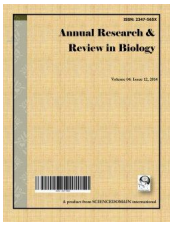

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Anti-inflammatory and Immuno-modulatory Effects of *Capparis spinosa* Flower Bud Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author NK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HB, SK, DM and AMA managed the analyses of the study. Author AS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Capparis spinosa* L. is a plant widely used in traditional medicine for its different purpose including the anti-inflammatory properties. The aim of this study was to evaluate the anti-inflammatory properties of this plant and to define its possible mechanism of action by verifying its effect on the production of some inflammatory mediators.
Methodology: The anti-inflammatory activity of *Capparis spinosa* bud methanolic extract was evaluated *in vivo*, using paw edema and air pouch inflammation models. *In vitro*, the ability of the extract to modulate the production of some pro and anti-inflammatory mediators such as TNF-α, IL-1β, IL-8 and IL-10 released from peripheral blood mononuclear cells stimulated by concanavalin A

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was evaluated. Moreover, the effect of the extract on LTB_4 and superoxide anion released from neutrophils was tested.

Results: Results showed that the oral administration of 200 and 400 mg/kg of *Capparis spinosa* methanolic extract reduced significantly carrageenan-induced paw edema. Above 2 h, both doses of the extract exerted a significant ($P < 0.001$) anti-edematous effect, with 52%-69%. In addition, this extract inhibited the neutrophil migration into the air pouch. The inhibition exerted by 1 mg/pouch of the extract (48.92%) was better than that exerted by indomethacin, used as reference. On the other hand, the extract inhibited significantly the production of TNF-α, IL-1β, LTB_4 and superoxide anion generation. At 100 µg/mL, the inhibition values were 21.28%, 38.04%, 20.84% and 71.16%, respectively. In contrast, the extract did not show any significant effect on the release of IL-8 and IL-10.

Conclusion: *Capparis spinosa* bud extract inhibited the inflammatory process by modulating the pro-inflammatory mediator release. Thus this extract can offer a new therapeutic strategy for the treatment of inflammatory disorders.

Keywords: Anti-inflammatory; Capparis spinosa; edema; cytokines; leukotrienes; superoxide anion.

1. INTRODUCTION

Inflammation is an innate immune response that protects the body against injury or infections. It involves the participation of various inflammatory cell types, including neutrophils and monocytes as the initial responders [1]. In the inflammatory site, these cells contribute in the immunological responses by recognizing and removing invading microorganisms, and producing a numerous inflammatory mediators such as cytokines, lipid mediators and reactive oxygen species (ROS) [2,3]. Under regulating inflammatory conditions, these mediators cooperate to achieve healing and to restore homeostasis. However, overproduction of these mediators is related with the excessive activation of the immune cells which promotes the exacerbation of the inflammatory process and the development of various inflammatory diseases such as neurodegenerative diseases, diabetes, atherosclerosis, arthritis and cancer [4,5,6]. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are the most pro-inflammatory cytokines that play a crucial role in the inflammatory process and immune response regulation [7]. Together, these cytokine share many pro-inflammatory properties, such as the regulation of the expression of many cellular adhesion molecules and the stimulation of the production of other pro-inflammatory cytokines and chemokines including IL-8 [8,9]. This chemokine is known for its chemotactic and pro-inflammatory activities [10]. In addition, elevated levels of these pro-inflammatory cytokines trigger immune cells to release anti-inflammatory cytokines, such as IL-10 [11,12]. This cytokine reduces macrophage functions by blocking the synthesis of other pro-inflammatory cytokines like TNF-α, IL-6, IL-12, IL-2 and IFN-γ, and reduces the harmful effects of excessive activation of this cell during inflammation [13,7]. On another hand, leukotriene B_4 (LTB_4) plays a pivotal role in inflammation by acting as a potent vasoactive and leucotactic agent, enhancing the expression of several adhesion molecules and inducing the recruitment of leukocytes to inflamed sites [14]. LTB_4 also promotes the production of a variety of inflammatory cytokines like TNF-α, and IL-1β [15]. In neutrophils, this mediator induces the release of lysosomal enzymes and superoxide anion ($O_2^{\cdot-}$) [16,14], which in turn produces other ROS.

