Investigation of Origanum compactum essential oil for analgesic and anti-inflammatory activities

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Abstract. *Origanum compactum* Benth. has been widely used in moroccan traditional medicine for various therapeutic treatments. Belonging to the same genus, *O. onites* was found to have marked analgesic and anti-inflammatory activities. The aim of this work is to evaluate theses pharmacological properties of the essential oil of O. *compactum* in order to provide a basis for the folkloric use of the plant. Aerial parts of plant were subjected to steam distillation, according to the French Pharmacopoeia. Male OF1 mice and male Wistar rats were used for these studies. The analgesic effect was done using Writhing test in mice and Tail-Flick test in rats. The mechanism investigation was evaluated employing an antagonism assay using naloxone, a specific antagonist of opiate receptors. Anti-inflammatory property has been studied using carrageenin and experimental trauma induced edema in rats. The essential oil of the aerial parts of *Origanum compactum* was found to exert central analgesic properties. Such a dose-dependent action was obtained against chemical and thermic stimuli, respectively, from the doses of 6.25 and 12.5 mg/kg and it was inhibited by a naloxone pretreatment, a specific morphinic antagonist compound. Significant and dose-dependent anti-inflammatory effects were observed on an acute inflammatory process from the dose of 100 mg/kg. **Key words**: *Origanum compactum*, Essential oil, Analgesic Activity, Anti-inflammatory Activity.

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1 Introduction

Herbal remedies have been used for centuries for the treatment and management of various diseases. Herbal medication is a promising alternative to modern synthetic drugs because of their low side effects. They are therefore considered safe and effective in the treatment of human diseases [1].

Several bioactive compounds are extracted of various medicinal plants. These products, belonging to different chemical families such as terpenoids, flavonoids, saponins, alkaloids phenolic acids and essential oils, have also been identified as promising molecules in the treatment of inflammation and pain [2-5].

Essential oils, extracted from plants, are recognised as active and remarkable pharmaceutical substances due to their various biological properties. We mention antibacterial, antifungal, antiviral, antioxidant, anticancer, immunomodulatory, analgesic, anti-inflammatory and other activities [6-8].

The analgesic and anti-inflammatory activities were also widely demonstrated in other Origanum species, including Origanum onites and O. majorana [9-11].

Origanum compactum Benth. (Lamiaceae) has been widely used in traditional medicine for various therapeutic treatments including its analgesic and anti-inflammatory indications [12, 13, 14]. The aim of this work is to evaluate these pharmacological activities for the plant's essential oil in order to provide a basis for its folkloric use and its use as an alternative to synthetic drugs.

2 Material and method

2.1. Plant material

Origanum compactum aerial parts were collected in the Rifian mountains (province of AL Hoceima, Morocco) and authenticated in the Department of Botany, National Scientific Institute, Rabat. A voucher of studied plant was deposited in the laboratory of Natural Products (Faculty of Medicine and Pharmacy of Rabat), under number O951.

2.2. Preparation of the extract

Air-dried, ground plant (200 g) was subjected to steam distillation, according to the French Pharmacopoeia [15], for 2 h, to obtain the essential oil (yield: 5.4 %).

2.3. Animals

Male Ico IOPS OF1 mice (Iffa credo: animals breeder, Indemnes d'Organismes Pathogènes Spécifiques: sanitary status, Oncins France 1: the strain, l'Arbresle, France), weighing 20 - 25 g and male Wistar rats (Iffa Credo) weighing 200-250 g were used for these studies. All animals were kept in standardized environmental conditions with a 12/12 light/dark cycle and fed standard rodent diet and water.

2.4. Analgesic activity

2.4.1. Writhing test in mice

Thirty min after receiving i.p. injection of the essential oil (3.125-200 mg/kg i.p.), each mouse was given i.p. 3 % acetic acid at the dose level of 300 mg/kg (10 ml/kg). Acetylsalicylic acid (68 mg/kg i.p.) and morphine (1.15 mg/kg i.p.) were respectively used as a reference peripheral and central analgesic compound. Immediately after the injection of the algic substance, each animal was isolated in an individual box to be observed. The number of writhings and stretchings was counted for 20 min, according to the method of Koster et al. [16]. A percentage of protection of extract and reference substances will be expressed using the following ratio: (control mean - treated mean) x 100 / control mean.

