Clinical Neurology and Neuroscience
2017; 1(3): 60-66
http://www.sciencepublishinggroup.com/j/cnn
doi: 10.11648/j.cnn.20170103.13

Analgesic and Anti-inflammatory Effects of *Tanacetum balsamita* Essential Oil and One of Its Major Constituents (Quercetin) in Male Rats

Mohammad Kazemzadeh[1], Parichehr Yaghmaei[2], Saeed Mohammadi[2, *]

[1]International Campus, Hamadan University of Medical Sciences, Hamadan, Iran

[2]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Email address:

smiauhphd.sm@gmail.com (S. Mohammadi)

[*]Corresponding author

To cite this article:

Mohammad Kazemzadeh, Parichehr Yaghmaei, Saeed Mohammadi. Analgesic and Anti-inflammatory Effects of *Tanacetum balsamita* Essential Oil and One of Its Major Constituents (Quercetin) in Male Rats. *Clinical Neurology and Neuroscience*. Vol. 1, No. 3, 2017, pp. 60-66. doi: 10.11648/j.cnn.20170103.13

Received: March 29, 2017; **Accepted**: April 13, 2017; **Published**: May 26, 2017

Abstract: *Tanacetum balsamita* is one of the important medicinal plants that is used by iranianan folk medicines. This study aimed to assess the phytochemical Screening, analgesic and anti-inflammatory effects of *Tanacetum balsamita* essential oil in rats. The essential oil of *Tanacetum balsamita* (EOTB) was treatment in doses of 10, 50, and 100 mg/kg. In addition, anti-nociceptive activity of EOTB was evaluated by formalin, writhing, and tail-flick tests. The EOTB was also combined with 2 mg/kg naloxone to determine the involvement of opioid mechanism. Anti-inflammatory reaction was evaluated via xylene-induced ear edema. In addition, the EOTB origin has been analyzed by a combination of GC and GC/MS. The EOTB at doses of 10-100 mg/kg have been shown significant analgesic effects ($P<0.05$). In compare of standard opioid agonist drug (morphine) and non-steroidal anti-inflammatory drug (indomethacine), Use of naloxone plus EOTB has been inhibited pain in all three models. The present results have been shown significant ($P<0.05$) anti-inflammatory effect of EOTB in the xylene-induced ear edema test in compare to dexamethasone. The major components in the tested oils were mainly contained quercetin, Isoquercitrin and luteolin. In conclusion, findings propose that EOTB probably have analgesic and anti-inflammatory effects. Existent of quercetin may be an important reason for mentioned effects.

Keywords: Pain, Inflammation, Medicinal Plant, *Tanacetum balsamita,* Quercetin

1. Introduction

Pain is a somatic sensation such as touch, proprioception, and pressure. Pain has always been a serious challenge in medicine as which has an important protective role in avoiding or treatment of actual or potential tissue damages. Although nonsteroidal anti-inflammatory drugs (NSAID) and opioid are mostly used to control pain, but these drugs have many adverse effects and sometimes causes renal and gastrointestinal disorders. Therefore, most people looks for new drugs that have fewer side effects and are cheaper and easily available [1]. There is also increasing evidence that in traditional medicine prescribing medicinal plants to treat a pain and an inflammation is prevalently but the origin and structure of such plants have often remained unknown. Therefore, information about the pharmaceutical effects of these medicinal plants can be applied as an agreeable research approach in order to discover new drugs [2, 3].

Essential oils are imperative regular items utilized as crude materials as a part of extensive sum fields, including: phytotherapy fragrances, fragrant healing, beauty care products, flavors and sustenance [4]. The genius Eryngium consists of 250 species that widely distribution in North Africa, North America, Australia and Eurasia [5, 6]. Tanacetum species have been used as a remedy in digestive, antitusive, diuretic, inflammatory, rheumatism and pain in traditional medicine systems [7, 8].

Tanacetum balsamita is medicinal plant that belongs to the copmosiate family [9]. This local plant is utilized as the part of Iranian society prescription for different purposes an

outstanding as a love potion, and expectorant [10]. There are multiple scientific evidences about the antioxidant, antimicrobial, cytotoxicity and, antibacterial effects of *Tanacetum balsamita* essential oil [11-13]. Considering the anti-inflammatory effects of some Eryngium genus [14] and the close relation of inflammatory processes with pain, and lack of studies on analgesic and anti-inflammatory effects of *Tanacetum balsamita* essential oil in major databases, for the first time a study to assess its aforementioned effects was needed.

