Study of Analgesic Activity of Methanolic Extracts of Cuminum Cyminum (L.) and Centratherum Anthelminticum (L.) in Mice

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Abstract

Objective: This study was conducted to investigate and compare the analgesic activity of methanolic extracts of seeds of Cuminum Cyminum (L) and Centratherum anthelminticum (L)in mice.
Methods: This study was conducted in the department of Pharmacology University of Karachi; 30 healthy mice were selected from animal house of Pharmacology department of University of Karachi for the study. All animals were divided into three groups, each group of 10 mice. Mice of Group 1 were given DMSO (Dimethyl sulphoxide) an organic solvent, mice of group 2 were given Methanolic extract of Cuminum Cyminum (L) and mice of group 3 were given Methanolic extract of Centratherum anthelminticum (L). The analgesic activity was investigated by tail flick test, heat is used to stimulate pain and observe analgesic activity, after a week of oral dosing of Methanolic extracts of seeds of Cuminum Cyminum (L) and Centratherum anthelminticum (L) in mice. Methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) were insoluble in water so Dimethyl sulphoxide (DMSO) i.e. an organic solvent was used in this study (it is used for solutes that are insoluble in water). The methanolic extracts of Cuminum cyminum(L), Centratherum anthelminticum (L) and DMSO (dimethyl sulphoxide) were administered orally to three groups of mice for a week and on 7th day of dosing analgesic activity was tested through tail flick test, response is checked after 30, 60, 90, 120, 150, 180 and 240 minutes of 7th day of dosing.
Results: The group of mice, which were given Methanolic extract of Cuminum cyminum(L)showed highly significant analgesic activity after 60, 90, 120, 150, 180 and 240 minutes of dosing. The group of mice which was given Methanolic extract of Centratherum anthelminticum (L) showed significant analgesic activity after 60, 90, 120, 150, 180 andhighly significant analgesic activity after 240 min. of dosing on 7th day.
Conclusion: The results indicate that both extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L)showed analgesic activity. On comparison Cuminum Cyminum (L) have more superior analgesic potential than Centratherum anthelminticum (L).
Keywords: Pain, dimethyl sulphoxide (DMSO), analgesics.
IRB: Approved by Board of Advanced Studies and Research (BASR), University of Karachi. Dated: 21st February 2014 and 3rd March 2014
Citation: Mahmood R, Jawed I, Nosheen, Najam R, Anjum N, Sayeed BZ, Zafar F. Study of Analgesic Activity of Methanolic Extracts of Cuminum Cyminum (L.) and Centratherum Anthelminticum (L.) in Mice [Online]. Annals ASH & KMDC 2019;24:.

(ASH & KMDC 24(2):90;2019)

Introduction

Pain is a subjective complaint. According to IASP (International Association for the Study of Pain), pain is an unpleasant sensory perception and emotional experience associated with tissue damage or potential to damage tissue. Pain is the safety alarm or response of the body before it get damaged with any harmful stimulus that can damage to normal tissue, and a nociceptor is the receptor that is sensitive to a stimulus which would become noxious if given for prolonged duration. Analgesic agents are basically the agents that reduce the pain in response to stimulation which would normally be painful, a person could be hypoalgesic means he has diminished response to pain. An increased response to a stimulus which is normally

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Date of Submission: 5th March 2019
Date of Acceptance: 25th July 2019

painful is called hyperalgesic or more sensitive to painful stimuli. There are many drugs for pain relief such as aspirin and morphine and many others are used as a single drug or in combination for analgesia. The analgesics ranged from narcotics to non-narcotics that are widely used recently to relieve pain. New generations of anti-inflammatory drugs are developed in an attempt to improve the analgesic and anti-inflammatory activities of classic non-steroidal anti-inflammatory drugs (NSAIDs), and to reduce the adverse effects caused by these agents. Selective cyclooxygenase-2 (COX-2) inhibitors, which were initially claimed to have less adverse effects than selective COX-1 or than nonselective COX inhibitors, when given at therapeutic dosage. Unfortunately, the first generation of selective COX-2 inhibitors did not show a better pharmacological profile than nonselective COX inhibitors and had unexpectedly severe adverse effects like increased myocardial infarction on prolong usage. All these problems lowered expectations for these new analgesics[1]. In most instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) relieve approximately 50% of the pain in patients[2]. The major reasons of limitation of the drugs are its serious side effects. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause major gastrointestinal disorders from heart burn to gastrointestinal bleed. So, research to discover other alternatives to treat pain is crucial. Medicinal herbs have been used for centuries for therapeutic purposes. Many of the herbs with analgesic activity are being used in hikmath or herbal treatment. Pain is a subjective and psychological experience, the animal models or the species most frequently used in pain research are rats and mice, to measure analgesic activity indirectly in animals for the study[3].

