

The Analgesic Effect of Ethanolic Extract of *Tanacetum Parthenium* in Acetic Acid Model

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Article information	Abstract
<p>Article history: Received: 14 Jan 2012 Accepted: 6 Aug 2012 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 22-25</p> <p>Keywords: Visceral pain Opioid Morphine</p> <p>*Corresponding author at: Medical Plants Research Center, Shahrekord University of Medicine Sciences, Shahrekord, Iran. E-mail: np285@yahoo.com</p>	<p>Background: Different <i>Tanacetum</i> species have been widely used in traditional medicine as a remedy for the pain and inflammation since ancient times. Because of the few studies conducted on the mechanism of <i>Tanacetum parthenium</i> (TP), this study has been conducted to determine the effects of TP on pain relief and its action mechanism.</p> <p>Materials and Methods: In this experimental study, 100 male mice (25-35 g) were randomly grouped into receivers of distilled water, morphine (0.5 mg/kg), ibuprofen (100 mg/kg), different doses of the extract including 10, 20, 30 and 40 mg/kg of the extract. In order to study the pain relief effect of this herb, two groups were also received naloxon (0.5 mg/kg) and naloxon together with the 40 mg/kg of the extract. Animals were injected with 0.9% acetic acid for visceral pain induction. 15 minutes after each injection antinociceptive effects were recorded by counting the number of writhes for 30 minutes. Achieved data were analyzed by SPSS statistical software, Kruskal-Wallis and Dunn post hoc test.</p> <p>Results: 40 mg/kg of the extract of TP caused a significant reduction in the pain response. Group receiving a dose of 40 mg/kg extract had higher antinociceptive effects than the group receiving ibuprofen ($p<0.001$) but it didn't have any significant difference with the group receiving morphine. Group receiving naloxone had a statistical significant difference with the group receiving 40 mg/kg extract with naloxone and the group receiving 40 mg/kg extract ($p<0.001$).</p> <p>Conclusion: Antinociceptive activity of TP extract is due to the activation of opioid system, however further studies are needed to be conducted for finding out the suitable position or the role of the antispasmodic effect of TP.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Human has used many different forms of treatment to relieve pain throughout the history, among which traditional medicine has a special place. In this field, the most common plant is poppy (*Papaver somniferum*) from which morphine is obtained and is considered as the leader of opioid analgesics. Such drugs are important, especially in the treatment of chronic pain [1]. Nowadays we can see a worldwide return to the drugs of natural and herbal origin after revealing the side effects and also severe harmful effects of chemical drugs. Also, using total extract of the herb has been considered instead of using a pure substance isolated from the herb [2]. Searching for the new analgesic compounds has started in the world since 1960s, because the drugs have still a wide range of unwanted effects that are sometimes passed on to the next generations. It is believed that the most natural compositions, especially medicinal herbs, can be a source of new compounds because some of the current known pharmaceutical compounds such as aspirin, atropine, morphine and cocaine have obtained from the plants used as an analgesic drug [3].

Antinociceptive effects of TP with the scientific name of *Tanacetum parthenium* and local name of feverfew from the family of Asteraceae has been considered [4]. This

herb was used to treat inflammatory conditions and migraine in the 1980s and several clinical studies have examined the effect of this herb on migraine. In general, this herb prevents the release of serotonin which causes the onset of migraine pains [4, 5]. Also this herb is used in traditional medicine in the countries like Denmark for the treatment of epilepsy. Evaluation of the effects of this herb on patients with epilepsy showed that ethanolic extract of this herb has a great affinity for the place of benzodiazepines on GABA receptor [6]. Medicinal compounds of this herb are extracted by alcohol - ether, chloroform and water. Its main ingredients are volatile oils. In addition, there is also a bitter substance called Tansyn (C₁₁H₁₆O₄) in this herb. These compounds are mainly in the flowers. This herb also has compounds such as terpene, sesquiterpene lactone (including partenolid and sesquiterpenes (including alpha-pinenes)). It is anti-migraine, anti-inflammatory and anti-rheumatism and has a bitter substance that is somewhat toxic which is used in the disposal of the intestinal parasites [4]. In a study conducted by Czyz et al. it was revealed that the Paratenoid which is one of the compounds of TP, has anti-migraine effects together with anti-cancer effects [7, 8]. Considering the mass consumption of analgesic drugs

in modern societies and side effects of these drugs on normal groups of patients and limitations of their use in the special groups, the necessity to study alternative herbal and synthetic drugs is strongly felt. Therefore, this study was conducted with the purpose of examining the analgesic effects of TP extract and its action mechanism using the model of acetic acid.

