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Analgesic Effect of Essential Oil (EO) from Carum Copticum in Mice

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Abstract: The purpose of this study was to examine anti nociceptive effect of essential oil from fruit of Carum Copticum which is a plant in ambelliferea family grow as a herb with anti cholinergic and anti histaminic activities. The major components in the fruit of this plant are an essence containing thymol and some other chemical components and essential oil. In this study formalin test was used as a standard pain inducing test and the analgesic effect of intrapritonial injection of 20 mg kg^{-1} of essential oil (prepared by steam distillation) were compared with different concentration of morphine (1&2 mg kg^{-1}). Our data show that essential oil has no effect on early response of pain but its effect on late phase of formalin test is significant (p=0.01). This effect was the same as 1 mg kg^{-1} of morphine solphate. According to our finding essential oil significantly reduced pain sensation in inflammatory phase of formalin test and it is may be due to the presence of thymol in essential oil and it seems that mechanisms other than opioid receptors involved in the analgesic activities because Naloxon an opioid antagonist could not reverse the analgesic effect observed in the formalin test.

Key words: Essential oil · carum copticum · pain · medicinal herb

INTRODUCTION

One of the important and effective part of herbal plants is essential oil (EO) and this substances are present in different part of plants (leaf&wall&fruit). Essential oil are components which are oil soluble that have effective smell and aroma and are separate by use of water and steam distillation and prepared by extraction with solvents and enzymatic hydrolysis [1]. The EO which is produced by different plants confirms analgesic and anti inflammatory activities which affects by different ways. The study of LINO[2] on activities of the essential oil of Ocimun show a possible analgesic effect on the acetic acid induced writhing and formalin test in mice. The EO demonstrated antinociceptive effects and pretreatment with naloxone did not reverse the antinociception, indicating that the opioid system is not involved. On the other hand, pretreatment with L-argenine reversed the antinociception. Suggesting involvement of nitric oxide system.

The essential oil obtained by hydrodistillation of the resin of Canarium schweinfurthii growing in Central African Republic, was analyzed by GC and GC/MS and its analgesic and antiinflammatory effects were studied by Koudou [3] show that the major constituents of the essential oil were octylacetate (60%) and nerolidol (14%). At the doses of 1, 2 and 3 mL kg^{-1} i.p. essential oil shows a significant analgesic effect using acetic acid-induced writhing and hot plate methods. However, its was unable to reduce inflammatory process in cotton pellet induced granuloma method.

The essential oils obtained by water distillation from aerial parts of Achillea schischkinii Sosn [4]. and Achillea aleppica DC. subsp. aleppica were analyzed by gas chromatography and gas chromatography/mass spectrometry. 1,8-Cineole (32.5 and 26.1%, respectively) was the main component in both oils. The oil of *A. aleppica* subsp. aleppica was also found to be rich in bisabolol and its derivates. When tested for their antimicrobial, antiinflammatory and antinociceptive activities the oil of *A. aleppica* subsp. aleppica showed significant antiinflammatory, antinociceptive and moderate antimicrobial activities.

The antinociceptive effects of the orally administered essential oil of *A. zerumbet* (EOAz) were evaluated [5] in male Swiss mice (20-25 g each). In the acetic acid-induced writhing test, EOAz (30, 100 and 300 mg kg^{-1} body wt.; n = 10, n = 13 and n = 15, respectively) was effective at all doses. In the hot-plate test, EOAz significantly increased the latency at doses of 100 and 300 mg kg^{-1} body wt., but

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not at 30 mg kg^{-1} body wt., at all observation times up to the 180th min (n = 10 for each dose). In the formalin test, EOAz significantly reduced paw licking time in the second phase of the test at 100 mg kg^{-1} body wt. (n = 10), but decreased it in both phases at 300 mg kg^{-1} body wt. (n = 10). At 30 mg kg^{-1} body wt., the effect of EOAz did not differ from control values in either phase of the formalin test (n = 10). Pretreatment with naloxone (5 mg kg^{-1} body wt., i.p.) caused a significant reversal of the analgesic effect of 300 mg kg^{-1} body wt. EOAz (n = 8) that was complete for the first phase, but only partial for the second phase of the formalin test. The data show that orally administered OEAz promotes a dose-dependent antinociceptive effect, with a mechanism of action which probably involves the participation of opiate receptors.