2. Materials and Methods

2.1. Plant Material Gathering

Some of new *Tanacetum Balsamita* petals were arranged and validated by a botanist and after that a voucher example number of the plant was saved in the herbarium (150-19-2) of the division in science, Bu Ali Sina University, Iran.

2.2. Essential Oil of Tanacetum Balsamita

Distillation of oil was completed by means of a refining and clevenger mechanical assembly. New petals of *Tanacetum Balsamita* were air dried at standard room temperature and drove into coarse powders by means of pestle and mortar. 800 grams of the powder were hydrodistilled and it yielded 22.28 g adding up to 3.9% w/w of the trademark illuminatin fragrant of the EOTB. The oils got were stacked in a lightproof jug and kept in an icebox until use. The relative thickness of the essential oil of *Tanacetum Balsamita* got was resolved by means of the 10 ml limit thickness bottle. The oil was emulsified by the 5% Tween 80 in a matter of seconds before organization [15].

2.3. Animals Experiments

Eighty-four grown-up male wistar rats (200–250 g) were acquired from Pasteur's organization of Iran. The creatures were housed 4 to 5 for each confine and limited at a standard controlled temperature of 23 ± 1 Centigrade degree under a light/dim cycle of 12:12 h with nourishment and faucet water open not obligatory. All tests were directed amid 10:00 and 16:00. All rats were dealt with empathetically and were directed in concordance by the entire of the IASP rules on the utilization of research facility creatures [16]. The creatures were arbitrarily separated into seven equivalent gatherings (N=6 rodent per bunch): control, EOTB (10, 50, and 100 mg/kg, i.p), morphine (10 mg/kg, i.p.), indomethacin (10 mg/kg, i.p.) and 100 mg/kg of EOTB in addition to naloxone (2 mg/kg, i.p.).

2.4. Gas Chromatography–Mass Spectrometry

For GC-MS overview, an Agilent 6890N arrangement gas chromatograph incorporate to a LECO time-of-flight mass spectrometer finder (Agilent Technology, Palo Alto, USA) was utilized. Mixes were unmistakable on a DB-5 hair like section with the accompanying temperature execution: broiler temperature was customized from 40 to 260°C at 4°C/minutes amid 20 minute, lastly up to 340°C for 20 minutes isothermally; injector and MS exchange line temperatures were put at 200 and 300 °C, separately. What's more, Helium was utilized as the transporter gas at a nonstop stream rate of 1 ml/min; split proportion, 1:20. A blend of the homologous arrangement of N-alkanes (C8-C20) in hexane was straight infused into the GC under the above temperature, keeping in mind the end goal to get assess the RIs of tops in the chromatograms. Significant unstable mixes were distinguished by co-organization with bona fide by looking at the reservation times of the chromatographic crests, and their MS fracture designs with those of immaculate mixes, of the ghastly index of the National Institute of Standards and Technology MS or from writing information [17].

2.5. Drugs and Chemicals

Morphine sulfate, naloxone, indomethacin, and dexamethasone were purchased from Darou pakhsh (Iran), acetic acid and formalin from Merck Inc (Germany). Also, quercetin extracted from *Tanacetum balsamita* with 95% purity has been bought from sigma company (USA).

2.6. Lethal Dose (LD_{50})

An intense poisonous quality test was directed as beforehand portrayed [18]. Rats were partitioned into six gatherings, every gathering comprising of five creatures. To start with gathering was given Tween 80 (1%) in ordinary saline (2 ml for each kg body weight). Alternate gatherings were treatment, individually with various dosages of 100, 200, 400, 800 and 1000 mg of EOTB per kg body weight. As indicated by unique reference, rats were seen for the following 24 hours to any behavioral changes or passing.