Materials and Methods

This clinical experiment which has been approved by the Ethics Committee of Shahr-e-Kord University of Medical Sciences, was conducted in spring and summer of 2009. 90 adult male mice weighing 5 ± 25 g were purchased from Tehran Pasteur Institutes. In order to comply with the new environment, mice were kept for one week in the whereabouts of animals of the Shahr-e-Kord University of Medical Sciences at the temperature of 21-25°C. During this period, there were no restrictions for the animals as far as standard food (pellets) and water are concerned.

Extraction (preparation of oil): Collected samples were prepared for the extraction after confirmation of genus and species using existed valid identification keys by the expert. Then the aerial parts of the herb were separated and dried away from light and high temperature conditions. Dried herbs were powdered and soaked in 70% ethyl alcohol for 2 days and the obtained extract was concentrated using filter paper, rotary or in a vacuum distillation unit. After evaporation of alcohol, the remaining substance was used to obtain the required concentration for the test [9-11]. In this study, 10 g was extracted from each 100 g of dry powder. Herbarium code of this herb in the Shahr-e-Kord University of Medical Sciences is 210.

Acetic acid test (visceral pain): In this test, 90 mice were randomly tested in the 9 groups of 10 mice. There were 6 groups including a group receiving 10, 20, 30 and 40 mg/kg of the extract, control group receiving distilled water controls, a group receiving naloxone group, a group receiving naloxone+effective dose of the extract, a group receiving morphine and a group receiving ibuprofen. Evaluation of the role of the extract:

Initially, all rats were weighed and their weights were recorded. Then, 50 mice were randomly divided into 4 groups of 10 recipients of the extract and a control group receiving distilled water. 60 mg/kg of 0.9% acetic acid were intraperitoneally injected to each mouse 5 minutes prior to the peak effect of the extract (approximately 10-15 minutes after the injection of extract) [12]. Abdominal writhing was initiated after 5-10 minutes and it was recorded up to 30 minutes after the commencement of writhing. Then also distilled water, at the same volume of the extract (0.3 ml) was injected to the 10 mice as the control group and after 5-10 minutes, the number of abdominal writhing was counted and recorded up to 30 minutes [12].

Evaluation of the role of opioid receptors: In this section of the study, 20 mice were used in two groups of receiving naloxone and naloxone+effective dose of the

extract in order to examine the role of opioid receptors in the analgesic effects. At first, 40 mg/kg dose of the extract which has the most effective analgesic effect was determined. In this section of the study, 0.5 mg/kg of naloxone, as the opioid receptor antagonist, together with the specified dose of the extract were subcutaneously injected with the interval of 10 to 15 minutes after the injection of extract. For this purpose, 10 mice were considered for the pain test.

In a series of the groups of 10 mice (sham group) only 0.5 mg/kg of naloxone was subcutaneously injected [12, 13]. Generally, the abdominal writhing induced by intraperitoneal injection of acetic acid is used as one of the standard tests used to evaluate the effectiveness of new drugs in the treatment of visceral pain [14]. Writhing test not only is considered as a standard test for abdominal writhing but is also used in gastrointestinal ileus which can be evaluated through direct observation and counting abdominal writhing [15].

Standard drugs test: In this part of the study, 20 mice were randomly divided into 2 groups. In the first group, 100 mg/kg ibuprofen was intraperitoneally injected. (similar to the tests performed on the extract). The effect of 0.5 mg/kg morphine (intraperitoneal injection) was similar to the group receiving ibuprofen [12, 13]. At the end of each section of this study mice were excluded while observing the ethical principles. Finally, obtained data was analyzed using the statistical software of SPSS-17, Kruskal-Wallis test and Dunn post hoc tests.

Results

Following testing, data analysis using Kruskal-Wallis test showed that repetitions of mice ratings in different understudy groups were not equal to each other and there were significant differences ($p<0.001$).

