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Original Article

IN VIVO ANTI-NOCICEPTIVE AND ANTI-INFLAMMATORY PROPERTIES OF *ORMENIS MIXTA* L. FOM MOROCCO

GHIZLANE HAJJAJ*[1], AZIZ BAHLOULI[2], MOUNA TAJANI[3], YAHIA CHERRAH[1], AMINA ZELLOU[1]

[1]Laboratory of Pharmacology and Toxicology, Department of Drugs Sciences, Faculty of Medicine and Pharmacy, Mohammed V University, ERTP, BP 6203, Rabat Instituts, Agdal, Rabat, Morocco, [2]Laboratory of Biotechnology, Environment and Quality (LABEQ), Department of Biology, Faculty of Science, Ibn Tofaïl University, BP 133;14000 Kenitra, Morocco, [3]Department of Biology, Faculty of Sciences, Ibn Tofail University, BP 133;14000 Kenitra, Morocco

Email: hajjajghizlane1@gmail.com

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ABSTRACT

Objective: *Ormenis mixta* L. (Asteraceae) is a traditional herbal medicine widely used as a mild sedative, spasmolytic and antibacterial agent. This paper aimed to examine possible anti-inflammatory and antinociceptive effects of *Ormenis mixta* L. in rodents.

Methods: Anti-inflammatory effect of *Ormenis mixta* L. was investigated with the carrageenan and experimental trauma-induced hind paw edema tests. *Ormenis mixta* L. essential oil (OMEO) by gavage at 100 and 200 mg/kg and aqueous extract (OMAE) at 200 and 400 mg/kg, were administered and compared with indomethacin, and control group. Antinociceptive effects of both extracts were evaluated using acetic acid-induced writhing and tail immersion tests and compared with standard drug (morphine and aspirin).

Results: It was found that pretreatment with OMEO and OMAE significantly ($P<0.05$) reduced paw swelling and diminished the nociceptive response in rodents.

Conclusion: *Ormenis mixta* L. essential oil and aqueous extract have a potent anti-inflammatory and an antinociceptive properties

Keywords: *Ormenis mixta* L. Anti-inflammatory, Antinociceptive, Morphine, Carrageenan

INTRODUCTION

Inflammatory and arthritic conditions are among those treated using traditional remedies, with considerable success. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Although several modern drugs are used to treat these types of disorders, their prolonged use may cause severe adverse side effects on chronic administration, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. It is worthwhile to note that most of the present day analgesic drugs also exert a wide range of side effects. Herbal medicines are popular as remedies for diseases by the vast majority of world's population. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth, and a large number of diverse types of plants grow in different parts of the country. There is considerable evidence that plant extracts have the potential to be developed into agents that can be used as preventative or treatment therapies for oral diseases. *Ormenis mixta* L. (Family: Asteraceae), wild Moroccan chamomile, is an aromatic plant known as the vernacular name "Hellâla" [1]. In Morocco, it's found in two disjunct areas, one between Tanger, Ouezzane, Souk Larbaa, Moulay Bousselham and Azilah, and the second between Kenitra, Sidi Slimane, Khemisset, and Rabat [2].

Ormenis mixta L., leaves and flowers infusion is used in Moroccan traditional medicine to treat different ailments. This plant is recommended as an anxiolytic and to rebalance the central nervous system, it's recommended in nervous breakdowns, for shortcomings liver, stomach light and colibacillaires colitis. The plant is well-known and cultivated for the extraction of essential oil from its aerial parts, which is sold and used in aromatherapy as an aphrodisiac, antibacterial, and anxiolytic [2-4]. To the best of our knowledge, studies with Ormenis mixta L. from Morocco were never done to assess its *in vivo* analgesic and anti-inflammatory activities. The present study was undertaken for the first time to investigate the analgesic and anti-inflammatory effects of *Ormenis mixta* L. essential oil and aqueous extract in different animal models.

MATERIALS AND METHODS

Collection of plant material

Ormenis mixta L. was collected between March and June 2014 from Oulad Sidhoum-Sidi Yahya (Morocco) and identified by a botanist from the Department of Plant Biology, Ibn Tofail University, Morocco.

Preparation of plant extracts

Preparation of essential oil

The aerial parts of *Ormenis mixta* L. were air-dried in shadow at room temperature for 48 h. The oils were obtained by hydro distillation using a Clevenger apparatus for up to 4 h, time which was necessary for a complete extraction. The oils obtained at the yield range of (0.42%, v/w), were dried over anhydrous sodium sulphate, filtrated and stored at+4 °C until tested and analysed.