2.4.2. Tail-Flick test in rats

In the manner of Jacob [17], the tail of the rat up to a constant level was immersed in a water bath at 55 °C. The reaction time to flick the tail from the liquid was recorded 30 min before injection of oil (3.125-200 mg/kg i.p.) and morphine (5 mg/kg i.p.) and at 15, 30, 45, 60 and 120 min after administration of these substances. The normal tail flick time is inferior to 2 sec. A previous selection of animals was necessary before the beginning of experimentation. Each rat was used for one essay. A certain adaptation of the heat may be developed. The pain threshold inhibition was 6 sec.

2.4.3. Mechanism of analgesia study

The mechanism of analgesia action of the test compound was investigated employing an antagonism assay using naloxone (Narcan®), a specific antagonist of opiate receptors. Experimental procedure is identical to that previously described at the time of analgesic property research by the tail flick test. Naloxone (1 mg/kg *s.c.*) was administered 15 min prior to oil (100 and 200 mg/kg i.p.) or morphine (5 mg/kg i.p.) treatment.

2.5. Anti-inflammatory activity

2.5.1. Carrageenin induced paw oedema in rats

The method used was similar to that described by Winter et al. [18]. Animals were fasted overnight prior to

dosing, but had free access to water. The oil was given (50-200 mg/kg i.p.) 1 h before a subplantar injection of carrageenin (0.1 ml at 1%) in the left hindpaw of rat. Indomethacin (indocid ®) was tested at 10 mg/kg i.p. as a reference anti-inflammatory substance.

2.5.2. Trauma induced paw oedema in rats

The Riesterer and Jacques's test was used [19]. In this test, the left hindpaw of rat was deposed under a vertical plexiglass tube. A weight of 50 g fall through the tube, above the paw. The oil (50-200 mg/kg i.p.) and indomethacin (20 mg/kg i.p.) were administered 1 h before the experimental trauma.

Right (R) and left (L) hindpaw' s volume of each rat were measured by an digital plethysmometer (LETICA 7500) at 1 h 30 min, 3 h 00 and 6 h 00 after chemical or mechanical induction of edema.

2.6. Statistical analysis

Results were expressed as mean values ± standard deviation. The significance between means was determined using the student's *t*-test and the results were regarded as significant when $p< 0.05$.

Table 1: Influence of essential oil, acetylsalicylic acid and morphine on acetic acid-induced pain in mice (writhing test)

Values are mean ± S.D., n = 10, * P < 0,05 vs. Control, Student's *t*- test.

Treatment	Doses (mg/kg i.p.)	Average cramps	Percentage of protection
Control	-	50.2 ± 3.0	-
O. compactum	3.125	46.8 ± 4.0	7
	6.25	37.4 ± 4.3 *	25
	12.5	22.5 ± 3.1 *	55
	25	18.4 ± 2.7 *	63
	50	7.3 ± 1.9 *	85
	100	5.1 ± 2.6 *	90
	200	0.0 ± 0.0 *	100
Acetylsalicylic acid	68	19.5 ± 2.0 *	61
Morphine	1.15	15.0 ± 1.7 *	70

3. Results

3.1 Peripheral analgesic activity

According to the results presented in Table 1, the essential oil significantly reduces the number of cramps induced by the injection of 3% acetic acid, as early as the dose of 6.25 mg/kg (percentage of protection compared to controls is 25%). This effect is dose-dependent and the percentage of protection obtained with the highest dose tested, 200 mg/kg, was 100%.

The results obtained with the reference substances, acetylsalicylic acid and morphine, show a significant and important protective effect, reducing the pain phenomena by more than 50% (respective percentages of protection: 61% and 70%).

Table 2: Influence of essential oil acetylsalicylic acid (ASA) and morphine (M) on heat-induced pain in rat (tail flick test)

Values are mean ± S.D., n = 10, * P < 0,05 vs. Control, Student's *t*- test.

