Chapter 7

In Vivo Potential Anti-Inflammatory Activity of Extracts from *Calendula arvensis* (*CA*) Flowers

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Abstract

Calendula arvensis (*CA*) had been reported in traditional Moroccan medicine to exhibit its extensive use to treat pain and inflammation. Therefore, the objective of this study was to evaluate the anti-inflammatory activity of *CA* flowers. The methanol, aqueous, and hexane extracts (ME, AE, and HE) were investigated for inflammatory effects by using two methods, namely, carrageenan and experimental trauma-induced hind paw edema in rats and using indomethacin (20 mg/kg body weight) as a standard drug. The results demonstrated that *Calendula Arvensis CA* extracts had significant anti-inflammatory activity where the HE at the doses of 300 and 500 mg/kg p.o. ($p < 0.001$) had the best significant reduction and inhibition of edema with 51.08, 71.33 and 63.38, 67.33% induced by carrageenan and on experimental trauma induced rat paw edema at third hour, respectively, and similar as compared with standard drug indomethacin 20 mg/kg body weight p.o. ($p < 0.001$). These results indicate that it could be suggested as contributory effects to the use of *CA* flowers in the management of inflammation and pain conditions.

Keywords: *Calendula arvensis*, anti-inflammatory activity, indomethacin, wistar male rats

1. Introduction

Morocco is known as the "emporium of medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones, and it has favored the proliferation of more than 42,000 species of plants, divided into 150 families and 940 genuses [1–4].

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Calendula arvensis (*CA*) (family asteraceae) [5] is found within a wide geographic distribution: central and southern Europe, northern Africa, southwestern Asia, and the Macaronesian region (the Azores Islands, the Madeira Islands, the Salvage Islands, the Canary Islands, and the Cape Verde Islands).

C. arvensis (*CA*) is an annual herb with tall reach to 10–50 cm, width of the leaves (5–20 mm), and is lance-shaped and borne on petioles from the slender. The inflorescence is a single flower head up to 4 cm wide, the color of the flowers are bright yellow to yellow-orange ray florets, and the fruit is an achene, which can take any of three shapes, including ring-shaped, that facilitate different methods of dispersal [6, 7].

Inflammation was described 2000 years ago by the four Latin words: rubor, calor, tumor, and dolor [8]. It is a healthy process resulting from some disturbances or disease and it is usually associated with pain as a secondary process resulting from the release of analgesic mediators: nonsteroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immunosuppressant drugs, which have been used usually in the relief of inflammatory diseases by people around the world for a long time [9].

Most anti-inflammatory drugs and antiarthritic drugs have wide applications in clinical conditions [10], and they are associated with several side effects such as gastrointestinal tract complications, ulcers, and cardiovascular problems [11, 12]. Therefore, alternative therapies from natural resources are ventured throughout the world.

In the recent years, inflammation is one of the major target research areas among biomedical researchers, which includes various cellular processes (e.g., phagocytosis, chemotaxis, mitosis, and cell differentiation).

Thus, the aim of this study is to evaluate the anti-inflammatory effect of the extracts of the flowers of *CA* and, therefore, to determine the scientific basis for its use in traditional medicine in the treatment of inflammation.

2. Materials and methods

2.1. Sample collection and authentication

Flowers of *CA* were collected based on ethnopharmacological information from the villages around the region Rabat-Khemisset, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was authenticated by Pr. M. Al-Saghir [botanist from Institute Scientific (IS) in Rabat], and a voucher specimen (N°RAB 78161) was deposited in the herbarium of the botany department.

2.2. Sample preparation and extraction

The aqueous extract (AE), 200 g of *CA* flowers powder was extracted in 500 mL of boiling water for 30 min. Then, the infusion was filtered and then freeze-dried [13].

The hexanolic and methanolic extracts (HE and ME) were, respectively, obtained by the method of soxhlet extraction of 200 g of *CA* flowers for 6 h in about 500 mL of solvents.

The filtrated extracts were evaporated using a rotator evaporator. After that, the extracts were concentrated to dryness and the residue was kept at 4°C [14].