2.7. Tests of Pain

2.7.1. Writhing Test

On the trial day, 30 min before running the experiments, the rat was sent into an experiment glass box to get used to the conditions. The EOTB was solved in saline and then injected intraperitoneally in doses of 10, 50, and 100 mg /kg. After 15 min, acetic acid with dose of 1mg/kg of the body weight (with density of 6%) was used and then the number of abdominal contractions (writhing) was counted for 30 minutes. It is also necessary to mention that each rat was used only once [19].

2.7.2. Tail-Flick Test

This valid model of pain is a reaction in animals, like the hot plate model. It was first described by D'Amour and Smith in 1941[20]. Routinely, a light steam is focused on the animal's tail and timer starts in continue a. When rats start moving or flicks its tail, the timer cease and the recorded time (latency) is a measure of the pain threshold. For the tail flick test, an analgesimeter apparatus (Manufactured by Borj Sanat Company, Iran) was used. Animals were separately put

in a restrainer and 30 minutes after ingestion, the standard response time was measured by centering a light stream on the distal 33% bit of the rats tail terminal. Every 15 minute interim, the response time was recorded until 2 hours. However 15 seconds cut off time was utilized for avoiding tissue damage. Also Percent of most extreme conceivable antinociceptive or %MPA impact was ascertained for every time.

2.7.3. Formalin Test

In order to evaluate the acute and chronic pains, the model proposed by Dubuisson and Dennis was used. One hour before the test, the rats were sent into the formalin test box in order to get used to the experimental condition. The box was made of Plexiglas (dimensions: 30 × 30 × 30 cm). Positioned in 45°, a mirror was inserted under the box and in front of the observer to observe the rat's behaviors more clearly. Thirty minute after drugs injection, formalin (50 µl, 2.5%) was infused subcutaneously in the sub plantar part of the left rear paw and afterward the rats were exchanged to the test box once more. The animal's behavior was observed and labeled for 60 min as follows: once every 15 seconds, the motor response to pain was rated and recorded on an amount of these numbers: 0, 1, 2, and 3. The numbers reveal the following reactions: number 0 for the animal moves with complete balance and its weight assort equally on both foot; number 1 for the animal could not undergo its body weight on the being-injected foot or head of that foot; number 2 for the animal raised the painful claw and has no contact with the box floor; and number 3 for the animal licks the painful claw, chewed or moved severely. The moderate of first 5 minute grades was considered as phase 1 or acute phase and the moderate of min 15 to 60 was considered as the phase 2 or chronic phase [21].

2.8. Anti-inflammatory Study

In this experimental research xylene-impelled ear edema was utilized to assess the inflammation. The rats were isolated into 7 bunches. Thirty minutes after i.p. Infusion of the dexamethasone as a solid mitigating drug, xylene (0.03 ml) was directed to the front and back portion of the right ear. The left ear was considered as a control group. Two hours after xylene application, the rodent was kicked the bucket and both ears were removed. Circular segments were taken, by means of a stopper borer (measurement of 7mm), and after that weighed. The rise in weight brought about by the inconvenience was measured by subtracting the heaviness of the under-treated left ear part from that of the treated right ear segments. Ordinary saline (10 ml/kg, i.p.) was utilized as control gathering and dexamethasone (15 mg/kg, i.p.) were managed as reference medications [22].

3. Results

3.1. Preparatory Chemical Composition of Essential Oil

The real parts in the tried oil of *Tanacetum balsamita*oil was introduced fundamentally contained quercetin, Isoquercitrin, luteolin7-O-β-D-glucopyranoside and scopoletin (Table 1).

