The range of scan mass was from 40-400 m/z at a sampling rate of 1.0 scan/s. Quantitative data were obtained from the electronic integration of the FID peak areas. The components

of TBEO were identified by their retention time, retention indices, relative to C_9-C_{28} n-alkanes, computer matching with the WILEY275.L library and as well as by comparison of their mass spectra with those of authentic samples or with data already available in the literature (Adams, 1995; Swigar, Silverstein, 1981). The percentage composition of the identified compounds was computed from the GC peaks areas without any correction factors and was calculated relatively.

Experimental animals

Male NMRI mice (20–25 g) were used throughout this study. Animals were housed in groups of 6-8 and were allowed free access to food and water except for the short time they were removed from their cages for testing. All experiments were conducted between 10.00 a.m. and 13.00 p.m., with normal room light (12 h regular light/dark cycle) and temperature (22 ± 1 °C). All procedures were carried out in line with the institutional guidelines for animal care and use (ethical approval number: 3183). Each mouse was used only once.

Analgesic activity

The hot plate assay method was used for the purpose of preferential assessment of a possible centrally mediated analgesic effect of TBEO (McDonald *et al.*, 1946). Morphine was also used as positive control substance. Six groups of six mice were fasted overnight prior to the start of the experiment, while given free access to water. For 3 consecutive days preceding the experiments, mice (20-25 g) were placed on a plate maintained at room temperature for 15 min each day. The vehicle (sweet almond oil, 10 mg/kg) was given to the first group by intraperitoneal injection. Morphine (5 mg/kg) and TBEO (50, 100, 200 and 400 mg/kg) were also administered to the second to sixth groups by the same route. Each animal was then placed gently on a 55 °C hot plate. Latency to exhibit nociceptive responses, such as licking paws or jumping off the hot plate, was determined at 15, 30, 45 and 60 min after administration of the test substances or vehicle (McDonald *et al.*, 1946).

Anti-inflammatory activity

Acute anti-inflammatory activity was assessed based on the inhibition of paw edema induced by the injection of 0.1 mL 2% carrageenan into the sub-plantar region of the right hind paw of the rat (Winter, Risley, Nuss, 1962). Male rats were divided into four different groups of 6

animals each that separately received the vehicle, sweet almond oil (10 mg/kg), Mefenamic acid (30 mg/kg) and TBEO (100, 150 and 250 mg/kg), intraperitoneally 1 h before the injection of carrageenan. Paw volume was measured after 0.5, 1, 2, 3 and 4 h after the carrageenan administration using a Plethysmometer (model PM 4500, Borj Sanat, Iran).

Statistical analysis

The data were expressed as mean values ± S.E.M. and tested using analysis of variance followed by the multiple comparison test of Tukey-Kramer.

RESULTS

Analgesic activity: Using the hot plate test, it was shown that intraperitoneal administration of 100 mg/kg dose of TBEO had significant anti-nociceptive activity in comparison to vehicle group only; the anti-nociceptive activity of TBEO was less than the standard drug, morphine. TBEO with low and high doses of 50 and

250 mg/kg practically did not increase the reaction time in tested animals against the thermal source (Figure 1a). Morphine significantly showed anti-nociceptive activity in hot-plate test. Intraperitoneal co-administration of low doses of morphine (0.5 mg) and effective dose of TBEO (100 mg) in rats was not associated with a significant response in hotplate latency in comparison to TBEO (100 mg) group (Figures 1b).

Vehicle, Morphine (5 mg/kg; IP) or TBEO (50, 100, 150 and 250 mg/kg, IP) were administered 15 min prior to the placement of the animal on the hot plate and reaction time of mice was measured at 15-min intervals for one hour.

Opioid independence analgesic action: Naloxone (5.0 mg/kg, IP) did not significantly change the anti-nociceptive effect of the TBEO in hot plate test (Figure 2). Whereas; morphine-induced analgesia (5.0 mg/kg) was completely reversed at the presence of naloxone.

