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ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF TANACETUM LINGULATUM SESQUITERPENE LACTONIC EXTRACTS IN RATS

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ABSTRACT

Plant natural products have contributed extraordinary to the discovery of drugs for use in modern medicine. The aim of this study was to assay the analgesic and anti-inflammatory effects of sesquiterpene lactone extracts of *Tanacetum lingulatum* flowers. In this study, rats were used and they divided into 10 groups. Formalin test was used to evaluate the analgesic effect of extracts. In analgesic test, the groups include: Negative control group (phosphate buffered saline and tween 80), positive control group (morphine 10 mg/kg), treatment groups(extracts 100, 200, 300 mg/kg).The groups for inflammation test include: positive control group (Dexamethasone 15 mg/kg), Negative control group (phosphate buffered saline and tween 80), treatment groups(extracts 100, 200, 300 mg/kg). The data were analyzed using ANOVA and LSD tests. The results were showed that the ethanolic extracts reduced significant the inflammation and pain at concentrations of 100, 200, 300 mg/kg ($p<0.001$). The maximum analgesic effects of the extracts were observed at concentration of 300 mg/kg and the maximum anti-Inflammatory effects of the extracts were observed at concentration of 200 mg/kg. The results obtained in this study indicate that sesquiterpene lactone and flavonoid compounds in this plant may be the reason of these effects.

Keywords: Analgesic, Anti-inflammation, Sesquiterpene lactone, *Tanacetum lingulatum*.

INTRODUCTION

Inflammation is the response to injury or infection, that allows protection from further

damage and characterized by redness, heat, swelling, loss og function and pain [1, 2]. Pain is the outcome of activation and sensitization of primary afferent nerve fibres. A great number of inflammatory mediators including amines, platlet-activating factor, leukotrienes, prostaglandins, kinins, purines, cytokines, chemikines have been found to act on specific targets and leading to the release of other mediators to the site inflammation [1].

Natural products used for inflammatory conditions such as fevers, pain, migraine, arthritis etc. These products are plant secondary metabolites, such as: terpenoids, flavonoids, polyphenolic compounds, sulfur-containing compounds etc[2, 3].

Plants from the genus *Tanacetum* L. (Asteraceae) have been used in the traditional medicine for a long time, of which *Tanacetum* species have been widely used as a treatment in pain and inflammatory conditions [3, 4]. *Tanacetum* is a rich source of secondary metabolites such as sesquiterpene lactones, flavonoids and terpenoid compounds [2]. Previous investigations revealed a large number of *Tanacetum* species possessing biologically active compounds [3-5]. Sesquiterpene lactone compounds a large and diverse group of biologically active plant chemicals that have been identified in several plant families such as Acanthaceae, Anacardiaceae, Apiaceae, Euphorbiaceae, Lauraceae, Magnoliaceae, Rutaceae, Winteraceae and Asteraceaeetc[6]. Sesquiterpene lactones are a class of naturally terpenoids that are formed from head-to-tail condensation of three isoprene units and a cis or trans-fused lactone [6].

Previous investigations revealed that the anti-inflammatory potential of *Tanacetum* species is mainly due to their high content of sesquiterpene lactones [2, 3]. Studies have revealed that *Tanacetum* species have anti-inflammatory activity and sesquiterpene lactones such as parthenolides are believed to mediate many of these effects of *Tanacetum*[2, 3].Parthenolide inhibits the expression of genes involved in inflammation such as pro-inflammatory cytokines IL-1, IL-4, IL-8. IL-12, TNF-α, nitric oxide synthase, Thus, they are inhibitors of the pro-inflammatory transcription factor NF- κB with is a key regulator of the immune response [2, 6]. Although numerous species of the genus *Tanacetum* are widely known for their pharmacological and medicinal properties [7], there have been no reports on pharmacological activities of *Tanacetumlingulatum*sesquiterpene lactone extracts found in Iran. Therefore, we reported

for the first time the analgesic and anti-inflammatory activities of the sesquiterpene lactone extract of flowers of the plant *T. lingulatum.*

MATERIALS AND METHODS

Collection of plants

The flowers of *Tanacetumlingulatum*were collected in Lashotor, province of Isfahan, Iran, in June 2013. The voucher specimen was deposited at the herbarium of the Research-Institute of Isfahan Forests and Rangelands.

Preparation of extracts

The dried flowers of*Tanacetumlingulatum*L.(40g) were finely ground and extracted at room temperature with cyclohexane- Et_2O-MeOH (1:1:1, 30:30:30 ml). The extract was then washed with brine (20 mg/ml), the aqueous layer was subjected to extraction once again with EtOAc, and the organic layer was dried with Na_2SO_4 and thus, aqueous layer and organic layer were mixed together and concentrated under reduced pressure [8]. The extracts were dissolved in phosphate buffered saline and tween 80 in ratio of 1 to 4 at the concentrations of 100, 200, 300 mg/kg body weight.

