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Original Article

IMMUNOSTIMULATORY ACTIVITY OF PHOENIX DACTYLIFERA

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ABSTRACT

Objective: The aim of our study was to evaluate *in vivo* the immunostimulatory properties of *Phoenix dactylifera* "AZARZA variety".

Methods: The immunostimulant potential of the plant extract of Phoenix *dactylifera* on the phagocytic activity was measured by the carbon clearance rate test. The anti-oxidant activity was measured by spectrophotometric determination of glutathione from liver's homogenate.

Results: Our results obtained in this study shown that the phagocytic and the anti-oxidant activities was increased significantly in animals injected with *Phoenix dactylifera* "AZARZA" extract at doses (30,50 and 100mg/kg) P<0,05. The clearance rate of carbon was significantly faster at the concentration of 50 mg/kg when is compared to the two concentrations 30 and 100mg/kg (P= 0,004) and the release of the GSH from the liver was significantly higher at the concentration of 50 mg/kg when is compared to the two concentrations 30 and 100mg/kg (P= 0,003).

Conclusion: The *Phoenix dactylifera* extract revealed an immune-stimulatory effect on the reticuloendothelial system and anti-oxidant activity with higher effect by the administration of 50 mg/kg.

Keywords: Phoenix dactylifera, Immunostimulatory activity, Carbon clearance rate, Glutathione.

INTRODUCTION

The term immunostimulation comprises a prophylactic or therapeutic concept which aims at the stimulation of our non-specific immune system. This implies primarily the non-antigen dependent stimulation of the function and efficiency of granulocytes, macrophages, complement and natural killer cells. In contrast to immunity achieved by immunization or antibody injection, this type of immunity, arising from unspecific immunostimulation, is termed paramunity and the agents responsible are known as paramunity inducers. It is characteristic for these agents that they do not affect immunological memory cells [1]. Immunostimulation is also indicated to counteract immunosuppression and ineffectively working immune system, manifesting itself for example by a reduced resistance against infectious diseases, which may be the consequences of serious infections, physical and psychological stress, alcoholism, environmental damages such as pesticides, excessively applied chemotherapy, or long term treatment with immunosuppressive drugs [1].

Herbal drugs are known to possess Immunomodulatory properties and generally act by stimulating both specific as well as non-specific immunity. Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens [2].

Immunostimulatory therapy is now being recognised as an alternative to conventional chemotherapy for a variety of disease conditions, involving the impaired immuno-response of the host [3].

Glutathione (L-g-glutamyl-L-cysteinylglycine) is the principal non protein thiol involved in the antioxidant cellular defense. It is a tripeptide composed of cysteine, glutamic acid and glycine, and its active group is represented by the thiol (–SH) of cysteine residue. Glutathione is a ubiquitous molecule that is produced in all organs, especially in the liver [4].

Glutathione reduced (GSH) plays an important role in many biological processes such as intracellular reduction–oxidation metabolic cycles, transportation, protein synthesis, catabolism, and metabolism [5].

The *Phoenix dactylifera* is a monocotyledonous woody perennial belonging to the Arecaceae family, which comprises 200 genera and 3000 species. The beneficial health and nutrition values of date palm, for human and animal consumption, have been claimed for centuries [6].

Algeria is the sixth important countries in date world production. During 2007, 468000 metric tons were produced in Algeria. The Algerian dates represented about 7.28% of the total world production as reported by FAO in 2009 [6].

Fruits of the date palm (*Phoenix dactylifera* Fruits) are commonly consumed in many parts of the world especially the Arabian countries. Date fruit are used as nutrient while the pollen grains used in the treatment of infertility [7]. Traditional medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions. The use of herbal medicine has become increasingly popular worldwide especially in the Asian and African countries. The various parts of *Phoenix dactylifera* widely are used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, and inflammation [8].

MATERIALS AND METHODS

Plant material

Collection

The jam was prepared from the date palm (*Phoenix dactylifera* AZARZA variety) which was collected from Ghardaïa (Algerian septentrional Sahara).

Preparation of the extract

The jam concentrations of 30, 50 and 100 mg/kg were diluted into 10 ml of Nacl (0,9%).

Animals

Adult male Mus Musculus mice (2-2.5 month old) were procured from central pharmacy Algeria. The animal experiments weighing (20–33 g) were used for determination of the phagocytic activity. The animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-7 with 12:12 light: dark cycles). Food was provided in the form of dry pellets (SARL Production Locale, Bouzaréah. Algeria) and water adlibitum. 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 guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Phagocytic activity

Phagocytic activity of reticuloendothelial systems (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticuloendothelial systems by carbon clearance test determined by a reported method (Biozzi et al.,1955). Animals were divided into four groups, GI, GII, GIII and GIV. Group I (Control) was given by i.p injection 0.9% NaCl (0.5 ml/mouse.), groups II, III and IV were administered with different concentrations of the Phoenix dactylifera extract (30, 50 and 100 mg/kg) respectively.