The major anti-inflammatory drugs used in the treatment of inflammatory diseases target the production of pro-inflammatory mediators [17,18]. However, the use of these agents is not innocuous since they mainly increase the risk of gastrointestinal and cardiovascular complications [17]. Therefore, many researchers have focused on the development of new anti-inflammatory drugs from natural sources with more powerful activity and with lesser side effects.

Capparis spinosa L. (*C. spinosa*) is a little perennial shrub that belongs to the *Capparaceae* family and has been used since ancient time in folk medicine for their anti-rheumatic, tonic, expectorant, anti-spasmodic, diuretic, analgesic and antihypertensive properties [19]. Few studies were reported comparing the anti-inflammatory activities of this plant by reducing inflammatory cell functions. Therefore, this study is aimed to explore some mechanisms underlying the anti-inflammatory effects of *C. spinosa* by using *in vivo* and *in vitro* different experimental models.

2. MATERIALS AND METHODS

2.1 Chemicals

Lithium heparin, dextran, Hypaque®-1077, Hanks' balanced salt solution (HBSS), Trypan blue, formyl-methionyl-leucyl-phenylalanine (fMLP), cytochalasin B (CB), cytochrome C, superoxide dismutase (SOD), λ-carrageenan, indomethacin, acetylsalicylic acid (aspirin), gallic acid and quercetin were purchased from Sigma (Germany). Concanavalin A (Con A) was purchased from Sigma (Vienna, Austria). Lymphocytes separated medium-1077, RPMI 1640, heat inactivated fetal bovine serum (FBS) and penicillin-streptomycin were purchased from PAA laboratories GmbH (Pasching, Austria). TNF-α, IL-1β, IL-8 and IL-10 enzyme-linked immunoassay (ELISA) kits were obtained from eBioscience (USA). LTB_4 ELISA kits were purchased from R&D system (USA). All other reagents were of the highest grade available from Sigma and Fluka (Germany).

2.2 Animals

Male Wistar rats weighing 150–200 g and *Swiss Albinos* mice weighing 20–25 g were purchased from Pasteur Institute of Algiers, Algeria. The animals were maintained under standard conditions with free access to food and water. One day before the experiment, the food was withdrawn, but free access to water was allowed. European Union Guidelines for Animals Experimentation (2007/526/EC) were followed to carry out this study.

2.3 Plant Material

Flower buds of *C. spinosa* were collected in June 2012 from Bejaia, Algeria. The plant material was identified by Prof H. Laouer and a voucher specimen (No. C.S. 2012-1) was deposited at local herbarium in the Department of Vegetal Biology and Ecology, University Ferhat Abbas, Setif 1, Algeria. The flower buds were air-dried at room temperature and then reduced to powder.

2.4 Extract Preparation

Methanol extract (Met. E) of *C. spinosa* buds was prepared by maceration of 100 g of powdered plant material with 80% methanol at room temperature for 24 h with frequent agitation. After filtration, the extract was concentrate under reduced pressure in a rotary evaporator (Buchi R-210, Switzerland) at 40°C to give a crude methanolic extract (yield was 24%). This extract was stored at -32°C until use.

2.5 Quantification of Total Phenolic Content

Total phenolic content in the extract was estimated by the Folin-Ciocalteu colorimetric assay [20]. Briefly, 500 µL of the standard Folin-Ciocalteu reagent (10% in distilled water) were added to 100 µL of extract solutions and allowed to react before adding 400 µL of 7.5% sodium bicarbonate. After 90 min of incubation, the absorbance of the mixture was measured at 765 nm. Gallic acid was used for the standard calibration curve and the results were expressed as milligram gallic acid equivalent (mg GAE)/g extract.