Table 1. *The compounds identified in the essential oil of Tanacetum balsamita.*

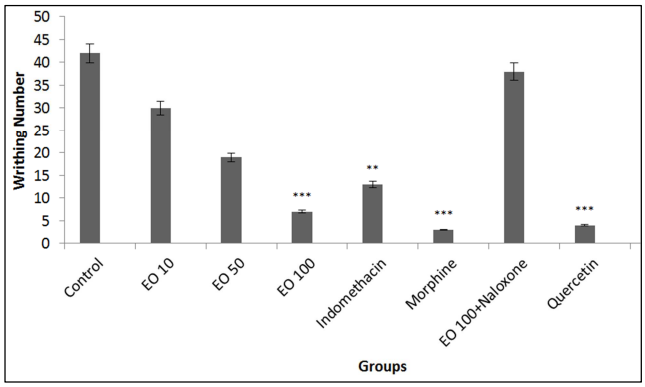
No.	Compound Name
1	quercetin (45.5%)
2	Scopoletin (7.8%)
3	Isoquercitrin (7%)
4	Falcarinolone(5.1%)
5	caffeic acid (4.1%)
6	P-pinene (4%)
7	luteolin7-O-β-D-glucopyranoside (3.3%)
8	kaempferol7-O-α-L-rhamnopyranoside (2.1%)
9	Oleanolic acid (3%)
10	Falcarinol (2.7%)
11	eryngiosides A (0.9%)
12	octanal (0.1%)
13	Decanal (0.7%)
14	Undecanal (0.5%)
15	a-phellandrene (0.4)
16	Nonanal (0.4%)
17	Campesterol (0.3%)
18	p-cymene (1.2%)
19	2, 4, 5- or 2, 4, 6-trimethyl benzaldehyde (1.4%)
20	unidentified compounds (12.9%)

3.2. Acute Test

In this experimental model, after seventy-two hours of injection of the different dosage of extract and quercetin no fatalities were recorded (data not shown).

3.3. Acetic Acid-Induced Writhing

EOTB with dosage of 100mg/kg significantly ($P<0.01$) lessened the quantity of acetic acid impelled writhes in rodent, however the rate protection was essentially ($P<0.05$) lower than the standard pain relieving utilized, morphine (Figure1). EOTB with dose of 10 and 50 mg/kg did not inhibit writhings in rats. Moreover treatment of rats with EOTB with dose of 100mg/kg +naloxone reversed the inhibitory effects of the EOTB in acetic acid-induced writhings.

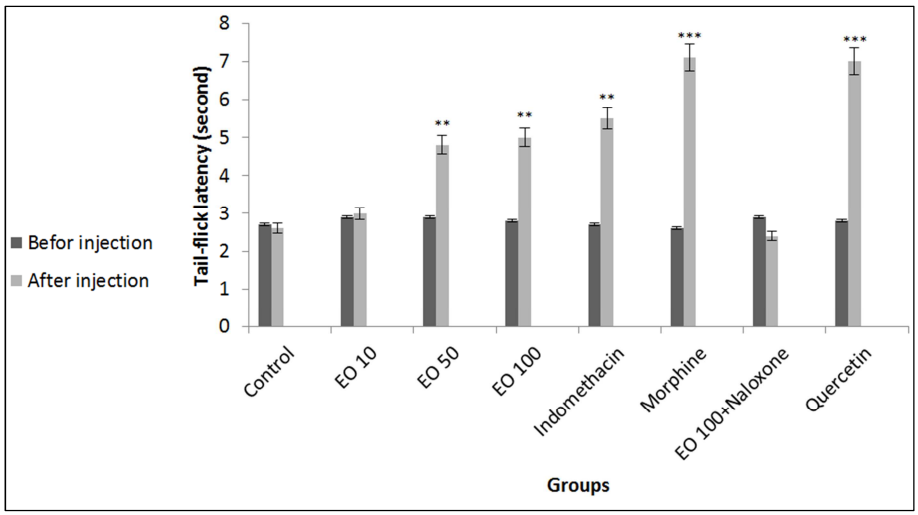


As compared with control: $^{**}P<0.01$ and $^{***}P<0.001$(n=6, means ± S. E. M).

Figure 1. *Effects of the different doses of essential oil of Tanacetum balsamita (EO) and its major constituent, quercetin (100mg/kg) on acetic acid induced writhing in rats.*

3.4. Tail Flick Test

According to Figure 2, groups of the EOTB with dose of 10, 50 and 100 mg/kg showed a significant increase of tail flick latency when compared to the control group (P<0.05, P<0.01 and P<0.01mg/kg, respectively). Injection of morphine and indomethacin were increased tail flick latency as well as (P <0.001 and P <0.01, respectively).



As compared with control: *P<0.05, **P<0.01 and ***P<0.001. (n=6, means ± S. E. M).