Animals

Male Wistar rats (120-200gr) were obtained from Iran Pastor Institute. They were maintained under controlled temperature, 12h light/12h dark conditions for 1 week before the start of the experiments for adaption to laboratory conditions. The procedures in this study were carried out in a accordance with the institution's scientific procedures for animals and was approved by the Institutional animal care.

Inflammation test

The animals were divided into 5 groups of 6 animals each, including: 1-negative control group that received phosphate buffered saline and tween 80, 2- positive control group (Dexamethasone 15 mg/kg), 3, 4 and 5-treatment groups (ethanolic extracts 100, 200, 300mg/kg). In this test, the xylene was used for inflammation effect of ethanolic extracts. So that, 15 minutes after treatment, 0.03 ml of Xylene was injected into the right ear of each animal, then, after 2 hours the animals were anesthetized and differences of ears weight were investigated [9].

Analgesic test

In this test also, the animals were divided into 5 groups of 6 animals each, including: 1-negative control group that received phosphate buffered saline and tween 80, 2-positive control group (Morphine 10 mg/kg), 3, 4 and 5- treatment groups (ethanolic extracts 100, 200, 300 mg/kg). Formalin test was used to evaluate the analgesic effect of

ethanolic extracts. So that, 30 minutes after treatment, 0.02 ml of 2.5% formalin solution was injected into the plantar surface of the right paw of the rats. The time period spent by the rats in licking the injected paw was measured as an index of pain [10].

Data analysis

The data were analyzed using ANOVA and Least Significant Difference (LSD) tests. Also, all data were presented as mean ± SEM.

RESULTS

According to the results, There was a significant difference between the groups receiving 100, 200, 300 mg/kg of *Tanacetumlingulatum*sesquiterpene lactone extracts and the group receiving phosphate buffered saline and tween 80 (p<0.001). Thus the group receiving 200 mg/kg showed a greater anti-inflammatory effect (Table 1).

Dexametazone was used for positive control group and the results showed that the group receiving Dexametazone has not a significant difference with the groups receiving 100, 200, 300 mg/kg of the extract (p<0.001), Thus, the effect of extract in reduce the inflammation was adjacent the Dexametazone.

There was a significant difference between the groups receiving 100, 200, 300 mg/kg of *Tanacetumlingulatum*sesquiterpene lactone extracts and the group receiving phosphate buffered saline and tween 80 (p<0.001). Thus the group receiving 300 mg/kg showed a greater analgesic effect (Table 2).

Morphine was used for positive control group and the results showed that the group receiving Morphine has not a significant difference with the groups receiving 100, 200, 300 mg/kg of the extract (p<0.001), Thus, the effect of extract in reduce the acute pain was adjacent the Morphine.

There was a significant difference between the groups receiving 100, 200, 300 mg/kg of *Tanacetum lingulatum* sesquiterpene lactone extracts and the group receiving phosphate buffered saline and tween 80 (p<0.001). Thus the group receiving 300 mg/kg showed a greater analgesic effect (Table 3).

Morphine was used for positive control group and the results showed that the group receiving Morphine has not a significant difference with the groups receiving 100, 200, 300 mg/kg of the extract (p<0.001), Thus, the effect of extract in reduce the acute pain was adjacent the Morphine.

Table 1: The mean±SD of ears weight difference in *Tanacetum lingulatum* flower sesqui terpene lactone extract (ANOVA-test)

Groups	Mean ears weight ±SD
Negative control group	0.0090±0.0020
Positive control group	0.0010±0.0063
sesquiterpene lactone extract (100 mg/kg)	0.0033±0.0010
sesquiterpene lactone extract (200 mg/kg)	0.0023±0.0005
sesquiterpene lactone extract (300 mg/kg)	0.0028±0.0007

Note: Value followed by different letters in column is significantly different where p < 0.001.