Treatment	Dose (mg/kg i.p.)	Reaction time to flick the tail (sec)					
		0 min	15 min	30 min	45 min	60 min	120 min
Control	-	2.0 ± 0.5	22 ± 0.5	2.0 ± 0.4	2.7 ± 0.6	2.9 ± 1.0	2.9 ± 1.2
	3.125	1.7 ± 0.2	2.0 ± 0.4	2.0 ± 0.3	2.3 ± 1.3	2.4 ± 0.9	2.7 ± 0.3
O. compactum							
	6.25	1.9 ± 0.1	2.0 ± 0.8	3.1 ± 0.9	3.5 ± 1.2	3.7 ± 0.6	3.3 ± 0.5
	12.5	1.8 ± 0.5	2.7 ± 0.5	3.8 ± 0.7	5.1 ± 0.9*	4.4 ± 1.2*	3.8 ± 0.9
	25	2.0 ± 0.3	2.8 ± 0.5	4.7 ± 0.5*	6.5 ± 0.7*	4.6 ± 0.9*	4.0 ± 0.2
	50	1.8 ± 0.1	3.6 ± 0.9	5.3 ± 0.4*	7.1 ± 1.3*	5.7 ± 0.8*	3.9 ± 1.4
	100	1.9 ± 0.2	5.3 ± 0.4*	6.7 ± 1.0*	8.5 ± 0.4*	6.1 ± 1.3*	5.0 ± 0.7*
	200	1.8 ± 0.3	5.6 ± 0.7*	8.8 ± 1.4*	9.7 ± 0.3*	7.3 ± 1.1*	5.3 ± 1.7*
ASA	68	2.0 ± 0.1	2.1 ± 0.6	2.0 ± 0.3	2.9 ± 0.9	3.0 ± 1.2	3.0 ± 0.6
M	1.15	1.7 ± 0.6	2.4 ± 1.0	4.1 ± 0.7*	5.5 ± 0.8*	4.6 ± 0.9*	3.2 ± 0.9
M	5	1.9 ± 0.4	3.2 ± 0.4	4.9 ± 0.6*	7.4 ± 0.5*	5.5 ± 0.6*	3.7 ± 0.3

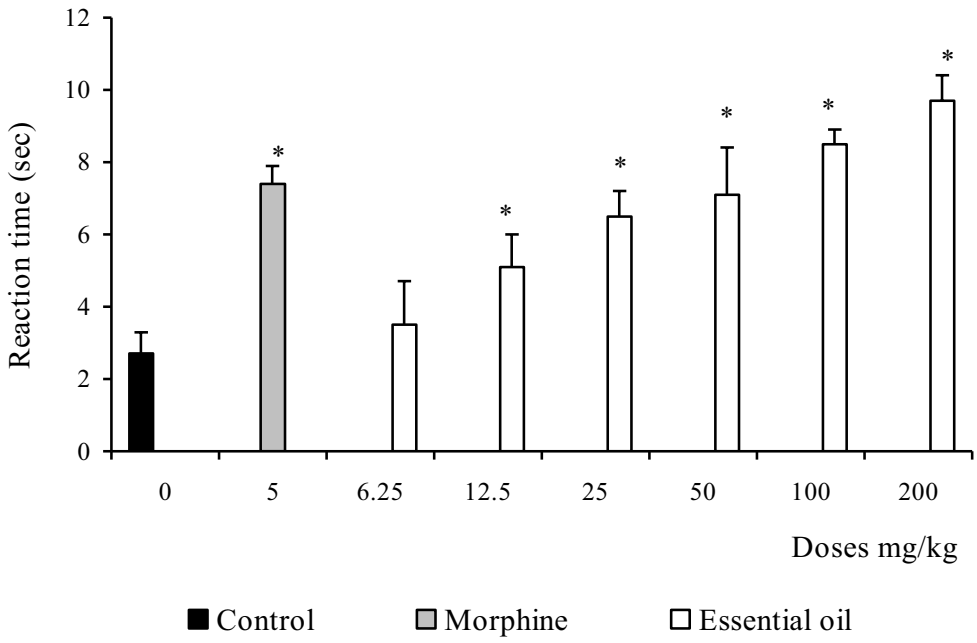

Fig. 1 Analgesic effects of essential oil and morphine at 45 min in the tail flick test.
n=10, *P < 0.05 vs control, Student's t-test.

