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Research Article

IMMUNOMODULATORY ACTIVITY OF ARGANIA SPINOSA SEEDS

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ABSTRACT

Objective: Various compounds of medicinal plants have been widely investigated since ancient times for their possible immunomodulatory properties in the body's immune system.

Methods: In the present study, the immunostimulatory effect of *Argania spinosa* crude extract was evaluated *in vivo*. The immunostimulant potential of crude extract on the phagocytic activity was measured by the carbon clearance rate test.

Results: Our research revealed that at different doses (30,50, 100 and 150 mg/kg), *Argania spinosa* crude extract increased the phagocytic activity in a dose dependant manner when compared with the control and thus the clearance rate of carbon was faster after the administration of the plant extract P=0,000.

Conclusion: *Argania spinosa* crude extract exhibited a dose-dependent immunostimulant effect on the reticuloendothelial system, which could be attributed to the presence of active principles in this crude extract.

Keywords: Immunomodulatory, *Argania spinosa* seeds, Phagocytic activity, Carbon Clearance rate, Reticuloendothelial system.

INTRODUCTION

The immune system is the most complex biological systems in the body. At the time of infection immune system go under the attack of a large number of viruses, bacteria and fungi[1]. There are two branches of immunity response: humoral immunity and cellular immunity [2].

Immunity disorders may affect both cellular and humoral components. An important role in the cellular immunity is played by reticuloendothelial system which mainly comprise of phagocytic cells whose function is to ensure elimination of senescent cells, pathogenic microorganisms and immune complex from blood and tissues and participate in inflammation. This way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions [3].

In order to perform phagocytic function, cells of reticuloendothelial system must be transformed to the active state. This specific ability is significantly suppressed by the action of physiological and pathological factors in nature. However, it is possible to influence this ability using certain immunomodulating agents [4, 5]

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions [6]. In the innate immune the nature killer cell plays an important role to the defiance against virus – infected and malignant cell to destroy the abnormal cell [7]

Medicinal plants which are used as immunomodulatory effect to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism. The use of plant product like polysaccharides, lectines, peptides, flavonoids and tannins has been the immune response or immune system in various *in –vitro* modals [8].

Argan tree (*Argania spinosa* (L.) Skeels) belongs to the *Sapotaceae* family and it is the only species of this tropical family, is endemic in South-western Morocco [9] and Algerian region of Tindouf [10, 11].

The fruit of the argan tree is a stone-fruit (as for walnut tree or almond tree); with pulp covering a lignified endocarp (the nut) containing one to three kernels (the seeds) which furnish edible and marketable oil [12].

Pharmacological studies have confirmed that *Argania spinosa* have several biological effects including: antiproliferative [13, 14, 15, 16] Hypolipidemic, hypocholesterolemic [17], antiatherogenic [18, 19, 20] antiradical [21, 22] and anti-inflammatory activities [23].

The present investigation was undertaken to evaluate the immunostimulatory effect of the crude extract obtained from *Argania spinosa* seeds using phagocytic responses by carbon clearance test *in vivo* experimental model.

MATERIALS AND METHODS

Plant Material

Seeds of *Argania spinosa* were collected from Tindouf (South –west of Algeria).

The fruits were cut into pieces to obtain seeds, and then the seeds were subjected to size reduction to a coarse powder using a mechanical grinder. The powder (crude extract) was then used for treatment preparations by dissolving it in normal saline (0.9%).

Animals

Adult male *Mus Musculus* mice (2.5- 3 month old) from central pharmacy Algeria, weighing(28- 35) were used for determination of the phagocytic activity.

The animals were kept under standard laboratory conditions of humidity, temperature (25± 1°C) and light (12h day :12h night), and allowed free access to food and water. 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).

Phagocytic index

The clearance rate of carbon was measured by the method of [24].

