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Preliminary Phytochemical Screening, Analgesic and Anti-inflammatory effect of Eryngium pyramidale Boiss. & Husson Essential Oil in Male Rat

Ali Reza Fallahzadeh[1], Mohammad Zarei[2] and Saeed Mohammadi[3]*

[1] *Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran*
[2] *Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
[3] *Department of Biology, Hamedan Branch, Islamic Azad University, Hamedan, Iran*
**Corresponding E-mail: smiauhphd.sm@gmail.com*

ABSTRACT

Objective: Eryngium pyramidale is one of the important medicinal plants that use in iranian folk medicine. In this study, we aimed to assess the Phytochemical Screening, analgesic and anti-inflammatory effects of Eryngium pyramidale essential oil in rats. Materials and methods: The essential oil of Eryngium pyramidale (EOEP) was treatment in doses of 10, 50, and 100 mg/kg. In addition, anti-nociceptive activity of EOEP was evaluated by formalin, writhing, and tail-flick tests. EOEP was also that combined with 2 mg/kg naloxone to determine the involvement of opioid mechanism. Anti-inflammatory reaction was evaluated via xylene-induced ear edema. Also the EOEP origin has been analyzed by a combination of GC and GC/MS. Results: The EOEP at dose 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 EOEP has been inhibited pain in all three models. The present results shown that anti-inflammatory effect of EOEP in the xylene-induced ear edema test is significant ($p<0.05$) in compare to dexamethasone. The major components in the tested oils was mainly contained quercetin, Isoquercitrin and luteolin. Conclusion: Our findings propose that EOEP probably have analgesic and anti-inflammatory effects. Existent of flavonoids may be an important reason for mentioned effects.

Keywords: Pain, Essential oil, Eryngium pyramidale, Medicinal plants

INTRODUCTION

Pain is a somatic sensation such as touch, proprioception, and pressure. Always pain has 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 (NSAID) drugs and opioid are mostly used to control pain, but these drugs have many adverse effects and cause renal disorders and gastrointestinal sometimes. 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 a 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].

Some species have been used to nation folk medicine. E. campestreis a notable therapeutic plant of the Apiaceae family and is utilized as a part of Turkish Ayurveda drug. Implantations of the airborne and root parts are utilized as a diuretic, antitussive, sexual enhancer, stimulant, and hors d'oeuvre [7-9]. E. creticum has been used in traditional medicine in Jordan as a relief for scorpion stings and as a hypoglycemic agent [10]. E. elegans Cham. & Schltdl.

Was declared to be used for diuretic uses in Argentina [11], and E.foetidumfor the relief of several anti-inflammatory disorders in China [12].

Eryngium pyramidale is a medicinal plant that member of the Apiaceae family. This local plant is utilized as a part of Iranian society prescription for different purposes an outstanding as a love potion, anti-spasmodic, and expectorant [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 Eryngium pyramidale in major databases, for the first time a study to assess its aforementioned effects was needed.

MATERIALS AND METHODS

Plant material gathering

Some of new Eryngium pyramidale 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 of science, Islamic Azad university of Hamadan, Iran.

Essential Oil of Eryngium pyramidale

Distillation of oil was completed by means of a refining and clevenger mechanical assembly. New petals of Eryngium pyramidale 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 EOEP. The oils got were stacked in a lightproof jug and kept in an icebox until use. The relative thickness of the key oil of Eryngium pyramidale 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].

Animal 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° C 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, EOEP (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 EOEP in addition to naloxone (2 mg/kg i.p.).

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 hairlike 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].

Drugs and chemicals

Morphine sulfate, naloxone, indomethacin, and dexamethasone were purchased from darou pakhsh (Iran), and acetic acid and formalin from Merck Inc (Germany).

Acute toxicity test (LD50)

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 EOEP per kg body weight. As indicated by unique reference, we watched rats for the following 24 hours to any behavioral changes or passing.

Tests of pain

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 EOEP 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 (or writhing) was counted for 30 min. It is also necessary to mention that each rat was used only once [19].

Tail Flick Test

This valid model of pain is a reaction in animals, like to 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 in continue a timer starts. When rats start to moved or flicks its tail, the timer cease and the recorded time (latency) is a measure of the pain threshold. For the tail flick test, we used a tail flick analgesimeter apparatus (Made in Borj Sanat, Iran Company). 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.