The leaf essential oil from Croton sonderianus (EOCS) was evaluated by Santos [6] for antinociceptive activity in mice using chemical and thermal models of nociception. Given orally, the essential oil at doses of 50, 100 and 200 mg kg^{-1} produced significant inhibitions on chemical nociception induced by intraperitoneal acetic acid and subplantar formalin or capsaicin injections. However, it evidenced no efficacy against thermal nociception in hot-plate test. More prominent inhibition of acetic acid-induced writhing and capsaicin-induced hind-paw licking responses was observed at 100 and 200 mg kg^{-1} of EOCS. At similar doses, the paw licking behavior in formalin test was more potently suppressed during the late phase (20-25 min, inflammatory) than in early phase (0-5 min, neurogenic). The EOCS-induced antinociception in both capsaicin and formalin tests was insensitive to naloxone (1 mg kg^{-1}, s.c.), but was significantly antagonized by glibenclamide (2 mg kg^{-1}, i.p.). In mice, the essential oil (100 and 200 mg kg^{-1}) neither significantly enhanced the pentobarbital-sleeping time nor impaired the motor performance in rota-rod test, indicating that the observed antinociception is unlikely due to sedation or motor abnormality. These results suggest that EOCS produces antinociception possibly involving glibenclamide-sensitive KATP+ channels, which merit further studies on its efficacy in more specific models of hyperalgesia and neuropathic pain.

The Satureja khuzistanica hydroalcoholic extract was prepared and its anti-inflammatory and anti-nociceptive effects were investigated using the carrageenan-induced rat paw edema and formalin test [7]. The extract showed anti-nociceptive activity in a dose-dependent (10-150 mg kg^{-1}, i.p.) manner at the second phase of formalin test which was comparable with morphine (3 mg kg^{-1}, i.p.). This study confirms that anti-inflammatory and anti-nociceptive properties of *S. khuzistanica* are comparable to those of indomethacin and morphine. Presence of flavonoids, steroids, essential oil, mainly carvacrol and tannin might be responsible for anti-inflammatory and anti-nociceptive activities of this plant.

The aqueous extract and the essential oil of *Satureja viminea* (Lamiaceae) were tested [8]. General physiologic effects were assessed through the Hippocratic screening test. A very clear and significant analgesic effect was observed with the oral administration of the essential oil of *S. viminea* (1000 mg kg^{-1}). This effect is compared to that of indomethacine.

The Dracocephalum kotschyi essential oil was isolated and studied on writhing test a visceral pain model in mice [9]. Different constituents of the essential oil were determined by gas chromatography mass spectrophotometry technique. Limonene, verbenone, alpha-terpineol, perillyl alcohol and caryophyllene were the major constituents of the essential oil. Hyoscine (1 mg kg^{-1}) and indomethacin (5 mg kg^{-1}) induced significant ($p<0.01$) reductions (74.9 and 76.7%, respectively) in pain response in comparison to control. This study confirms that antinociceptive properties of *D. kotschyi* are comparable to those of hyoscine and indomethacin used. Presence of limonene and alpha-terpineol might be responsible for antinociceptive properties of this essential oil.

The steam-distilled essential oil of Iranian black cumin seed (*Nigella sativa* L.) was investigated for its composition and analgesic and antiinflammatory properties [10]. After oil analysis by GC/MS, 20 compounds were identified in the oil, obtained in 0.4% (v/w) yield. Among them, para-cymene (37.3%) and thymoquinone (13.7%) were the major components. Acetic acid-induced writhing, formalin and light tail flick tests were used for assessment of analgesic activity. Black cumin seed essential oil (BCSEO) was found to produce a significant analgesic effect in acetic acid-induced writhing, formalin and light tail flick tests. Naloxone, an opioid antagonist, could not reverse the analgesic effect observed in the formalin test.

Antinociceptive effect of hydroalcoholic extract and essential oil of Zataria multiflora was studied using writhing, tail flick and formalin tests [11]. In tail flick test, the hydroalcholic extract (500 mg kg^{-1}, i.p.) and the essential oil (0.3 mL kg^{-1}, i.p.) of the plant showed antinociceptive activity ($p<0.05$). Moreover, they showed antinociceptive activity in writhing and formalin tests.

