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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *MATRICARIA CHAMOMILLA* L. ESSENTIAL OIL IN VIVO FROM MOROCCO

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ABSTRACT

The present work was carried out to evaluate the anti-inflammatory activity of *Matricaria chamomilla* L. essential oil in rodents. The flowers of *Matricaria chamomilla* L. was subjected to extraction by hydrodistillation and the chemical composition of the essential oil was assessed through gas chromatography coupled to mass spectrometry (GC/MS). The effect of the essential oil against acute inflammation was studied using carrageenan and experimental trauma-induced hind paw edema in rats at different doses (100, 200 mg/kg p.o.) and compared with standard drug (indomethacin) and with control group. *Matricaria chamomilla* L. essential oil contained Chamazulene (25.21 %), Cis-beta-farnesene (12.51 %), Eucalyptol (9.19 %), Coumarin (7.72 %), Galaxolide (6.28 %) and Camphor (4.3 %) this essential oil produced significant anti-inflammatory effect ($P<0.001$) at all doses studied in both models when compared with control group. This study revealed that *M. chamomilla* L. essential oil has a potent anti-inflammatory activity. This effect, at least in part, depend upon the chemical composition properties of the essential oil of this medicinal plant from Morocco.

Keywords: Inflammation; carrageenan; paw edema; *Matricaria chamomilla* L.; essential oil.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Most of the tribal people still depend on local medicinal plants for the treatment of different diseases using the knowledge of herbal treatment they have inherited from their forefathers. In recent years interest in herbal medicines has increased considerably both at home and abroad as they are believed to be comparatively less toxic than the synthetics [1]. Essential oils (EO) are natural complex mixtures of terpenic and non-terpenic compounds. In general monoterpenes and sesquiterpenes as well as their oxygenated derivatives are the predominant constituents but phenylpropanoids, fatty acids and their esters may also occur [2]. Aromatic plants and their EO have traditionally been used since antiquity for their biological properties (bactericidal, fungicidal, virucidal, antiparasitical, insecticidal), as well as for cosmetic and medicinal applications. Nowadays many EO are commercially valued in the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries [2]. Chamomile, *Matricaria chamomilla* L. (Family: Asteraceae) is a well-known medicinal plant in folk medicine cultivated all over

the world. EO of chamomile is widely used in food industries, cosmetic, and pharmaceutic. The pharmacological effects of *Matricaria chamomilla* L. is mainly connected with its EO for its spasmolytic, antimicrobial and disinfective properties [3]. In several studies *Matricaria chamomilla* L. has been reported to exhibit anti-inflammatory, antispasmodic, anti-oxidative, antibacterial, antifungal, anti-cancer, anti-allergic, hypoglycemic, analgesic, immunomodulatory, antistress, , antiulcerogenic, antihypertensive, CNS depressant, hepatoprotective, chemopreventive, radioprotective, antitumor and anti-pyretic [4]. In our previous studies we have investigated the central nervous system (CNS) activity of the essential oil of *Matricaria chamomilla* L. and we have reported the anti-inflammatory activity of the aqueous extract and the analgesic activities of both extracts (essential oil and aqueous extract) in rodents. [5-7]. In the present study, we determined the constituents of the essential oil by a gas chromatography mass spectroscopy (GC-MS) method and then we evaluated the anti-inflammatory effect of *Matricaria chamomilla* L. essential oil (MCEO) by using carrageenan and experimental trauma-induced hind paw edema in rats to test the validity of the scientific basis for the use of this plant uses in Morocco.

MATERIALS AND METHODS

Plant Material: *Matricaria chamomilla* L. was purchased at a farmer's market in Hay Nahda-Rabat, between March and April 2013, and was identified with botanist 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 [7].

Preparation of the Essential Oil: The aerial parts of *Matricaria chamomilla* L. were hydrodistilled in Clevenger apparatus for 4 hours after the oils were dried over hydrous K2CO3; they were stored at +4∘C until used for GC-MS analysis. The yield of extraction (ratio weight of EO/weight of dry plant) was 0.5% [5].

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 Polaris Q 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 [5-7].