Preparation of aqueous extract

Flowers of *Ormenis mixta* L. were dried under a shade and pulverized. The coarse powder (50 gm) was macerated for 24 h in 500 ml of distilled water. The extract was dried using a rotary vacuum evaporator and stored in a desiccator until further use. The percentage yield of aqueous extract of *Ormenis mixta* L. was 7.84% w/w.

Animals

Rats (180-220 gm each) and mice (20-25 gm each) of either sex used in the study were housed in the animal center of Mohammed V University, Medicine and Pharmacy Faculty, Rabat, Morocco. Animals were given tap water *ad libitum* and maintained under standard conditions at 23±1 °C and relative humidity 60–70% and 12h-dark/12h-light cycle. The investigation was conducted in accordance with the Official Journal of the European Committee in 1991 and approved by the Institutional Research Committee regarding the care and use of animals for the experimental procedure in 2010;CEE509 [5-6].

Acute toxicity studies

LD50 values were determined as described by OECD 423 this method is preferred because fewer animals are required [7]. Median lethal dose (LD50) of the aqueous extract and essential oil were determined using female Swiss mice. The aqueous extract was dissolved in distilled water, and essential oil was dissolved in peanut oil and given by orally way in a single dose (2 gm/kg, p. o.) of body weight. Mice were observed for clinical effects and mortality.

***In vivo* analgesic activity**

Acetic acid-induced writhing response in mice

The test was performed according to Koster test [8]. Nociception was induced by an intraperitoneal (i. p.) injection of 0.1mL/10 gm acetic acid solution (3% with 300 mg/kg). Positive control animals were pretreated with aspirin (200 mg/kg, p. o.) 30 min before acetic acid. The essential oil of *Ormenis mixta* L. was administered in different doses at 100,200 and 300 mg/kg p. o. and aqueous extract of *Ormenis mixta* L. at doses of 200,400 and 600 mg/kg p. o. to the Swiss mice after an overnight fast. Five minutes after the i. p. injection of acetic acid, the number of writhing and stretching was recorded during 20 min.

Tail immersion test

This experiment was done based on the previous observation demonstrating that morphine-like analgesics prolongs the tail withdrawal latency from hot water in rodents [9-10]. Rats that showed tail withdrawal time between 1.5 and 2 s were selected for this experiment, and the pretreatment latency was recorded. Then the animals were pretreated with morphine or (EO/AE) and 4 to 5 cm of their tail was immersed in the warm water with a constant temperature of 55 °C. The time between tail submersion and tail deflection was recorded at 15, 30, 45, 60 and 120min after the treatment with standard drug or extract. A cut-off time of 10 s was maintained to avoid tail tissue damage in the rodents.

***In vivo* anti-inflammatory activity**

Carrageenan-induced rat paw edema

Carrageenan-induced hind paw edema model was used to determine the anti-inflammatory activity [11-12]. Animals were treated with the aqueous extract (200,400 mg/kg p. o.), essential oil (100, 200 mg/kg p. o.), Indomethacin (10 mg/kg) or normal control, 60 min prior to injection of 1% carrageenan (50 µl) in the plantar side of left hind paws of the rats. Paw volume was measured after Carrageenan injection at 1h30, 3h, and 6 h using a plethysmometer (model 7500, Ugo Basile).

Percent inhibition of the edema was calculated as:

% of inhibition= mean [v Left _v Right] control-[v Left _v Right] treated / [v Left _v Right] control × 100.

V Left means the 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 properties were investigated by using mechanical stimuli (Riesterer and Jaques test) [13]. Rats were given indomethacin (20 mg/kg p. o.), aqueous extract (200,400 mg/kg p. o.) and essential oil (100, 200 mg/kg p. o.) 1 h before dropping a weight of 50 gm onto the dorsum of the left hind paw of all rats. The right hind paw is not treated; it is taken as a witness. The paw volume was measured immediately before and at 1h30, 3h and 6h after treatment by means of volume displacement methods using a 7500 Ugo Basile Plethysmometer. The difference between the left paw and right paw volumes indicated the degree of inflammation. The average percentage increase in paw volume of each group was calculated and compared with the control group and the indomethacin 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

Values were presented as mean±SEM and were analyzed by one-way analysis of variance (ANOVA), following by student's t-test. All differences showing a *p<0.05 were accepted as statistically significant.

RESULTS

The acute toxicity studies of *Ormenis mixta* L. essential oil and aqueous extract on mice shows that no animal died within 24 h after taking the treatment with the extracts, and the LD50 was greater than 2 gm/kg b.w. Again, no death was recorded among all the dose groups throughout the two weeks experimental period.