In the current investigation [12], we evaluated the analgesic and anti-inflammatory effects of essential oil

extracts from three species of Eucalyptus employing various standard experimental test models. Using acetic acid-induced writhes in mice and hot plate thermal stimulation in rats, it was shown that the essential oils of *Eucalyptus citriodora* (EC), *Eucalyptus tereticornis* (ET) and *Eucalyptus globulus* (EG) induced analgesic effects in both models, the data suggest that essential oil extracts of EC, ET and EG possess central and peripheral analgesic effects as well as neutrophil-dependent and independent anti-inflammatory activities. These initial observations provide support for the reported use of the eucalyptus plant in Brazilian folk medicine. Further investigation is warranted for possible development of new classes of analgesic and anti-inflammatory drugs from components of the essential oils of the eucalyptus species.

For evaluation of its probable analgesic and anti-inflammatory effects, hydroalcoholic extract, polyphenolic fraction and essential oil of the leaves of the *Lavandula angustifolia* Mill. (Lamiaceae) were prepared [13] and their analgesic effects were studied in mice using formalin and acetic acid-induced writhing tests. Results showed that while the hydroalcoholic extract (400-1600 mg kg^{-1}, p.o.) inhibited only the second phase of formalin test, the polyphenolic fraction (800 and 1600 mg kg^{-1}, p.o.) and essential oil (100 and 200 mg kg^{-1}, p.o.) suppressed both phases. In acetic acid-induced writhing test, polyphenolic fraction (400 and 800 mg kg^{-1}, p.o.) and essential oil (100 and 200 mg kg^{-1}, p.o.) reduced the number of abdominal constrictions.

The effects of eugenol on synaptic transmission and long-term potentiation (LTP) were investigated [14]. Population spikes (PS) were recorded in the stratum pyramidal following stimulation of stratum fibers. To induce LTP, eight episodes of theta pattern primed-bursts (PBs) were delivered. Eugenol decreased the amplitude of PS in a concentration-dependent manner. The effect was fast and completely reversible. Eugenol had no effect on PBs-induced LTP of PS. It is concluded that while eugenol depresses synaptic transmission it does not affect the ability of CA1 synapses for tetanus-induced LTP and plasticity.

Present study is a investigation on EO separate from Carum Copticum by steam distillation and the major components in the fruit of this plant is an essence containing thymol substance which used to measure the analgesic activities by formalin test.

MATERIALS AND METHODS

A: Type of study - this is an experimental method on 20 mice which is choice randomly that are present in the same situation of light, air and nutrition and are separate into 4 groups of control, test and two groups of sham.

B: Preparation of sample- For preparing EO, 40 g powdered of Carum Copticum added to 200 mL of D water and then by use of steam water distillation which is a popular method for separation of EO and then the sample with concentration of 20 mg kg^{-1} body weight is ready and by use of Tween 80 as a cosurfactant and cosolvant [15-17] that is able to increase viscosity or absorption of surfactant on the crystal surface. The sample is injected intrapritonial (20 mg kg^{-1}) and the analgesic effects are measured by formalin test [18, 19] and the mean score results of paw licking behavior were compared with different concentration of morphine (1&2 mg kg^{-1}) and also for assessing the receptors of EO, pretreatment of naloxone (5 mg kg^{-1}) an opioid antagonist were examined.

C: Formalin test is one of the standard test for assessment of pain which is used in animal model. In this method injection of formalin leads to a biphasic pain response. (The paw licking behavior) The first phase is the fast response which is follow by one late response.

D: Method of data analyze:- The data were collected every 5 min and during one hour, the pain score were calculated and by use of t-test and analyze variance the $p>0.05$ is significant.

RESULTS

The data show a biphasic response in paw licking behavior in formalin test that the maximum pain score is belong to the first 5 min (2.31±0.23) and the paw licking behavior was more potently suppressed during the late phase (20-25 min, inflammatory) than in early phase (0-5 min, neurogenic). Our findings show that EO has no effect on early response of pain but its effect on late phase of formalin test is significant ($p=0.01$) In the formalin test, EO significantly reduced paw licking time in the second phase of the test at 20 mg kg^{-1} body wt. ($n = 5$), but decreased it in both phases the effect of EO did not differ from control values in late phase of the formalin test during 20-60 min (0.42±0.24). This effect was the same as 1 mg kg^{-1} of morphine sulphate ($p = 0.12$) (Table 1). Essential oil was found to produce a significant analgesic effect in formalin tests. It seems that mechanisms other than opioid receptors involved in the analgesic activities because Pretreatment with naloxone (5 mg kg^{-1} body wt., i.p.) an opioid antagonist, could not reverse the analgesic effect observed in the formalin test.

