IN VIVO ANALGESIC ACTIVITY OF ESSENTIAL OIL AND AQUEOUS EXTRACT OF MATRICARIA CHAMOMILLA L. (ASTERACEAE)

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ABSTRACT

Natural products have served as a source of medicines for centuries, and about half of the pharmaceuticals in use today are derived from natural products. Matricaria chamomilla L. (asteraceae) has been widely used in traditional medicine to treat wounds, fever, intestinal worms, headache, inflammations, menstrual problems, sore throat etc. The aim of this study is to investigate the acute peripheral activity of the essential oil (EO) and aqueous extracts (AE) of Matricaria chamomilla L. aerial parts by acetic acid induced writhing reflex test in mice and acute central analgesic activity by tail immersion method in rats. The essential oil obtained by hydrodistillation was analyzed by gas chromatography–mass spectrometry (GC-MS). Matricaria chamomilla L. essential oil major constituents were: Chamazulene (25.21 %), Cis-beta-farnesene (12.51 %), Eucalyptol (9.19 %), Coumarin (7.72 %), Galaxolide (6.28 %), Camphor (4.3 %) and Salicylic Acid (2.62 %). From the results of this study essential oil of Matricaria chamomilla L (100, 200 and 300 mg/kg p.o.) and aqueous extract (200, 400 and 600 mg/kg p.o.) have exhibited analgesic activity in the tested models. In the acetic acid-induced writhing model, both extracts had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control and reference drug. For the tail immersion method
EOMC exhibited significant analgesic activity at low dose of 200 mg/kg comparable to the aqueous extract at higher a dose of 600 mg/kg. The finding showed that the Matricaria chamomilla L. extracts shows a potent central and peripheral analgesic effect comparable to the reference drug (morphine and aspirin) (P<0.05). Our results suggest that Matricaria chamomilla L. extracts presents analgesic properties, which are mediated through central inhibitory mechanism.

**Key words:** Matricaria chamomilla L., Analgesic activity, Essential oil, Writhing response.

**INTRODUCTION**

Pain is a disabling accompaniment of many medical conditions it represents the symptom for the diagnosis of several diseases and conditions. It has a protective function and its widely accepted as one of the most important determinants of quality of life because of its widespread adverse effects, including diminishing mental health and wellbeing and impairing the individual’s ability to perform daily activities. Chronic pain impacts upon a large proportion of the adult population, including the working age population, and is strongly associated with markers of social disadvantage [1]. For thousands of years medicine and natural products have been closely linked prominently through the use of traditional medicines [2-4]. The Medicinal Plants are a rich source of medicines because they produce wide range array of bioactive molecules. Throughout history man has used many different forms of therapy for the relief of pain, among them medicinal herbs such as Papaver somniferum from which morphine was isolated. Developing treatments for pain relief has been the motivating factor behind many studies carried out in response to the demand for powerful analgesics and that exhibit their pharmacological response through new mechanisms of action and with less side effects [5].

*Matricaria chamomilla* L. is a widely recognized herb in Western culture and it is known as Babounj in Morocco. Its medicinal usage dates back to antiquity where such notables as Hippocrates, Galen, and Asclepius made written reference to it [6].

It is known to have numerous pharmacological activities, such as hypoglycemic, antistress, immunomodulatory, analgesic, antipyretic, anti-inflammatory, antiulcerogenic, antihypertensive, CNS depressant, hepatoprotective, chemopreventive, radioprotective, antitumor and antibacterial activities [7-8].
Although *Matricaria chamomilla* L. has traditionally been used in the treatment of many types of pain and inflammatory conditions in Morocco, an extensive search of the literature reveals the anti-nociceptive actions of chamomile in mice [30]. In our previous studies, we investigated the central nervous system (CNS) activity of the Essential Oil of *Matricaria chamomilla* L. and we have proven the efficacy of the aqueous extract of this plant against inflammations [10, 12]. We now report on the analgesic activities of *Matricaria chamomilla* L. extracts to validate these folkloric uses in Morocco as a part of our continuing studies on this medicinal plant in rodents using two experimental pain models.