2.3. Drugs and chemicals

The following drugs and chemicals were used in the studies: carrageenan (Sigma, St. Louis, Missouri, USA), PGE2 (Fluka Chemie AG), p-benzoquinone (Merck), indomethacin 20 mg/kg; all the plant extracts were dissolved in a mixture of arabic gum 5%, and then they were given to the test animals by oral mouth, and also the control group received the same treatment.

Indomethacin 20 mg/kg in 5% of gum arabic was used as the reference drug.

2.4. Animals

The study was performed on adult male rats (180–220 g), bred at the Laboratory of Pharmacology, Faculty of Medicine and Pharmacy of Rabat. All animals were kept in a room maintained under environmentally controlled conditions of 23 ′ 1°C and 12 h light–12 h dark cycle. The food was withdrawn on the day before the experiment; however, they were allowed free access to water and standard diet throughout the experiments, the animals were handled according to the prescribed ethical guidelines for laboratory animals.

2.5. Anti-inflammatory tests

In both methods, all animals were fasted 18 h before testing and received 5 mL of distilled water by gavages to minimize individual variations in response to the swelling of the paws. The left hind paw (LP) is not treated, and it is taken as control.

2.5.1. Carrageenan model

The carrageenan-induced hind paw edema model was used for the determination of anti-inflammatory activity [13–15]. Six animals were used for each extract dose, as well as the control and reference groups.

Note that 300 and 500 mg/kg doses were administered of extracts into the subplantar tissue of right hind paw of each rat that was injected with 35 μL of 30 mg/mL of freshly prepared carrageenan in physiological saline (0.9% NaCl). Note that 30 μL of saline was injected into subplantar tissue of left hind paw of control groups. Then, after the injection, the paw edema was measured at 1.5, 3, and 6 h.

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:

$$\% \text{ of inhibition} = \frac{\text{mean}\,[V_{left} - V_{right}]_{control} - [V_{left} - V_{right}]_{treated}}{[V_{left} - V_{right}]_{control}} * 100 \quad (1)$$

where V_{left} is the mean volume of edema on the left hind paw and V_{right} is the mean volume of edema on the right hind paw.

2.5.2. Experimental trauma model

This assay was determined as described by Riesterer and Jacques test [16].

The test groups of rats were given orally 300 and 500 mg/kg of each extract dose, the control group received 5 mL/kg of distilled water, and the standard group received the reference drug indomethacin 20 mg/kg.

One hour after oral administration of different substances dropping weight of 50 g 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 difference volume of two paws was measured and taken as the edema value by using digital plethysmometer LE750 at 1 h 30 min, 3, and 6 h after induction of inflammation [17].

The percentages of inhibition of inflammation were calculated according to the following formula 2 where the mean differences of treated groups were compared with the mean differences of the control groups.

$$\% \text{ of inhibition} = \frac{\text{mean}\,[V_{left} - V_{right}]_{control} - [V_{left} - V_{right}]_{treated}}{[V_{left} - V_{right}]_{control}} * 100 \quad (2)$$

where V_{left} is the mean volume of edema on the left hind paw and V_{right} is the mean volume of edema on the right hind paw.

2.5.3. Statistical analysis

The results are expressed as mean ' SEM and analyzed by one-way analysis of variance (ANOVA) followed by student's t-test. A value of $p < 0.001$ was considered significant.

3. Results and discussion

3.1. Carrageenan-induced rat paw edema

The results of the effect of the flowers *CA* extracts on carrageenan induced edema are shown in **Table 1** and **Figure 1** at doses of 300 and 500 mg/kg comparable to that of the control and standard drug indomethacin 20 mg/kg, p.o., and *CA* extracts exhibited significant ($p < 0.001$) anti-inflammatory activity as compared to the standard drug indomethacin 20 mg/kg (**Table 1** and **Figure 1**).

