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Original Article

IMMUNOMODULATORY EFFECTS OF ALGERIAN CAPER

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ABSTRACT

Objective: The aim of this study is to investigate the immunomodulatory properties of fruits and leaves of *Capparis spinosa* in mice.

Methods: The effect of methanolic extracts of *C. spinosa* on immune system were assessed by applying several approaches such as Lymphocyte proliferation assay in presence of mitogen (Concanavalin A), delayed-type hypersensitivity (DTH) response, humoral response and Cyclophosphamide-induced immunosuppression.

Results: Administration of methanolic extracts at doses 100 and 200 mg/kg produced statistically significant results as evidenced by the increase in delayed type hypersensitivity (DTH) response ($P<0.05$), enhanced the total WBC level in the cyclophosphamide induced myelosuppression model ($p<0.01$). These extracts also showed significant increase in humoral antibody (HA) titre ($P<0.05$, $P<0.01$) at dose 200 mg/ml. Equally, *C. spinosa* extracts evoked a significant ($p<0.05$, $P<0.01$) increase in mitogen-induced lymphocyte proliferation.

Conclusions: The results demonstrated that both the plant parts extracts exert a marked immunostimulatory effect on the mouse immune system.

Keywords: *Capparis spinosa,* Immunomodulatory properties, Hypersensitivity, Humoral response, Lymphocyte proliferation.

INTRODUCTION

The immune system is involved in the etiology as well as pathophysiologic mechanisms of many diseases. Modulation of the immune responses to alleviate the diseases has been of great interest for many years [1]. A number of medicinal plants and various substances have been claimed to possess immunomodulatory activity. Furthermore, these plants generally act by stimulating both specific and nonspecific immunity [2], and may play an important role in modern health care, particularly where satisfactory treatment is not available. Recently, it has been reported that caper possesses some medicinal properties and antioxidant activities [3]. *Capparis spinosa* L, Capparidaceae family, popularly known as (Kabar) in Algeria, is a shrub plant growing easily in poor and stony soils. This plant is widely used for its various pharmacological effects. Roots, leaves, buds, fruit, bark and seeds of caper were used by ancient people for medicinal purposes, to treat some diseases such as rheumatism, stomach problems, headache and toothache [3].

Actually, in Morocco the fruit of *C. spinosa* is used to cure diabetic [4, 5]. In India, buds and roots of *C. spinosa* are useful in the treatment of boils; leaves are used as counter-irritant and as a cataplasm in swellings; roots are used to treat fever, rheumatism, paralysis, toothache and kill worm in the ear; the bark is used in the treatment of coughs, asthma and inflammation [6]. Plants belonging to Capparacea family are well known for their traditional uses because to their important medicinal properties and have been used extensively as immunostimulants [7]. Besides, immunostimulatory activity was shown with a methanolic extract of the buds [8]. This extract may contribute in improving immune surveillance of human peripheral blood mononuclear cells toward virus infection by up-regulating expression of peculiar pro-inflammatory cytokines; it suppressed the replication of a herpes simplex virus type 2 and increased the expression of pro-inflammatory cytokines including interleukin-12, interferon-γ and tumor necrosis factor-α [8]. Apart the work carried out by Arena *et al.* [8] on buds, there is no scientific report available in the literature on the immunomodulatory activity of *Capparis spinosa*, particularly of fruits and leaves extracts. Therefore, the present study was undertaken to assess the *in vitro* and *in vivo* immunomodulating activities of the methanolic extract of the fruits and leaves of *Capparis spinosa* in relation with its medicinal in folklore use.

MATERIALS AND METHODS

Plant material

The fruits and leaves of *Capparis spinosa* were collected on May and June 2014 from the region of Beni-Aziz, Setif (northeast of Algeria; 36 ° 28′ North and 5° 39′ East). The samples were transported to the laboratory to be cleaned manually and identified by Prof H. Laouer and a voucher specimen was deposited at the Department of vegetal biology and Ecology, University Ferhat Abbas, Setif 1, Algeria. The extract was prepared as reported previously [9].

Animals

Male Wistar mice weighing between 25 and 30 g were used in all experiments. The animals obtained from 'Institute Pasteur d'Algérie', were maintained under standard laboratory conditions: temperature of 25 °C and a photoperiod of 12 h and received standard mouse food and water *ad libitum*. The animal studies were conducted after obtaining clearance from Institutional Animal Ethics Committee, and the experiments were conducted in strict compliance according to ethical principles and provided by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA).

