ANTI-INFLAMMATORY EVALUATION OF AQUEOUS EXTRACT OF
MATRICARIA CHAMOMILLA L. (ASTERACEAE) IN
EXPERIMENTAL ANIMAL MODELS FROM MOROCCO

Ghizlane Hajjaj*, 1 Amina Bounihi1, Mouna Tajani2, Yahia Cherrah1, and
Amina Zellou1

1Laboratory of Pharmacology and Toxicology, Department of Drugs Sciences, Faculty of
Medicine and Pharmacy, Mohammed V Souissi University, ERTP, BP 6203, Rabat Instituts,
Agdal, Rabat, Morocco.

2Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.

ABSTRACT

Matricaria chamomilla L. has been used in Moroccan traditional
medicine to relieve pain, fever, inflammation. It is also used as a mild
laxative and is antispasmodic, and bactericidal. The present study
explored the anti-inflammatory potential of aqueous extract of
Matricaria chamomilla L. (AEMC) (Asteraceae) in rodents, using
standard laboratory models. This study was carried out by using female
Swiss mice (25-30g) and Wistar male rats (180-220g). The aqueous
extract was prepared by using maceration at room temperature (25°)
over period 24 hours. The acute toxicity studies were carried out based
on OECD guidelines 423. The LD50 of AEMC was found to be more
than 2g/kg and did not produce mortality or changes in general
behaviour of the test animals. The aqueous extract of MC was screened
for it anti-inflammatory activity properties by using carrageenan and
experimental trauma-induced hind paw oedema in rodents at 300 and
500 mg/kg. Indomethacin at 10 and 20 mg/kg was used as standard. From the results obtained
the aqueous extract of Matricaria chamomilla L. showed significant activity comparable to
the control and reference drug used in both models.

This study showed the justification of the use of Matricaria chamomilla L. in the treatment
of inflammatory disease conditions in Morocco. However pharmacodynamics studies should

*Correspondence for
Author:
Ghizlane Hajjaj
Laboratory of Pharmacology and Toxicology, Department of
Drugs Sciences, Faculty of
Medicine and Pharmacy, Mohammed V Souissi
University, ERTP, BP 6203,
Rabat Instituts, Agdal, Rabat,
Morocco
hajjajghizlane1@gmail.com
be undertaken to establish the mechanism of action of the plant extract and the active chemical constituents when isolated will be added to the present anti-inflammatory agents.

**Key words:** *Matricaria chamomilla* L., Anti-inflammatory activity, Carrageenan, Indomethacin, Rat paw oedema.

**INTRODUCTION**

Inflammation is a defensive response that begins after cellular injury, which may be caused by microbes, physical agents (burns, radiation, and trauma), chemicals (toxins, caustic substances), necrotic tissue and/or immunological reactions [1]. The classical signs of inflammation are pain, heat, redness, swelling, and loss of function.

Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. However if inflammation remains unchecked, it leads to the onset of disease such as vasomotor rhinitis and atherosclerosis [2]. Although several agents are known to treat inflammatory diseases and pain, their prolonged use often leads to serious side-effects such as gastric intolerance and water and salt retention. It is therefore, inevitable to search for new, less and more effective anti-inflammatory and analgesic agents.

Naturally originated agents with very little side-effects can substitute chemical therapeutics. Medicinal plants are used by 80 % of the world population, and it has become imperative to investigate the acclaimed ones for their possible therapeutic benefits, especially nowadays, when treatment of many serious diseases still faces diverse challenges [3].

Chamomile is a beneficial herbal drug that is used as an anti-inflammatory, sedative and anti-allergic Agent in Morocco. The present investigation was carried out to find the effect of aqueous extract of *Matricaria chamomilla* L. (Asteraceae) for its anti-inflammatory activity in rodents.

**MATERIALS AND METHODS**

**Plant material**

Samples of *Matricaria chamomilla* L. (MC) were purchased at a farmer’s market in Hay Nahda-Rabat, between March and April 2012. They were identified by the Department of Plant Biology in Ibn Tofail University, Morocco.
Plant Extraction
Dried Stems and flowers of *Matricaria chamomilla* *L.* were reduced to a fine powder with a mechanical grinder. The powder plant material (50 g) was extracted by maceration for 24 h in 500 ml of distilled water, the mixture was then heated for 30 min in the water bath at 80 °C and concentrated to dryness using a rotary evaporator attached to a vacuum pump and yielded 16.76 % (w/ w) then it was stored at a temperature of 4°C until use.

