INVESTIGATING ANTINOCICEPTIVE POTENTIAL OF *Origanum vulgare* L. EXTRACT USING ANIMAL MODELS OF PAIN

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ABSTRACT: *The study was aimed to investigate the anti-nociceptive potential of Origanum vulgare methanolic extract, a traditional herb mainly recognized for its culinary property. The activity was assessed using chemical, mechanical and thermal nociceptive models which revealed that the extract when administered orally at doses 15 and 30 mg/kg showed significant increase (p< 0.001) in the response latency to noxious stimuli with most potent activity displayed at 30mg/kg which is comparable to the positive control in all models of nociception. However, dose 7.5 mg/kg was devoid of any analgesic effects. In view of the results obtained it could be suggested that Origanum vulgare methanolic extract has analgesic properties mediated by both peripheral and central mechanisms which needs further investigation.*

Key words: Origanum vulgare, analgesic, anti-nociceptive

INTRODUCTION*:*

Pain is an unpleasant sensation elicited as a warning response provoked by any noxious stimulus [1]. Pain sensations vary widely in their origin and intensity but in any form pain tends to severely affect daily life routine, quality of work and personal physical and psychological status [2]. During the last few decades prevalence of pain has increased too much [3]. In Pakistan, where living is stressful, people are very prone to pain sensitivity [4]. The existence of numerous pain killers in the market (OTCs) is not a true solution to the problem since these analgesic agents are not only associated with serious adverse effects at the same time few of them are too expensive to be in the reach of a common man [5, 6]. Need to explore natural origin remedial moieties with minimal adverse effects is getting more indispensible day by day. Relieving pain in many pathological states play a key role in reducing a patients' suffering from the disease.

Origanum vulgare is a popular culinary herb that is used to add flavor and aroma to the food [7]. It is the essential constituents of the herb that make it popular. Traditionally it was used as diuretic, in cold and cough, digestive problems and stomach ache [8]. Further studies revealed this herb to be strong antimicrobial, antioxidant, anti-mutagenic, antiviral and anti-diabetic agent [9].

A little data on the analgesic potential of the methanolic extract of the herb prompted us to investigate the pain subsiding effects of the extract and to our knowledge no study has fully elucidated the analgesic effects of methanolic extract of Origanum vulgare.

MATERIAL AND METHOD:

Plant material:

Aerial parts of Origanum vulgare were obtained from local market in Karachi. The plant material was identified by Dr. Aftab Saeed and for future reference a sample was submitted in University of Karachi herbarium (86523).

Extraction method:

The plant material was then subjected to shade drying for 5 days and later on was finely grounded with subsequent sieving to remove fibrous material. For extraction 1.5 kg of plant material was soaked in 3500 ml of 100% methanol in tightly sealed vessel and after 4 days it was filtered through muslin cloth. The marc obtained was then re-soaked in methanol for same number of days and similar procedure was conducted two more time. Finally all the four filtrates were combined and subjected to drying at 77-80ºC in the rotary evaporator. The finally obtained extract was then weighed and kept in refrigerator in sealed vials for further use.

Dosing and administration:

All the extract solutions were freshly prepared before the test and administered according to animal body weight orally, 60 mins prior to the test. Morphine when used as positive control was administered subcutaneously 30 minutes before the test.

Animals and grouping:

NMRI mice of either sex were obtained from Hafiz Mohammad Ilyas Institute of Pharmacology and Herbal Sciences randomly placed into groups Control (saline), Positive Control (acetylsalicylic acid or morphine), MeOV 7.5 mg/kg, 15 mg/kg and 30 mg/ kg. Six animals were selected for each group. A total of 120 mice were utilized in different analgesic models.

