Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: www.informahealthcare.com/journals/iphb20

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To cite this article: Hamama Bouriche, Laid Selloum, Chafia Tigrine & Chahra Boudoukha (2003) Effect of Cleome arabica Leaf Extract on Rat Paw Edema and Human Neutrophil Migration, Pharmaceutical Biology, 41:1, 10-15, DOI: 10.1076/phbi.41.1.10.14698

To link to this article: https://doi.org/10.1076/phbi.41.1.10.14698

Pharmaceutical Biology
2003, Vol. 41, No. 1, pp. 10–15

1388-0209/03/4101-010$16.00

Effect of *Cleome arabica* Leaf Extract on Rat Paw Edema and Human Neutrophil Migration

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Abstract

The anti-inflammatory activity of C*leome arabica* leaf extract was studied *in vivo* and *in vitro*. Firstly, the extract was examined for its anti-inflammatory activity using carrageenan-induced rat paw edema as a model of acute inflammation. A subplantar injection of 0.1 ml of carrageenan 1% induced a progressive swelling of the rat paw in all time points, that reached a maximal volume in placebo group within 5 h. Results showed that pre-treatment of rats by *Cleome arabica* leaf extract, 1 h prior the injection of the phlogogenic agent, prevented the increase of the edema in dose-dependent manner with an ED_{50} of 231 mg/kg, body weight. The extract doses 100, 200 and 300 mg/kg, reduced edema to 65.54 ± 5.2%, 57.86 ± 8%, and 41.54 ± 3.6%, respectively, 5 h after the carrageenan injection. Secondly, we have examined the effect *of Cleome arabica* leaf extract on human neutrophil migration induced by fMLP (10^{-7} M), using 48-well chemotaxis chamber. Results showed that the extract inhibited neutrophil chemotaxis significantly ($p < 0.01$) and in a dose-dependent manner. Neutrophil migration was reduced to 16.71 ± 4.6% in presence of 50 µg/ml of *Cleome arabica* leaf extract. It appears that the anti-inflammatory activity of *Cleome arabica* leaf extract, observed *in vivo* as well as *in vitro*, could be due to its high flavonoid content (19%). These results may contribute to explain the use of this plant in folk medicine.

Keywords: Acute inflammation, anti-inflammatory activity, *Cleome arabica*, neutrophil, chemotaxis, flavonoid, carrageenan.

Introduction

Polymorphonuclear neutrophils (PMNs) play an important role in protecting the host against invasion by micro-organisms. Activated by inflammatory signals, blood PMNs adhere to the endothelium, and then migrate and accumulate within inflammatory sites where they release proteolytic enzymes, arachidonic acid metabolites and generate reactive oxygen species (Witko-Sarsat et al., 2000; Gompertz & Stockley, 2000). All these factors gathered participate in killing and eliminating pathogen agents. Although this process is usually self limiting, it may, in some circumstances, become continuous or excessive, thus contributing to tissue damage. It is well-known that PMNs are involved in pathogenesis of wide range of inflammatory diseases such as chronic destructive lung disease and rheumatoid arthritis (Llewellyn-Jones et al., 1994; Gompertz & Stockley, 2000; Schiller et al., 2000).

As PMNs play an important role in the development of acute and chronic inflammation, inhibition of their migration may account for parts of the anti-inflammatory effect exerted by steroidal and non steroidal anti-inflammatory drugs. However, several adverse effects limit the clinical usefulness of these drugs. Therefore, much clinical and pharmacological research efforts continue to be expended in the search for effective anti-inflammatory drugs with fewer adverse side effects. In this regard, medicinal plants could provide a useful source of such compounds. Plant leaves of some *Cleome* species are reported in folk medicine to have an almost immediate effect on relieving abdominal and rheumatic pains (Sharaf et al., 1992). The genus *Cleome*, belonging to the family *Capparidaceae*, is abundantly distributed in the north of Africa (Wollenweber & Dorr, 1992). The leaf extract of some *Cleome* species contains various polyphenolic compounds such as flavonoids (Sharaf et al., 1992). These latter compounds are a group of low molecular weight polyphenolic secondary plant metabolites which display many pharmacological properties (Kim et al., 1996; Middleton et al.,

Accepted: April 19, 2002

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2000). Flavonoids have long been considered to possess antioxidant and anti-inflammatory activities (Robak & Marcinkiewicz, 1995; Kim et al., 1996; Packer et al., 1999). They showed a profound effect on the function of inflammatory cells as determined by a large number and variety of *in vivo* and *in vitro* investigations (Pagonis et al., 1986; Lee et al., 1995; Middleton et al., 2000).