Animals
The animals used in this study were female Swiss mice weighing between 25 and 30g in the acute toxicity for estimation of the LD50, and male Wistar rats weighing between 180 and 220g for Anti-inflammatory studies. The rodents were obtained from the animal experimental centre of Mohammed V Souissi University, Medicine and Pharmacy Faculty, Rabat.
Animals were grouped in clean polycrylic cages and maintained at standard laboratory condition (temp 23±1°C) and relative humidity (50±5%) with dark and light cycles (12/12 hrs). All animals had free access to water and standard diet ad libitum.
Rodents were kept fasting for 18 h with free access to water prior to each experiment.
Six animals were used in treated & controlled groups respectively in Anti-inflammatory studies.

Drugs
Carrageenan and indomethacin were used for the experiments. All drugs were dissolved in distilled water.

Acute toxicity study
Acute toxicity study was carried out to determine the LD50 value in experimental animals. The test was performed according to the Organization for Economic Co-operation and Development (OECD) guidelines 423 [4].

Experiments were performed using healthy young adult female Swiss mice, nulliparous, non-pregnant and weighing 25-30 g. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 300 and 2000 mg/kg body weight by intragastric tube and observed for 14 days. The aim of performing the acute toxicity study is to establish the therapeutic index of a particular drug and to ensure its safety in vivo.
Anti-inflammatory activity

The evaluation of the anti-inflammatory activity of *Matricaria chamomilla* L. aqueous extract was carried out by using two different methods that used mechanical stimuli (Riesterer and Jaques test), and chemical stimuli (winter test) induced paw oedema in rats.

Carrageenan-induced left hind paw oedema in rat

Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay [5-6]. The test groups of rats were given orally 300 and 500 mg/kg of aqueous extract of *Matricaria chamomilla* L. before the carrageenan injection. After One hour, 0.05 ml of 1% of fresh carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the left hind paw. Indomethacin (10 mg/kg, p.o.) was administered orally as reference drug while distilled water (5ml/kg, p.o.) was used as negative control. The right hind paw is not treated; it is taken as a witness. The difference volume of two paws was considered as the inflammation induced by carrageenan. The volume of each paw was measured using a plethysmometer Digitals 7500 immediately, 1h30, 3h and 6 hour after induction of inflammation. Mean differences of treated groups were compared with the mean differences of the control group.

Percent inhibition of the oedema was calculated as:

\[
\text{% of inhibition} = \frac{\text{mean } [v_{\text{Left}} - v_{\text{Right}}] \text{ control} - [v_{\text{Left}} - v_{\text{Right}}] \text{ treated}}{[v_{\text{Left}} - v_{\text{Right}}] \text{ control}} \times 100.
\]

*V Left* means volume of oedema on the left hind paw and *v Right* mean volume of oedema on the right hind paw.

Experimental trauma induced left hind paw oedema in rat

In this test the anti-inflammatory activity was evaluated using the Experimental trauma induced rat paw oedema according to the method described by the Riesterer and Jaques test 1970 [7].

For each experiment, the rodents were divided into 4 groups with 6 animals in each group.

- The control group received 5ml/kg of distilled water p.o.
- The test group 1 received MC aqueous extract 300 mg/kg p.o.
- The test group 2 received MC aqueous extract 500 mg/kg p.o.
- The standard group received indomethacin 20mg/kg.p.o.
In this method, the substances to be tested were administered by stomach tubing 1 hour before eliciting the traumatic oedema. A weight of 50 g was made to fall onto the dorsum of the left hind-paw of all animals. The right hind paw is not treated; it is taken as a witness. The ensuing paw oedema was recorded volumetrically by using a plethysmometer Digitals 7500 at 1h30, 3 and 6 h after induction of inflammation [8]. Mean differences of treated groups were compared with the mean differences of the control group.

The percentages of inhibition of inflammation were calculated according to the following formula:

\[
\% \text{ of inhibition} = \frac{\text{mean } [v \text{ Left } - v \text{ Right}] \text{ control } - [v \text{ Left } - v \text{ Right}] \text{ treated}}{[v \text{ Left } - v \text{ Right}] \text{ control}} \times 100
\]

**Statistical analysis**

All the values in the test are expressed as mean ± SEM [Standard error of the Mean]. Statistical difference between the mean of the various groups were analyzed by using Student’s “t” test, followed by One-way ANOVA. P values < 0.05 or less were considered as significant.

**RESULTS**

**Oral acute toxicity study**

We observe no mortality at any dose (up to 2g/kg) after 14 days. This result suggests that the LD50 is more than 2 g/kg.