Figure 2. *Effect of different doses of Essential oil of Tanacetum balsamita (EO) and its major constituent, quercetin (100mg/kg) on the reaction time of rats.*

3.5. Formalin Test

The essential oil in 100 mg/kg (i.p.) hindered paw-licking time at the two stages in contrasted with control group. Additionally indomethacin (10 mg/kg, i.p.) a strong non-steroidal anti-inflammatory drugs (NSAID) and morphine (10 mg/kg, i.p.) a powerful opioid likewise has been gave pain relieving hint. Besides the essential oil at dose of 50 mg/kg (i.p.) indicated restraint in the second stage as acquired with control. Pretreatment with EOTB+Naloxone essentially diminished their pain relieving impacts at the two stages (Fig. 3).

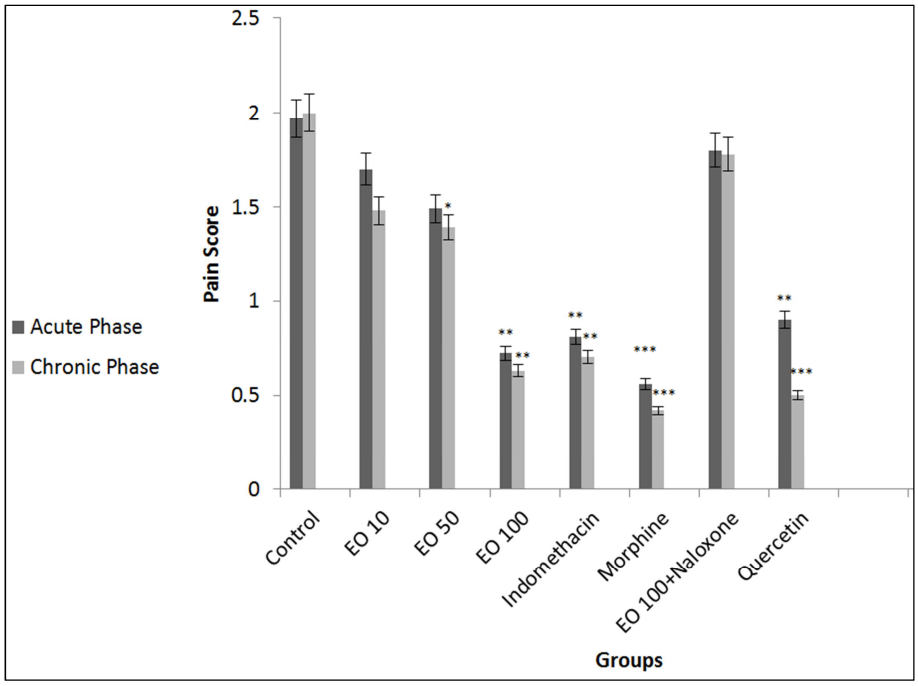


Figure 3. *Effects of Essential oil of Tanacetum balsamita (EO) and its major constituent, quercetin (100mg/kg) on formalin-induced nociception in rats.*

As compared with control: *P<0.05, **P<0.01 and ***P<0.001. (n=6, means ± S. E. M).
Values are the mean ±S. E. M. for 7 rat, P<0.05*, P<0.01**, and P<0.001***, compared to control (normal saline).

3.6. Xylene-Induced

EOTB in dosage of 50 and 100 mg/kg fundamentally ($P<0.05$, $P<0.01$, separately) decreased the heaviness of xylene-actuated ear edema in rodent with a figured restraint of 35.2, 50.7%, while the littler dosage created no noteworthy impact (Table 2).

Table 2. *Effects of the intraperitoneal doses of Tanacetum balsamita essential oil (EO) and dexamethasone on xylene-induced ear swelling in male rats.*

Treatment	Dose	Ear Swelling (mg)	Inhibation (%)
Control	10 ml/kg	7.6 ±0.6	--------
Dexamethasone	15 mg/kg	3.2 ±0.3***	58.2
EO	10 mg/kg	6.4 ±0.4	15.9
EO	50 mg/kg	5.1.8 ±0.3*	35.2
EO	100 mg/kg	3.2 ±0.3**	50.7

4. Discussion

The general aftereffects of the present examination demonstrated that the EOTB and its major constituent, Quercetin has critical antinociceptive and anti-inflammatory movement. Looking at the outcomes got by the entire of three distinctive exploratory models of nociception (tail-flick, writhing and formalin test), it can conjecture that EOTB acts both at the peripheral and central levels of pain.