Medicinal herbs are the potential source of drugs, and playing significant role for therapeutic health system all over the world. It is frequently used by number of individuals and number of populations approximately 25-50% of people all over the world for maintaining health and treating diseases by using herbs or herbal based medicines[4]. The biological properties of the medicinal herbs are being investigated scientifically. The major sources of archiving active contents of allopathic medicines are isolated from plants and herbs. The medicines are synthetically derived from major potential sources to decrease its toxicity and made it more effective with decreased side effects. The scientific advancement in isolating active compounds are playing vital role in development of new drugs. The cost, side effects and regular monitoring of allopathic drugs are attracting people towards traditional medicines. Today approximately 55-75% of developing countries population depends on traditional medicines. In herbal medicines, it is made up of part of plant for example capsule of fruit or whole fruit or leaves and stem or only leave or only stem. The herbs Cuminum Cyminum (L) and Centratherum anthelminticum (L), these herbs are used in old Indian medicines for treating different diseases of GIT like anorexia, nausea, vomiting, colic dyspepsia etc[5,6]. Cuminum Cyminum (L) and Centratherum anthelminticum (L) are used in South East Asian foods for aroma and flavor. It has been proved through number of studies that phytochemicals, phenolic content of the herbs has antioxidant and disease curing potential[7,8].

Cuminum Cyminum (L) is a flowering plant of Apiaceae family, common name in India is Kalazera or Siyahzera. Its medicinal use in Ayurvedic system is for enhancing appetite, taste perception and digestion[6]. Centratherum anthelminticum (L) belongs to family Astraceae. It is also used in Ayurvedic medicine to cure ulcers and as bowel astringent, it can eradicate worm infestation e.g. earthworm and tapeworm infestations9. The whole plant has been reported to have antimicrobial and contraceptive activity[10,11].

The tail flick and hot plate models have conventionally been used to study centrally acting analgesics[5,12]. Although both methods employed thermal stimuli, the tail-flick response indicates spinally mediated reflex while the paw-licking hot plate response is due to complex supraspinal integrated

behavior. Prolonged the reaction time in the tail-flick method might indicate a higher sensitivity of the spinally mediated reflex response in the tail-flick method[1].

In the present study, we investigated and compared the analgesic activity of the two herbs in tail flick model of mice with Methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L).

Subjects and Methods

The seeds were collected from herbal market of Karachi, Pakistan. The seeds were identified and authenticated the specimens of seeds are deposited in Pharmacognosy Herbal Museum, of Pharmacognosy department, University of Karachi for future reference with voucher # 00111 for Cuminum Cyminum (L) and voucher # 00112 for Centratherum anthelminticum (L).

The one-kilogram seeds of Cuminum Cyminum (L) and Centratherum anthelminticum (L) were soaked in two to three liters of methanol till it get fully dipped in methanol for fifteen days. After fifteen days the solvent was obtained through filtration with filter paper and methanol is evaporated through rotator evaporator and concentrated extracts were obtained with the help of rotary evaporator.

Healthy albino mice weighing from 25 - 30 gm were selected for the study irrespective of gender. All animals were randomly distributed into three groups of ten mice each. One group served as control and the other two as treated groups. Housing of all was at 26 ± 2^{o} C room temperature with 12/12 hours light/dark cycle i.e. light on from 08.00 am to 08.00 p.m. All animals had free access to food and water ad labitum. They were housed under standard conditions and kept for one week before starting the dosing to acclimatize with the surroundings. All animals were handled as per Helsinki's Resolution 1964. This study was approved vide resolution # 10(P) 11 dated: 21-02-2014 & 03-03-2014.