**MATERIAL AND METHODS**

**Plant material**

*M. chamomilla* L. was purchased at a farmer’s market in Hay Nahda-Rabat, between March and April 2013, and was identified at the Department of Plant Biology, Ibn Tofail University, Morocco. A voucher specimen (N° Rab78995) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat.

**Preparation of the Essential Oil**

The essential oil was extracted from samples of the aerial parts of *Matricaria chamomilla* L. by steam distillation using Clevenger apparatus. The distillations were carried out on sample size of 300g with a distillation time of 240 min after the oil was dried over anhydrous K2CO3. Oil volume was recorded and oil yield was calculated as the volume (milliliters) of oil per weight (gram) of fresh basil tissue. The final yield (0.5%) was stored in a refrigerator at + 4°C [9] and protected against light and heat until used for GC-MS analysis and then suspended in peanut oil to prepare suspension in desired concentration just before use [10-11].

**Preparation of the Aqueous Extract**

In the extraction process, Stems and flowers were air-dried and ground into a fine powder. 100g of *Matricaria chamomilla* L. powder was macerated for 24 hrs in 500ml of distilled water. A percentage yield of 16.76 % was obtained after extraction and concentration under reduced pressure on a rotary evaporator attached to a vacuum pump and stored at a temperature of 4°C until use [12].
Phytochemical analysis of Matricaria chamomilla L. essential oil by combined gas chromatography-mass spectrometry (GC-MS).

Gas chromatography combined with mass spectrometry was used for the identification of the components of EOMC. The analysis was performed on a PolarisQ quadrupole ion trap mass spectrometer coupled with a TRACE GC Ultra gas chromatograph equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed from 40 to 300°C at 5°C/min. Helium was used as the carrier gas at a flow rate of 2 ml/min. The injector and detector temperature was 220°C. The MS operating parameters were: ionization voltage 70 eV, ion source temperature 200°C. Identification of the oil components was based on the retention indices relative to n-alkanes (C8-C24) and computer matching with NIST and Wiley 275 libraries, as well as by the comparison of fragmentation patterns of the mass spectra with those reported in the literature [10-14].

Animals

Adult wistar rats weighing 150 to 200g and Adult swiss mice weighing 25 to 30g of either sex were used for this study. The animals were obtained from the animal centre of Mohammed V Souissi University, Medicine and Pharmacy Faculty, Rabat, Morocco.

They were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions at 23 ± 1°C and relative humidity 60 – 70% and 12h-dark/12h-light cycle. All animals were fed the standard rodent pellet diet and water ad libitum. They were acclimatized at least one week before the experiments were started. The animals submitted to oral administration of the extracts or drugs were fasting for 18 h before the experiment (water was available). All experiments were conducted in accordance with the Official Journal of the European Committee in 1991. The experiment protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509 [10, 11-15, 16].

Chemical and reagents

The Chemicals used in the present study were Aspirin, Acetic acid and Morphine.

Acetic acid-induced writhing response in mice

The peripheral analgesic effect was assessed according to Koster test (using chemical stimuli) [17]. In this method, acetic acid is administered intraperitoneally to the animals to create pain sensation.
The essential oil of MC was administered in different doses at 100, 200 and 300 mg/kg p.o. and aqueous extract of MC at doses of 200, 400 and 600 mg/kg p.o. to the Swiss Albino mice after an overnight fast.

Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of acetic acid solution (3% with 300 mg/kg). Each mouse of all groups were observed individually, the number of writhing they made in 20 minutes were counted starting just 5 minutes after the intraperitoneal administration of acetic acid solution.

The number of cramps and stretching were also recorded and allowed us to express the percentage of protection using the following ratio \((\text{control mean} - \text{treated mean}) \times 100/\text{control mean}\).

A significant reduction in number of writhes by drug treated as compared to vehicle treated animal which was considered as a positive analgesic response while aspirin (200 mg/kg, p.o.) was used as a reference substance (positive control).