A literature survey indicates a lack of pharmacological investigations on *Cleome arabica* extract. In the present work, this extract was investigated for its anti-inflammatory properties. Firstly, we have evaluated its anti-inflammatory activity *in vivo*, using λ-carrageenan-induced rat paw edema. Secondly, we have studied the effect of this extract on human neutrophil chemotaxis induced by the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP).

Materials and methods

Chemicals

Lambda carrageenan, aspirin, formyl-methionyl-leucyl-phenylalanine (fMLP), cytochalasin B, Histopaque®-1077, dextran, carboxymethyl cellulose (CMC) and dimethyl sulfoxide (DMSO) were obtained from Sigma (Germany). Solvents were from Prolabo (France). All other reagents and chemicals were from Sigma (Germany), and were of analytical grade.

Preparation of plant extract

Cleome arabica was collected from Boussaâda (Algeria) in April, 1998. Identification of plant material was made by Prof. M. Kaâbache (University of Setif, Algeria). The air-dried leaves (100 g) were powdered and extracted three times with 200 ml of methanol/water (7/3, v/v) for 24 h. The collected filtrates were concentrated under reduced pressure, and the boiled water was added to residue and incubated for 24 h at 4 °C. Then, the addition of ethyl acetate to aqueous layer yielded to the formation of a yellow precipitate. This latter was washed with distilled water and lyophilised. The yellow extract was investigated for its antiinflammatory activity. The extract was dissolved in CMC (in experiments with rats) or in DMSO (in experiments with neutrophils).

Total polyphenol contents

Total polyphenol contents were determined by the colorimetric method adapted from Bahorun et al. (1996). A half ml of the extract aqueous solution was added to 7 ml of distilled water and 0.5 ml of Follin-Ciocalteu reagent. After 3 min, 2 ml of 20% Na_2Co_3 were added and the mixture was incubated at 100 °C for 1 min. After cooling in darkness, the absorption was measured at 685 nm (Shimadzu, 1601). Results were expressed in g per 100 g of dry matter with respect to tannic acid serving as standard.

Total flavonoid contents

The flavonoid contents were estimated by the aluminium chloride method (Bahorun et al., 1996). One ml of methanol extract is added to 1 ml of 2% methanolic $AlCl_3$. After 10 min, the absorption was read at 430 nm. Results were expressed in g per 100 g of dry matter with respect to rutin serving as standard.

Animals

Male and female Albino Wistar rats weighing between 150 and 250 g were obtained from Pasteur Institute of Algeria. They were housed in polypropylene cages with free access to water and food, under standard conditions. Animals were acclimatised at least for 1 week prior to use, and fasted overnight before treatment.

Anti-inflammatory activity

To evaluate the anti-inflammatory activity of *Cleome arabica* leaf extract against acute inflammation, carrageenan-induced rat paw edema test was used. The plant extract, prepared in 1% CMC (w/v), was administered orally at different doses (100, 200 and 300 mg/kg). Aspirin, used as a standard anti-inflammatory drug, was administered orally at 100 mg/kg body weight, 1 h prior the injection of 0.1 ml of carrageenan 1 % (w/v) into the subplantar region of the right hind paw (Winter et al., 1962). The placebo group was injected by 0.1 ml of carrageenan, and received orally the vehicle in a comparable volume (1 ml/100 g body weight). The control group was injected by 0.1 ml of sterile chloride saline and received orally the vehicle. The volume of the injected paw was measured initially and 1, 2, 3, 4, 5, and 6 h after carrageenan injection, using a Plethysmometer (UGO Basile, Germany). The volume of edema was expressed for each animal as percentage change in rat paw volume after carrageenan injection, compared to placebo group set to 100%.