Humoral antibody response

Sheep red blood cells (SRBC) were used as antigen for humoral antibody reaction. SRBC collected in Elsevier's solution [10], were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to 5×10^9 cells/ml for injection.

Animals (7 groups of 5 mice each) were immunized intraperitoneally (i. p.) with 5.10^9 SRBC on day 0. The first group received vehicle (normal saline) and groups II to IV were treated with different doses of fruit extract (50, 100 and 200 mg/kg of body weight: bwt, respectively) administrated on days-2,-1, 1 and 2 of immunization. The mice of groups V, VI and VII were treated with leaf extract (50, 100 and 200 mg/kg bwt) on days-2,-1, 1 and 2 of immunization. Blood samples were obtained from each mouse on day 7 for the evaluation of humoral response. Antibody titer was determined by haemagglutination test [11]. A volume of 25 µl of 0.1% SRBC suspension (5×10^9 cells/ml) was added to 25 µl of two-fold diluted serum samples in microtitration plates. After 1 h of incubation, the last dilution of serum samples which caused haemagglutination was considered as antibody titer.

Delayed type hypersensitivity response

Mice were divided into 7 groups of 5 mice. Different concentrations (50, 100 and 200 mg/kg bwt) of the fruit extract were injected intraperitoneally in groups (II, III and IV) at days-2,-1, 0, 1 and 2. The animals of groups V, VI and VII were given leaf extract (50, 100 and 200 mg/kg bwt, respectively) on days-2,-1, 1 and 2 from the first subcutaneous immunisation with 10^8 SRBC/100 µl (day 0). The vehicle (normal saline) was injected at the same days to the group I as a control. The treated mice were then challenged by injection with 0.05 ml of 2×10^8 SRBCs in right hind foot pad at day 7. The thickness of the right hind foot pad was measured using Vernier caliper after 24 h [12].

Myelosuppression induction with cyclophosphamide

In cyclophosphamide induced myelosuppression, mice were divided into 8 groups of 5 animals. Group I (control group) and group II (cyclophosphamide group) received the vehicle for a period of 13 d. The animals of treatment group, III to V, were given fruit extract (50, 100 and 200 mg/kg, respectively) either for 13 d. The groups VI, VII and VIII, were given leaf extracts (50, 100 and 200 mg/kg, respectively) for 13 d. The mice of groups II to VIII were subsequently injected with cyclophosphamide (30 mg/kg, i. p.) on the 11th, 12th and 13th days, 1 h after the administration of the respective treatment. Blood samples were collected on the 14th day of the experiment, and the total white blood cells (WBC) count was determined with haemocytometer [13].

Lymphoproliferation assay

Blood (20 ml) was obtained from four healthy volunteer donors. The blood sample was diluted with the same volume of phosphate-buffered saline PBS containing ethylene glycol bis (β-aminoethyl ether)-N, N, N', N'-tetraacetic acid (EGTA, 1 mM) [14]. Peripheral blood mononuclear cells (PBMCs), including T lymphocytes, were isolated from the blood using HISTOPAQUE-1077 (Sigma) gradient centrifugation. Briefly, the diluted blood sample was carefully layered on HISTOPAQUE-1077. The mixture was centrifuged under at 400x g for 35 min at 20 °C. The lymphocyte layer was carefully transferred out. The lymphocyte was washed and pelleted down with three volume of PBS-EGTA for twice, After washing, cell concentration was adjusted to 10^6 cells mL-1 in the culture medium containing Dulbecco's Modified Eagle's Medium (DMEM, Sigma), 10% v/v Fetal Bovine Serum (FBS), (Sigma), penicillin (100U/ml) and streptomycin (100 mg/ml). The viability of the cells was determined using a trypan blue exclusion dye test. For proliferation assay, Cell suspension solution (100µl) introduced into each well of a 96-well flat-bottomed plate with or without *Capparis spinosa* at (100, 200, 400 µg/ml) and 10 µg mL-1 concanavalin A (ConA, Sigma) were co-cultured with the cells. The plates were maintained at 37 °C in a 5% CO2 humidified atmosphere for 72 h. Lymphocyte proliferation was determined by [3(4,5-di methylthiazol2yl)2,5-diphenyl tetrazolium bromide] (MTT, Sigma) assay as previously described [15]. Briefly, MTT was dissolved in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) at 5 mg/ml and added to all wells of the microplate, and plates were incubated at 37 °C for 4 h. Acid-isopropanol (100 µl of 0.04 N HCI in isopropanol, Sigma) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes, the plates were read on a microplate reader at 570 nm against a reference wavelength of 630 nm. The results are presented as absorbance by mean±SEM, and all reactions were performed in quadruplicate.