2.6 Quantification of Total Flavonoid Content

Total flavonoid content in the extract was determined according to the aluminum chloride colorimetric method [21]. Briefly, 1 mL of 2% of aluminum trichloride (AlCl3) in methanol was mixed with the same volume of extract. After 10 min of incubation, the absorbance was measured at 430 nm. Quercetin was used for the standard calibration curve and the results were expressed as milligram quercetin equivalent (mg QE)/g extract.

2.7 Carrageenan-Induced Paw Edema in Rats

Paw edema was induced by injecting 0.1 mL of 1% λ-carrageenan into the sub-plantar region of the right hind paw of rats [22]. One hour before carrageenan injection, rats received orally 200 and 400 mg/kg of the extract or 100 mg/kg of aspirin (suspended in CMC 1%). Rats of control group were injected with 0.1 mL of λ-carrageenan and received orally only the vehicle before the injection. The edema was assessed by measuring the injected paw initially (V_0) and 1, 2, 3, 4, 5, and 6 h after carrageenan injection (V_t), using a plethysmometer (UGO Basile, Varese, Italy). Inflammation was calculated as the increase in volume of the paw after treatment subtracted of the basal volume. Results were expressed as a percentage of inhibition of edema, calculated according to the following equation:

% inhibition = $[(V_t-V_0)_{control} - (V_t-V_0)_{treated}/(V_t-V_0)_{control}] \times 100$

2.8 Air Pouch Induced in Mice

The air pouches were raised on the dorsum by subcutaneous injection of 3 mL of sterile air, as previously described [23]. To maintain the air pouches potency, 1.5 mL of the sterile air were injected every 2 days. On day 7, the pouches were injected with 1 mL of methanolic extract (1 mg/mL), indomethacin (0.1 mg/mL) or 1 mL of sterile saline solution (control) 1 h prior the injection of 0.1 mL of λ-carrageenan (1%). After 4 h of treatment, the mice were scarified by cervical dislocation and the pouches were opened and washed twice by 1mL of sterile saline solution. Aliquots were diluted (1:10) with Turk's solution and the polymorphonuclear leukocytes were counted in a standard hemocytometer chamber.

2.9 Cells Preparation

Polymorphonuclear Neutrophils were isolated from heparinized venous blood of healthy adult donors declared that they were non-smokers and were not taking any medication. As described by [24], cells were obtained by dextran sedimentation coupled to differential centrifugation over Ficoll-Hypaque® gradient. Contaminating erythrocytes were removed by hypotonic lysis. The purity of the neutrophils preparation was 95%.

Peripheral blood mononuclear cells (PBMCs) were isolated according to [25]. Briefly, the heparinized blood was diluted 1:1 with RPMI-1640 and layered on a Ficol-Hypaque gradient. The gradient was centrifuged at 1250 rpm for 15 min at room temperature, and the buffy coat containing PBMCs was collected and washed twice in RPMI-1640. Following isolation, the cells (neutrophils or PBMCs) were suspended in an appropriate medium, such as (Ca^{2+} and Mg^{2+})-free HBSS at pH 7.4 or RPMI 1640 medium and were maintained at 4°C before use. The cells viability was more than 90% as judged by Trypan blue exclusion test.

2.9.1 Cell viability

Prior the investigation of the effects of *C. spinosa* extract on the release of some inflammatory mediators by activated PBMCs and neutrophil cells, the cytotoxic effect on these cells were tested using Trypan bleu exclusion test. This test was performed after incubating cells for 30 min or 24 hours at 37°C with the maximal concentration (100 µg/mL) of *C. spinosa* bud extract. Cells were incubated with Trypan blue (0.4%) for 10 min and the resulting percentage of blue cells, indicating a capture of the colorant due to plasma membrane rupture, were counted. Normal cell viability was considered to be 90-95% colorless cells.

2.9.2 Cytokines release

Peripheral blood mononuclear cells (2 x 10^5 cell/mL) were incubated overnight in a 24-well plate in RPMI 1640 supplemented with 10% of FBS and 5% of penicillin-streptomycin in the absence (control) or presence of different concentrations of *C. spinosa* extract (1, 10, 50 and 100 µg/mL) and Con A (5 µg/mL) at 37°C with 5% of CO_2. Cytokines (TNF-α, IL-1β, IL-8 and IL-10) measurement in cell culture supernatants were performed using appropriates commercial ELISA kits (eBioscience, San Diego, CA). The amount of each mediator was expressed in pg/mL as indicated in the manufacturer's prescription. The sensitivity of TNF-α, IL-1β was 4 pg/mL, whereas that of IL-8 and IL-10 was 2 pg/mL.