Table 1: The mean and standard deviation of pain score during one hour (every 5 min) in case (Essential oil) and control and compared with morphine (1&2 mg kg^{-1})

Morphine 2 mg kg^{-1}		Morphine 1 mg kg^{-1}		Essential oil 20 mg kg^{-1}		Control N saline		G Time
M	S.D	M	S.D	M	S.D	M	S.D	(min)
3/15	0/45	2/19	0/23	2/31	0/23	2/48	0/46	5
0/98	0/25	0/51	0/28	0.91	0/46	1/31	0/43	10
0/32	0/2	0/32	0/17	0/46	0/30	1/17	0/55	15
0/25	0/1	0/65	0/33	0/12	0/21	0/94	0/83	20
0/32	0/25	0/55	0/25	0/27	0/20	1/33	0/51	25
0/22	0/14	0/39	0/21	0/69	0/76	1/53	0/12	30
0/59	0/18	0/76	0/3	0/84	0/82	1/34	0/55	35
0/3	0/16	0/65	0/3	0/42	0/38	1/33	0/68	40
1	0/54	1/1	0/06	0/19	0/23	1/43	0/4	45
0/77	0/43	0/57	0/12	0/40	0/75	1/52	0/36	50
0/69	0/23	0/52	0/19	0/63	0/81	1/42	0/55	55
0/63	0/32	0/49	0/18	0/23	0/18	1/54	0/5	60

DISCUSSION

Our findings show that the modulating of the pain is lead by different system and also support other study indicating analgesic effect for essential oil that significantly reduced pain sensation in inflammatory phase of formalin test the study of LINO in 2005 on activities of the essential oil of Ocimun show a possible analgesic effect and the opioid system is not involved [2]. On the other hand, pretreatment with L-argenine reversed the antinociception. Suggesting involvement of nitric oxide system.The antinociceptive effects of the orally administered essential oil of *A. zerumbet* (EOAz) were evaluated [5]. The effect of EOAz did not differ from control values in either phase of the formalin test (n = 10). Pretreatment with naloxone (5 mg kg^{-1} body wt., i.p.) caused a significant reversal of the analgesic effect with a mechanism of action which probably involves the participation of opiate receptors and involvement of nitric oxide system. The leaf essential oil from Croton sonderianus (EOCS) was evaluated by Santos [6] for antinociceptive activity in mice. These results suggest that EOCS produces antinociception possibly involving glibenclamide-sensitive KATP + channels, The Satureja khuzistanica hydroalcoholic extract was prepared and its anti-inflammatory and anti-nociceptive effects were investigated [7].This study confirms that anti-inflammatory and anti-nociceptive properties of *S. khuzistanica* are comparable to those of indomethacin and morphine. Presence of flavonoids, steroids, essential oil, mainly carvacrol and tannin might be responsible for anti-inflammatory and anti-nociceptive activities of this plant. The *Dracocephalum kotschyi* essential oil was isolated and studied on writhing test a visceral pain model in mice [9]. This study confirms that antinociceptive properties of *D. kotschyi*. Presence of limonene and alpha-terpineol might be responsible for antinociceptive properties of this essential oil.The steam-distilled essential oil of Iranian black cumin seed (*Nigella sativa* L.) was investigated for its composition and analgesic and antiinflammatory properties [10]. Naloxone, an opioid antagonist, could not reverse the analgesic effect observed in the formalin test. The effects of eugenol on synaptic transmission and long-term potentiation (LTP) were investigated [14]. It is concluded that eugenol depresses synaptic transmission. Cholinergic system is also one of important mechanism which also interfere in modulation of pain [20] and it can reverse by atropine therefore due to the presence of cholinomimetice activity in carum Copticum [21] it may be antinociceptive action of essential oil is due to cholinergic system and also study of Gedney [22] show that aromatherapy may not elicit a direct analgesic effect but instead may alter affective appraisal of the experience and consequent retrospective evaluation of treatment therefore thymol which is the main part of essential oil with smell and aroma may help the analgesic property of essential oil.

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