One of the most important chemical assessment tests is writhing test, which used to evaluate possible antinociceptive mixtures. In this test acetic acid is a chemical stimulation which is comprehensive used to screen peripheral antinociceptive activity [23]. The EOTB prevented abdominal constriction caused by acetic acid therefore, it is imagined that its attenuate effects are supported by the environmental mechanisms. Intraperitoneal administration of acetic acid can cause the acute inflammation of the peritoneum [24, 25]. In this standard trial, it seems that peripheral antinociceptive effects of EOTB are indirectly due to internal mediators for instance, serotonin, histamine, substance-P, bradykinin, and prostaglandins. It is give reasons for that all of these mediators are related with the stimulation of peripheral nociceptive neurons [17, 26].

The tail-flick model or tail flick test makes utilization of high-power light emission target a rat's tail to recognize torment. What's more, it is utilized to fundamental torment examination and to quantify the viability of analgesics medications, by watching the response to warm. In this study, treatment of rats with EOTB at all three measurements essentially diminished torment edge. Since tail flick test is performed to assess the spinal reflexes reaction and the focal pain relieving pathways level along these lines, it appears that the antinociceptive impact of the fundamental oil includes a focal apprehensive segment which might be evoked from a few characterized ranges in the CNS [27].

Between several tests of persistent nociception, formalin test has been established as an acceptable pain test for study of anti-inflammatory and antinociceptive agents that act through central pain route from peripheral pain [21, 27]. In this test, the use of formalin can shows some signs of pain such as licking (phase 1), and subsequently a quiet period was described by less torment practices and late hyperalgesic segments (stage 2) that keep going for roughly 60 minutes. The essential stage or neurogenic nociception comes about direct enactment of fringe nociceptors, while the second stage due to provocative nociception that reflect incitement of focal refinement, the improvement of irritation and broadening of responsive fields. Likewise the simultaneous nearness of low level cans contribution from both huge and little afferents. The results demonstrated that EOTB have an inhibitory impact over the torment. The EOTB under scrutiny in this examination indicated critical pain relieving movement in both periods of formaldehyde-impelled agony. It was observed that its diminishing impact is more incessant stage than the intense stage. Restraint of the perpetual period of the formalin test by EOTB can be an intense in the consequence of aggravation, so that part of the antinociceptive impact is by all accounts intervened by discharging prostaglandins, for example, E2 and F2α that in a few sums sharpened by focal nociceptive terminal [28].

To evaluation probably the role of opioid system in analgesic effect of EOTB, naloxone was used as a reference antagonist of opioid system. This amazing drug can inhibit the activation of opioid receptors (especially mu receptor) [29, 30]. The results indicate that naloxone can attenuate the antinociceptive effect of EOTB. Therefore, it seems that the effect of EOTB in pain relief partly is due to the opioid receptors

Biologic or therapeutic activity of herbs has a close relationship with their chemical combinations (28). According to present results from phytochemical analysis', *Tanacetum balsamita*contains has several compounds such as flavonoids, isoflavonoid derivatives, coumarins, hydroxycinnamic acid (caffeic acid), and terpenoids. Earlier papers reported that flavonoids have an analgesic effect [31]. In fact, flavonoids in one possible mechanism can inhibit NMDA receptors and meanwhile cut intracellular calcium down. Consequently, the synthesizer enzyme of phospholipase A_2 and calcium-related nitric oxide diminished and with the reduction of prostaglandins and nitric oxide, particularly the prostaglandin $F_{2\alpha}$ and E_2 revealed its analgesic effects [32] One of the important flavonoids families is quercetin. Recently kaur et al in 2005 has been shown that quercetin can stimulute analgesic effect and which this effect contain primarily the modulation of adrenergic pathways [33] Furthermore, diverse in vitro examination have shown that quercetin represses nitric oxide generation and iNOS expression, which has likewise been affirmed in vivo related creatures ponders [34]. In this manner, might be one of the components in charge of its anti-inflammatory effect perhaps due to cease of iNOS system by quercetin. Another bioactive compound that exists in the plant was caffeic acid. Buzzi et al in 2009 has been shown that caffeic acid can decrease pain threshold in both writhing and formalin tests [35]. Furthermore, anti-inflammatory effect of caffeic acid has

been shown [36]. The other components that present in EOTB are cumarins. In new investigation, park et al shown that coumarin derivation can specifically activate the TRPV1 or nociceptors transient receptor potential vanilloid-1 channels and can reverse the inflammatory pain in rodent through channel desensitization [37].