However, Dunn post hoc test showed that totally 30 minutes rating count, among the groups receiving the extract, 40mg/kg extract has the most analgesic effect which has no significant difference with the other doses ($p<0.001$) and analgesic effects of this dose was significantly different from the control group receiving distilled water ($p<0.001$) (Fig. 1). Dose of 40 mg/kg of the extract was significantly different from the other doses of the extract in the control group in reducing the frequency of rating ($p<0.001$).

However, the group receiving a dose of 40 mg/kg extract was not statistically different from groups receiving morphine, but the group receiving ibuprofen was statistically different from the group receiving 10 mg/kg extract. In the group receiving a dose of 40 mg/kg of extract, antinociception effects were more observed ($p<0.001$) (Fig. 2).

There was a significant difference between the group receiving 40 mg/kg of the extract and the group receiving profen ($p<0.001$). Naloxone was used to examine the mechanism of the analgesic effect of the TP extract and the results showed that the group receiving naloxone has alone a significant difference with the groups receiving 40 mg/kg of the extract+naloxone and the group receiving

only 10 mg/kg of the extract ($p < 0.001$). Thus the group receiving 40 mg/kg showed a greater analgesic effect (Fig. 3).

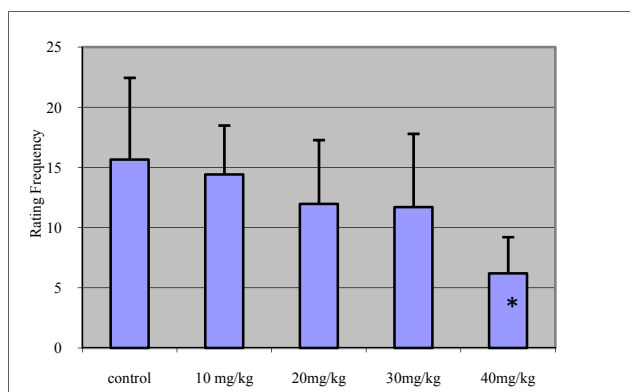


Figure 1. Compares the rating frequency (stretching the body) in different groups receiving extract using acetic acid method

*The most effective dose in reducing the frequency of rating ($p < 0.001$).

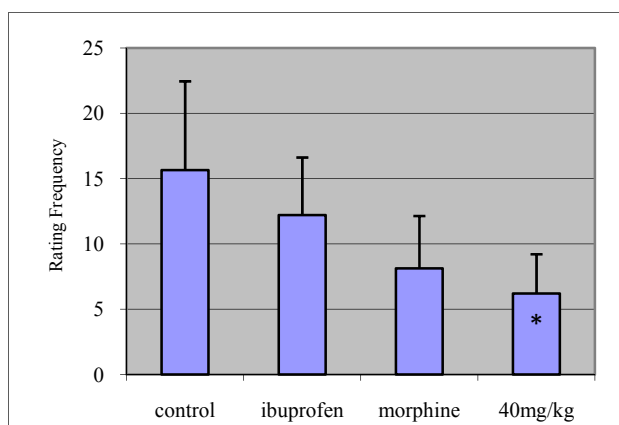


Figure 2. Comparison of the rating frequencies (stretching the body) in different groups of standard and control (distilled water) using the acetic acid method

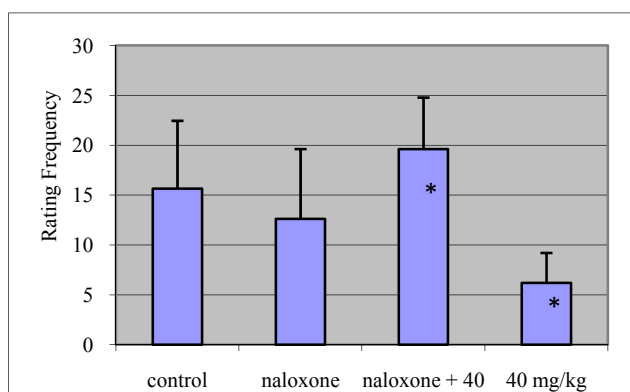


Figure 3. Comparison of the rating frequency (stretching the body) in the studied groups in the evaluation of the opioid mechanism

* There is a statistically significant difference between the group receiving naloxone and the group receiving 40 mg/kg extract + naloxone and the group receiving 40 mg/kg extract ($p < 0.001$).