Anti-nociceptive models:

Different anti-nociceptive models utilizing chemical, mechanical and thermal noxious stimuli were employed to assess the analgesic property as well as to evaluate the probable pathway involved. Following models were included in the study

Acetic acid induced writhing: Similar to method used by others [10], different doses of the test drugs and controls were administered orally through oral gavage tube. After 60 minutes of the administration acetic acid induced writhing test was performed by injecting 0.6% w/v acetic acid (0.1ml/10gm) in the peritoneal cavity of the animals The number of writhes produced for a period of 5-30 mins were counted. Abdominal writhing is characterized by abdominal contraction, twisting of trunk and stretching of the limbs.

Heffner's tail clip test: was used to evaluate the analgesic activity of MeOV by applying a mechanical stimulus in the form of metal artery clip at the rear end of the tail (up to 2 cm). The latency to respond to this painful stimulus by making an attempt to dislodge itself was recorded 60 minutes after treatment of different doses of test drug [11] with cut off limit of 30 s to avoid tissue damage [12].

Hot plate test: A slightly modified method as described by [13] was used. The test involved applying a thermal noxious stimulus to animal one hour after p.o. treatment of the doses of extract and control group while 30 mins post treatment to positive control group. Each mouse was individually placed

on a hot plate, the temperature of which was maintained at 50±1ºC by a thermostat. Jumping and licking were noted as a response to the heat and the latency to respond was measured in seconds for each mouse. Cut off limit was set to 60 s [14].

Tail immersion test: In this test the rear 5cm part of each rodent's tail was individually immersed in hot water (55±1ºC) sixty minutes after treatment to control and extract group while 30 minutes after morphine treatment to the positive control group and the response latency was noted when each animal withdrew its tail with an abrupt movement. Method was adopted from [15] with a cut-off limit of 60 seconds [16]

STATISTICAL ANALYSIS:

All the results were represented as mean ± SEM (standard error of mean). One way ANOVA followed by post hoc LSD was used to statistically evaluate the obtained results. Statistical significance was considered at p value less than 0.05

RESULTS:

One way ANOVA studies in acetic acid induced writhing test displayed a significant fall in the no of writhes by MeOV at doses 15 and 30 mg/kg as compared to saline. A maximum of 75.3% fall in the writhes was noted by MeOV. Post hoc analysis showed that the activity of 30 mg/kg MeOV was comparable to the acetyl salicylic acid treated group activity. However, no noticeable analgesic activity was exhibited by 7.5 mg/kg with mean no. of writhes

In tail clip test the prolongation in the response latency by MeOV was significant in comparison to control. Doses 15 and 30 mg/kg showed a significant percent rise as compared to control group (4.16±0.74 s) as inferred from one way ANOVA. Post hoc studies (LSD) is suggestive of dose 30 mg/kg being most potent of all three doses since it not only displayed rise in response latency twice that produced by control, but also the activity of the same dose is significantly different (p <0.001) with 83.82% rise as compared to morphine treated group. Dose 7.5 mg/kg displayed lack of any sort of analgesic effects

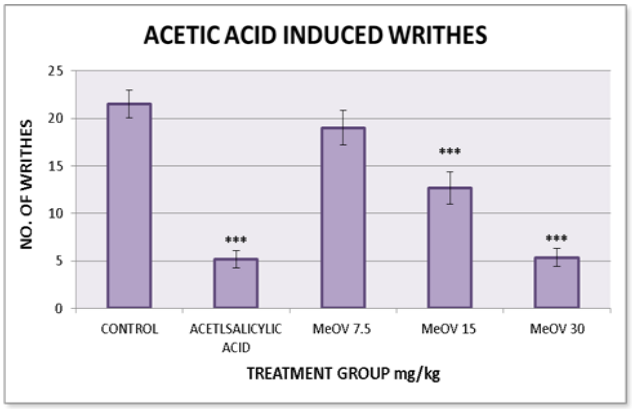

FIGURE 1: Effect of oral administration of MeOV at doses 7.5,15 and 30 mg/kg on no. of writhes induced by acetic acid in treated mice tested. Results are being expressed as mean±SEM (n=6 per group) where *p <0.05, **p <0.005 and ***p <0.001 [ANOVA followed by post hoc LSD]. MeOV (*Origanum vulgare*methanolic extract