2.9.3 LTB_4 release

LTB_4 concentration was measured in the supernatant of neutrophils (4 x 10^5 cell/mL) suspended in RPMI 1640 culture medium supplemented with 10% of FBS and 5% of penicillin-streptomycin and incubated overnight in a 24-well plate with *C. spinosa* extract (1, 10, 50 and 100 µg/mL) or RPMI (control) in absence or presence of concanavalin A (5 µg/mL) at 37°C with 5% of CO_2. The amount of LTB_4 in cells culture supernatants was expressed in pg/mL as indicated in the manufacturer's prescription of the commercial ELISA kit. The sensitivity of LTB_4 was 2 pg/mL.

2.9.4 Superoxide anion assay

Neutrophils superoxide anion ($O_2^{\cdot-}$) generation was measured spectrophotometriclly using SOD-inhibitable cytochrome C reduction assay described by [26]. Briefly, neutrophils (2.5 x 10^6 cells/mL) pre-incubated for 10 min at 37°C in the absence (control) or presence of *C. spinosa* extract (1,10,50 and 100 µg/mL) were stimulated with fMLP (10^{-7} M) supplemented with cytochalasin B (10^{-5} M) for 15 min at 37°C in the absence or presence of 330 µU /mL SOD. Prior fMLP activation, an aliquot of HBSS2 containing

0.2 mg/mL of cytochrome C was added to the mixture. After centrifugation (3000/5 min/4°C), the absorbance measured at 550 nm. The results were expressed as the percentage inhibition of NADH activity in comparison to the control considered as 100% of NADH activity.

2.10 Statistical Analysis

Results *in vivo* were expressed as mean ± SEM, while *in vitro* results were expressed as mean ± SD. Results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons using the Prism 5.01 computer software (GraphPad, San Diego, USA). Statistical differences were considered to be significant at $P < 0.05$.

3. RESULTS

3.1 Quantification of Polyphenolic Content

Phytochemical screening indicated that the methanolic extract of *C. spinosa* contained high amounts of polyphenols (151.54 mg GAE/g extract) and flavonoids (24.35 mg QE/g extract).

3.2 Effect of *C. spinosa* Extract on Carrageenan-Induced Paw Edema

The injection of 0.1 mL of carrageenan (1%) produced a progressive swelling of the rat paw. The oral administration of 200 and 400 mg/kg of the extract reduced significantly carrageenan-induced paw edema. After 1 h post-carrageenan injection, the extract at dose of 400 mg/kg showed significant ($P < 0.05$) anti-inflammatory effect as aspirin (100 mg/kg). Above 2 h, both doses of the extract exerted a significant ($P < 0.001$) anti-edematous effect, with 52%-69% (Fig. 1).

3.3 Effect of *C. spinosa* Extract on Air Pouch

In air pouch model, the mice of the control group developed after 4 hours an inflammation with infiltration of 7.56 ± 0.74 x 10^6 cells/mL into air pouch exudates (Fig. 2). Treatment with 1 mg/pouch of extract of *C. spinosa* induced a significant ($P < 0.001$) reduction in the number of infiltrating leukocytes (3.94 ± 0.58 x10^6 cells/mL) compared to the control group. This value corresponds to an inhibition of 48.92%, which is better than that obtained with 0.1 mg/pouch of indomethacin (41.03%).