3.2 Central analgesic activity

The essential oil significantly increases the reaction time of the rat to heat, from a dose of 12.5 mg/kg. This effect is dose-dependent (Table 2, Figure 1). As for the reference products, while acetylsalicylic acid does not protect against heat, morphine produces a dose-dependent protective effect as in the case of essential oil.

3.3 Action of naloxone

The effects of naloxone on the analgesic activity of the essential oil and morphine are reported in Table 3. According to the results obtained, this morphine antagonist has no protective effect of its own against heat. However, it completely suppresses the analgesic effects of the plant extract and the reference opoid substance.

Table 3: Influence of naloxone (1 mg/kg s.c.) on the analgesic activities of essential oil (EO) and morphine (M) in rats (tail flick test)

Values are mean ± S.D., n = 10.

Treatment	Dose (mg/kg i.p.)	Reaction time to flick the tail (sec)					
		0 min	15 min	30 min	45 min	60 min	120 min
Control	-	1.9 ± 0.4	2.0 ± 0.2	3.0 ± 0.9	3.3 ± 1.1	2.9 ± 0.6	3.0 ± 1.2
naloxone							
Naloxone	100	1.8 ± 0.4	2.2 ± 0.9	2.4 ± 0.7	4.1 ± 0.3	3.1 ± 0.4	2.3 ± 0.7
+ E.O.							
Naloxone + E.O.	200	1.8 ± 1.2	3.2 ± 1.4	3.7 ± 1.9	3.9 ± 0.7	4.2 ± 0.6	3.8 ± 0.3
Naloxone + M	5	2.1 ± 0.2	2.4 ± 0.8	3.1 ± 0.2	3.5 ± 1.2	3.4 ± 0.8	3.0 ± 0.9

3.4 Anti-inflammatory action on carrageenan oedema

The differences in volume between the left and right paws of the control and treated animals, obtained at the different readings, are reported in Table 4. The percentages of oedema inhibition obtained for the treated batches and at the different readings are presented in figure 2. As a preventive measure, the reference product chosen, indomethacin, significantly reduced oedema to carrageenan from 1 hour 30 minutes. Maximum inhibition is obtained three hours after the injection of the inflammatory agent (percentage of inhibition is 55.4%). This protective action remains relatively stable until six hours, when the percentage of inhibition is 53.3%.

The essential oil also reduces oedema in a progressive and dose-dependent manner. This protective effect is significantly and maximally manifested with doses of 100 and 200 mg/kg of the plant extract, after three hours. These doses cause an inhibition of 56.1% and 83.4% respectively. However, this action regresses but remains significant after six hours (32.9% and 64.7% inhibition respectively).

Table 4: Influence of essential oil and indomethacin on carrageenan and trauma-induced oedema.

Values are mean ± S.D., n = 12, * P < 0.05 vs. Control, Student's *t*- test.

Treatment	Dose (mg/kg i.p.)	Hindpaws average volumes (L-R) (ml)		
		1 h 30	3 h 00	6 h 00
Carrageenan-induced oedema				
Control	-	0.50 ± 0.09	1.39 ± 0.13	2.55 ± 0.27
O. compactum	50	0,52 ± 0.07	1.30 ± 0.07	2.00 ± 0.11*
	100	0.44 ± 0.07	0.61 ± 010*	1.71 ± 0.18*
	200	0.46 ± 0.08	0.23 ± 0.09*	0.90 ± 0.13*
Indomethacin	10	0.41± 0.11*	0.62 ± 0.12 *	1.19 ± 0.15*
Trauma-induced oedema				
Control	-	0.89 ± 0.16	0.77 ± 0.09	0.74 ± 0.20
O. compactum	50	0.84 ± 0.05	0.57 ± 0.10	0.69 ± 0.07
	100	0.86 ± 0.07	0.25 ± 0.04*	0.48 ± 0.06*
	200	0.81 ± 0.11	0.16 ± 0.05*	0.45 ± 0.10*
Indomethacin	20	0.71 ± 0.08*	0.32 ± 0.07*	0.29 ± 0.11*