Statistical analysis

Data are expressed as mean±SEM. Differences between groups were analyzed by using the one-way analysis of variance (ANOVA) with Dunnett's t-test. A *p*-value of ≤ 0.05 was considered statistically significant using Graph Pad Software.

RESULTS

Effect of extracts on lymphocyte proliferation

The immunomodulatory effects of methanolic extracts of *Capparis spinosa* fruits and leaves on T lymphocytes were assessed *in vitro* in the presence of Con A (10 µg/ml). Results presented in fig. 1 showed that the extract dose of 400 µg/ml of both fruits ($p<0.05$) and leaves ($p<0.01$), exhibited significant increases in proliferation of these cells.

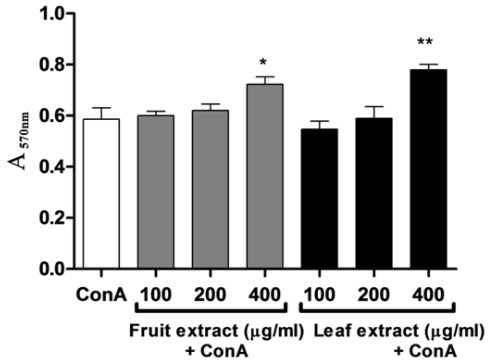

Fig. 1: Lymphocytes proliferation by fruit and leaf methanolic extracts of *Capparis spinosa* in the presence of ConA (10 µg/ml), for 72 h at 37 OC and 5% CO2. *p<0.05 and **p<0.01 compared to ConA positive control (10 µg/ml). Results presented as the average and standard deviation of four experiments performed in quadruplicate

Effects of extracts on antibody generation and DTH response

The effect of the different extract treatments on the production of anti-SRBC antibodies in mice was tested. As shown in fig. 2, significant differences between treated and control animals were observed only when the extract was administered intraperitoneally at a concentration of 200 mg/kg.

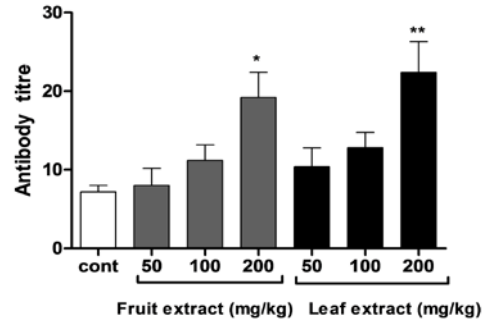

Fig. 2: Effect of *Capparis spinosa* extracts on SRBC-induced humoral antibody titres in mice. Each value represents the mean±SEM of triplicates; *p<0.05, **p<0.01. Cont: control

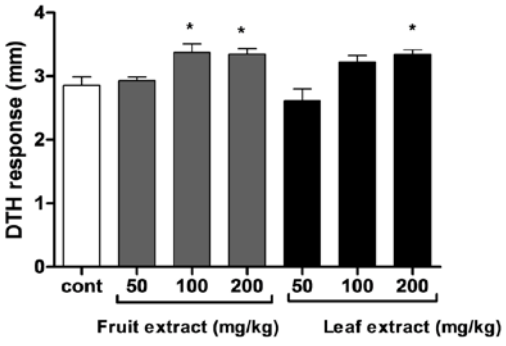

Fig. 3: Effect of *Capparis spinosa* on delayed-type hypersensitivity response in mice immunised with sheep red blood cells (SRBC). Values represent mean±SEM (*p<0.05) of footpad thickness after immunisation of mice groups, cont: control

In the DTH response test, the result in fig. 3 revealed that mice treated with fruit methanolic extract at the doses of 100 and 200 mg/kg, and treated with 200 mg/kg of a methanolic extract of leaves showed a statistically significant increase in footpad swelling paw after 24 h of a challenge with SRBC.

Effect of extracts on cyclophosphamide treated mice

Administration of cyclophosphamide at a dose of 30 mg/kg (group II) has significantly lowered the levels of total white blood cells (WBC) as compared to control group. The two doses (100 and 200 mg/kg) of both methanolic extracts combined with cyclo-phosphamide showed a significant increase in levels of WBC as compared with a group of mice treated with cyclophosphamide alone (table 1).

DISCUSSION

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions, it is named immunostimulatory drug which primarily implies stimulation of specific and nonspecific system, i.e. granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immuno-suppression implies mainly to reduce resistance against infections, stress and may occur on account of an environmental or chemotherapeutic factor [16].