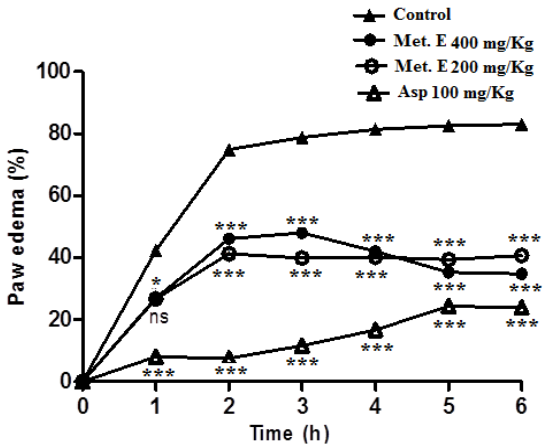

Fig. 1. Time course of carrageenan-induced rat paw edema

*The edema was induced by sub-plantar injection of 0.1 mL of carrageenan 1% in rat pre-treated orally with 200 and 400 mg/kg of methanol extract (Met. E), 100 mg/kg of aspirin (Asp) or vehicle (control). Each value represents the percentage increase in volume of the injected paw at different times after injection of carrageenan compared with the control group set to 100%. Values are means ± SEM (n = 6). * P < 0.05; *** P < 0.001; ns: not significant versus control*

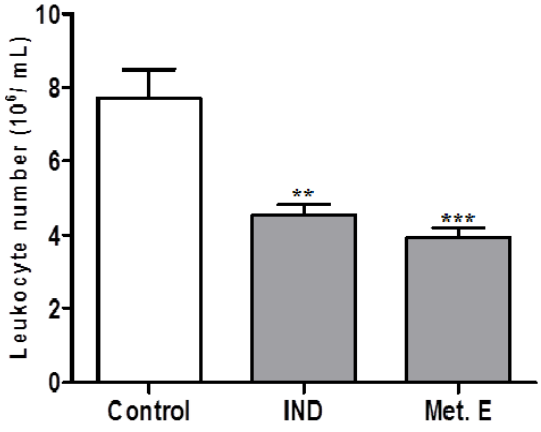

Fig. 2. Effect of *C. spinosa* bud extract on leukocytes infiltrated into air pouch

*The pouch inflammation was induced by 0.1 mL of carrageenan (1%). One hour before the induction of inflammation, mice were treated by 1 mg/pouch of methanol extract (Met. E) or 0.1 mg/pouch of indomethacin (IND). The comparison was made with respect to the control group (without treatment). Values are means ± SEM (n = 6). **P < 0.01; ***P < 0.001 significant versus control*

3.4 Effect of *C. spinosa* extract on Inflammatory Cytokine Production

The basal concentrations of TNF-α, IL-1β and IL-8 were 2.8 ± 2.62 pg/mL, 7.14 ± 0.72 pg/mL and 645.79 ± 32.24 pg/mL, respectively. The treatment of the cells with 5 µg/mL of Con A increase significantly the production these cytokines and IL-10 to 45.03 ± 4.99 pg/mL, 18.68 ± 1.02 pg/mL, 803.32 ± 21.67 pg/Ml and 10.77 ± 0.72 pg/mL, respectively.

As illustrated in Fig. 3, the treatment of the cells with the methanol extract reduced the production of TNF-α and IL-1β. At 50 and 100 µg/mL, the extract inhibited the release of TNF-α by 26.77% and 21.28% and IL-1β by 38.04% and 41.31%, respectively. In contrast, the extract did not show any significant effect on IL-8 and IL-10 production when compared with the unstimulated cells.

3.5 Effect of *C. spinosa* Extract on LTB_4 Production

Basal concentration of secreted LTB_4 by neutrophils was 125.81 ± 11.93 pg/mL. The treatment of the cells with 5 µg/mL of Con A increase significantly the production of LTB_4 to 169.19 ± 8.49 pg/mL. The treatment of the cells with *C. spinosa* methanolic extract (1, 10, 50 and 100 µg/mL) inhibited significantly and similarly the production of LTB4 at all tested concentration. At 100 µg/mL, this inhibition was 21% (Fig. 4).