5. Conclusion

Finally, these results suggest that EOTB and one of its major constituent, Quercetin can decrease nociceptive behaviours and inflammation in these algesimetric tests. A possible modulation of opioid receptors at least in part, could be involved in the effect of essential oil. In addition, it can help resist pain and reduce sensitivity to tonic phase as well as acute pain cause a reduction in pain through inhibition of inflammatory mediators.

References

[1] Zarei M, Mohammadi S, Abolhassani N, Nematian MA. 2015. The Antinociceptive Effects of Hydroalcoholic Extract of Bryonia dioica in Male Rats. Avicenna Journal of Neuro Psych Physiology; 2 (1): e25761.

[2] Chin Y-W, Balunas MJ, Chai HB, Kinghorn AD. 2006. Drug discovery from natural sources. The AAPS journal; 8: 239-53.

[3] Calixto JB. 2005. Twenty-five years of research on medicinal plants in Latin America: a personal view. J Ethnopharmacol; 100: 131-4.

[4] Afolayan A, Jimoh F. 2009. Nutritional quality of some wild leafy vegetables in South Africa. International journal of food sciences and nutrition; 60: 424-31.

[5] WORZ A. 2004. On the distribution and relationships of the South-West Asian species of Eryngium L. (Apiaceae-Saniculoideae). Turkish Journal of Botany; 28: 85-92.

[6] Calvino CI, Martínez SG, Downie SR. 2008. The evolutionary history of Eryngium (Apiaceae, Saniculoideae): Rapid radiations, long distance dispersals, and hybridizations. Molecular phylogenetics and evolution; 46: 1129-50.

[7] Olanj N, Garnatje T, Sonboli A, Vallès J, Garcia S. 2015. The striking and unexpected cytogenetic diversity of genus Tanacetum L.(Asteraceae): a cytometric and fluorescent in situ hybridisation study of Iranian taxa. BMC plant biology; 15: 174.

[8] Kumar V, Tyagi D. 2013. Chemical composition and biological activities of essential oils of genus Tanacetum-a review. Journal of Pharmacognosy and Phytochemistry; 2: 121-9.

[9] Attighi Lorestani F, Khashaveh A, Attighi Lorestani R. 2013. Fumigant toxicity of essential oil from Tanacetum balsamita L. (Compositae) against adults and eggs of Callosobruchus maculatus F. (Coleoptera: Bruchidae). Archives of Phytopathology and Plant Protection; 46: 2080-6.

[10] Zargari A. Medicinal plants: Tehran University Publications. ISBN; 1995.

[11] Bączek KB, Kosakowska O, Przybył JL, Pióro-Jabrucka E, Costa R, Mondello L, et al. 2017. Antibacterial and antioxidant activity of essential oils and extracts from costmary (Tanacetum balsamita L.) and tansy (Tanacetum vulgare L.). Industrial Crops and Products; 102: 154-63.

[12] Yousefzadi M, Ebrahimi SN, Sonboli A, Miraghasi F, Ghiasi S, Arman M, et al. 2009. Cytotoxicity, antimicrobial activity and composition of essential oil from Tanacetum balsamita L. subsp. balsamita. Natural product communications; 4: 119-22.

[13] Bagci E, Kursat M, Kocak A, Gur S. 2008. Composition and antimicrobial activity of the essential oils of Tanacetum balsamita L. subsp. balsamita and T. chiliophyllum (Fisch. et Mey.) Schultz Bip. var. chiliophyllum (Asteraceae) from Turkey. Journal of Essential Oil Bearing Plants; 11: 476-84.

[14] Dawilai S, Muangnoi C, Praengamthanachoti P, Tuntipopipat S. 2013. Anti-inflammatory activity of bioaccessible fraction from Eryngium foetidum leaves. BioMed research international; 12: 321-5.

