



Biological Evaluation of Anti-Inflammatory Activity of *Artemisia campestris* L. and *Spitzelia coronopifolia* Desf Ethanolic Leaves Extract

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ABSTRACT

Artemisia campestris L. and *Spitzelia coronopifolia* Desf. are medicinal plants widely distributed in southern Algeria and used by populations in several Saharan regions to treat various diseases. The anti-inflammatory activity of an ethanolic extracts of *Artemisia campestris* L. et *spitzelia coronopifolia* Desf. leaves was investigated in rats using formaldehyde induced paw oedema. Experimental animals received 150 and 300 mg/kg (orally) of the extracts or saline (control group) and the reference group received 50 mg/kg of acetylsalicylic acid. The ethanol extract of plants studied leaves at the dose level of 150 and 300 mg/kg decreased the edema significantly ($p < 0.001$). The percent inhibition at the end of experiment showed that the extract of *Artemisia campestris* L. at the dose level of 300 mg /kg was more active (67.15%) followed by *Spitzelia coronopifolia* Desf. extract at the Dose level of 300 mg/kg (48.39%). These two leave extracts at the concentration of 300 mg / kg are more effective than the standard, acetylsalicylic acid (50 mg/kg). While the lowest percentage of paw edema inhibition was represented by the *Artemisia campestris* L. extract at the dose level of 150 mg/kg (34.61%). The effect is therefore dose dependent.

Keywords: Anti-inflammatory activity; Formaldehyde; Extracts; *Artemisia*; *Spitzelia*; Oedema

INTRODUCTION

Sometimes inflammation can be harmful because of the aggressiveness of the pathogen, its persistence, the site of inflammation, anomalies in the regulation of the inflammatory process, or quantitative or qualitative anomalies in the cells involved in the inflammatory process. However, the use of anti-inflammatory synthetic chemicals is always accompanied by undesirable side effects, whereas the use of phytochemicals is useful and without side effects [1]. Different species of plants are used in traditional medicine in several countries. *Artemisia campestris* L. and *Spitzelia coronopifolia* Desf. are medicinal plants widely distributed in southern Algeria and used by populations in several Saharan regions to treat various diseases. Nonetheless, this use does not follow precise rules and does not take into account the new necessities of the current therapeutics. Many studies have focused on the study of plants used in traditional medicine. Therefore, we are interested in evaluating the anti-inflammatory activity of the alcoholic extracts of *Artemisia campestris* L. et *Spitzelia coronopifolia* Desf., Two plants widely used in traditional Algerian medicine as an astringent, expectorant and healing agent.

MATERIALS AND METHODS

Plant Material

The species *Artemisia campestris* L. and *Spitzelia coronopifolia* Desf. were collected in February 2017 in the region of oued rig (Algeria). The plant materials from the different species were identified in Educational laboratory of life sciences and nature university Elshahid Hama Lakder of El Oued.

Experimental Animals

Healthy albino wistar rats (150 ± 200 g) were procured from the Pasteur Institute of Algiers. Rats were randomly divided into 6 groups, each group consisting of 4 individuals.

Preparation of Crude Extracts

The test plants (20 g) were macerated in a water-alcohol mixture (ethanol/water, 70/30, v/v). Maceration is repeated 3 times with solvent renewal (×50 ml). The three extracts are combined after filtration by Wattman paper filter N 3. The resulting filtrate was concentrated by a rotary evaporator type Büchi at a temperature of 60°C. The brown powder obtained constitutes the total extract

This extract were re-dissolved in physiological solution and stored in sterile brown glass bottles in a freezer at -20°C until bioassayed.

Anti-inflammatory Activity Assay: Formaldehyde-induced Paw Edema in Rats

Wistar albino rats of either sex (150-200 g) in 6 groups (each n=4) were fasted for 12 hours prior to the induction of edema, but water was available ad libitum. Rats were deprived of water only during the experiment to ensure uniform hydration and to minimize variability in edematous response. Inflammation of the hind paw was induced by injecting 0.2 mL of formaldehyde (1% weight/volume; w/v) [2], in a normal isotonic saline solution into the subplantar region of the right-hind paw. The control group orally received a saline solution (0.1 mL), and the positive standard groups received acetylsalicylic acid (50 mg/kg) orally). The groups treated with plant extracts received doses at 150 mg/kg and 300 mg/kg. All drug treatments were given 1 hour before the inflammatory injection. Edema was measured with a digital micrometer before and after the inflammatory injection at one-hour intervals for six hours. The average paw volume was measured and compared with control and standard groups. Reduction in the paw volume in plant extracts pretreated groups compared with the control animals was considered as anti-inflammatory response. The percentage of paw edema inhibition was calculated by using the following formula [3]:

$$\text{Inhibition of Paw edema (\%)} = \frac{O_c - O_t}{O_c} \times 100$$

Where 'O_c' is edema volume of control group and 'O_t' is edema volume of treated groups.

Statistical Analysis

The data were analyzed using student's t-test statistical methods.