The main objective of the present study was to explore the immunomodulatory potential of *Capparis spinosa* methanolic extracts of leaves and fruits. The *in vitro* proliferation of human T lymphocytes was carried out in the presence of Con A as a mitogen and monitored by MTT assay. The results demonstrated that both parts showed an immunostimulant effect on human lymphocytes. The result is in agreement with that obtained for the buds in the same plant where the *in vitro* exposure of human peripheral blood mononuclear cells (PBMCs) treated with *C. spinosa* methanolic extract has shown an immunostimulant effect against infection with *Herpes simplex virus* type 1 [8].

Table 1: Effect of leaf and fruit methanolic extracts of *Capparis spinosa* on cyclophosphamide induced myelosuppression in mice

Animal groups	Treatment	Total WBC count (10^3/µl)
Group I	Control	4.1±0.33
Group II	Cyclophosphamide (30 mg/kg)	3.64±0.11
Group III	Fruits extract 50 mg/kg	3.29±0.9
Group IV	Fruits extract 100 mg/kg	4.33±0.08**
Group V	Fruits extract 200 mg/kg	4.19±0.08*
Group VI	Leaf extract 50 mg/kg	3.12±0.14
Group VII	Leaf extract 100 mg/kg	4.19±0.12*
Group VIII	Leaf extract 200 mg/kg	4.40±0.24**

Results are expressed as mean±SEM, n=5, *P<0.05 and **P<0.01 when groups III to VIII were compared with group II

Hemagglutination test was carried out to determine the effect of *Capparis spinosa* on the humoral immune system. In this system, when the B lymphocytes bind the foreign antigen, they multiply by mitosis, and then they turn and differentiate into antibody-secreting cells. These antibodies are involved in the mechanisms of antigen elimination namely; complement activation, opsonization, neutralization of toxins, etc [17]. In the experiments undertaken to study the effect of *C. spinosa* on antibody titre against SRBC, it was observed that both fruit and leaf extracts showed a remarkable increase on circulating antibody titre. This clearly explains the increase in the humoral response by *Capparis spinosa* by enhancing the responsiveness of macrophages, T and/or B lymphocyte subsets involved in antibody synthesis [18].

Delayed-type hypersensitivity is a fourth type of hypersensitivity reaction where, in the sensitization phase after initial contact with antigen, T_H cells proliferate and differentiate into T_H1 cells. In the effector phase after subsequent exposure of sensitized T_H cells to an antigen, T_H1 cells secrete a variety of cytokines and chemokines. These factors attract and activate macrophages and other nonspecific inflammatory cells [19]. Activated macrophages are more effective in presenting antigen, thus perpetuating the DTH response, and function as the primary effector cells in this reaction [20]. Therefore, an increase in DTH reaction in mice in response to T cell dependent antigen revealed the stimulatory effect of methanolic extracts of *Capparis spinosa* on T lymphocytes.

Immunomodulatory activity of *Capparis spinosa* leaf and fruit methanolic extracts was also explored by evaluating their effects on cyclophosphamide induced myelosuppression in mice. Results of this study revealed an attenuating effect on the cyclophosphamide-induced bone marrow suppression by an increase in total WBC. This indicates the eventual effect of the extract to restore and enhance immune system depleted by cyclophosphamide [21]. Previous reports and phytochemicals screening of alcoholic extracts of *Capparis spinosa* have shown the presence of alkaloids, glycosides, carbohydrates, tannins, phenolics, flavonoids and triterpenoids while the aqueous extract showed the presence of steroids, glycosides, carbohydrates, flavonoids and saponins [22, 23]. Also, several works indicated that more types of flavonols stimulate human peripheral blood leucocyte proliferation and increase the activity of helper T cells, interleukin 2 and macrophage and are thereby useful in the treatment of several diseases caused by immune dysfunction [24]. Moreover, it has been demonstrated that the leaf and fruit methanolic extracts have the highest antioxidant effects due to their polyphenols components, especially flavonoids [9, 25].

CONCLUSION

It is evident that the immunostimulatory effect produced by the both methanol extracts of *Capparis spinosa*, containing flavonoids, may be responsible for increasing the humoral and cellular immune response. It can, therefore, be concluded that the methanolic extract of fruit and leaf Algerian *Capparis spinosa* is a potent immuno-stimulant and can be used as a complimentary therapeutic agent in immuno-deficiency treatment.

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CONFLICT OF INTERESTS

Declared none

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