Animals: Male Wistar rats (180 - 220 g) were used for the present study. The animals were obtained from the animal centre of Mohammed V University, Medicine and Pharmacy Faculty, Rabat, Morocco. All animals were housed in groups of six under standard laboratory conditions of temperature (23 ± 1 °C) and 12/12 hr light/dark cycle. They were provided with standard pellets and tap water *ad libitum*. Rodents were kept fasting for 18 hr with free access to water prior to each experiment. The study protocol was approved by the Institutional Research Committee regarding the care and use of animals for experimental procedure in 2010; CEE509 [8-9].

***In Vivo* Anti-Inflammatory Activity:** The evaluation of the anti-inflammatory activity of MCEO was carried out by using two different methods that used mechanical stimuli (Riesterer and Jaques test), and chemical stimuli (winter test) induced paw edema in rats. In both methods, all animals received 5 ml of distilled water by gavages to minimize individual variations in response to the swelling of the paws [10-11].

Carrageenan-Induced Rat Paw Edema: Rats were divided in four groups of 6 animals each. Group A: vehicle control; Group B: 10 mg/kg indomethacin; Group C: essential oil – 100 mg/kg; Group D: essential oil – 200 mg/kg. One hour after the oral administration of drugs, acute paw edema was induced by injecting 0.05 ml of 1% of fresh carrageenan suspension in 0.9% NaCl solution into the sub-plantar tissue of the left hind paw. The right hind paw is not treated; it is taken as a witness.

Paw volume was measured with the help of plethysmometer Digitals 7500 immediately at 1h30, 3h and 6 hours. Mean differences of treated groups were compared with the mean differences of the control group.

The percentage inhibition of paw edema in treated groups was then calculated by using the formula: **% of inhibition=mean [v Left _v Right] control - [v**

Left _v Right] treated / [vLeft _v Right] control × 100.
V Left means volume of edema on the left hind paw and **v Right** mean volume of edema on the right hind paw.

Experimental Trauma-Induced Rat Paw Edema: In this test the anti-inflammatory activity was evaluated by experimental trauma-induced rat paw edema using plethysmometer Digitals 7500 to measure the paw volume. The rats were divided into four groups of six animals each. The different groups were treated with essential oil of *Matricaria chamomilla* L. (100 and 200mg/kg b.w.,p.o.), indomethacin (20 mg/kg, p.o.) and vehicle control (peanut oil) p.o., and the paw volume was measured at 1hr30, 3h and 6hr after trauma-induced rat paw edema using a plathysmometer.

The rodents were pretreated with the extract 1hr before eliciting the traumatic edema. 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. 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:
% of inhibition=mean [v Left _ v Right] control [v Left _ v Right] treated / [v Left _ v Right] control × 100

Statistical analysis : All data were expressed as a mean ± SEM. Comparison in all test groups was made using one way ANOVA followed by student's t- test. The significance level was $*P < 0.001$.