Animals were divided into five groups, consisting of six mice in GI, GII, GIII, GIV and GV. Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II-III-VI and V were administered by i.p injection with different concentrations of *Argania spinosa crude* extract (30, 50, 100 and 150 mg/kg/) respectively.

After 48h of i.p injection, the mice were administered with carbon ink suspension at a dose of (0.1ml/10g through the tail vein ; the mixture consisted of black carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml.

Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample drops (14) were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer.

The animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

The phagocytic activity is expressed by the phagocytic index K which measures all the reticuloendothelial system function in the contact with the circulating blood and by corrected phagocytic index α which expresses this activity by unit of active weight organs: liver and spleen. The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). These are calculated by means of the following equations [25, 26]:

$$K = \frac{\ln OD1 - \ln OD2}{t2 - t1}$$

$$\alpha = \sqrt[3]{K} \times \frac{\text{Body weight of animal}}{\text{Liver wt} + \text{spleen wt}}$$

$$t_{1/2} = 0.693/K$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Statistical Analysis

Results were analysed for differences between the groups across dietary treatments by one –way ANOVA test and Tukey's multiple comparison tests (SPSS version 9).

RESULTS

The present data showed that there is a highly significantly difference in the means for the phagocytic index (K) between groups (GI, GII, GIII, GIV and GV) P= 0,000 and the group V is Highly significantly different from groups (GI,GII,GIII and GIV) at P=0,000.

This indicates that *Argania spinosa* crude extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependant manner **(Figure 1).**

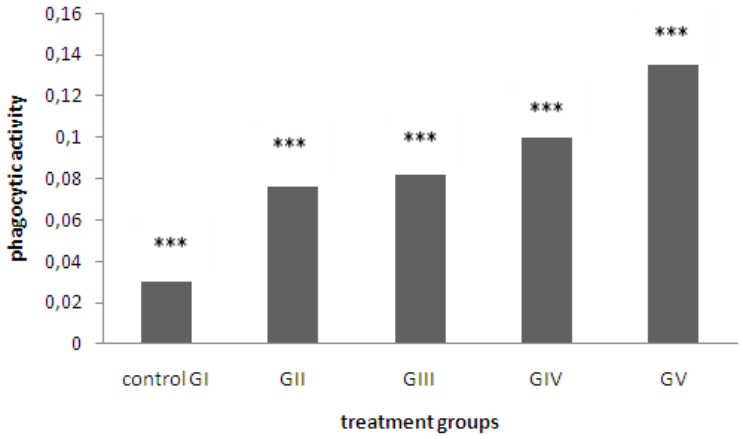

Fig. 1: Effect of *Argania spinosa* crude extract on phagocytic activity.

As shown in **Figure 2**, the half-time of colloidal carbon was highly significantly faster at 48h, after the administration of *Argania spinosa* crude extract between groups P= 0,000 and the clearance rate was decreased highly and significantly in groups (GII,GIII,GIV and V) when it is compared to the control group(GI) P=0,000. This indicates that the extract reduces the elimination time of carbon particles from blood and affirms that *Argania spinosa* crude extract enhanced the phagocytic activity.

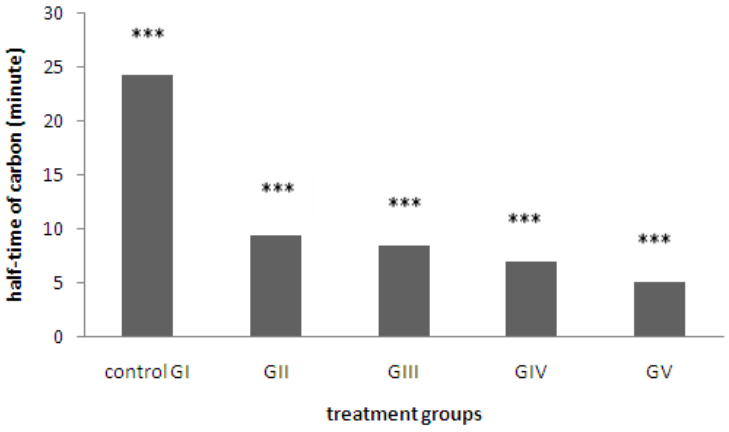

Fig. 2: Effect of *Argania spinosa* crude extract on half time t1/2 of carbon in blood.

