Effects of *Sophora alopecuroides* L., *Zingiber officinale* Rosc. and *Melissa officinalis* L. in Formalin and Straub Tail Tests

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Abstract

Background: ***S. alopecuroides, Z. officinale* and *M. officinalis* are used traditionally in the treatment of pain.**

Objective: To evaluate the plants effects in the rat formalin test and their activity on the opioid receptors.

Methods: Each rat was placed individually in a cage for 30 min to get acclimated. Then 0.05 mL of 10% formalin was injected subcutaneously into the dorsal surface of the right hind paw of the rat. Afterward, the animal was returned to the cage for observation. Pain responses were scored at 30 and 60 min after formalin injection. 90% ethanol extract was injected subcutaneously 30 min before formalin injection. To evaluate the role of opioid receptors in the analgesic effect of the extracts inhibiting the early phase of the test, naloxone was injected subcutaneously 30 min before extract injection. Moreover, the extracts effects on the Straub reaction were studied in mice. Mice were placed in individual cages immediately after extract injection for observation. The positive Straub tail response was considered as a persistent elevation of the tail at an angle more than 45º, 30 min after extract injection.

Results: *S. alopecuroides* and *M. officinalis* inhibited both phases of the formalin test ($p<0.05$), but *Z. officinale* inhibited only the late phase ($p<0.05$). Naloxone did not reverse the effects of *S. alopecuroides* and *M. officinalis* in the formalin test. Further, the extracts did not cause Straub reaction.

Conclusion: The plants have analgesic effect without opioid including μ_2 receptor activity and may not produce the side effects caused by activation of μ_2 receptors.

Keywords: Adverse drug reaction, Analgesia, Traditional medicine

Introduction

Pain is an unpleasant sensory and emotional experience caused by tissue damage. Pain can be classified by its pathophysiology and location as somatic, visceral and neuropathic [1]. Pain is commonly the main reason for seeking healthcare. Different pharmacological modalities exist for the treatment of pain such as using acetaminophen, nonsteroidal anti-inflammatory drugs, opioids, anticonvulsants, antidepressants, local anesthetics, cannabinoids, ketamine and others [2]. Pain treatment is usually inadequate and less than ideal. Undertreated pain has important clinical, economic and human consequences [3]. Multiple neurotransmitters and other mediators modulate pain signaling, providing potential targets for pharmacological interventions. The use of multiple analgesic agents with different mechanisms provide comparable or superior analgesic efficacy with lower doses of the individual agents and reduced incidence of side effects [4, 5]. Current guidelines support multiple-mechanism strategies for both acute and chronic pain management [4, 5]. Furthermore, current drugs used to relieve pain have important adverse effects which limit their use [6-8]. Thus, safer and more efficacious analgesic medications are urgently needed [8, 9]. The analgesic effects of many plants and plant-derived compounds have been demonstrated. Plants such as *Papaver somniferum*, *Cannabis sativa* and those of the *Capsicum* and *Salix* species have caused the development of drugs useful for the management of pain. There is a continuing search for safe and effective analgesic medications from natural sources [10]. Numerous plants are used in the traditional medicine as analgesic remedies. *Sophora alopecuroides* L., *Zingiber officinale* Rosc. and *Melissa officinalis* L. are among the most popular of such plants [11-13]. *Z. officinale* has had analgesic effects in the mice hot-plate and acetic acid tests [14] and rat tail-flick test [15]. *M. officinalis* has inhibited acetic acid-induced visceral pain in mice and the early (neurogenic pain) and late (inflammatory pain) phases of rat formalin test [13]. However, the analgesic effect of *S. alopecuroides* has not been studied so far. Further, as yet the effects of *Z. officinale* in the formalin test have not been evaluated. Notably, the potential of these plants for causing adverse effects of opioid analgesics has not been determined so far. The formalin test has been widely used in rats and mice to rapidly evaluate the efficacy of potential analgesic agents [16, 17, 18]. Thus, in the present study, the effects of *S. alopecuroides*, *Z. officinale* and *M. officinalis* in the rat formalin test were evaluated. Moreover, involvement of the opioid receptors in the action mechanism of the plants inhibiting the early phase of the test was investigated. Since the Straub reaction is one of the main parameters used to assess opioid activity [19, 20], the Straub tail effects of the plants were studied in mice.

Materials and methods

Plant material

The seeds of *S. alopecuroides* L. were collected at fruiting stages from the Kerman province of Iran. The aerial parts of *M. officinalis* and roots of *Z. officinale* were obtained from the Research Institute of Medicinal Plants (ACECR) in June 2012 and were air-dried at room temperature.

Samples were authenticated by a botanist (Y. Ajani), and voucher specimens were preserved in the Central Herbarium of the Research Institute of Medicinal Plants.