Formalin test

In this test, suggested a model of Dubuisson and Dennis, was used in order to evaluate the acute and chronic pain. One hour before the test, the rats were sent into the box of formalin test 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 rats behaviors more clearly. Thirty minute after drugs injection, formalin (50 µl, 2.5%) was infused subcutaneously in the subplantar 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].

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. Besides the left ear was considered as a control bunch. 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].

RESULTS

Preparatory chemical composition of essential oils

The real parts in the tried oils are introduced in Table 1. Eryngium pyramidale oil fundamentally contained quercetin, Isoquercitrin and luteolin7-O-β-D-glucopyranoside and coumarins (scopoletin) (Table 1).

Table1. The compounds identified in the essential oil of Eryngium pyramidale

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

Acetic Acid-Induced Writhings

EOEP with dosage of 100mg/kg significantlly (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 (Fig.1). EOEP with dose of 10 and 50 mg/kg did not inhibit writhings in rats. Moreover treatment of rats with EOEP with dose of 100mg/kg +naloxone reversed the inhibitory effects of the EOEP in acetic acid-induced writhings.

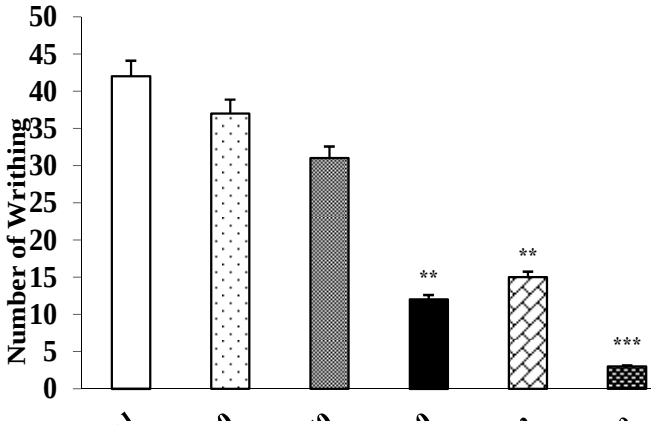

Fig1. Effect of different doses of essential oil of Eryngium pyramidale (EO) on acetic acid induced writhing in rats .As compared with control: **P<0.01 and ***P<0.001(n=6, means ± S.E.M).

Tail flick test

According to Fig.2, groups of the EOEP 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).

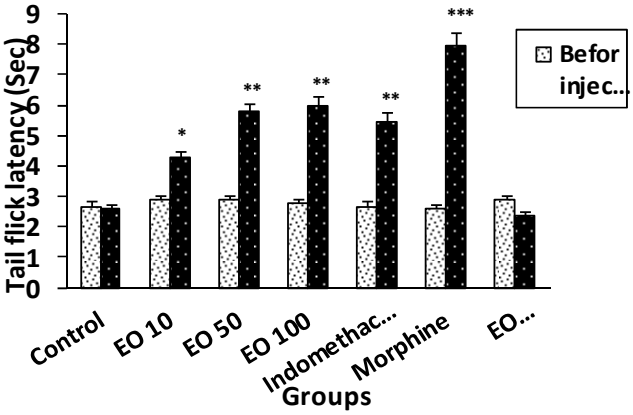

Fig 2. Effect of different doses Essential oil of Eryngium pyramidale (EO) on the reaction time of rats. As compared with control: *P<0.05, **P<0.01 and ***P<0.001. (n=6, means ± S.E.M).

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 antiinflammatory drugs (NSAID) and morphine (10 mg/kg, i.p.) a powerful opioid likewise has been gave pain relieving hint. Besides the key oil at the dosage of 50 mg/kg (i.p.) indicated restraint in the second stage as acquired with control. Pretreatment with EOEP+Naloxone essentially diminished their pain relieving impacts at the two stages (Fig 3).