Discussion

In this study, the analgesic effect of TG was studied in the acetic acid model. The results showed that the group receiving 40 mg/kg extract had more analgesic effects than other groups. However, the group receiving a dose of 40mg/kg of the extract had no statistically significant difference with the group receiving morphine, but the analgesic effect of this dose was better than ibuprofen. Naloxone reduced antinociception effects of the dose of 40 mg/kg of the extract which indicates the possibility of analgesic effects of the extract using the opioid mechanism. As the results of this study showed that naloxone reduced the analgesic effects of the extract, it seems that the effect of this herb on pain relief is probably due to the opioid receptors, because this effect has been greatly weakened in the groups receiving naloxone which are the antagonist of the opioid receptors (μ , δ , GABA) and naloxone injection has likely led to the blockage of opioid receptors [16].

In a study conducted by Iger, it was found that ethanol extract of the TG has great affinity for benzodiazepine sites on GABA receptor and inhibits and blocks the transmission of pain signals and reduces the pain [6]. Among the known and effective compounds of this herb, Paratinolid can be mentioned which has anti-migraine effects. It is a peripheral analgesic compound because of inhibiting the production of thromboxane and leukotrienes. However, this compound is similar to the serotonergic receptor antagonists [12].

In a study, Capasso evaluated the effect of aqueous extract of TG on arachidonic acid metabolism in the in-vitro conditions. In this study both oxygenase and lipooxygenase cycles were examined. The results showed that the metabolic products of both cycles are inhibited by the highest concentrations of the extract, but the lowest dose of the TG extract is effective on the arachidonic acid metabolism [17]. In this study, the dose of 40 mg/kg of the extract showed a better effect on reducing the pain which is consistent with Capasso, who stated that the highest concentration of the extract of the metabolic products inhibits both cycles. In this study which was conducted using different doses of the extract, abdominal writhing induced by acetic acid was reduced. In fact, the injection of the extract was led to the dose-dependent reduction in the abdominal writhing. Presumably, the analgesic effect of the TG herb on visceral pain can be achieved by inhibiting prostaglandins, similar to the non-steroidal anti-inflammatory drugs. The best known aspect of non-steroidal anti-inflammatory analgesic drugs (NSAIDs) is their effect on inflammation. Prostaglandin E2 stimulates pain receptors both directly and by enhancing their sensitivity to the other factors such as bradykinin.

Therefore, NSAIDs may cause short-term and immediate recovery. NSAIDs analgesic effects are beyond their effect on inflammation and include wide central and peripheral actions. There are evidences of the effect of cyclooxygenase and prostaglandins in the central mechanisms of pain [18]. In the studies conducted by

Christine et al. on the biological properties of flavonoids in the TG herb, the presence of 6-4 hydroxy-flavonol methyl ether in the extract of the leaf, flower and seeds were proved and anti-inflammatory properties of the most flavonoids called tannins were clearly specified in this herb [19]. Therefore, given the presence of flavonoids in this herb, part of its analgesic effects is probably related to these compounds with the mentioned mechanism. Jain et al. also studied the analgesic effect of the extract of TG herb on inflammation and pain in rats and mice in the framework of acetic acid model and it was specified that the oral intake of the extract of this herb is associated with the analgesic and anti-inflammatory properties and the responses were dose-dependent. The results of this study about the pain reduction and the effect of the each doses of the extract are consistent with the results of the present study. But in the study conducted by Jain et al. the mechanism of the analgesic effect was not evaluated in accordance with the present study and no comparison made with the standard drugs, so, in this regard, it is not comparable with the present study. Evaluation of the parthenolide extracted from TG herb also showed that this compound also has anti-inflammatory and analgesic properties [12] which may explain the analgesic effects of this herb.

According to this study, TG herb has analgesic properties and it seems that steroids and especially

flavonoids in the extract of this herb may prevent the formation of prostaglandins through Cyclooxygenase inhibition in the inflamed tissue. Compounds of this herb, especially flavonoids in the extract of this herb, reduce pain and inflammation by activating numerous neural pathways. Therefore further studies are needed to be done in this field and it is recommended more analgesic mechanisms to be evaluated in the future studies and molecular tests also be considered to evaluate the effects of agonists and antagonists

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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