Extraction

The dried powder of each plant (40 g) was extracted using percolation method with 90% ethanol at room temperature. Solvents were completely removed by drying under reduced pressure at 40 ºC in a rotary evaporator. The samples were stored at 4 °C until use.

Drugs

Morphine sulfate and naloxone hydrochloride were purchased from the Darou Pakhsh (Tehran, Iran) and Sigma-Aldrich companies respectively. Formalin was obtained from the Merk company. For dilution, all drugs and extracts were dissolved in normal saline. All drugs and extracts were prepared immediately before use and injected subcutaneously in a volume of 0.5 mL in rats and 0.1 mL in mice. The doses of morphine and extracts were found experimentally as follows. Morphine: 5 mg/kg; *S. alopecuroides*: 100, 300 and 600 mg/kg and both *Z. officinale* and *M. officinalis*: 100, 500 and 1000 mg/kg.

Animals

Male Wistar rats and male albino mice weighing between 200 - 300 g and 25 - 30 g respectively from our own breeding colony were used. Animals were maintained under standard environmental conditions and had access to standard rodent feed and water ad lib. To minimize effects of noise, the experimental room was kept silent. The number of animals used for each dose of the extracts or morphine was 10 in the formalin test [21-25] and 30 in the Straub tail test [20]. All animals were used only once.

Formalin test

In the beginning, each rat was placed individually in a clear, transparent, polypropylene cage for 30 min to get acclimated. Then 0.05 mL of 10% formalin was injected subcutaneously into the dorsal surface of the right hind paw of the rat. Immediately afterward, the animal was returned to the clear, transparent, polypropylene cage for observation. The formalin injection elicited a series of responses such as elevation and licking of the paw. Readings were taken at 30 and 60 min after formalin injection and scored according to the following scale:

0 = full weight was placed on the paw.

1 = the injected paw rested lightly on the floor and bore no weight.

2 = when the injected paw was selectively elevated and was not in contact with any other surface.

3 = when the paw was licked or bitten by the animal.

Analgesic response or protection was indicated when both the paws were resting on the floor with no obvious favoring of the injected paw. In view of the formalin volume and concentration injected, the first and second 30 min after formalin injection represent the first and late phases respectively.

Each extract or standard drug (morphine) was injected subcutaneously 30 min before formalin injection [21-25] (Table 1). To evaluate the role of opioid receptors in the analgesic effect of the extracts inhibiting the early phase of the test, naloxone was injected subcutaneously 30 min before extract injection (Table 2). Moreover, the extracts effects on the Straub reaction were studied in mice (Table 3).

Straub tail test

Mice were placed in individual cages immediately after morphine or extract injection for observation. The positive Straub tail response was considered as a persistent

Table 1- Effects of *S. alopecuroides*, *Z. officinale*, *M. officinalis* and morphine compared with saline in the formalin test

Treatment (dose, mg/kg, s.c.) (N = 10 in each group)	Early phase score	P-value compared to saline	Late phase score	P-value compared to saline
Saline	2.2 ± 1	-	2.2 ± 0.5	-
Morphine (5)	0.6 ± 0.5	<0.001	0.2 ± 0.5	<0.001
S. alopecuroides (100)	1± 0	0.010	1 ± 0	0.002
S. alopecuroides (300)	0.5 ± 0.5	<0.001	0.5 ± 0.7	<0.001
S. alopecuroides (600)	0.3 ± 0.7	<0.001	0.4 ± 0.7	<0.001
Z. officinale (100)	2.4 ± 0.5	0.992	1 ± 0.7	0.008
Z. officinale (500)	2 ± 0.7	0.990	0.7 ± 0.3	<0.001
Z. officinale (1000)	1.7 ± 0.5	0.927	0.3 ± 0.7	<0.001
M. officinalis (100)	0.9 ± 0.6	0.003	1.1 ± 0.6	0.030
M. officinalis (500)	0.7 ± 0.7	0.001	1.1 ± 0.8	0.031
M. officinalis (1000)	0.5 ± 0.8	0.003	0.9 ± 0.8	0.002

Table 2- Effects of naloxone on the early and late phase scores of *S. alopecuroides*, *M. officinalis* and morphine compared with saline in the formalin test

Treatment (dose, mg/kg, s.c.) (N = 10 in each group)	Early phase score	P-value compared to saline	Late phase score	P-value compared to saline
Saline + Saline	2.6 ± 0.5	-	2.2 ± 0.5	-
Morphine (5) + Saline	0.7 ± 0.7	0.002	0.5 ± 0.7	<0.001
Morphine (5) + Naloxone (5)	2.1 ± 0.8	0.997	2.4 ± 0.5	0.997
S. alopecuroides (600) + Naloxone (5)	0.7 ± 0.5	0.002	0.9 ± 0.8	0.001
M. officinalis (1000) + Naloxone (5)	1 ± 0.7	0.015	1.1 ± 1	0.007

Table 3- Dose-response relationship of *S. alopecuroides*, *Z. officinale* and *M. officinalis* along with morphine and saline in the Straub tail test.