RESULTS

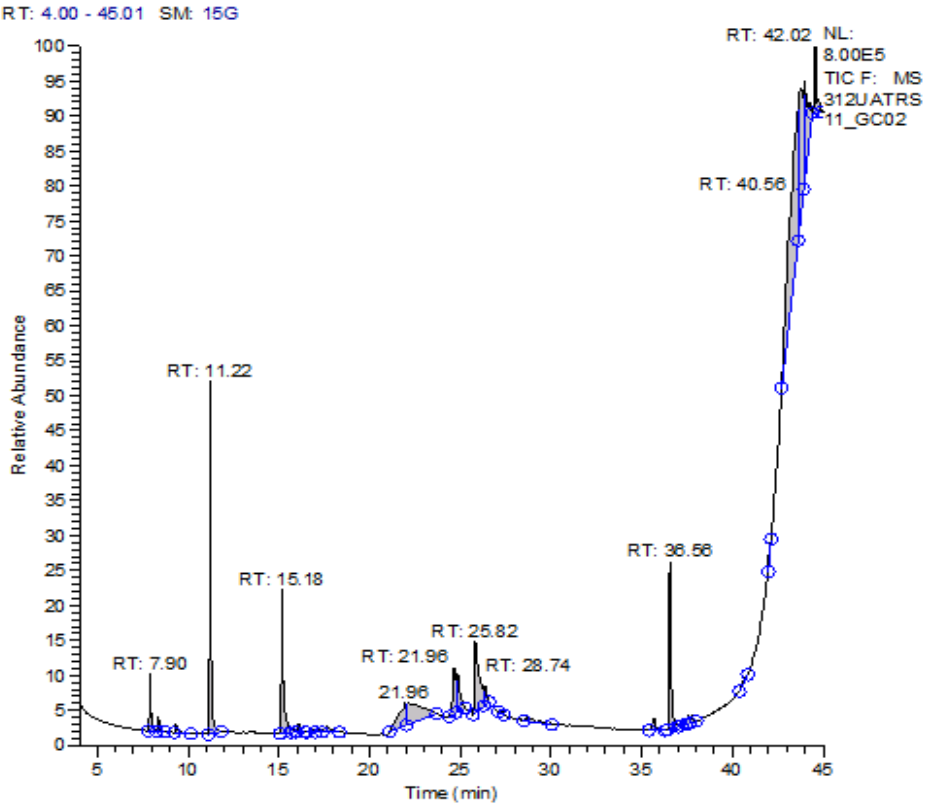

Figure 1: Gas chromatography-mass spectrometry (GC-MS) of *Matricaria chamomilla* L. essential oil.

Table 1: Effect of essential oil of *Matricaria chamomilla* L. on carrageenan-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Mean volume of edema (left paw-right paw) induced by carrageenan (mL)		
		1h30min	3h	6h
Control		0.386±0.01	0.581±0.00	0.478±0.01
Indomethacin	10	0.115±0.003*	0.15±0.006*	0.165±0.008*
EOMC	100	0.02±0.02*	0.025±0.007*	0.02±0.01*
EOMC	200	0.03±0.007*	0.02±0.006*	0.01±0.023*

Values are expressed as mean ± S.E.M. (n = 6), EOMC: essential oil of *Matricaria chamomilla* L., P< 0.001 statistically significant compared to the control and reference drug (Indomethacin).

Table 2: Percentage of inhibition of inflammation of essential oil of *Matricaria chamomilla* L. using carrageenan-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Percentage of inhibition of inflammation induced by carrageenan (%)		
		1h30min	3h	6h
Indomethacin	10	69.24	74.14	63.59
EOMC	100	79.44	94.75	92.65
EOMC	200	85.96	93.86	94.94

n= 6; these results compared with standard drug (Indomethacin, 10mg/kg,p.o.) were administered by the oral route.
Table 3: Effect of essential oil of *Matricaria chamomilla* L. on experimental trauma-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Mean volume of edema (left paw-right paw) induced by experimental trauma (mL)		
		1h30min	3h	6h
Control		0.441±0.01	0.693±0.01	0.563±0.01
Indomethacin	20	0.09±0.006*	0.102±0.008*	0.142±0.006*
EOMC	100	0.06±0.06*	0.08±0.007*	0.06±0.04*
EOMC	200	0.04±0.003*	0.03±0.004*	0.023±0.04*

Values are expressed as mean ± S.E.M. (n = 6), EOMC: essential oil of *Matricaria chamomilla* L., P< 0.001 statistically significant compared to the control and, reference drug (Indomethacin).

Table 4: Percentage of inhibition of inflammation of essential oil of *Matricaria chamomilla* L. using experimental trauma-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	Percentage of inhibition of inflammation induced by experimental trauma (%)		
		1h30min	3h	6h
Indomethacin	20	79.55	83.62	75.16
EOMC	100	94.78	94.60	91.21
EOMC	200	79.55	83.62	75.16

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

Chemical Composition of the Essential Oil: Gas chromatography mass spectroscopy analysis of MCEO resulted in the identification of six major compounds accounting for 65.21% of the total oil. Chamazulene was the predominant compound (25.21 %), followed by Cis-beta-farnesene (12.51 %), Eucalyptol (9.19 %), Coumarin (7.72 %), Galaxolide (6.28 %), and Camphor (4.3 %) (Fig. 1) [5].

Carrageenan-Induced Rat Paw Edema: The effect of the essential oil on carrageenan-induced rat paw edema test are shown in Tables 1-2. EOMC (100 and 200 mg/kg) significantly ($P<0.001$) inhibited carrageenan-induced paw edema. The reduction of paw edema was observed at 1h30, 3h and 6 hours after carrageenan injection where it caused (79.44%, 94.75% and 92.65%) at dose of 100 mg/kg and (85.96%, 93.86%, 94.94%) at dose of 200mg/kg inhibition in increase in paw volume as compared to that of 10 mg/kg Indomethacin.