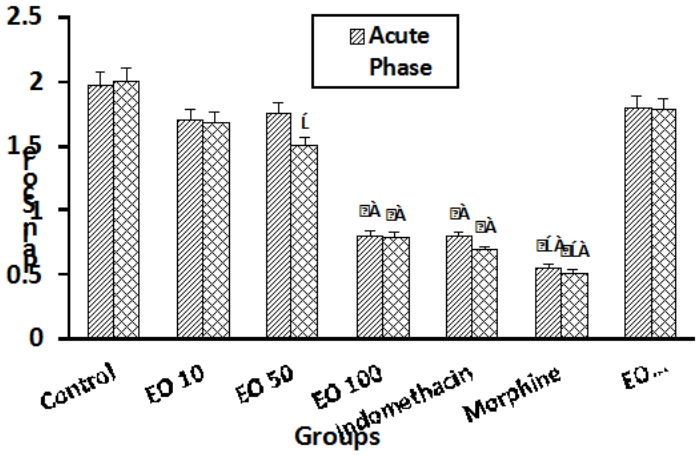

Fig3. Effect of Essential oil of Eryngium pyramidale (EO) on formalin-induced nociception in rats. As compared with control: *P<0.05, **P<0.01 and ***P<0.001. (n=6, means ± S.E.M).

xylene-induced

EOEP 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. Effect of the intraperitoneal doses of Eryngium pyramidale essential oil and dexa methasone on xylene-induced ear swelling in male rat

Treatment	Dose	Ear Swelling (mg)	Inhibation (%)
Control	10ml/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

The increase in weight caused by the irritant (xylene) was measured by subtracting the weight of the untreated left ear section from that of the treated right ear sections. Values are the mean ±S.E.M. for 7 rat, P<0.05* and P<0.001***, compared to control (normal saline), Tukey.

DISCUSSION

The general aftereffects of the present examination demonstrated that the EOEP has critical antinociceptive and antiinflammatory movement. Looking at the outcomes got by the entire of three distinctive exploratory models of nociception (Tail-flick, writhings and formalin test) we can conjecture that EOEP acts both at the peripheral and central levels of pain.

One of the most important 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 EOEP prevented abdominal constriction caused by acetic acid therefore, it is imagined that its attenuate effects are supported by the environmental mechanisms. I.p administration of acetic acid can cause the acute inflammation of the peritoneum. In this standard trial, it seems that peripheral antinociceptive effects of EOEP 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 [1]. The tail-flick model or tail flick test make utilization of high-power light emission target a rats 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 EOEP 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 [24].
Between several test 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. in this test 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, improvement of irritation and broadening of responsive fields. Likewise the simultaneous nearness of low level contribution from both huge and little afferents.The results demonstrated that EOEP have an inhibitory impact over the torment. The EOEP 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 EOEP 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 neurons [25,26].

To evaluate opioid system interference with analgesic effect of EOEP, we were used one of the important antagonist of opioid system, naloxone. This amazing drug inhibite the activation of opioid receptors (especially mu receptor). The results indicate that naloxone can attenuates the antinociceptive effect of EOEP. Therefore, it seems that the effect of EOEP in pain relief is due to the opioid receptors [27].

Biologic or therapeutic activity of herbs has a close relationship with their chemical combinations [28]. According to our present results from phytochemical analysis', *Eryngium pyramidale* contains several compounds such as flavonoids, isoflavonoid derivatives, coumarins, hydroxycinnamic acid (caffeic acid), and terpenoids. Earlier papers reported that flavonoids have an analgesic effect [29-31]. 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 family 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 [34], which has likewise been affirmed in vivo related creatures ponders [35]. In this manner, might be one of the components in charge of its antiinflammatory 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 [36]. Furthermore, anti-inflammatory effect of caffeic acid has been shown [37]. The other components that presented in EOEP was cumarins. In new investigashion, park et al has shown that coumarin derivation can specifically activate the TRPV1 or nociceptor transient receptor potential vanilloid 1 channels and can reverse the inflammatory pain in rodent through channel desensitization [38].

CONCLUSION

It concludes that the antinociceptive and anti-inflammatory effects of EOEP might be identified with their phytochemical bioactive segment, for example, flavonoids. Besides we reason that crucial oil have pain relieving impact that is likely because of restraint of prostaglandin combination and hindrance of the focal and fringe sensory system. So crucial oil of this plant could possibly be utilized to control the difficult infection.

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