Treatment groups	Dose mg/kg p.o.	1 h 30 min	3 h 00min	6 h 00min
Control		0.458 ± 0.003	0.71 ± 0.009	0.55 ± 0.002
IND	20	0.06 ± 0.001*	0.113 ± 0.007*	0.135 ± 0.001*
EM	300	0.375 ± 0.001*	0.473 ± 0.002*	0.426 ± 0.005*
EM	500	0.318 ± 0.005*	0.363 ± 0.003*	0.325 ± 0.004*
EA	300	0.273 ± 0.003*	0.34 ± 0.005*	0.291 ± 0.001*
EA	500	0.2 ± 0.007*	0.251 ± 0.001*	0.235 ± 0.003*
EH	300	0.22 ± 0.005*	0.26 ± 0.007*	0.24 ± 0.001*
EH	500	0.123 ± 0.003*	0.158 ± 0.001*	0.136 ± 0.002*

Notes: Values are expressed as mean ± S.E.M. ($n = 6$), extracts of *CA* flowers, $^{*}p < 0.001$ statistically significant compared to the control and reference drug (indomethacin 20 mg/mL).

Table 1. Effect of extracts of *CA* flowers on carrageenan-induced rat paw edema.

The hexanolic extract showed maximum reduction and inhibition of edema by 51.08 and 71.43% at 300 and 500 mg/kg, respectively, compared to the aqeuose and methanolic extracts 48.26, 65.14, and 35.96, 52.63% respectively, and similar to standard drug indomethacin (20 mg/kg) by 72.36% during the same time (**Figure 1**).

3.2. Experimental trauma-induced rat paw edema

The effect of two doses 300 and 500 mg/kg p.o.of the *CA* extracts on experimental trauma-induced inflammation is shown in **Table 2** and **Figure 2**, and the results are comparable to

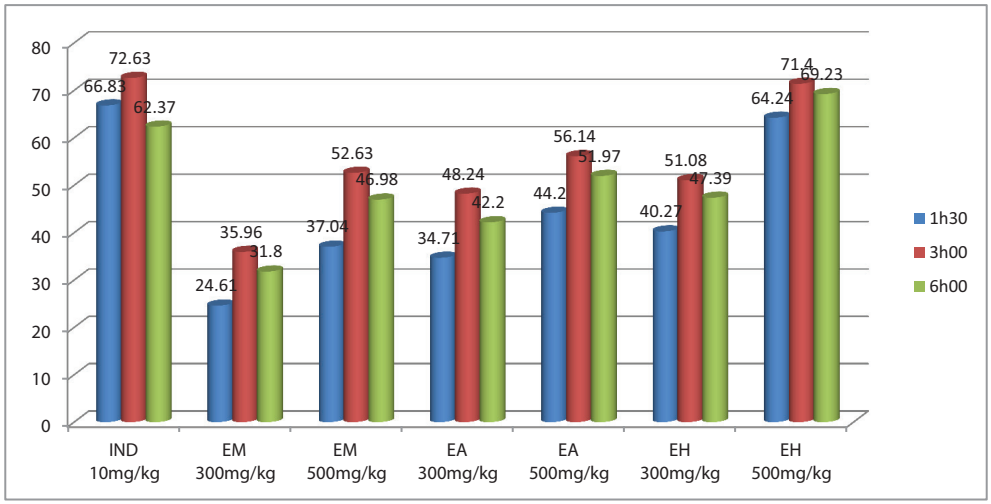


Figure 1. Percentage of inhibition of inflammation of extracts of *CA* flowers using carrageenan-induced rat paw edema.

Treatment groups	Dose mg/kg p.o.	1 h 30 min	3 h 00 min	6 h 00 min
Control		0.458 ± 0.003	0.71 ± 0.009	0.55 ± 0.002
IND	20	0.06 ± 0.001*	0.113 ± 0.007*	0.135 ± 0.001*
EM	300	0.375 ± 0.001*	0.473 ± 0.002*	0.426 ± 0.005*
EM	500	0.318 ± 0.005*	0.363 ± 0.003*	0.325 ± 0.004*
EA	300	0.273 ± 0.003*	0.34 ± 0.005*	0.291 ± 0.001*
EA	500	0.2 ± 0.007*	0.251 ± 0.001*	0.235 ± 0.003*
EH	300	0.22 ± 0.005*	0.26 ± 0.007*	0.24 ± 0.001*
EH	500	0.123 ± 0.003*	0.158 ± 0.001*	0.136 ± 0.002*

Notes: Values are expressed as mean ± S.E.M. ($n = 6$), extracts of *CA* flower, $^{*}p < 0.001$ statistically significant compared to the control and reference drug (indomethacin 20 mg/mL).