RESULTS AND DISCUSSION

In control animals, the sub plantar injection of formaldehyde produced a local edema that increased progressively to reach a maximal intensity 3 hours after injection (0.069 ± 0.02 ml). This value corresponds to an increase percentage of 78.65% compared to the initial volume of the foot. From the third hour the volume of the edema of the paws of the control mice begins to decrease progressively (Figures 1 and 2). For the standard group, the results obtained show that administration of the anti-inflammatory agent of acetylsalicylic acid (50 mg/kg) causes a reduction in the volume of the edema of the rats during the six hours of the experiment. Indeed, this significant reduction is observed from the second hour and continues until the end of the experiment. The ethanol extract of *Artemisia campestris* L. leaves at the dose level of 150 and 300 mg/kg decreased the edema significantly ($p < 0.001$) from the second hour after administration of the extract when compared to the control group. At the dose level of 300 mg/kg, the effect was strong when compared to the activity produced by standard acetylsalicylic acid in different hours of experiment. The ethanolic extract of *Spitzelia coronopifolia* Desf. administered under the same conditions as before also considerably reduces the edema. Results are not significant for the two concentrations ($P < 0.01$ and $P < 0.001$) at the majority of hours after the induction of the edema, but significant compared to the control rats.

The percent inhibition showed that the extract of *Artemisia campestris* L. at the dose level of 300 mg/kg was more active (67.15%) followed by *Spitzelia coronopifolia* Desf. extract at the Dose level of 300 mg/kg (48.39%). These two plants at the concentration of 300 mg/kg are more effective than the standard, acetylsalicylic acid (50 mg/kg). While the lowest percentage of paw edema inhibition was represented by the *Artemisia campestris* L. extract at the dose level of 150 mg/kg (34.61%). The effect is therefore dose dependent.

Under the experimental conditions, the formalin caused the edema whose volume is maximal after three hours [4,5]. Formalin causes local inflammation when injected into the sub plantar region of right hind paw [2,6,7] as well as carrageenan [8,9]. Carrageenan edema is also known to be a multimediated phenomenon that liberates a great number of mediators. Thus, the first phase (1 h) implicates the release of serotonin and histamine while the second

phase (over 1 h) is mediated by prostaglandins, cyclo-oxygenase products and the continuity between the two phases is provided by kinins [10-12].

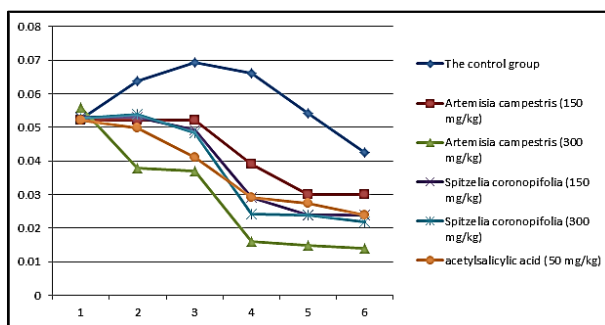


Figure 1: Edema evolution in the presence of per os pretreatment after injection of formalin (1%)

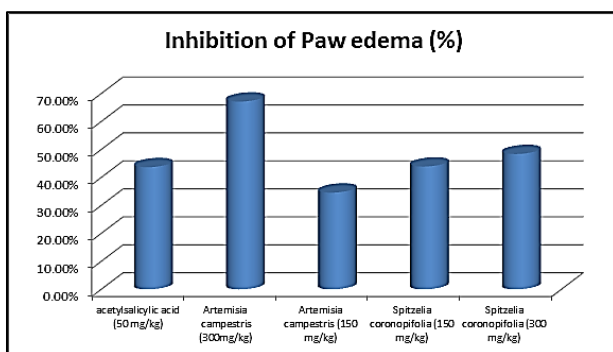


Figure 2: Percent inhibition of *Artemisia campestris* et *spitzelia coronopifolia* Desf. leaves extract on paw edema induced by formalin

In the present study, ethanolic extracts of *Artemisia campestris* L. and *Spitzelia coronopifolia* Desf. leaves possessed varying degree of antiinflammatory activity. This effect could be explained by the inhibition of the synthesis of pro-inflammatory substances. This suggests the significant anti-inflammatory effect of the extract of plants; it could be due to the richness of the methanol extract in bioactifs compounds, mostly polyphenols, flavonoids and others.

These results are consistent with several studies showing that the anti-inflammatory activity of the extract can be explained in part by the presence in the leaves of polyphenolic compounds such as tannins and flavonoids [13]. Also, other studies have shown that many species of the family Lamiaceae such as *Thymus vulgaris* L., *Rosmarinus officinalis* develop an anti-inflammatory activity *in vivo* [14,15].

Moreover, numerous studies suggest that flavonoids possess anti-inflammatory properties and that they are able to modulate the functioning of the immune system by inhibiting the activity of the enzymes which may be responsible for inflammations [16]. Thus, Kim et al. [17] demonstrated that flavonoids are capable of inhibiting histamine, and flavones and flavonols in glycosylated or free form such as quercetin, kaempferol and myrecetin have inhibitory activity of Cyclooxygenase [18].

CONCLUSION

The subplantar injection of formaldehyde produced a local edema. The ethanolic extracts of *Artemisia campestris* and *spitzelia coronopifolia* Desf. leaves inhibit in a way time and dose-dependent edema induced by formaldehyde (1%). The results achieved in the present study constitute an indication that *Artemisia campestris* and *spitzelia coronopifolia* Desf. can be effective in acute inflammatory disorders and in that it showed significant result ($p < 0.001$) with both of the 150 mg/kg and 300 mg/kg dose level. Therefore, *these palnts* have anti-inflammatory active ingredients that should be extracted, purified and characterized.

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