**Anti inflammatory activity**

**Carrageenan induced left hind paw oedema in rat**

In this method we have tested the anti-inflammatory activity of the aqueous extract of MC in rodents. We administered per os either vehicle (control group), aqueous extract (300 mg/kg or 500 mg/kg) or Indomethacin (10mg/kg, p.o.) 1 hour before an oedema was induced in the rat-paw by subcutaneous injection of carrageenin. The rat-paw volume was measured 1h30, 3h and 6h after injection of carrageenin.

In control group, the carrageenin increases the development of oedema of the rat-paw at 1h30, 3h and 6h. The Indomethacin, at a dose of 10 mg/kg, reduced significantly paw volume by 69.24%, 74.14 % and 63.59% at 1h30, 3 and 6 hours respectively.
At a dose of 300 mg/kg the aqueous extract of MC inhibited significantly the development of oedema at 1h30, 3 and 6 hours (reduction by 79.33%, 72.48% and 74.52% respectively). Similar results were obtained with the dose of 500 mg/kg after carrageenin injection. The aqueous extract reduced significantly paw volume by 80.90%, 83.14% and 79.38% at 1, 3 and 6 hours respectively (Table 1 and Table 2).

**Experimental trauma-induced left hind paw oedema**

The aqueous extract of *Matricaria chamomilla* L. was evaluated for experimental trauma induced paw oedema anti-inflammatory activity in experimental animal model. Doses of 300 and 500 mg/kg of the extract were significant inhibited in different reaction time. The inhibition was observed during the different exposures.

The reduction was statistically significant (p<0.05) at 1h30, 3 and 6 hours (reduction by 64.34%, 92.97% and 63.14% respectively) at dose of 300mg/kg and at a dose of 500 mg/kg the aqueous extract of *Matricaria chamomilla* L. reduced significantly paw volume by 60.27%, 93.24% and 68.90% at 1h30, 3 and 6 hours respectively. These results are comparable to the standard drug Indomethacin at dose of 20mg/kg p.o. (Table 3 and Table 4).

**Table 1. Anti inflammatory effect of Matricaria chamomilla L. aqueous extract on carrageenan induced hind paw oedema in rats.**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose mg/kg p.o.</th>
<th>Mean volume of oedema(paw left-paw right) induced by carrageenan(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h30</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.386±0.01</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.115±0.003</td>
</tr>
<tr>
<td>MCAE</td>
<td>300</td>
<td>0.086±0.006</td>
</tr>
<tr>
<td>MCAE</td>
<td>500</td>
<td>0.05±0.008</td>
</tr>
</tbody>
</table>

The data represent the mean volume of oedema (paw left - paw right) ± standard error mean (Mean±S.E.M), Control (vehicle)-distilled water; MCAE: *Matricaria chamomilla* L. aqueous extract (300 and 500mg/kg, p.o.), P<0.05 compared with control and reference drug (indomethacin 10mg/kg, p.o.).
Table 2. Percent of inhibition of inflammation of *Matricaria chamomilla* L. aqueous extract using carrageenan induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment groups n=6</th>
<th>Dose mg/kg p.o.</th>
<th>Percent of inhibition of inflammation induced by carrageenan (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h30</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>69.24</td>
</tr>
<tr>
<td>MCAE</td>
<td>300</td>
<td>79.33</td>
</tr>
<tr>
<td>MCAE</td>
<td>500</td>
<td>80.90</td>
</tr>
</tbody>
</table>

N=6; these results compared with standard drug (indomethacin 10mg/kg, p.o.) were administered by the oral route.

Table 3. Anti inflammatory effect of *Matricaria chamomilla* L. aqueous extract on experimental trauma induced hind paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment groups n=6</th>
<th>Dose mg/kg p.o.</th>
<th>Mean volume of oedema(paw left-paw right) induced by experimental trauma(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1h30</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.441±0.01</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>20</td>
<td>0.09±0.006</td>
</tr>
<tr>
<td>MCAE</td>
<td>300</td>
<td>0.186±0.008</td>
</tr>
<tr>
<td>MCAE</td>
<td>500</td>
<td>0.087±0.005</td>
</tr>
</tbody>
</table>

The data represent the mean volume of oedema (paw left - paw right) ± standard error mean (Mean±S.E.M), Control (vehicle)-distilled water; MCAE: *Matricaria chamomilla* L. aqueous extract (300 and 500 mg/kg, p.o.), P<0.05 compared with control and reference drug (indomethacin 20 mg/kg, p.o.).
Table 4. Percent of inhibition of inflammation using experimental trauma induced paw oedema in rats of *Matricaria chamomilla* L. aqueous extract.