Experimental Trauma-Induced Rat Paw Edema: The essential oil (100, and 200 mg/kg) exhibited a significant inhibition on experimental trauma-induced rat paw edema. Table 3-4 shows the results on antiedematous effect of orally administered EOMC on experimental trauma paw edema in rats. At dose of 100 mg/kg, p.o., EOMC produced a greater inhibition of edema development by 94.78%, 94.60% and 91.21% respectively. At dose of 200 mg/kg MCEO caused a significant reduction in paw edema by 79.55% (1h30), 83.62% (3h) and 75.16% (6h) respectively. While edema reduction by the standard drug, indomethacin (20mg/kg), was (79.55% (1h30) ,83.62% (3h) and 75.16% (6h).

DISCUSSION

In recent years, many medicinal herbs have been used as a form of therapy for the relief of pain and inflammation throughout the world without any adverse effects. It is therefore essential and efforts should be made to introduce new medicinal plants to develop drugs which are cheaper, safer and more effective such as *Matricaria chamomilla L.* in Morocco.

Matricaria chamomilla L. (Asteraceae) also called German chamomile is one of the most widely used and well-documented medicinal plant in the world. It has been used in traditional medicine to relieve muscle spasms, menstrual disorders, ulcers, wounds, gastrointestinal disorders and rheumatic pain which imply anti-inflammatory activity for the plant [12]. The acute toxicity study in mice of *Matricaria chamomilla* L. essential oil at the doses of 2000 and 5000 mg/kg, p.o., revealed no toxicity and no changes in the weight gain between the control and the treated group. This result indicates that the LD_{50} of MCEO by the oral route was higher than 2g/kg in mice. These results were previously reported by Hajjaj et al. [5]. Twenty-five compounds were found in the volatile composition of essential oil from *M. chamomilla* L., and the largest class of compounds identified belonged to sesquiterpenes, represented by (trans-caryophyllene, α-Cedrol, chamazulene, Cis-beta-farnesene), which constituted approximately 49.47% of the total area of the chromatogram peaks. It was followed by monoterpenes represented by (cis-Ocimene, Camphene, 2-α-pinene, Eucalyptol, Camphre, Thymol, terpinene-4-ol, b-pinene) with percentage of 25.27% [5].

In the present study, we examined the anti-inflammatory effect of *Matricaria chamomilla* L. *in vivo* using 2 different models. This activity was evaluated by observing hind paw edema by oral administration of essential oil. Administration of *Matricaria chamomilla* L. showed significant inhibition of edema. Carrageenan and experimental trauma-induced paw edema in rats are a suitable experimental animal model to evaluate the antiedematous effect of diverse bioactive compounds such as plant extracts and essential oils [13]. The development of the edema depends upon the kinins and polymorphonuclear leukocytes. The development of paw edema for every hour attributes to various factors such as histamine and serotonin, prostaglandin release [14-15].

Anti-inflammatory activity of *Matricaria chamomilla* L. aqueous extract (MCAE) in rats had been reported in our previous study. The aqueous extract at doses (300 and 500 mg/kg p.o.) significantly decreased carrageenan and experimental trauma-induced paw edema in rats [6]. It is known that Chamazulene, flavonoids, apigenin, Cis-beta-farnesene, eucalyptol are related to various biological activities, such as antimicrobial anti-inflammatory, anti-allergic effect and anti-oxidant properties which could be the reason for its observed high activity.

The main anti-inflammatory activity is due to chamazulene, which is formed during the distillation of the oil of matricine, and (-)-α-bisabolol [16], but also bisabololoxide A and B play a role [17]. Janmejai K *et al.* (2009) suggest that chamomile works by a mechanism of action similar to that attributed to non-steroidal anti-inflammatory drugs. In this study the mechanism of action of chamomile on the inhibition of PGE2 production was due to the suppression of the COX-2 gene expression and direct

inhibition of COX-2 enzyme activity [18]. These results suggest that essential oil of *Matricaria chamomilla* L. from Morocco has potential clinical benefits for the treatment of edema. Further anti-inflammatory activity in vitro study are needed and planned in our laboratories to understand the mechanism of action underline the above mention activity.

Conflict of interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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