The dosing was done with methanolic extracts, which were insoluble in water so it was dissolved in 10% DMSO (dimethyl sulph oxide) an organic solvent. The average weight of the mice was 25 gms so each mouse in the group 1 (Control group) was administered with DMSO 0.25 ml of 10% DMSO orally, group 2 of mice were given 12.5 mg/25 gms of methanolic extract of Cuminumcyminum(L), by making the solution 500 mg/ 10 ml and 0.25 ml was given to each mouse. The dose of methanolic extract of Cuminum Cyminum (L) was 500 mg/kg. Each mouse in the group 3 was administered 0.5 mg of methanolic extract of Centratherum anthelminticum (L), by making the solution 200 mg/ 10 ml and 0.25 ml was given to each mousedaily. The dose of methanolic extract of Centratherum anthelminticum (L) was 200 mg/kg. These doses were administered through feeding tube one week before performing the test.

Thermal method of tail flick test was used for analgesic activity. In tail flick test water bath apparatus is used, water temperature was maintained at 55ºC. Tail of mice up to 3-5 cm is immersed in hot water and tail flick time response was noted with stop watch. The analgesic activity increases the time to flick the tail. The analgesic response was believed to be related with spinally mediated nociceptive responce and the effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain perception.

After giving DMSO and the Methanolic extracts of Cuminumcyminum (L), Centratherum anthelminticum (L) on 7th day the response to pain was checked after 30, 60, 120, 150, 180 & 240 minutes through tail flick test, MRT (mean response time).

The percentage increase in the mean reaction time (MRT) which indicates the degree of analgesia produced was calculated using the following formula.

$$\text{Percentage increase in MRT} = \frac{\text{MRT in test standard - MRT in control} \times 100}{\text{Mean time in control}}$$

The data obtained from present study was analyzed through SPSS version 19. All results were expressed as mean ± S.D (Standard deviation). The

Table 1. Analgesic activity - tail flick test

Group	0 min MRT	30 min MRT	60 min MRT	90 min MRT	120min MRT	150min MRT	180min MRT	240min MRT
Control(DMSO)	2.30 ±0.17	2.31 ±0.027	2.30 ±0.11	2.29 ±0.18	2.29 ±0.05	2.30 ±0.28	2.33 ±0.07	2.30 ±0.18
CA	2.61 ±0.28	2.74 ±0.12	3.50 ±0.18*	3.68 ±0.05*	3.71 ±0.28*	3.73 ±0.06*	3.76 ±0.29*	4.47 ±0.68**
CC	3.30 ±0.38*	3.44 ±0.20*	4.52 ±0.13**	4.69 ±0.07**	5.15 ±0.09**	5.20 ±0.26**	5.23 ±0.44**	5.18 ±0.74**

n=10, MRT (mean response time) Values are mean ± S.D (standard deviation), significance calculated by using one way ANOVA followed by post hoc tukey's test and LSD, significant * p<0.05, highly significant **p<0.01.

significance of difference between mean were calculated by applying two way ANOVA, Post hoc analysis and LSD. An effect was defined as significant $p < 0.05$, highly significant $p < 0.01$.

Results

The data are expressed as mean±SD of observations of tests. In tail flick test the response time of flicking the tail after immersion of tail in hot water bath were recorded in seconds with the help of stop watch,it is the response of flicking of the tail with stimulation of pain due to thermal heat in animals of all three groups receiving DMSO, extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) for a week from test day. The group of animals receiving Methanolic extract of Cuminum Cyminum (L) showed highly significant ($p<0.01$) increased duration of tail flick time (MRT) as compared to control from 60 min. to 240 min. after dosing i.e. (3.30 ± 0.38, 3.44 ± 0.20, 4.52 ± 0.13, 4.69 ± 0.07, 5.15 ± 0.09, 5.20 ± 0.26, 5.23 ± 0.44, 5.18 ± 0.74 at 0, 30, 60, 90, 120, 150, 180 and 240 minutes after 7th day of dosing. The group of mice those who were given Methanolic extract of Centratherum anthelminticum (L) showed significant ($p<0.05$) increased duration of mean tail flick time as compared to control on 60 to 180 minutes and highly significant ($p<0.01$) increased duration of mean tail flick time as compared to control on 240 minutes after 7th day of dosingi.e. (2.61 ± 0.28, 2.74 ± 0.12, 3.50 ± 0.18, 3.68 ± 0.05, 3.71 ± 0.28, 3.73 ± 0.06, 3.76 ± 0.29, 4.47 ± 0.68 to 3.97) after receiving a week dosing.

n=10, MRT (mean response time) Values are mean ± S.D (standard deviation), significance calculated by using one way ANOVA followed by post hoc tukey's test and LSD, significant * p<0.05, highly significant **p<0.01.