**Tail immersion test**

The central analgesic activity of *Matricaria chamomilla* L. was evaluated using thermal stimuli method (Tail immersion test) [18, 19]. Rats divided into groups of six each. They were held in position in a suitable restrainer with the tail extending out. Area of the extremity of the tail 4-5 cm was marked and immersed in the water bath thermo-statistically maintained at 55°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut-off time for immersion was 10 seconds to avoid the injury of tail tissues. Distilled water was administered to control animals, essential oil at 100, 200 and 300 mg/kg and aqueous extract at 200, 400 and 600 mg/kg were given orally by intubation. The initial reading started immediately before administration of test and standard drugs (Morphine 5 mg/kg s.c) and then 15, 30, 45, 60 and 120 minutes after the administration.

**Statistical analysis**

The results were expressed as mean ± SEM. The data were subjected to one-way ANOVA followed by student’s t-test. A value of \(P < 0.05\) was considered significant.
RESULTS

Acetic acid induced writhing

The peripheral analgesic effect for the writhing test was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of the animals.

As seen in Table 1 and 2, the mean writhes of the mouse decreases as the dose of the extract increases from 100 to 300 mg/kg for the essential oil of *Matricaria chamomilla* L. and from 200 to 600 mg/kg for the aqueous extract.

Essential oil extract of *Matricaria chamomilla* L. at doses (100, 200 and 300 mg/kg p.o.) produced a dose-dependent decrease in acetic acid induced writhing with the highest dose producing an effect of 100% inhibition of writhing which was comparable to that of 200 mg/kg of aspirin (61.56% inhibition of writhing).

The aqueous extract also reduced the number of writhing with the highest dose (600mg/kg p.o.) producing an effect of 96.56% inhibition of writhing in mice. These results indicate that *Matricaria chamomilla* L. extract has a potent peripheral analgesic activity (Figure 1 and 2).

Effect of essential oil and aqueous extract on Tail immersion test

The tail immersion method indicated that the central analgesic effect of the EO and AE of test drug was significant and dose dependent as revealed by the increased reaction time after giving thermal stimulus to the rats as compared to the control group (Figure 3 and 4).

The EO and AE at the three doses showed significant analgesic action peaking at 60 min for the essential oil at 200 mg/kg p.o. and 45 min for aqueous extract at dose of 600 mg/kg p.o whereas the morphine- induced analgesic effect peaked at 45 min.

Table 1: Effect of Essential Oil of *Matricaria chamomilla* L. on acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose, route</th>
<th>No. of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control</td>
<td>0.5 ml/mouse, p.o</td>
<td>45±2.58</td>
</tr>
<tr>
<td>Group-II</td>
<td>Aspirin</td>
<td>200 mg/kg, p.o</td>
<td>19.6±2.88*</td>
</tr>
<tr>
<td>Group-III</td>
<td>EOMC</td>
<td>100 mg/kg, p.o</td>
<td>26.25±2.21*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>EOMC</td>
<td>200 mg/kg, p.o</td>
<td>0.25±0.5*</td>
</tr>
<tr>
<td>Group-V</td>
<td>EOMC</td>
<td>300 mg/kg, p.o</td>
<td>0±0*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. * P < 0.05, significantly different from control; Student’s t-test (n = 6).
Figure. 1. Effect of Essential Oil of *Matricaria chamomilla* L. and aspirin on acetic acid-induced writhing response in mice.

Table 2: Effect of Aqueous Extract of *Matricaria chamomilla* L. on acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose, route</th>
<th>No. of writhing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Control</td>
<td>0.5 ml/mouse, p.o</td>
<td>51±2.8</td>
</tr>
<tr>
<td>Group-II</td>
<td>Aspirin</td>
<td>200 mg/kg, p.o</td>
<td>19.6±2.88*</td>
</tr>
<tr>
<td>Group-III</td>
<td>AEMC</td>
<td>200 mg/kg, p.o</td>
<td>40.8±3.11*</td>
</tr>
<tr>
<td>Group-IV</td>
<td>AEMC</td>
<td>400 mg/kg, p.o</td>
<td>10±2.6*</td>
</tr>
<tr>
<td>Group-V</td>
<td>AEMC</td>
<td>600 mg/kg, p.o</td>
<td>1.75±2.06*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. * P < 0.05, significantly different from control; Student’s t-test (n = 6).