Table 2: The mean±SD of time of feet lick (acute pain) in *Tanacetumlingulatum* flowersesquiterpene lactone extract (ANOVA-test)

Groups	Mean ears weight ±SD
Negative control group	91.166±20.701
Positive control group	0±0
sesquiterpene lactone extract (100 mg/kg)	13±3.346
sesquiterpene lactone extract (200 mg/kg)	8±1.414
sesquiterpene lactone extract (300 mg/kg)	3.833±1.169

Table 3: The mean±SD of time of feet lick (chronic pain) in *Tanacetum lingulatum* flower sesquiterpene lactone extract (ANOVA-test)

Groups	Mean ears weight ±SD
Negative control group	78.666±9.912
Positive control group	0±0
sesquiterpene lactone extract (100 mg/kg)	4.666±1.366
sesquiterpene lactone extract (200 mg/kg)	3.833±0.983
sesquiterpene lactone extract (300 mg/kg)	1.833±0.752

DISCUSSION

The present study showed that *Tanacetum lingulatum* flowers sesquiterpene lactone extract possesses anti inflammatory and analgesic activity at concentrations of 100, 200, 300 mg/kg.As the results of this study showed that the effect of *Tanacetum lingulatum* sesquiterpene lactone extract on pain relief is probably due to the opioid receptors, because this effect has been greatly reduced in the groups receiving Morphine which are the antagonist of the opioid receptors and Morphine injection has likely led to the blockage of opioid receptors. likely, the analgesic effect of the *Tanacetum lingulatum* herb on visceral pain can be attained by inhibiting prostaglandins, similar to the non-steroidal anti-inflammatory drugs [11].

Murray *et al.*(1988) and Hunnkaar and Hole (1987) have been demonstrated that formalin induced pain involves two distinct phases; in the first phase (Neurogenic phase) the pain is caused due to direct stimulation of the sensory nerve fiber by formalin and the second phase (Inflammatory phase) the pain is due to release of inflammatory mediators such as serotonin, histamine, prostaglandin

and bradykinin [12, 13]. The effect of the crude extract sesquiterpene lactone compounds of *T. lingulatum*, was dominant in the second phase of the formalin test, similar to diclofenac sodium, indicating their peripheral action of analgesia via inhibition of the formalin induced inflammation.

Tanacetum species are widely used herbal treatment for conditions such as arthritis, fever, migraine, vertigo, menstrual disorders, toothache etc[3,5]. Studies have revealed that *Tanacetum* extracts have anti-inflammatory and analgesic activities and sesquiterpene lactones such as parthenolide and flavonoids such as catechin, epicatechin and taxifolin are believed to mediate many of these effects of *Tanacetum*[14, 15]. These activities are explained through: the expression of transcription factors such as AP-1, NF-κB and the expression of key pro-inflammatory molecules such as inducible NO synthase, cyclooxygenase, cytokines [1]. Parthenolide is the major sesquiterpene lactone present in several medicinal plants that have been used in folk medicine for their analgesic and anti-inflammatory activities [2]. Parthenolide has a strong anti-inflammatory activity *in vivo* [6]. Several studies have demonstrated that a great part of the anti-inflammatory effect of this compound is ability to inhibit the NF-κBpatway[16]. NF-κB is a ubiquitous and an regular of expression of pro-inflammatory proteins. The inhibition of NF-κB by parthenolide is believed to be a primary mechanism of action for the anti-inflammatory effect of the botanical extract [5]. In a study conducted by Petrovic*et al.* (2003), they were showed that the anti-inflammatory effect of *Tanacetum lavatum* extract was due to the presence parthenolidesesquiterpene lactone [3]. According to the researchs the ability of *Tanacetum parthenium* extract to reduce inflammation is due to the high concentration of parthenolide sesquiterpene lactone that this effect is dose-dependent [17]. Many investigations have proven that sesquiterpene lactones exhibit analgesic and anti-inflammatory effects [15, 16]. Moreover, the flowers of this plant contains a high amount of sesquiterpene lactone compounds (such as parthenolide) and flavonoids, that anti-inflammatory and analgesic activities of *Tanacetum lingulatum* flowers sesquiterpene lactone extract could be altributed of these compounds. Baltaci*et al.* (2011) evaluated the anti-inflammatory activity of *Anthemisaciphylla* sesquiterpene lactone extract [8]. They showed that, the sesquiterpene lactone extract (200 mg/kg) exhibited significant anti-inflammatory activity (76%) comparable to that of

indometacin (81%). Their studies demonstrated that, secondary metabolites in sesquiterpene lactone extract included sesquiterpene lactones, flavonoids and saponins in high concentrations [8]. This results were similar with our results, we reported that the sesquiterpene lactone extracts of flowers of *Tanacetum lingulatum* showed significant analgesic and anti-inflammatory activities.

CONCLUSION

Tanacetum lingulatum contains many sesquiterpene lactones, with higher concentration of parthenolide lipophilic and flavonoids in the flower heads. Flowers showed significant analgesic and anti-inflammatory activities, which confirmed the folk use of *Tanacetum lingulatum* herb for treatment of fever, arthritis, migraine etc, and these effects are attributed to flowers mainly due to the presence of sesquiterpene lactones and flavonoids. Thus, further investigation in this area may lead to the development of safer drugs for the management of inflammatory processes. This provides a complementary value for this plant and supports its achieving popularity as a botanical supplement.

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