Isolation of human neutrophils

Human polymorphonuclear neutrophils (PMNs) were isolated from freshly heparinized (10 U/ml) blood from healthy donors according to Arnhold et al. (1999). The isolation procedure included a dextran-enhanced sedimentation, Ficoll-Hypaque-density centrifugation, lysis of the remaining red blood cells with distilled water and washing of cells with Hanks' balanced salt solution (HBSS). PMNs were suspended in HBSS (pH 7.4) medium at a concentration of 2×10^6 cells/ml in an ice bath. The cells were used within 2 h after isolation. Cells isolated through this technique were more than 95% viable, as determined by trypan blue exclusion test, and more than 95% of cells were PMNs.

Neutrophil chemotaxis

The chemotaxis assay was performed using the 48-well chemotaxis chamber (Neuro probe, inc, USA) as described

by Falk et al. (1980). The lower wells were filled with 25 µl of 10^{-7} M fMLP. Test compounds at various concentrations, *Cleome arabica* leaf extract (5, 10, 25, 50 µg/ml) or aspirin (10, 25, 50, 100 µM), were placed in the lower wells of the chamber in presence of the chemoattractant. Then, 45 µl of PMNs (1.5×10^6 cells/ml), suspended in HBSS supplemented with 0.25% Ovalbumin, were placed in the upper wells of the chamber. Upper and lower wells were separated by a 5 µm pore polycarbonate filter (Neuro probe, inc., USA). The chemotaxis chamber was incubated for 90 min in a humidified atmosphere rich in CO_2. Thereafter, the filter was removed, fixed in absolute methanol, stained with Wright's stain and subsequently mounted in immersion oil under cover slip. Cells adherent to the lower surface were counted at X400 magnification for five random fields for each of three replicate wells. A mean value was obtained for each well. The average number of cells migration in the negative control wells (lower wells contained medium only) was subtracted as background from the number migrating in test wells, to yield the net number of neutrophils migrating per field. Positive controls assessing chemotaxis to fMLP were conducted in parallel with experimental groups. Chemotaxis to a test substance was expressed as a percentage of the maximal chemotaxis to fMLP in the same experiment.

The cytotoxic effect of the extract

The cytotoxic effect of the extract on neutrophils was determined by the release of the cytoplasmic enzyme lactate dehydrogenase LDH (EC 1.1.1.27). PMNs (2.5×10^6 cells/ml) were incubated with the extract (100 µg/ml) for 30 min at 37 °C. LDH activity in supernatant was measured at 340 nm using 1.6 mM pyruvate and 0.2 mM NADH. All concentrations are final ones. Enzyme release was expressed as the percentage of the maximum value, obtained after treatment of the cells with 0.2% Triton-X100 (Komura et al., 1995).

Statistical analysis

Results were presented as mean ± SEM. The significance of the difference between test and control group was analysed using Student's *t*-test.

Results

Crude extract analysis

In this study, a yellow crude precipitate was obtained from dry leaves of *Cleome arabica* using methanol/water (7/3, v/v) and ethyl acetate, successively. The amount of this extract was about 1 g per 100 g of the dry raw material. The preliminary analysis of the crude extract was carried by determining the total polyphenol and flavonoid contents. The quantitative estimation of these natural compounds present in the extract, using Folin-Ciocalteu and $AlCl_3$ reagents, showed that the extract was relatively rich in polyphenols, particularly flavonoids. Indeed, the crude extract contained 32.2 ± 0.5 g of polyphenols per 100 g of dry matter, using acid tannic as standard. The content of flavonoids was 19.33 ± 0.7 g per 100 g of dry matter, using the natural flavonol rutin as standard.