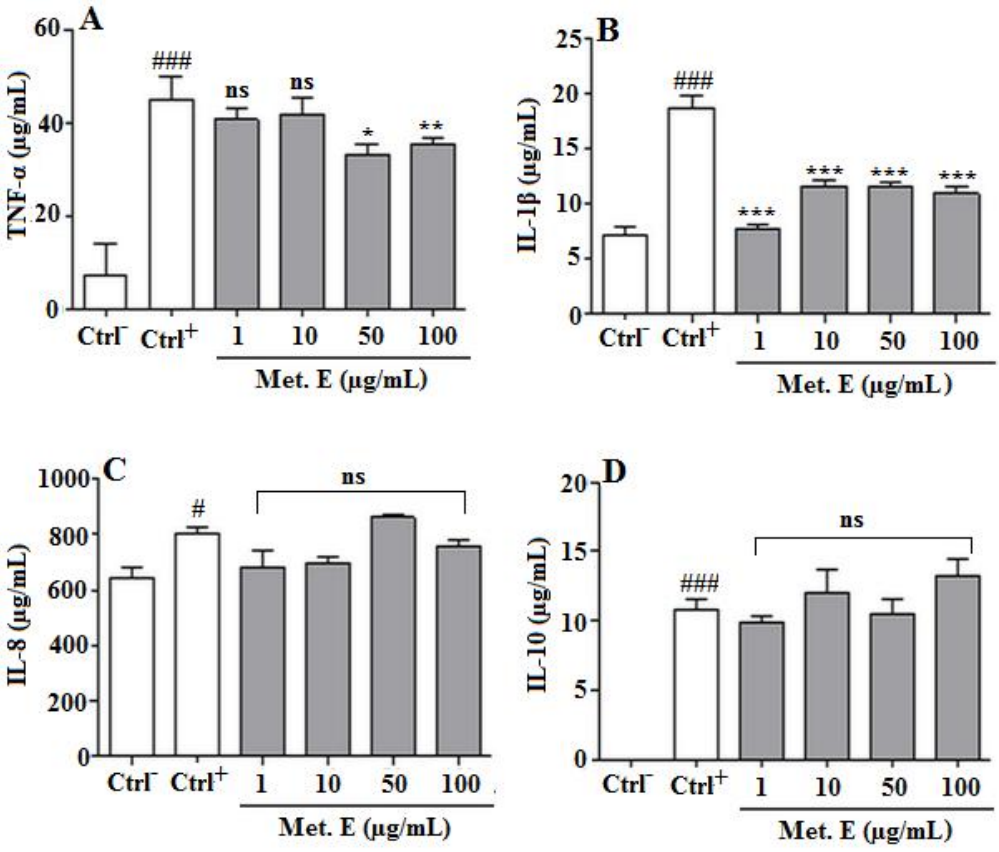

Fig. 3. Effect of *C. spinosa* bud extract on cytokines production

*TNF-α (A), IL-1β (B), IL-8 (C) and IL-10 (D) were measured in the supernatant of PBMCs (2 x 10^5 cells/mL) incubated overnight in the absence ($Ctrl^+$) or presence of different concentrations of C. spinosa methanol extract (Met. E) (1, 10, 50 and 100 µg/mL) and Con A (5 µg/mL). Results are expressed as mean ± SD. ** P < 0.01; *** P < 0.001 significant versus control ($Ctrl^+$); ns: not significant; $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$ significant versus the basal concentrations of secreted cytokines ($Ctrl^-$)*

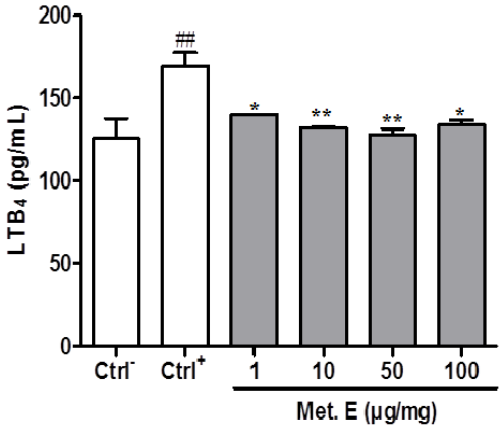

Fig. 4. Effect of *C. spinosa* bud extract on LTB_4 production

*LTB_4 was measured in the supernatant of neutrophils (4 x 10^5 cells/mL) incubated overnight in the absence ($Ctrl^+$) or presence of different concentrations of C. spinosa methanol extract (Met. E) (1, 10, 50 and 100 µg/mL) and Con A (5 µg/mL). Results are expressed as mean ± SD. (n = 3).*P < 0.05; **P < 0.001 significant versus control ($Ctrl^+$); ## P < 0.01 compared to the basal concentrations of secreted LTB4 ($Ctrl^-$)*