Treatment (dose, mg/kg, s.c.) (N = 30 in each group)	Number of mice responded at 30 min (>45º) /Number tested
Saline	0/30
Morphine (50)	30/30
S. alopecuroides (100)	0/30
S. alopecuroides (300)	0/30
S. alopecuroides (600)	0/30
Z. officinale (100)	0/30
Z. officinale (500)	0/30
Z. officinale (1000)	0/30
M. officinalis (100)	0/30
M. officinalis (500)	0/30
M. officinalis (1000)	0/30

elevation of the tail at an angle more than 45º, 30 min after morphine or extract injection. The Straub tail response was expressed in terms of the number of animals tested showing the effect [20] (Table 3).

Statistical analysis

The formalin test results were expressed as means ± S.D. and analyzed with the One-Way ANOVA followed by the tukey post hoc test. $p < 0.05$ was taken as significant [22, 26]. The Straub tail test results were given as the number of mice responded at 30 min (>45º)/number tested. The results of the Straub tail test were analyzed by the Chi-squared test [20, 26].

Results

While morphine, *S. alopecuroides* and *M. officinalis* inhibited both phases of the formalin test significantly ($p<0.05$), *Z. officinale* inhibited only the late phase significantly ($p<0.05$) (Table 1). Naloxone antagonized the effects of morphine in the formaline test. However, naloxone did not reverse the effects of *S. alopecuroides* and *M. officinalis* in the formalin test (Table 2). Moreover, all morphine injected mice demonstrated Straub tail effect ($p<0.001$), but the extracts did not cause Straub reaction ($p>0.05$) (Table 3).

Discussion

The results show that the 3 plants have analgesic effect in the rat formalin test and confirm the traditional use of the plants as analgesics. Lack of antagonism of the analgesic effects of *S. alopecuroides* and *M. officinalis* by naloxone indicates that their analgesic effects are not through opioid receptors. Moreover, none of the plants caused Straub reaction. Therefore, the plants do not have opioidergic activity. The results of the present study concur with a report stating that opioid receptors are not involved in the analgesic effects of *M. officinalis* [13]. In that work, rosmarinic acid as the chief constituent of *M. officinalis*, muscarinic and nicotinic receptors and the L-arginine-nitric oxide pathway were implicated in the analgesic effect of *M. officinalis* [13]. Two constituents called (6)-gingerol and (6)-shogaol and inhibition of cyclooxygenase-2 may be involved in the analgesic effects of *Z. officinale* [27, 28]. Quinolizidine alkaloids are the main active constituents of *S. alopecuroides*. Matrine is the major quinolizidine alkaloid of *S. alopecuroides* [29] with antinoceptive effect in mice [30-34]. A study reported that (+)-matrine produced an antinociceptive effect mainly through the activation of κ (kappa) opioid receptors and partially through μ (mu) opioid receptors [30], but another study stated that the antinociceptive effect of (+)-matrine is mediated through both μ and κ opioid receptors [31] and in a later study κ receptors have been implicated in the antinociceptive effect of (+)-matrine [33]. Further, according to a report μ, κ and δ (delta) opioid receptors have no role in the antinociceptive effect of (+)-matrine and its antinociceptive effect is through activation of cholinergic receptors in the central nervous system [34]. The present work is in line with the just mentioned study [32]. Of note, morphine produces analgesia and the Straub reaction by acting at different subtypes of μ opioid receptors. The Straub reaction is mediated by central μ_2 opioid receptors while analgesia involves μ_1 opioid recptors. This fact regarding Straub reaction is of relevance to the screening of opioid drugs [20]. It is important to assess the μ_2 opioid

receptor activity of an opioid because these receptors are involved in opioid side effects such as physical dependence, respiratory depression and constipation [20, 35]. As the Straub reaction is a simple observation appearing within the analgesic dose range and has an added advantage over other methods for the testing of other μ_2 effects which involve cumbersome experiments, it has been suggested that the Straub reaction in mice may be considered as a reliable parameter to test the μ_2 opioid receptor activity [20]. Thus, the present study also indicates that *S. alopecuroides* and the other 2 plants do not activate μ_2 opioid receptors and thus may not produce the side effects caused by μ_2 receptor activation. Finally, it seems that further studies on the mechanism (s) of analgesic effect of *S. alopecuroides* and conduction of trials concerning the analgesic effects of the 3 plants are warranted.

Conclusion

S. alopecuroides, *Z. officinale* and *M. officinalis* have analgesic effects in the rat formalin test not mediated by the opioid receptors. Moreover, they do not activate μ_2 receptors and therefore, may not produce the side effects caused by μ_2 receptor activation.

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