Anti-inflammatory activity of *Cleome arabica* leaf extract

Subplantar injection of 0.1 ml of λ-carrageenan 1% induced progressive edema of the rat paw in all time point, that reached a maximal volume in placebo group within 5 h. In contrast, the control group paws did not swell. In fact, upon reabsorption of saline, the paw recovered its previous volume after 1 h. Figure 1 shows the time course of edema in animals treated with placebo, aspirin (100 mg/kg) and leaf extract (100 mg/kg). Our results showed that the extract as well as aspirin exerted an antiphleogenic activity. Pretreatment of rat with *Cleome arabica* leaf extract at 100, 200 and 300 mg/kg reduced edema at each time point compared with placebo. Indeed, percentages of edema inhibition observed after 5 h were 34.46 ± 5.2%, 42.14 ± 8% and 58.46 ± 3.6% at 100, 200 and 300 mg/kg doses, respectively, with an effective dose 50% (ED_{50}) of 231 mg/kg body weight. This anti-inflammatory activity was significant ($p < 0.05$) compared with placebo, and dose-dependent. Whereas, 100 mg/kg of aspirin, used as a standard anti-inflammatory drug, showed a great inhibition of edema, 73.63 ± 4.0% (Fig. 2).

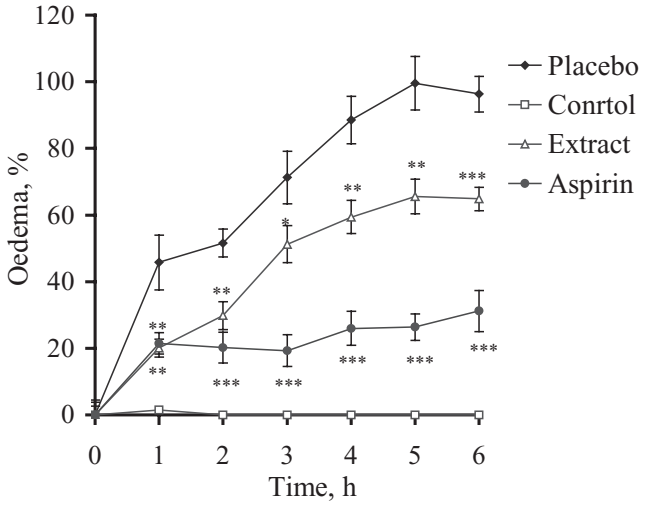

Figure 1. Time course of the paw edema induced by subplantar injection of 0.1 ml of carrageenan 1% in rat pre-treated orally with vehicle (◆, placebo), extract (△, 100 mg/kg body weight) or aspirin (●, 100 mg/kg body weight). The control group is injected with 0.1 ml of saline solution and pre-treated orally with the vehicle (□). Each point represents the percentage increase in volume of the injected paw compared with the maximal volume of placebo group set to 100%. Symbols are means ± SEM of 7 animals for each group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

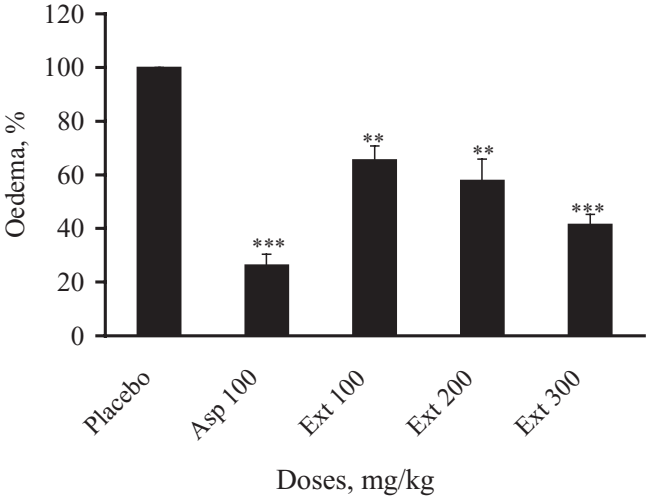

Figure 2. Effects of *Cleome arabica* leaf extract (Ext, 100, 200 and 300 mg/kg) and aspirin (Asp, 100 mg/kg) on the inflammatory response measured 5 h after subplantar injection of 1% carrageenan into the right hind paw. Drugs were given orally 1 h before carrageenan injection. Results are expressed as the percentage of edema compared with placebo group set to 100%. Values are means ± SEM of 7 animals for each group. **p < 0.01, ***p < 0.001.