[15] Vale T, Matos F, De Lima T, Viana G. 1999. Behavioral effects of essential oils from Lippia alba (Mill.) NE Brown chemotypes. J Ethnopharmacol; 67: 127-33.

[16] Carbone L, Austin J. 2016. Pain and laboratory animals: Publication practices for better data reproducibility and better animal welfare. PloS one; 11: e0155001.

[17] Fallahzadeh AR, Zarei M, Mohammadi S. 2016. Preliminary Phytochemical Screening, Analgesic and Anti-inflammatory effect of Eryngium pyramidale Boiss. & Husson Essential Oil in Male Rat. Entomology and Applied Science Letters; 3: 140-7.

[18] Lorke D. 1983. A new approach to practical acute toxicity testing. Archives of toxicology; 54: 275-87.

[19] Collier H, Dinneen L, JOHNSON CA, Schneider C. 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. British journal of pharmacology and chemotherapy; 32: 295-310.

[20] D'AMOUR FE, SMITH DL. 1941. A method for determining loss of pain sensation. Journal of Pharmacology and Experimental Therapeutics; 72: 74-9.

[21] Dubuisson D, Dennis SG. 1978. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain; 4: 161-74.

[22] Mohammadi S, Zarei M, Mahmoodi M, Zarei MM, Nematian MA. 2015. In Vivo Antinociceptive Effects of Persian Shallot (Allium hirtifolium) in Male Rat. Avicenna Journal of Neuro Psych Physiology; 2 (1): e27149.

[23] Gawade S. 2012. Acetic acid induced painful endogenous infliction in writhing test on mice. Journal of Pharmacology and Pharmacotherapeutics; 3: 348.

[24] Koster R, Anderson M, De Beer E. 1959. Acetic acid-induced analgesic screening. FEDERATION PROCEEDINGS; 11: 21-8.

[25] [25] Golshani Y, Zarei M, Mohammadi S. 2015. Acute/Chronic Pain Relief: Is Althaea officinalis Essential Oil Effective? Avicenna Journal of Neuro Psych Physiology; 2 (4): e36586.

[26] Negus SS, Vanderah TW, Brandt MR, Bilsky EJ, Becerra L, Borsook D. 2006. Preclinical assessment of candidate analgesic drugs: recent advances and future challenges. Journal of Pharmacology and Experimental therapeutics; 319: 507-14.

[27] Tjølsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. 1992. The formalin test: an evaluation of the method. Pain; 51: 5-17.

[28] Mahmoodi M, Mohammadi S, Enayati F. 2016. Evaluation of the Antinociceptive Effect of Hydroalcoholic Extract of Potentilla Reptans L. in the Adult Male Rat. SSU_Journals; 24: 201-210.

[29] Borras M, Becerra L, Ploghaus A, Gostic J, DaSilva A, Gonzalez R, et al. 2004. fMRI measurement of CNS responses to naloxone infusion and subsequent mild noxious thermal stimuli in healthy volunteers. Journal of neurophysiology; 91: 2723-33.

[30] Vilela FC, de Mesquita Padilha M, dos Santos-e-Silva L, Alves-da-Silva G, Giusti-Paiva A. 2009. Evaluation of the antinociceptive activity of extracts of Sonchus oleraceus L. in mice. J Ethnopharmacol; 124: 306-10.

[31] Woodman OL, Chan EC. 2004. Vascular and anti‐oxidant actions of flavonols and flavones. Clinical and Experimental Pharmacology and Physiology; 31: 786-90.

[32] Kaur R, Singh D, Chopra K. 2005. Participation of α 2 Receptors in the Antinociceptive Activity of Quercetin. Journal of medicinal food; 8: 529-32.

[33] Michaluart P, Masferrer JL, Carothers AM, Subbaramaiah K, Zweifel BS, Koboldt C, et al. 1999. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer research; 59: 2347-52.

[34] Park S-H, Sim Y-B, Kang Y-J, Kim S-S, Kim C-H, Kim S-J, et al. 2013. Antinociceptive profiles and mechanisms of orally administered coumarin in mice. Biological and Pharmaceutical Bulletin; 36: 925-30.