After 48 h of i.p injection, Carbon ink suspension was injected via the tail vein to each mouse at a dose of 0.1 ml/10g, the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3% gelatin solution 4 ml. Blood samples (≈14 drops or 25µl) were then withdrawn from the retro-orbital plexus at 5 and 15 minutes after injection of colloidal carbon ink via an heparin glass capillaries and lysed in 0.1% sodium carbonate solution (4ml). The optical density was measured spectrophotometrically at 676nm.

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 (t1/2, min) [9].These parameters are calculated using the following formulas:

$$K = \frac{\log OD\ 1 - \log OD\ 2}{t2 - t1}$$

$$t\frac{1}{2} = \frac{0.693}{K}$$

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

OD1 and OD2 are the optical densities at time t1 and t2, respectively.

Glutathione assay (GSH)

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

Preparation of the homogenate

The weight of 0,5g of the liver was homogenized in 2ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4). Then the homogenates were centrifuged at 9000 g for15 min at 4°C after that the supernatant was used for determination of glutathione reduced (GSH).

Method

The glutathione reduced content in the liver was measured spectrophotometrically by using 5,5′-dithiobis-(2 nitrobenzoic acid) (DTNB) as a coloring reagent, following the method of Weckbekeret al.,1988 [10].

Statistical Analysis

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

P<0,001, P< 0,01,P< 0,05 were considered to indicate the significant levels.

RESULTS

The present data showed that there is a significant difference in the means for the phagocytic index (K) between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,003 and the group 50 mg has the Highest significantly difference from groups (NaCl, 30 mg and 100 mg) at P=0,002.This indicates that *Phoenix dactylifera* enhanced the phagocytic activity by stimulating the reticuloendothelial system (Figure 1).

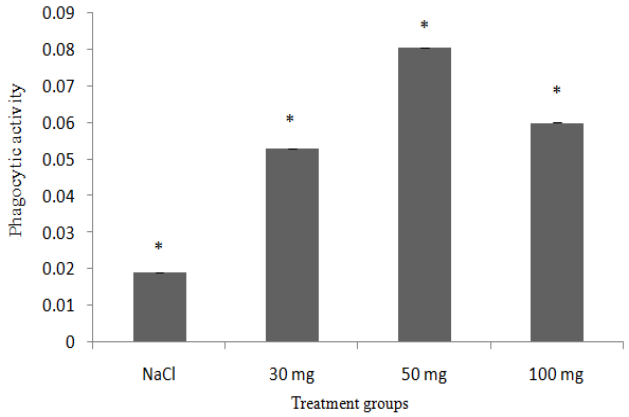

Fig. 1: It shows the effect of Phoenix dactylifera extract on phagocytic activity.

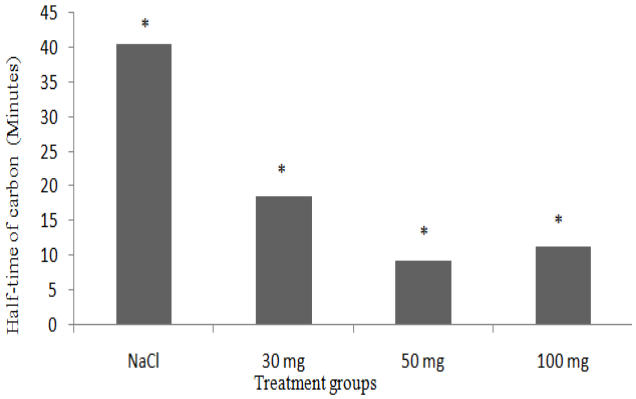

Fig. 2: It shows the effect of *Phoenix dactylifera* extract on half time t1/2 of carbon in blood.

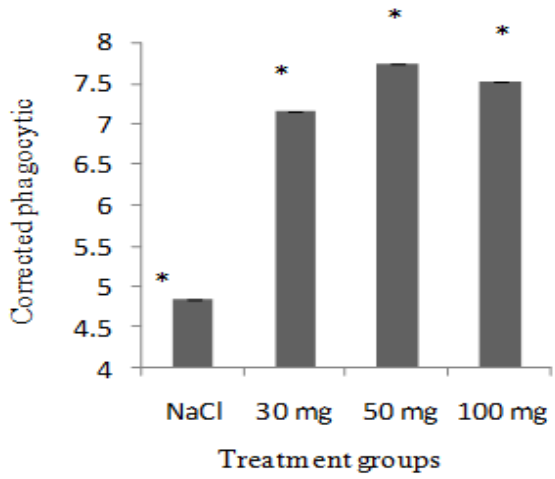

Fig. 3: It shows the Effect of *Phoenix dactylifera* extract on corrected phagocytic index α.