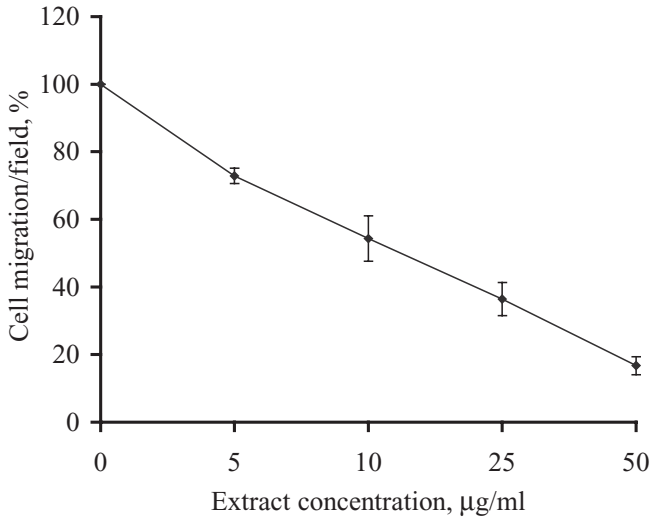

Figure 3. Effect of *Cleome arabica* leaf extract on neutrophil chemotaxis. The chemotaxis assay was performed in the 48-well chemotaxis chamber, using a 5 µm pore polycarbonate filter. PMNs (1.5×10^6 cells/ml) were incubated in presence of the chemoatractant with or without extract. After incubation for 90 min at 37 °C, cells adherent to the lower surface of the filter were counted at X400 magnification. Results are expressed as the percentage of neutrophils migrating per field compared with neutrophil chemotaxis to 10^{-7} M fMLP without extract set to 100%. Values represent the mean ± SEM of three independent experiments performed in triplicate. A significant dose response is seen ($p < 0.01$).

Effect of the extract on neutrophil migration

The effect of *Cleome arabica* leaf extract on human neutrophil migration induced by fMLP, compared with the effect of aspirin, was examined. Control experiments showed that neutrophils migrated in a large number (80.2 ± 3.36 cells/field) in response to the chemoattractant (fMLP, 10^{-7} M). The migration was highly inhibited in presence of cytochalasin B at a concentration of 1.5 µM. Our results showed that *Cleome arabica* leaf extract exerted a significant ($p < 0.001$) inhibitory effect on neutrophil chemotaxis to fMLP. This inhibition was dose-dependent (Fig. 3). Indeed, the inhibitory percentage of neutrophil migration was 83.29 ± 4.6% in presence of the extract at 50 µg/ml. Whereas, this value was 66.23 ± 4.6% in the presence of 50 µM aspirin (Fig. 4). Under these assay conditions, the extract, up to 100 µg/ml concentration, did not induce cell toxicity as assessed by LDH release. Therefore, these drug effects are not due to a cytotoxic action.

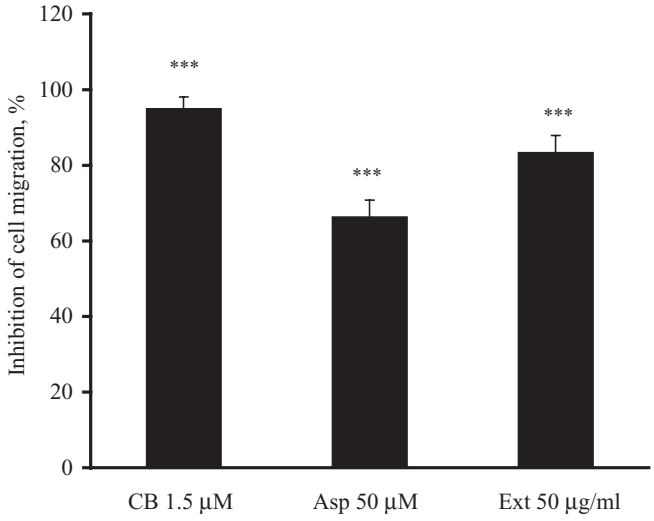

Figure 4. Effect of 50 µg/ml *Cleome arabica* leaf extract (Ext), 50 µM aspirin (Asp) and 1.5 µM cytochalasin B (CB) on neutrophil chemotaxis to 10^{-7} M fMLP. All other experimental conditions are the same as in Figure 3. Results are expressed as the inhibition percentage of neutrophil migration. Each histogram is the mean ± SEM of three separate experiments performed in triplicate. A significant effect is seen (***p < 0.001).