3.6 Effect of *C. spinosa* Extract on $O_2^{\cdot-}$ Production

Treatment of the neutrophils with fMLP/CB (10^{-7} M/10^{-5} M) produced an important production of $O_2^{\cdot-}$ (9.06 µM/10^6 cells) compared to unstimulated cells, which produce 3.31 µM/10^6 cells. The treatment of the cells with *C. spinosa* extract (10, 50 and 100 µg/mL) inhibited significantly and in dose dependent-manner the production of $O_2^{\cdot-}$. At 100 µg/mL, this inhibition was 71.16% (Fig. 5).

4. DISCUSSION

Many of the anti-inflammatory drugs have their origin from plant material, which has recently become a major interest of scientific research for their pharmacological properties. In the present study the anti-inflammatory potential of *C. spinosa* bud methanolic extract was studied by using *in vivo* and *in vitro* anti-inflammatory models.

In carrageenan-induced paw edema model, the edema induced by the injection of carrageenan remains located in the area of administration [27]. This acute inflammation is characterized by biphasic event, in which various mediators operate in a sequence to produce inflammatory response. The early phase begins immediately is related to the production of histamine, leukotrienes and cyclooxygenase products.

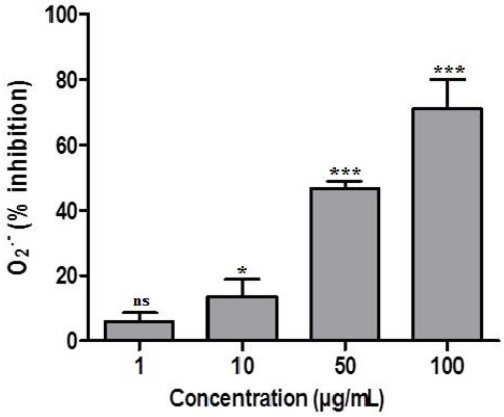

Fig. 5. Effect of *C. spinosa* bud extract on superoxide anion production

*Neutrophils (4 x 10^6 cells/mL) were incubated 10 min in the absence or presence of various concentrations of the methanol extract of C. spinosa (1, 10, 50 and 100 µg/mL) and then stimulated by fMLP/CB (10^{-7} M/10^{-5} M). The production of $O_2^{\cdot-}$ was measured in the cell supernatant by using superoxide dismutase-inhibitable cytochrome C reduction. Results are mean ± SD (n = 3). *P < 0.05; ***P < 0.001 compared to the control set to100%*

The late phase begins after 1 h of the inflammation induction, and is related to the neutrophils infiltration and the production of various types of inflammatory mediators including ROS and TNF-α and IL-1β [28,29]. The treatment with methanolic extract of *C. spinosa* bud exerted a significant inhibitory effect on edema formation during early and late phases. This result indicates that the studied extract may reduce the synthesis and/or the release of inflammatory mediators implicated in this event. The significant effect of the extract in the late phase demonstrated the ability of this extract to inhibit neutrophil migration and the blockage of the release of the inflammatory mediators implicated in this phase. Several studies reported the anti-edematous effect of plant extracts [1,29]. According to [30], the significant inhibitory effect of methanolic extract of the flower buds and the fruit of *Capparis ovate* is probably due to the suppression of the production of kinine-like substances, leukotiernes, protease, lysosome and especially prostaglandins. This activity may be attributed to the presence of bioactive molecules such as polyphenols, alkaloids, lipid and terpenes [31,32].