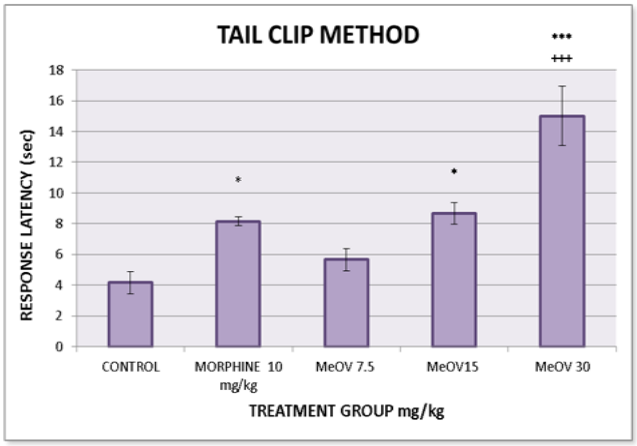

FIGURE 2: Effect of oral administration of MeOV at doses 7.5,15 and 30 mg/kg on response latency of treated mice tested by tail clip method. Results are being expressed as mean±SEM (n=6 per group) where *p <0.05 and ***p <0.001. Also +++p <0.001 compared to Morphine. MeOV (*Origanum vulgare* methanolic extract).

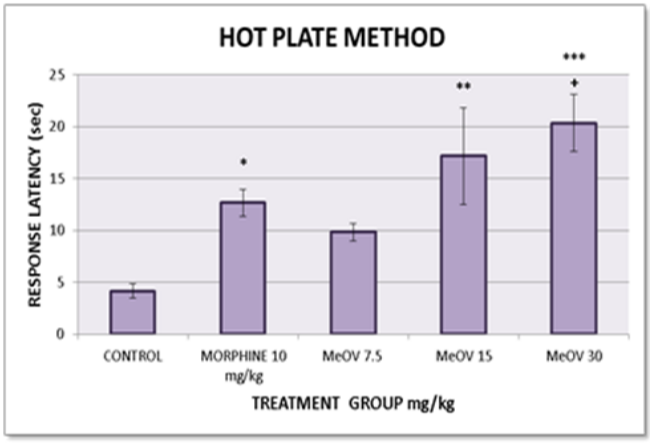

FIGURE 3: Anti-nociceptive activity of MeOV at doses 7.5,15 and 30 mg/kg as obtained by increase in response latency (secs) of treated mice subjected to hot plate method of nociception. Results are being expressed as mean±SEM (n=6 per group) where *p <0.05, **p <0.005 and ***p <0.001 [ANOVA followed by post hoc LSD]. Also +p <0.05, ++p <0.005 and +++p <0.001 compared to Morphine. MeOV (Origanum vulgare methanolic extract).

As compared to saline, MeOV at doses 15 and 30 mg/kg significantly elevated the response latency duration to a maximum rise of 20.33± 2.75 s in hot plate test, while itincreased to a maximum of 18.33±3.38 s in tail immersion test as shown in figures 3 and 4 respectively.

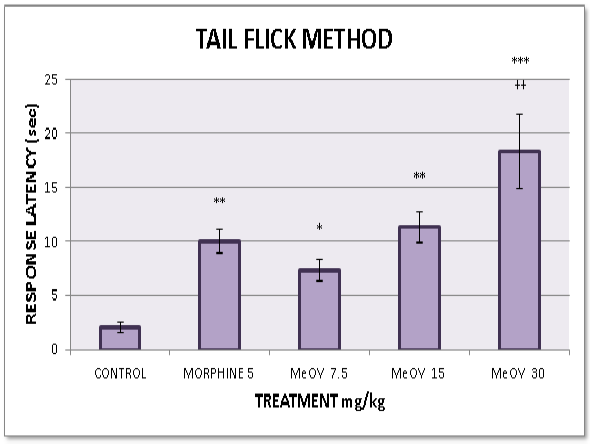

Figure 4: Effect of oral administration of OVME at doses 7.5,15 and 30 mg/kg on response latency of treated mice tested by tail flick method. Results are being expressed as mean±SEM (n=6 per group) where *p <0.05, **p <0.005 and ***p <0.001 [ANOVA followed by post hoc LSD]. Also +p <0.05, ++p <0.005 and +++p <0.001 compared to Morphine. MeOV (Origanum vulgare methanolic extract).