In the acetic acid-induced writhing test in mice the OMEO (100, 200 and 300 mg/kg, p. o.) and OMAE (200, 400 and 600 mg/kg, p. o.) significantly ($p<0.05$) reduced the nociceptive response at all tested doses with an inhibition percentage of 57.04%, 76.66% and 77.77% respectively of essential oil and with an inhibition percentage of 61.27%, 62.74% and 63.72% respectively of aqueous extract, compared to Aspirin (51%) as positive control (table 1) on the other hand in the tail immersion in rats test morphine (5 mg/kg, i. p.) significantly increased the latency time (7.49±0.15 sec) at 45 min in the thermal stimulus.

The EO at dose of 100 mg/kg, p. o. significantly ($p<0.05$) increased the reaction time (6.49 sec±0.13) at 60 min in the thermal stimulus and AE at dose of 400 mg/kg, p. o. significantly ($p<0.05$) increased the reaction time (6.92 sec±0.27) at 30 min in the thermal stimulus (table 2).

The essential oil (100 mg/kg and 200 mg/kg, p. o.) showed reduction in the carrageenan-induced paw edema in rat at 1h30, 3h and 6h after carrageenan injection with (87.25%, 94.14% and 94.07%) of the inhibition respectively at dose of 100 mg/kg and with (90.31%, 93.70% and 94.90%) of the inhibition respectively at the dose of 200 mg/kg, the results are presented in (table 3). Aqueous extract of *Ormenis mixta* L. (200 mg/kg, p. o.) also inhibited paw edema in the rat after carragenan injection with 92.40%, 95.22% and 94.71% of the inhibition respectively and at a dose of 400 mg/kg, p. o. the inhibition was 94.00%, 95.22% and 95.88% respectively.

Table 1: Effect of *Ormenis mixta* L. extracts on acetic acid induced writhing in mice

Treatment groups	Dose mg/kg p. o.	No. of writhing	Percent of inhibition (%)
Control	0,5 ml/mouse	45±2.58	-
Aspirin	200	19.6±2.88*	61.56
OMEO	100	19.33±2.08*	57.04
OMEO	200	10.5±3.53*	76.66
OMEO	300	10±1.41*	77.77
OMAE	200	19.75±1.31*	61.27
OMAE	400	19±0.8*	62.74
OMAE	600	18.5±1.53*	63.72

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

The effects of *Ormenis mixta* L. on rat paw edema induced by experimental trauma are reported in the table (4). The results showed that both extracts at all doses exhibited a significant (P<0.05) reduction of average edema compare to the control.

The inhibition were 89.49%, 95.70% and 96.07% respectively for essential oil of *Ormenis mixta* L. with 100 mg/kg and at the dose of 200 mg/kg, the EO inhibited the paw swelling with (90.98%,94.93%, and 95.48 % inhibition) at 1h30, 3h and 6 h after the experimental traum-induced rat paw edema.

At 1h30, 3h and 6h treatment with aqueous extract at dose of 200 mg/kg the inhibitions were (92.65%, 94.76% and 96.76%) after experimental trauma–induced rat paw edema. At dose (400 mg/kg), AE caused a significant inhibition of 92.52% (1h30), 95.87% (3h) and 95.01% (6 h).

Table 2: Central analgesic activity of *Ormenis mixta* L. extracts by tail immersion test

	Reaction time in seconds					
Treatment/Dose	**0 min**	**15 min**	**30 min**	**45 min**	**60 min**	**120 min**
Control	1.57±0.13	1.62±0.1	1.63±0.09	1.65±0.13	1.54±0.14	1.45±0.14
Morphine 5 mg/kg	1.78±0.19	3±0.15	5.5±0.19	7.49±0.15*	5.05±0.14	3±0.13
OMEO 100 mg/kg	1.96±0.16	3.91±0.2	3.9±0.23	4.32±0.15	6.49±0.13*	2.54±0.2
OMEO 200 mg/kg	1.85±0.09	4.55±0.14	5.71±0.17	4.8±0.13	4.15±0.15	3.98±0.19
OMEO 300 mg/kg	1.2±0.2	3.87±0.16	4.75±0.13	4.37±0.08	5.23±0.56	4.04±0.15
OMAE 200 mg/kg	2±0.11	4.8±0.08	4.1±0.98	3.3±0.14	3.32±0.4	2.45±0.12
OMAE 400 mg/kg	2.06±0.17	3.09±0.19	6.92±0.27*	3.61±0.6	4.01±0.16	3.17±0.07
OMAE 600 mg/kg	2.15±0.2	4.2±0.14	5.71±0.23	4.27±0.15	3.37±0.17	2.58±0.13

Values are means±SEM *P<0.05, significantly different from control; Student's t-test (n= 6). OMEO: *Ormenis mixta* L. Essential Oil, OMAE: *Ormenis mixta* L. Aqueous Extract.