Table 2. Effect of extracts of *CA* flowers on experimental trauma-induced rat paw edema.

that of the control and standard drug indomethacin 20 mg/kg, p.o. *CA* extracts exhibited significant ($p < 0.001$) anti-inflammatory activity as compared to the standard drug indomethacin 20 mg/kg (**Table 2** and **Figure 2**).

The hexanolic extract showed maximum reduction and inhibition of edema by 63.38 and 76.33% at 300 and 500 mg/kg, respectively, compared to the aqeuose and methanolic extracts

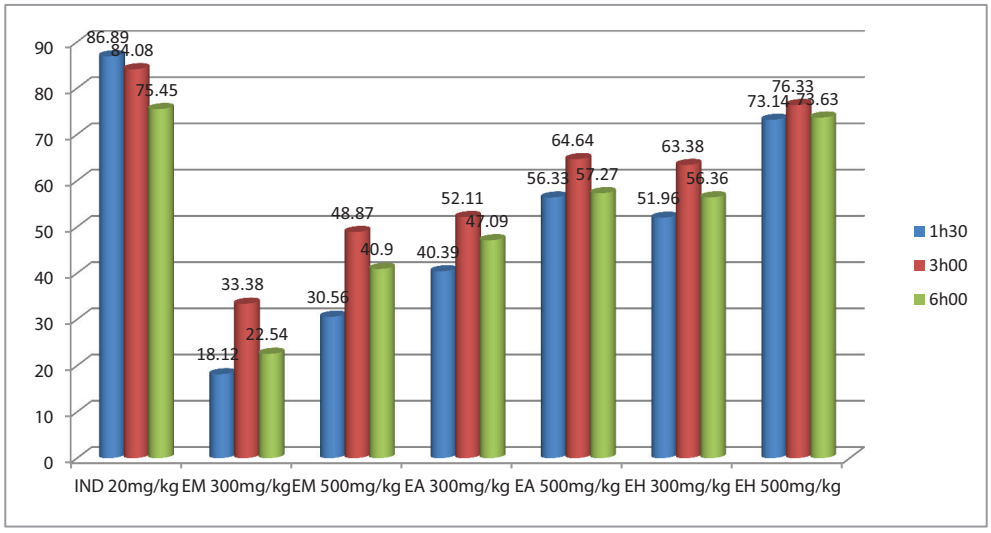


Figure 2. Percentage of inhibition of inflammation of extracts of *CA* flowers using experimental trauma-induced rat paw edema.

52.11, 64.64 and 33.38, and 48.87%, respectively, and similar to standard drug indomethacin 20 mg/kg by 86.89% during the same time (**Figure 1**).

4. Discussion

Medicinal plant extracts have been used for thousands of years in the world by numerous civilizations.

Carrageenan-induced rat paw edema in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis [18]. It plays a major role in the development of second phase of inflammatory reaction, which is measured at the third hour [19].

The anti-inflammatory activity of extracts of *CA* flowers is attributed to the present phytochemical constituents of these extracts, which include phenolic terpenoids, tannins, flavonoids; this results confirm our previously published results [20] and this is in agreement with many literature studies reporting that many plants containing these chemical classes of compounds have been reported to possess potent anti-inflammatory properties that act through inhibiting prostaglandin pathways [21].

5. Conclusion

Our study demonstrates significant anti-inflammatory activity where the hexanolic extract showed maximum inhibition of edema similar to the standard drug indomethacin (20 mg/kg) on carrageenan-induced paw edema and experimental trauma-induced rat paw edema in a dose-dependent fashion. Aqueous and methanolic extracts of *CA* flowers showed modest anti-inflammatory activity. This investigation suggests that *CA* flowers are a potential candidate for the discovery of new anti-inflammatory agents.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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