As shown in the figure 2, the half time of colloidal carbon was decreased significantly between groups P= 0,003 however at the concentration of 50mg/kg was faster when it is compared to the other groups P= 0,004.

The results of this study showed that there is a significant difference in the means for the corrected phagocytic index α between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,004 and the corrected phagocytic index α was increased significantly in groups (30 mg, 50 mg and 100 mg) when it is compared to the control group (NaCl) P<0,05 but at the concentration of 50mg /kg the corrected phagocytic index α was higher than the other groups P= 0,006 (Figure 3).

The last part of this study showed that there is a significant difference in the means for the Glutathione values between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,002 and the Glutathione values was decreased highly and significantly in groups (30 mg, 50 mg, and 100 mg) when it is compared to the control group (NaCl) P<0,05 however the glutathione reduced was lower than the other groups P= 0,003 (figure 4). This indicates that the extract liberates the glutathione particles from liver and affirms that *Phoenix dactylifera* enhanced the anti-oxidant activity.

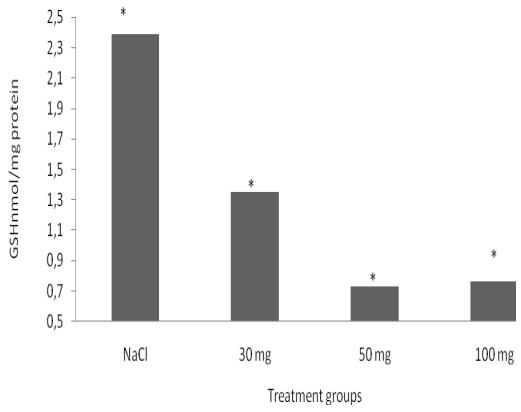

Fig. 4: It shows the effect of *Phoenix dactylifera* on Glutathione reduced values.

DISCUSSION

Today, the use (which we can say 'return to nature') of traditional herbal medicines, herbal health products, pharmaceuticals food supplement, cosmetics, etc. is increasing due to the growing recognition that natural products are safe, have either no or negotiable side effects[11].

Some of these plant products are believed to enhance the natural resistance of the body to infection, on the basis of their constituents like polysaccharides, lectins, saponins and flavonoids etc. Some of these stimulate both 'humoral and cell mediated immunity', while others activate only the cellular components of the immune system [12]. Immunostimulatory molecules intensify and modify the lymphocyte mediated immune response and its duration. Such molecules can, therefore, be potentially applied as adjuvants in vaccines and allergy preparations [13].

The activity was investigated by phagocytic carbon clearance by the phagocytic function of the reticuloendothelial system which is known to be important in the removal and destruction of pathogenic organisms from the tissues and blood [14].

Glutathione is a major antioxidant and a vital component of host defenses. In addition to protecting against free radical injury, it is important in the activation of lymphocytes, critical for the function of natural killer cells and lymphocyte-mediated cytotoxicity, and may have a role in the protection of neutrophils and macrophages against oxidative damage [15].

From ages dates are consumed by humans for its beneficial health and nutritional values [16].

In this study we observed that the animals administered with the extract of *Phoenix dactylifera* stimulates the phagocytic index at different concentration. So, this result agrees with those of Gokani *et al*. [17] and Aribi *et al* [18] who reported that the administration of extraction of *Clerodendrum phlomidis* and *Premna integrifolia* roots and *Argania spinosa* respectively in the mouse are increased the phagocytic index at different concentrations.

Treatment by the extract of *Phoenix dactylifera* enhanced the rate of carbon clearance from the blood when it is compared to the control group. Cells of the reticuloendothelial systems play important role in the clearance of particles from the blood stream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is increased during the treatment of rats by the methanolic extract of *Morus Alba* Linn (Mulberry) leaves. [19]. Also the jam reduces the glutathione particles from liver and affirms that Phoenix dactylifera enhanced the glutathione reduced concentration and anti-oxidant activity. This result agrees with those of Hasnaoui *et al* [20].

CONCLUSION

In vivo investigations showed that the jam of *Phoenix dactylifera* at concentration of 50mg/kg increased the phagocytic index, corrected phagocytic index α and decreased the half time of carbon and the concentration of the glutathione reduced. This Immunomodulatory effect of *Phoenix dactylifera* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E-C family) and phenolic compounds [21].

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