The results of this study showed that there is a highly significantly difference in the means for the corrected α between groups (GI, GII,GIII, GIV and GV) P= 0,000 and the corrected α was increased highly and significantly in groups (GII,GIII,GIV and V) when it is compared to the control group (GI)P=0,000. **Figure 3**

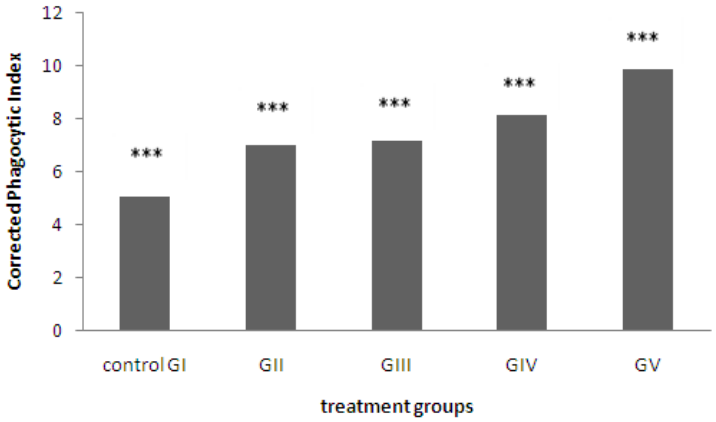

Fig. 3: Effect of crude extract of *Argania spinosa* seeds on corrected phagocytic index

DISCUSSSION

Due to high cost of antiretroviral drugs have caused researchers to turn to plants as prospective therapies in the search of alternative anti HIV or immunomodulatory compounds [27].

There are a number of natural agents (herbs) which are used for the enhancing of the body's response to disease. In recent time a large number of drugs extracted from the plants are coming in to the marked by proper clinical trials. When taking any of these agents take proper advice on dose, length of treatment [1].

In this study we observed that the animals administered with the crude extract of *Argania spinosa* stimulates the phagocytic index at different concentration. So, this result agrees with those of Shuklaa et al. [28] and Benmebarek et al. [29] who reported that the oral administration of ethanolic extraction of *Caesalpinia bonducella and S. mialhesi* respectively in the mouse are increased the phagocytic index at different concentration.

Also Gaoa et al. Have reported that the extract of Curcumin from Curcuma *longa is* inhibited the IL-2 induced proliferation of spleen cells completely at concentrations of 25mmol/L [30]. The study of [31] confirmed that the methanolic extract of *S. mahagoni* seeds has therapeutic potential and could be served as an effective immunomodulatory candidate without any side effects

Treatment by Argania *spinosa* crude extract enhanced the rate of carbon clearance from the blood when it is compared to the control group. This reflects the enhancement of the phagocytic activity of phagocytes and non specific immunity, which includes opsonisation of the foreign particulate matter with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matter from the blood [32].

[33] Showed the immunomodulatory activity of Isoprinosine on chicken infected by three different viruses : Newcastle disease, fowl plague and avian infectious bronchitis. A stimulatory influence on primary anti-Newcastle disease virus antibody response was observed. In the avian model the Isoprinosine antiviral effect appears as due mainly to the enhancement of interferon production and to a synergistic interferon –isoprinosine interaction.

CONCLUSION

In vivo investigations showed that the crude extract of *Argania spinosa* at concentrations of 150mg/kg increased the phgocytic index, corrected α and decreased the rate of carbon clearance this immunomodulatory effect of *Argania spinosa* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E family), phenolic compounds, triterpenoids, sterols and saponins [34, 35].

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