Anti-inflammatory activity: The anti-inflammatory effect of TBEO in rat paw edema induced by carrageenan was established using the essential oil administered intraperitoneally (Figure 3). TBEO with lower doses of
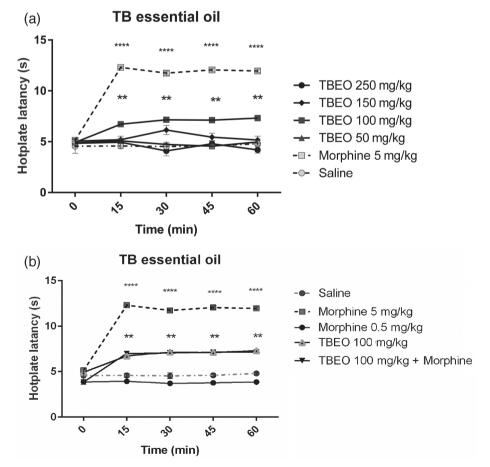

FIGURE 1 - Anti-nociceptive activity of TBEO in the Hot Plate Test. Data represent Mean ± SEM of six to eight animals in each group **P<0.01. ****P < 0.0001, compared with the control group.
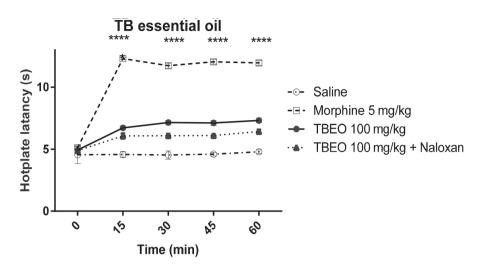
FIGURE 2 - Opioid independence analgesic activity of TBEO. Data represent Mean ± SEM of six to eight animals in each group. ****$P < 0.0001$ compared with the control group.
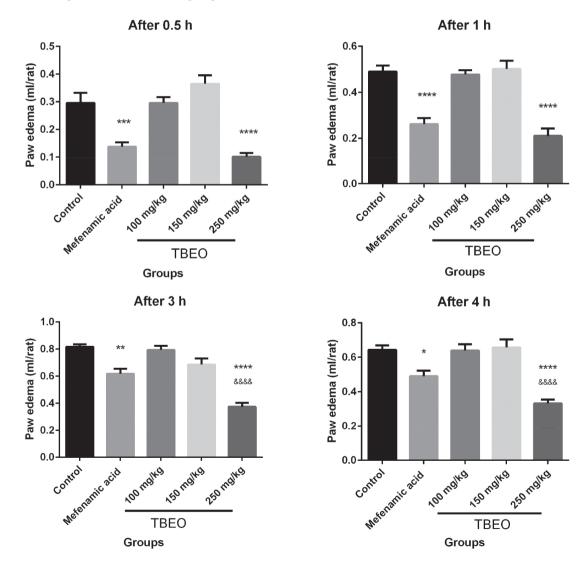

FIGURE 3 - Effects of TBEO on Carrageenan induced inflammation in the 0.5, 1, 3 and 4 hour after the injection of carrageenan. Values represent Mean ± SEM (n = 6 - 8). *$P < 0.05$, **$P<0.01$, ***$P<0.001$ and ****$P<0.0001$ in compared to control, &&&& $P<0.0001$ in compared to Mefenamic acid.

TABLE I - Effect of TBEO on the carrageenan induced inflammation

Groups	Dose (mg/kg)	0.5h	1h	2h	3h	4h
Mefenamic Acid	**30**	53.59	46.68	40.61	24.46	23.79
TBEO	**100**	0.08	0.03	4.16	2.87	0.47
TBEO	**150**	0	0	12.45	16.21	0
TBEO	**250**	65.91	57.14	59.18	54.13	48.29

Each value represents the mean ± SEM (%inhibition) of 6 rats

100 and 150 mg/kg practically did not inhibit the paw edema induced by carrageenan in comparison to control. TBEO (250 mg/kg) as well as Mefenamic acid (30 mg/kg) significantly inhibited ($p<0.05$) the carrageenan-induced rat paw edema formation which was determined at the third hour of the experiment (peak of edema formation) by 54.91 and 24.46%, respectively. At the fourth hour of experiment, this edema inhibition has reached 48.29 and 23.79%.