To gain more information on the underlying mechanisms of the anti-inflammatory effect of the studied extract, its efficacy on the neutrophil migration was carried out by using carrageneanan-induced air pouch model. In this model, the injection of the carragenan induced an acute inflammatory response characterized with an increase of total neutrophil number infiltrated in the inflammatory site. According to [33], this effect is occurred via an indirect mechanism that involves the activation of macrophages and the release of pro-inflammatory cytokines such as IL-1β and TNF-α. The treatment of the mice with methanolic extract of *C. spinosa* decreased significantly the cell migration into the air pouch. This result explains the anti-inflammatory effect of the studied extract. Indeed, [34] reported that the histological analysis of the skin section from the inflamed ear tissue of mice revealed that the pretreatment of mice by *C. spinosa* extracts prevent the development of the different features of the inflammation especially immune cells infiltration.

Recruitment of leukocytes from circulation to sites of inflammation and infection involves numerous soluble factors that mediate interaction between circulating leukocytes and vascular endothelia [35]. The attenuation of leukocytes influx into air pouch cavity can be explained as results of curtailed production of these inflammatory mediators. Among these mediators, TNF-α, IL-1β and IL-8 are considered to play a pivotal role not only in the inflammatory response, but also in the pathogenesis of several inflammatory diseases [18]. Hence, the inhibition of these pro-inflammatory cytokines offers a new therapeutic strategy for the treatment of such diseases [18,36]. Results of this study showed that *C. spinosa* methanolic extract exerted an anti-inflammatory effect by inhibiting cell migration and the production of TNF-α and IL-1β by PBMCs. In agreement, it has been reported that extracts from *C. spinosa* decrease significantly the expression of pro-inflammatory cytokines IFNγ and IL-17 *in vivo* [34]. These extracts are also able to promote the expression of the anti-inflammatory cytokine IL-4 in PBMCs [37]. In contrast, the studied extract did not show any significant effect on the release of the IL-8, which indicates that the anti-edematous and the anti-chemotactic effect of the extract found *in vivo* are not related to IL-8 production. Moreover, although the extract did not show any significant effect on the production of this mediator, this extract could be beneficial to the overall inflammatory cell response since the local microenvironment is depleted of TNF-α and IL-1β. On the other hand, since LTB_4 is a potent chemoattract agent, the ability of *C. spinosa* methanolic extract to reduce the production of this mediator can explained the attenuation of the neutrophil migration into the air-pouch cavity and prove also anti-inflammatory efficacy of the extract.

During the inflammatory process, the production of ROS in adequate amounts from neutrophils is essential to kill microbes and to keep the integrity of the organism. These powerful oxidants contribute to cell injury during excessive inflammatory reaction [38]. Therefore, the modulation of the production of the $O_2^{\cdot-}$ (precursor of ROS) from neutrophils seems to be an important therapeutic approach. Several anti-inflammatory agents were found to exert their effects by inhibiting the production of ROS from the activated neutrophils [39]. In this study, the pretreatment of neutrophils with *C. spinosa* bud extract inhibited significantly the generation of $O_2^{\cdot-}$. This effect can explain the anti-inflammatory effects of the extract observed *in vivo* and *in vitro*, since this oxidant induces the release of pro-inflammatory mediators. Indeed, ROS enhance the secretion of TNF-α, IL-8 and other pro-inflammatory cytokines [40]. Thus, *C. spinosa* extract by serving as a natural antioxidant can protect cells against inflammatory damages.

Several studies suggested that plant materials containing flavonoids and phenolic acids possess good anti-inflammatory activities [41,42]. Effectively, it has been reported that phenolic acid and flavonoids identified in *C. spinosa* extracts exhibit significant anti-inflammatory effect [43,44,45]. In this study, phytochemical analysis revealed the presence of high amounts of polyphenols and flavonoids in *C. spinosa* bud extract. Therefore, the anti-inflammatory activity of the plant extract may be due to the presence of these active compounds.

5. CONCLUSION

Capparis spinosa buds possess significant anti-inflammatory properties by modulating the pro-inflammatory mediator production. Therefore, this part of plant can constitute a promising source of natural bioactive compound to treat of inflammatory disorders.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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