Figure. 2. Effect of Aqueous Extract of *Matricaria chamomilla* L. and aspirin on acetic acid-induced writhing response in mice.
Figure 3. Central Analgesic activity of Essential Oil of Matricaria chamomilla L. by Tail immersion test

Each value represents the mean ± SEM (n = 6) p<0.05, statistically significant relative to control at 45 min.

Figure 4. Central Analgesic activity of Aqueous Extract of Matricaria chamomilla L. by Tail immersion test

Each value represents the mean ± SEM (n = 6) p<0.05, statistically significant relative to control at 45 min.
DISCUSSION

Pain is a protective mechanism its occurs whenever any tissues are being stressed, and it causes the individual to react to remove the pain stimulus to avoid damage [20]. The sensation of pain can be initiated either peripherally or through the central nervous system. Peripherally mediated pain can be inhibited by NSAIDs (non-steroidal anti-inflammatory drugs produce analgesia or reduce inflammation by inhibition of the release and synthesis of prostaglandin) by this action, NSAIDs reduce the sensitization of afferent neuron by prostaglandins to the algesic actions of bradykinin and other pain provoking stimuli [21] which blocks the anti-inflammatory pathways responsible for pain. On the other hand, opioid analgesics are useful for the management of centrally acting pain in which opioid analgesics act by inhibition of opioid receptors [22].

The Acetic-acid-induced writhing represents peripherally acting pain sensation. The response is thought to involve local peritoneal cells [24]. Intraperitoneal administration of acetic acid mediate pain response through the release of inflammatory mediators, mainly prostacyclin (PGI2) [23].
The tail flick response predominantly consider being selective for centrally acting analgesics. The response of the tail flick is believed to be a spinally mediated reflex, which is mediated by a supraspinal inhibitory mechanism [25-26].

In this study *Matricaria chamomilla* L. essential oil and aqueous extract were tested on the mice for the peripheral analgesic activity according to the test of Koster and also on the rats for the central analgesic activity based on tail immersion test.

The essential oil (100, 200 and 300 mg/kg) and the aqueous extract (200, 400 and 600 mg/kg) showed significant analgesic activity (P<0.05) in both tail flick and the acetic acid induced writhing models.

The percentage inhibition of writhing at the higher doses of EOMC (200 and 300 mg/kg) and AEMC (400 and 600 mg/kg) indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard pure drug Aspirin within 20 minutes of test.

In the tail immersion method, the analgesic effect of EO and AE extracts of *Matricaria chamomilla* L. are dose dependent. Prolongation of reaction time in tail immersion test confirmed that EO and AE extracts possesses central analgesic action. The results showed significant analgesic activity against thermal stimuli.

Chamomile contains volatile oils (apigenin, apigenin-7-Oglucoside and its acetylated derivatives), flavonoids and glycosides that have anti-spasmodic and anti-inflammatory action, which can give a relief from the nervous tension and stomach cramps associated with anxiety [27, 28].

In a previous study we reported that the essential oil of *Matricaria chamomilla* L. revealed a mixture of monoterpenes (25.27%) and sesquiterpenes (Chamazulene 25.21 %, coumarin 7.72%, Trans- caryophyllene 6.85%, Salicylic Acid 2.72% etc) as the main components [10, 11].

The significant analgesic activity of *Matricaria chamomilla* L. in this study is often attributed to the phytochemicals presents in the extracts, which inhibited the synthesis by inhibiting cyclooxygenase and lipoxygenase [29], release of receptor responses in prostaglandin
mediated effects. These substances in which contribute in synergy to the overall effect, which will be different from that of any one in isolation.

CONCLUSION
The results of this study demonstrate the pharmacological importance of *Matricaria chamomilla* L. in Morocco highlighting the possibility of the medical use especially for the analgesic activity that this plant showed during our study. Further, works like isolation, structural elucidation and screening of above active principles need to be done to pin the activity of this drug. Therefore its worth to do research on this plant so that the claimed properties could be scientifically proved and possible new compounds or even effective formulations could be finally released for the public use at a low cost.

Conflict of interests
The authors declare that there are no conflicts of interests in this study.

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REFERENCES