Anti-inflammatory activity was revealed as the inhibition percent of the edema when compared with the control group (Table I).

TBEO was analyzed by GC/MS to determine the possible compounds responsible for the observed analgesic and anti-inflammatory activities. As shown in Table II, forty four components were identified in this oil which presented about 84.1% of the total chromatographic material. The

TABLE II - GC-MS analysis of TBEO

NO.	Compound[a]	KI[b]	KI[c]	Content (%)
1.	α-Pinene	938	939	**0.1**
2.	Camphene	951	954	**0.1**
3.	Verbenene	970	968	**0.1**
4.	Sabinene	973	975	**0.3**
5.	β-Pinene	979	979	**0.1**
6.	α-Terpinene	1019	1017	**0.1**
7.	ρ-Cymene	1022	1025	**1.0**
8.	Limonene	1030	1029	**1.5**
9.	γ-Terpinene	1057	1060	**0.3**
10.	α-Thujone	1099	1102	**11.9**
11.	β-Thujone	1117	1114	**2.2**
12.	ρ-Mentha-2,8-diene-1-ol	1120	1123	**1.2**
13.	*trans*-Pinicarveol	1142	1139	**0.2**
14.	Isoborneol	1153	1156	**0.5**
15.	4-Terpineol	1179	1177	**0.6**
16.	*trans*-ρ-Mentha-1(7),8-diene-2-ol	1181	1185	**0.6**
17.	dihydrocarvone	1198	1193	**1.1**
18.	*trans*-Carveol	1215	1217	**1.5**
19.	Carvone	1239	1242	**39.8**
20.	Chrysanthenyl acetate	1167	1262	**0.4**
21.	Carvacrol	1302	1299	**0.6**
22.	α-Cubebene	1349	1351	**0.1**
23.	Carvyl acetate	1355	1352	**0.3**
24.	α-Copaene	1380	1377	**0.3**
25.	β-Damascenone	1392	1391	**0.1**

TABLE II - GC-MS analysis of TBEO (cont.)

NO.	Compound[a]	KI[b]	KI[c]	Content (%)
26.	*cis*-Jasmone	1398	1393	0.1
27.	Gurjunene	1414	1410	0.1
28.	β-Caryophyllene	1422	1418	0.4
29.	β-Farnesene	1455	1457	0.3
30.	Germacrene D	1483	1485	3.0
31.	α-Murrolene	1503	1500	0.1
32.	β-Bisabolene	1510	1506	5.0
33.	δ-Cadinene	1529	1524	2.2
34.	*trans*-γ-Bisabolene	1539	1541	1.0
35.	*cis*-α-Bisabolene	1555	1551	0.3
36.	Nerolidol	1559	1563	0.3
37.	Spathulenol	1577	1578	0.6
38.	Caryophyllene oxide	1586	1583	0.2
39.	Dillapiol	1619	1622	0.2
40.	α-Cadinol	1628	1626	2.1
41.	t-Muurolol	1650	1646	1.3
42.	2,5,10-trimethyl-6,7,8,9-	1732	-	1.2
43.	tetradehydrocyclopentadecenone-6,10,14-Trimethyl-2-pentadecanone	1851	1846	0.2
44.	Phytol	2119	2114	0.5
	Total			84.1

[a]Compounds listed in order of elution. [b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the Materials and Methods section. [c]KI, (Kovats index) from literature.

major constituents of the oil were characterized as Carvone (39.8%) and α-Thujone (11.9%).