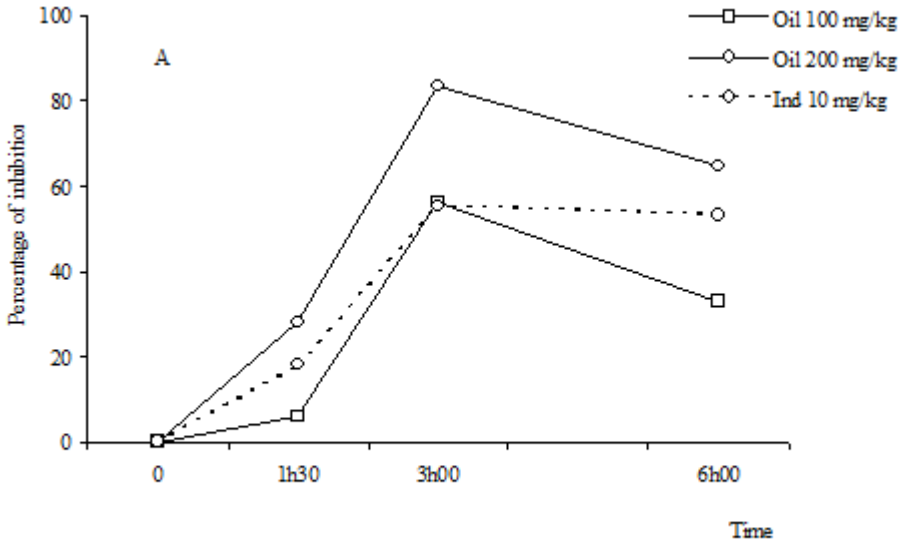

Fig. 2. Influence of essential oil and indomethacin on carrageenan-induced oedema in rat.
The values represent the percentage of inhibition of oedema, n = 12.

3.5 Anti-inflammatory action on experimental trauma oedema

The experimental trauma oedema induction test was used to confirm the anti-inflammatory action of the essential oil, previously proven in the carrageenan oedema test. According to the results presented in Table 4 and Figure 3, administration of ndometacin at a dose of 20 mg/kg induced a significant reduction in oedema one and a half hours after trauma to the rat paw. Maximum inhibition of inflammation was achieved after three hours (percentage of inhibition was 58.4%).

Treatment with essential oil at doses above 50 mg/kg also significantly inhibits the development of oedema in a dose-dependent manner.

Even in this experimental model, the activity profile of the essential oil shows that the inhibitory activity remains relatively stable throughout the experiment.

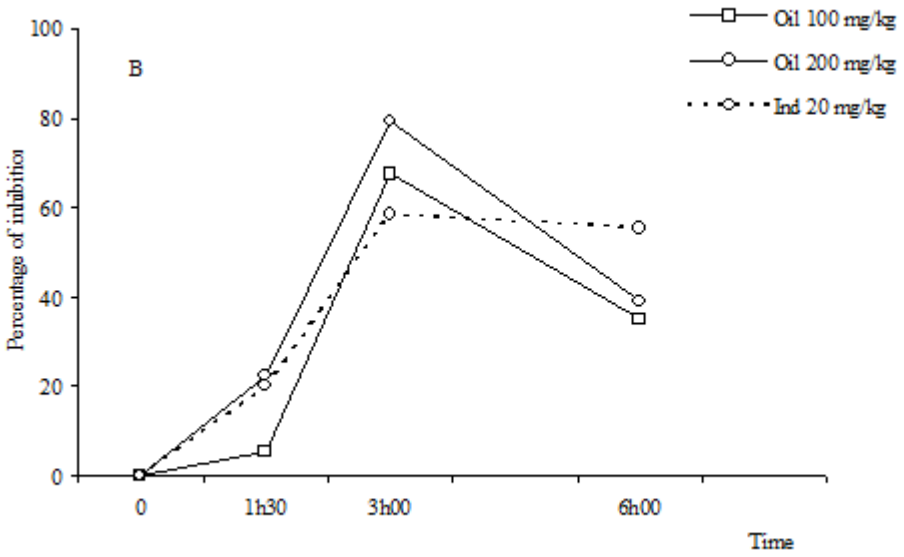

Fig. 3. Influence of essential oil and indomethacin on trauma-induced oedema in rat.