Discussion

In the present work, we observed that the subplantar injection of carrageenan induced a local oedema which reached a maximal intensity within 5 h in the placebo group. This model of inflammation is a standard model for screening anti-inflammatory compounds (Winter et al., 1962; Grau et al., 1991). According to Cuzzocrea et al. (1998), it seems that the early phase of carrageenan-induced oedema is related to the production of inflammatory mediators such as histamine,

platelet-activating factor (PAF), and arachidonic acid metabolites. While, the delayed phase of inflammatory response has been linked to neutrophil migration and accumulation within inflammatory site where they release reactive oxygen species, proteolytic enzymes as well as other neutrophil-derived mediators. Pre-treatment of rats by *Cleome arabica* leaf extract inhibited edema formation in dose-dependent manner. This inhibition may be explained, at least, by the inhibition of inflammatory mediators production. The anti-inflammatory activity of the extract could be due to the presence of certain compounds such as flavonoids. Indeed, the extract presented a high flavonoid contents (19%). In fact, it has been reporeted that flavonoids exerted an inhibitory effect on cyclooxygenase/lipooxygenase or phospholipase A_2 activities (Moroney et al., 1988; Kim et al., 1996; Kang et al., 1999). Furthemore, Borissova et al. (1994) reported that rutin prevented edema formation induced by histamine and serotonin.

Because migration is of decisive importance for neutrophils to reach their destination, where they play an important role in the development of inflammation, any inhibition in their migration affect inflammation. In the present study, we examined the effect of *Cleome arabica* leaf extract on human neutrophils by assessing their chemotaxis response. In this way, neutrophils were allowed to migrate through a micropore filter toward either a control medium or a medium containing a chemoattractant alone or a chemoattractant in the presence of a test substance. In control experiments, neutrophils migrate in a large number in response to fMLP (10^{-7} M). It is known that at low concentrations the chemotactic effect of fMLP predominates, while at higher concentrations ($>10^{-7}$ M) its stimulating effects predominate (Hoffman et al., 1991). In the presence of a chemoattractant, leukocytes move in unidirectional manner in response to the concentration gradient of the attractant. The force for producing PMN motility is generated by reorganization of cytoskeletal elements, primarily actin (Hoffman et al., 1991). It has been reported (Harvarth, 1990) that cytochalasin B, which binds to the barbed ends of actin filaments, inhibits actin polymerization/depolymerization in fMLP-stimulated neutrophils. Indeed, in a control experiment we observed that the addition of 1.5 µM cytochalasin B to fMLP abolished drastically neutrophil migration.

Our results showed that *Cleome arabica* leaf extract has an inhibitory effect on neutrophil chemotaxis to fMLP in a dose-dependant manner. This effect could be attributed to the presence of flavonoids. In fact, Li et al. (2000) reported that the flavonoid baicalin, purified from the medicinal plant *Scutellaria baicalensis*, inhibited cell migration by selective binding to chemokine ligands. Many works mentioned that flavonoids exerted an inhibitory effect on enzymes involved in cell signalling such as protein kinase C (PKC) and phosphoinositide 3-kinase (PI3-Kinase) (Gamet-Payrastre et al., 1999; Vlahos et al., 1994). PI3-Kinase has a central role in the regulation of directed cell migration, through reorganization of actin cytoskeleton (Payrastre et al., 2001).

In conclusion, it appears that the anti-inflammatory activity of *Cleome arabica* leaf extract, observed *in vivo* as well as *in vitro*, could be due to its high flavonoid contents. These results may contribute to explain the use of this plant in folk medicine. However, further investigations are essential to identify molecular compound(s) of the crude extract responsible for such properties. Their isolation and characterization is now under investigation in our Laboratory.

Acknowledgemnents

This study was supported by grants from the International Foundation for Science (IFS), Sweden (F/ 2825-1), from ANDRS, Algeria (05/ 01/ 02/ 98 006) and from the Ministry of High Education and Scientific Research, Algeria (F 1901/01/96).

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