Discussion

According to the International Association for the Study of Pain (IASP), pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage"[13]. Despite recent developments in pain therapies, the medical community still needs safe, effective, and potent analgesic drugs for the treatment of different painful conditions especially the chronic pain. Thousands of patients with intense pain, such as that resulting from cancer or severe injury, must depend on current regimes (peripheral or centrally acting) like morphine, aspirin, and nonsteroidal anti-inflammatory drugs[14]. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders. For that, the discovery of other alternatives to treat pain is crucial9. Herbal therapy could be an interesting option because of the opioid dependence and withdrawal, Analgesic herbs are used orally or topically as tinctures, either singly or in combination, for the symptomatic relief of generalized discomfort and aches[15]. Analgesics are de-

fined as a drug that relieves pain. Analgesics are classified as opioids and non-opioids (e.g. NSAIDs). Co-analgesic drugs have a primary indication other than pain but give relief in some conditions. For example, antidepressants and anticonvulsants reduce nociceptive transmission in neuropathic pain.In old age people pain killers are frequently used, due to arthritis and more susceptibility to trauma. Allopathic medicines are replaced with traditional medicines due to its side effects like GI upsets. In this study, it was demonstrated that methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) have analgesic potential. Further research is required to evaluate the exact mechanism of action of Cuminum Cyminum (L) and Centratherum anthelminticum (L) extracts. Analgesics are drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness[1]. Centrally acting analgesics act by raising the threshold for pain and also altering the physiological response to pain. On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain[16]. The animal models employed for screening of analgesic activity in this study are pain-state model of tail-flick. The tail-flick method mediates a spinal reflex to a nociceptive stimulus[17]. In tail-flick model, the methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) exhibited significant analgesic activity by increasing the reaction time of the mice compared to control (DMSO treated mice) at all time points. Analgesic drugs which are centrally acting elevate pain threshold of animals towards heat and pressure[11]. Therefore, the analgesic effect of the Methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) on this pain-state model indicates that it might be centrally acting. Tail-flick is one of the several methods available for evaluating central analgesic activity, the tailflick response indicates spinally mediated reflex[2]. A number of alkaloids, flavanoids, steroids, and tannin isolated from these medicinal herbs could be responsible to possess significant analgesic activity[1]. The major constituent of the extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L), observed analgesic activity with these extracts might be due to the presence of the flavonoids and tannins. Furthermore, there are reports on the role of tannin in analgesic activity[16], So the preliminary phytochemicals which were screened from extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) are responsible for the observed analgesic activity.

The slow onset and long duration of analgesic activity of the extract suggested an active metabolite that could be more effective than the prodrug in the extract and/or binding to plasma protein. The centrally mediated analgesia integrated response is affected mostly by opioids receptors, and since tail flick is a spinal reflex[18]. Methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum could exhibit a significant and potential analgesic activity via activation of opioid receptors in the central nervous system. Another research suggested that the presence of tannin and flavonoid in the methanol extract of Cuminum Cyminum (L) and Centratherum anthelminticum (L) seeds seems to inhibit prostaglandin synthesis and exerts the anti-inflammatory and analgesic effects. The pain induction occurs by liberating endogenous substances as well as some other pain mediators such as arachidonic acid metabolites via cyclooxygenases, such as prostaglandins[19].

Conclusion

The Methanolic extracts of Cuminum Cyminum (L) and Centratherum anthelminticum (L) both have analgesic activity. Cuminumcyminum (L) has more potential than Centratherum anthelminticum (L). Further studies will be required to investigate neurobiological mechanisms of action and possible interactions of Cuminum Cyminum (L) and Centratherum anthelminticum (L) with neurotransmitters.

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