<table>
<thead>
<tr>
<th>Treatment groups n=6</th>
<th>Dose p.o.</th>
<th>mg/kg</th>
<th>Percent of inhibition of inflammation induced by experimental trauma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1h30</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>20</td>
<td>79.55</td>
<td>83.62</td>
</tr>
<tr>
<td>MCAE</td>
<td>300</td>
<td>64.34</td>
<td>92.97</td>
</tr>
<tr>
<td>MCAE</td>
<td>500</td>
<td>60.27</td>
<td>93.24</td>
</tr>
</tbody>
</table>

N=6; these results compared standard drug (indomethacin 20 mg/kg, p.o.) were administered by the oral route.

**DISCUSSION**

Inflammation is one of the most important problems of pathology and various therapeutic procedures. Oedema is one of the main effects of inflammation although it is a defensive reaction of biologic organisms, but sometimes it represents the main pathology.

The anti-inflammatory drugs would ideally control the squeal of inflammation. Consequently anti-inflammatory drugs are extensively employed in virtually all branches of medicine. The routine use of synthetic anti-inflammatory drugs is challenged because of negative effects on the health of consumers that have been recently awarded. Thus, new natural substances of plant origin equipped with anti-inflammatory drugs are sought.

*Matricaria chamomilla* L. is a medicinal plant used for the treatment of various body pains in traditional medicine in Morocco. The aim of this study is to find out the anti-inflammatory activity of *Matricaria Chamomilla* L. aqueous extract by using experimental animals.

Our results show that the LD50 values of AEMC were 2000 mg/kg (p.o.). Consequently, no apparent behavioral side effects were observed in rodents during our studies. The high LD50 values also suggest that the aqueous extracts were relatively safe and nontoxic to the animals. The effect of aqueous extract of MC at different doses (300 and 500mg/kg) on carrageenan and on experimental trauma -induced rat paw oedema at different hours (1h30, 3h, 6h) was
compared to the control and reference drug (indomethacin) for the evaluation of anti-inflammatory activity on the basis of percent inhibition of paw oedema volume. The experiment showed that the extract exhibited statistically significant (p<0.05) inhibition of paw volume. Significant inhibition of paw oedema was observed with both doses tested till the six hour. This study has provided some justification for the folkloric use of the plant in Morocco for conditions such as stomachache, pain and inflammations.

Inflammation produced by Carrageenan and experimental trauma-induced paw oedema in rats is believed to be triphasic response. The first phase (1hr after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cycloxygenase products and lipoxygenase products [9]. The metabolites of arachidonic acid formed via the cycloxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cycloxygenase pathway) especially prostaglandin E2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B4 (product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade [10].

Many phenolic compounds of plant origin, especially flavonoids, possess anti-inflammatory effects. Flavonoids are known to target prostaglandin, which is involved in the late phase of inflammation [11]. Prostaglandins have two major actions: they are mediators of inflammation and they also sensitize nerve endings, lowering their threshold of response to stimuli of both mechanical and chemical, allowing the other mediators of inflammation, e.g. histamine, serotonin, bradykinin, to intensify the activation of the sensory endings [12].

Chamomile contains many chemical constituents such as flavonoids epigenin and quercetin luteolin, which posses anti-inflammatory properties [13-14] with well defined selective COX-2 inhibitory activity [15] and it induce a pain relieving effect [16].

Based on these reports, it can be inferred that the inhibitory effect of the extract of MC on carrageenin and experimental trauma-induced inflammation in rats may be due to inhibition of these mediators responsible for inflammation.

Previous studies have demonstrated that individual constituents of chamomile such as chalmuzene, luteolin and apigenin are efficacious in inhibiting COX-2, iNOS and leukotriene
expression in cell culture [17]. A freeze-dried extract of chamomile has been shown to suppress the inflammatory effects and leukocyte infiltration in hind paw edema test induced by simultaneous administration of carrageenan and prostaglandin E1 in Wistar albino rats [18].

Al-Hindawi et al. (1989) showed that Intraperitoneal Injection of ethanol chamomile extract (400 mg/kg) significantly decreased carrageenan-induced paw oedema in rats [19]. These data are in accordance with the results of our study showing anti-oedematic effect of MC aqueous extract. Indeed some in vitro studies have revealed a possible molecular basis of the anti-inflammatory effects of chamomile and its extracts [20].

CONCLUSION
From the above consideration we can conclude that the aqueous extract of *Matricaria chamomilla* L. has the anti-inflammatory activity at lower dose (300mg/kg) which is comparable with the indomethacin (standard drug). However, further studies are required to determine the constituents responsible for its anti-inflammatory activity and further authenticate its mechanism of action.

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REFERENCES