DISCUSSION

The obtained results showed that the TBEO had the most significant anti-inflammatory effect at the dose of 250 mg/kg among experimental doses (100, 150 and 250 mg/kg).

The essential oil in the dose of 250 mg/kg had significant anti-inflammatory effects in comparison to the control group. It also had significantly meaningful anti-inflammatory effect at the 3rd and 4th hours after the induction of the inflammation rather than standard drug Mefenamic acid (30 mg/kg).

In hot plate test, TBEO could meaningfully increase the reaction time against thermal stimulant at the dose of 100 mg/kg. Nevertheless, in comparison to the standard drug (Morphine 5 mg/kg), the essential oil was less effective at different times. In order to investigate whether or not the opioid system had a role in anti-nociceptive effects, some complementary experiments were conducted. We injected 100 mg/kg of the examined essential oil plus an ineffective dose of Morphine (0.5 mg/kg) into the mice. The results showed no significant changes in anti-nociceptive effects. This test revealed that the anti-noceptive mechanism of the essential oil at this dose is probably non-opioid. Injecting Naloxane (opioid receptor antagonist) plus essential oil 100 mg/kg confirmed this suggestion.

Previous study reported that the *Tanacetum* sp. oil possessed digestive, diuretic, antitussive, analgesic/anti-inflammatory and antimicrobial effects (Lotfipour *et al.*, 2008; Yousefzadi *et al.*, 2009). In support of our findings, it has been demonstrated that *Tanacetum* sp. oil possessed analgesic and anti-inflammatory effects (Lotfipour *et al.*, 2008; Yousefzadi *et al.*, 2009). Some of the medicinal *Tanacetum* species such as *T. microphyllum* and *T. larvatum* were useful for treating various inflammatory disorders and have shown protective activities against indomethacin-induced ulcerogenesis, respectively (Petrovic *et al.*, 2003). The analgesic and anti-inflammatory property was also found in extract of other *Tanacetum* genus such as *T. parthenium*.

It was previously mentioned that the monoterpenoid carvone was the main component found in many plant species. Previous studies showed that carvone (Gonçalves *et al.*, 2008) and carvone derivatives, such as rotundifolone (Sousa *et al.*, 2007) and hydroxydihydrocarvone (de Sousa *et al.*, 2006) possessed antinociceptive and analgesic properties. In line with our study, Gonçalves *et al.* (2008) reported that the analgesic effect of (-)-carvone was not reversed by the opiate antagonist naloxone in the acetic acid-induced writhing test. The anti-nociceptive effects of (-)-carvone represented by inhibition of the licking response of mice in both phases of the formalin test also revealed the involvement of central or peripheral mechanisms in this way (Gonçalves *et al.*, 2008). Moreover, previous studies indicated that Carvone, had intestinal antispasmodic and anti-nociceptive activity (Sousa *et al.*, 2007). In a previous study, the antispasmodic effect of (-)-carvone as a potent calcium channel blocker, in the guinea pig ileum was reported (Souza *et al.*, 2013).

Research on experimental animals confirmed the anti-inflammatory and anti-nociceptive effects of the synthetic compound α and β epoxy carvone (da Rocha *et al.*, 2013) and cyane-carvone (Marques *et al.*, 2014). Carvone decreases the formation of inflammatory cytokines and it also has a role in oxidative stress mechanisms. The anti-inflammatory effects of carvone in carrageenan induced edema and the measurement of Myeloperoxidase showed that the most important cytokines having a role in inflammatory mechanisms are IL-β1 and TNFα (Marques *et al.*, 2014).

As already assumed, carvone, as the main component of the essential oil, has been reported several times to have anti-inflammatory and anti-nociceptive effects. Thus, the observed anti-inflammatory and anti-nociceptive effects were probably caused by the high content of carvone in the essential oil. TBEO could be an appropriate choice to develop new anti-nociceptive and anti-inflammatory drugs which had fewer side effects than the typical drugs, though sub-chronic and dermal toxicity tests are strongly recommended to be done on the essential oil.

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