The values represent the percentage of inhibition of oedema, n = 12

4 Discussion

The present results indicate that the essential oil of *Origanum compactum* exhibits central analgesic properties, since it exerted a significant and dose-dependent protective effect on chemical (acetic acid injection) and thermic (heat) painful stimuli, from the respective doses of 6.25 and 25 mg/kg i.p. After being given the oil, the mean numbers of cramps were decreased and the pain thresholds were prolonged respectively in the writhing test and the tail-flick test. Such an efficacy on these two stimuli is characteristic of central analgesics like morphine [20]. Peripheral analgesics, as acetylsalicylic acid, are known to be inactive on thermic painful stimuli [21].

The analgesic activity of the oil may be compared to that of peripheral analgesic compounds (acetylsalicylic acid) and central ones (morphine). So, in the case of the chemical pain, the protection developed by the dose of 25 mg/kg i.p. of oil (63 %) may be reproached of that manifested by acetylsalicylic acid tested at the dose of 68 mg/kg i.p. (61 %). On the other hand, the dose of 1.15 mg/kg i.p. of morphine exercise a protective effect, which is lightly superior (70 %). In the case of the thermic pain, towards which the peripheral analgesics are ineffective, the dose of 50 mg/kg i.p. of oil increase the threshold of sensitivity of animals, in the same way that morphine used at 5 mg/kg.

A pre-treatment by naloxone (1mg/kg), which was not efficient by itself on heat-induced pain, abolished the analgesic effects of oil and morphine. The inhibitory effects of this specific opioid antagonist could suggest a putative morphine-like activity profile for *O. compactum.*

The study of anti-inflammatory effects on an acute inflammatory process (carrageenan-induced oedema in rat paw) permitted to discover a significant and dose-dependent effects of the oil in reduction oedema from the dose of 50 mg/kg i.p., 3 hours after the injection of the phlogistic compound As compared to the efficacy of indomethacin, the maximal inhibition of oedema obtained 3 hours after carrageenan injection was similar to the one induced respectively by the plant oil dose 100 mg/kg i.p.

The trauma-induced oedema test has been used to confirm the anti-inflammatory activity previously demonstrated with carrageenan-induced oedema test. Actually, a treatment by essential oil at doses above 50 mg/kg i.p. permit to inhibit significantly the development of oedema.

In both experimental models (carrageenan and trauma oedema), the activity profile of *O. compactum* extract differs from that of indomethacin, since the inhibitory effect of this NSAI remained relatively steady during all experimentation. On the other hand, plant oil induced a transient protector action. In fact, a pre-treatment by *O. compactum* showed a maximal inhibition of oedema 3 hours after its induction; then, this effect was reduced but still remained significant 6 hours after the injection of the irritant compound. Similar results have been observed with another species, *Peumus boldus* [22].

In inflammation caused by carrageenan injection, the authors established a biphasic development of oedema over 4 or more hours, and indomethacin was inefficient in the first phase [23]. This which has been confirmed in this study in both oedema induced by carrageenan and trauma. The early phase involves the release of histamine and serotonin while the late phase is mediated by products of arachidonic acid metabolism, via cycloxygenase (prostaglandins) and lipoxygenase (leucotrienes) [24]. Essential oil, which is devoid of anti-inflammatory activity in the first phase of inflammation evolution (chemical or mechanical), could acted on prostaglandins and/or leucotrienes synthesis or release.

5 Conclusion

We can affirm that the essential oil of O. compactum is endowed with central analgesic properties, associated to anti-inflammatory effects on acute inflammatory processes. Such an association is well-known for various NSAI compounds, but also for morphine which can inhibit the bradykinin synthesis, an inflammatory mediator [25-27].

Thus, further investigations will be necessary to obtain more information about this putative morphine-like activity profile for O. compactum, such as the research of the mechanism of action of the oil or its principle(s) responsible for this property and the study of phenomena of tolerance/dependence, well-known for opiate products.

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