Table 3: Effect of *Ormenis mixta* L. extracts on carrageenan-induced rat paw edema

Treatment groups	**Dose mg/kg p. o.**	**Mean volume of edema (left paw right paw) (ml) and percentage of inhibition (%) of inflammation induced by carrageenan**		
		1h30 min	**3h**	**6h**
Control		0.386±0.01	0.581±0.00	0.478±0.01
Indometacin	10	0.115±0.003* (69.24%)	0.15±0.006* (74.14%)	0.165±0.008* (63.59%)
OMEO	100	0.03±0.009* (87.25%)	0.02±0.003* (94.14%)	0.02±0.01* (94.07%)
OMEO	200	0.02±0.01* (90.31%)	0.02±0.008* (93.70%)	0.01±0.006* (94.90%)
OMAE	200	0.02±0.002* (92.40%)	0.01±0.001* (95.22%)	0.01±0.03* (94.71%)
OMAE	400	0.016±0.04* (94.00%)	0.015±0.005* (95.22%)	0.013±0.008* (95.88%)

Values are expressed as mean±SEM (n = 6), OMEO: *Ormenis mixta* L. Essential Oil, OMAE: *Ormenis mixta L.* Aqueous Extract, *P<0.05 statistically significant compared to the control and reference drug (Indomethacin).

Table 4: Effect of *Ormenis mixta* L. extracts on experimental trauma-induced rat paw edema

Treatment groups	**Dose mg/kg p. o.**	**Mean volume of edema (left paw right paw) (ml) and percentage of inhibition of inflammation (%) induced by induced experimental trauma**		
		1h30 min	**3h**	**6h**
Control		0.441±0.01	0.693±0.01	0.563±0.01
Indometacin	20	0.09±0.006* (79.55%)	0.102±0.008* (83.62%)	0.142±0.006* (75.16%)
OMEO	100	0.03±0.06* (89.49%)	0.01±0.007* (95.70%)	0.015±0.01* (96.07%)
OMEO	200	0.02±0.007* (90.98%)	0.02±0.004* (94.93%)	0.01±0.01* (95.48%)
OMAE	200	0.02±0.001* (92.65%)	0.02±0.002* (94.76%)	0.01±0.01* (96.76%)
OMAE	400	0.02±0.007* (92.52%)	0.016±0.004* (95.87%)	0.02±0.005* (95.01%)

Values are expressed as mean±SEM (n = 6), OMEO: *Ormenis mixta* L. Essential Oil, OMAE: *Ormenis mixta* L. Aqueous Extract, P < 0.05 statistically significant compared to the control and, reference drug (Indomethacin).

DISCUSSION

The results of the present study have shown that the essential oil and aqueous extract of the investigated plant exhibited very high anti-inflammatory and analgesic activities. In the preliminary acute toxicity study, the LD50, being greater than 2g/kg b.w., is thought to be safe as reported by Lorke [14]. Again, the absence of death among mice in all the dose groups throughout the two weeks of the experimental seems to support this claim.

The acetic acid-induced writhing assay is used to detect peripheral analgesia whereas tail immersion test assay is more sensitive to supraspinally acting analgesics [15-16].

The carrageenan and experimental-Trauma induced paw edema are suitable experimental animal models of acute inflammation and is believed to be biphasic [17-18]. The initial phase of carrageenan paw edema is mediated by histamine and serotonin while the mediators in the later phase are suspected to be arachidonate metabolites (prostaglandins, leukotrienes) producing an edema dependent on the mobilisation of neutrophils [19-20].

Previous study on the phytochemical screening of *Ormenis mixta* L. has shown that the leaves of the plant contain essential oils, in which Santolina alcohol, alpha pinene, germacrene D, yomogi alcohol, and (E)–beta-farnasene have been identified as the main components [21].

This study showed that *Ormenis mixta* L. extracts possess anti-inflammatory and analgesic activities in rodents. The observation that the essential oil exhibited greater anti-inflammatory and analgesic activities with low dose than the aqueous extract, this could be due to one or combination of the components of this plant and/or more of the active principle (s) responsible for those activities might be present in higher concentration in the essential oil than in the aqueous extract.

CONCLUSION

To our knowledge, this is the first study on the anti-inflammatory and analgesic activities of essential oil and aqueous extract of *Ormenis mixta* L. in Morocco. From the results of this study, *Ormenis mixta* L. is a safe plant to use in traditional medicine. Therapeutical doses needs to be determined for clinical applications. However, further, *in vitro* studies are required for thorough investigation of the anti-inflammatory and analgesic activities.

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ABBREVIATION

OMEO, *Ormenis mixta* L. essential oil; OMAE, *Ormenis mixta* L. aqueous extract; LD50, lethal dose of a drug for 50 % of the population;

CONFLICT OF INTERESTS

Declared none

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