DISCUSSION:

Current study was conducted to explore and contribute to the available literature, knowledge regarding the anti-nociceptive activity of Origanum vulgare methanolic extract. Origanum vulgare is famous for its culinary purpose in addition to its traditional use in respiratory disorders, digestive problems and urinary system complaints. Although, little literature has evidenced its use in Persian medicine for the treatment of headache [17]. Still the data available indicating its use in folklore medicine as pain and inflammation alleviating agent is scarce.

Different anti-nociceptive mouse models were employed that used chemical, mechanical and thermal noxious stimuli to expose the analgesic potential of the herb. In writhing test, a noxious chemical stimulus (acetic acid) administered intra-peritoneally resulted in abdominal constriction [18]. This peripheral response is generated due to release of various local inflammatory mediators including prostaglandins, cytokines, histamine, substance P, bradykinin and others [19] MeOV displayed a significant decline in the number of writhes at dose 15 and 30 mg/kg as compared to the saline treated group while 7.5 mg/kg dose failed to produce any significant effects. The decrease in the writhes is suggestive of the peripheral analgesic activity of the extract which ought to be either due to interference in the synthesis /release of these endogenous chemicals, their receptor site blockade or complete COX pathway inhibition.[20]

In contrast to writhing test, other animal models of pain (tail clip, hot plate and tail immersion test) elucidate centrally mediated analgesic mechanisms [11,21] that generate spinally and supra spinally integrated [22, 23]. In both hot plate and tail flick test MeOV extract prolonged the response latencies at 15 and 30 mg/kg with no notable effects at 7.5 mg/kg. Thus the analgesia produced by the extract in both models may reflect the probable central pain pathway involvement. Central analgesic effect is considered to be produced mainly due to the opioidergic activity at μ, δ, κ receptors but the involvement of dopaminergic and descending serotonergic or noradrenergic systems cannot be excluded. [24]

Similar results were obtained in tail clip test where MeOV extract produced a marked extension in the latency to respond when rodent's tail was clogged by a metal artery clip. Significant results were obtained at 15 and 30 mg/kg with insignificant activity at 7.5 mg/kg.

Numerous studies on phytochemicals have exhibited analgesic effects through a different or sometimes more than one mechanism .Phenolic acids for example not only interfere with the COX pathway but also exert some effect on the opioid receptors [25], similarly flavonoids along with an inhibitory effect on COX and lipoxygenase cascades interfere with descending serotonergic transmission [26] and the presence of chemical moieties like flavonoids, alkaloids, phenolic acids, saponins in the polar extract of the herb as revealed by various phytochemical studies could by the probable explanation of dual (central and peripheral) analgesic activity

CONCLUSION:

On a conclusive note, it can be inferred from the above results that the methanolic extract of Origanum vulgare could act as a potent anti-nociceptive agent at doses 15 and 30 mg/kg. The analgesia produced could be attributed to the presence of various chemical moieties which probably are responsible for both peripheral and central level anti-nociception. A definitive postulation however, cannot make about the pathway and receptors involved in the mechanism. Also, it is noteworthy to state here that 30 mg/kg dose of MeOV increased pain threshold in animals much more effectively than the commonly used analgesic agents i.e. acetyl salicylic acid and morphine employed in the experiment. Hence, taken together the possibility that Origanum vulgare extract can be used for subsiding pain adds to the current scientific trend of utilizing natural origin remedies for treating ailments that are not only cost effective but are also commonly available and are accompanied with minimum side effects.

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