



Immunomodulatory Activity of Intracellular Crude, Extracted from *Nocardiosis dassonvillei*

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ABSTRACT

Various compounds of secondary metabolites of actinomycetes have been widely investigated since ancient times for their possible immunomodulatory properties in the body's immune system. In the present study, the immunostimulatory effect of crude extract from *Nocardiosis dassonvillei* (Vic8) strains was evaluated *in vivo*. The immuno stimulant potential of crude extraction on the phagocytic activity was measured by the carbon clearance rate test. Our research revealed that at different doses (30, 50 and 100 mg/kg), *Nocardiosis dassonvillei* 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 actinomycete crude extract P< 0,0001. *Nocardiosis dassonvillei* 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, *Nocardiosis dassonvillei*, 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 fungi¹. There are two branches of immunity response: humoral immunity and cellular immunity².

A number of factors can influence the immune system development, maintenance and optimal functioning³. Therefore, modulation by either suppressing or stimulating the immune responsiveness of an organism against the invading antigens and alleviating the diseases, has been of interest for many years⁴. 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^{5,6}.

Furthermore, many of the presently available immunomodulators such as glucans, telonones, L-fucose and levamisole as well as Coryne bacterium parvum bacterium suffer from side effects such as fever, neutropenia, leucopenia and, at times, allergic reactions⁷. Hence, screening for better agents and evaluating their immunomodulatory potential is becoming a field of major interest all over the world.

Actino bacteria are Gram-positive bacteria characterized by a genome with a high G + C content. Most members of this group are soil-dwelling saprophytic microorganisms⁸ and are abundant in the rhizosphere⁹ which are well

known for their ability to produce antibiotics, pharmacologic agents and lytic enzymes¹⁰.

Several Actinobacteria have been isolated from Algerian Saharan soils. Some of them belong to rare genre. The Vic8 actinomycete strain have been isolated and identified by Aouar¹¹ from rhizospheri quealgerian soil.

The present investigation was undertaken to evaluate the immunostimulatory effect of the crude extract obtained from *Nocardiosis dassonvillei* (Vic8) using phagocytic responses by carbon clearance test *in vivo* experimental model.

MATERIALS AND METHODS

Actinomycetestrain and fermentation

The strain Vic8, was isolated from Algerian semi-arid soil and identified such as *Nocardiosis dassonvillei* by Aouar¹¹. A loopful of mycelia from a slant culture of the strain grown on yeast-malt extract agar (ISP2) (4g/l glucose, 4g/l yeast extract, 10g/l malt extract, and 10g/l agar, pH 7.0) was transferred to a 50-ml test tube containing 10 ml of a medium (4g/l glucose, 4g/l yeast extract and 10g/l malt extract, pH 7.0). The tube was shaken on a reciprocal shaker at 27°C for 3days. Tenmilliliters of the culture was transferred into erlenmeyer flask containing 1000 ml of the same medium and incubated on a rotary shaker (200 rpm) for 6daysat 27°C.

Crude extraction

The cells were harvested, washed 3 times in 0.01 M phosphate buffered saline (PBS), pH 7.2, and suspended to obtain a 20% suspension. The cells were disintegrated

by ultrasonic vibrations at a power of 50 to 60 watts intermittently for 10 min. This suspension was centrifuged at 20,000 x g for 30 Min at 4°C and the supernatant thus obtained was dried under lyophilization and stored in the freezer at -20°C.

Animals

Mus Musculus male mice weighing (25-35g) were housed under hygienic conditions in the departmental animal house. Animals were housed under standard conditions of temperature (21±1°C) and up to 12 h of light daily, fed with standard pellet diet and had free access to water. All the experiments were performed in accordance with the institutional animal ethics committee.

Phagocytic index

The clearance rate of carbon was measured by the published method¹². Animals were divided into four groups, consisting of seven mice in GI, GII, GIII, and GIV. Group I (control) was given 0.9% NaCl (0.5 ml/mouse i.p.), Group II-III and VI were administered by i.p injection with different concentrations of *Nocardiosis dassonvillei* strains intracellular crude extract (30, 50, and 100 mg/kg) respectively.

After 48h of i.p injection, the mice were administered with carbon ink suspension (carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml), at a dose of (0.1ml/10g) through the tail vein. 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^{12,13}:

$$K = \frac{(\ln OD_1 - \ln OD_2)}{(t_2 - t_1)}, \quad t_{1/2} = 0.693 / K,$$

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

Where OD₁ and OD₂ are the optical densities at times t₁ and t₂ respectively.

Statistical analysis

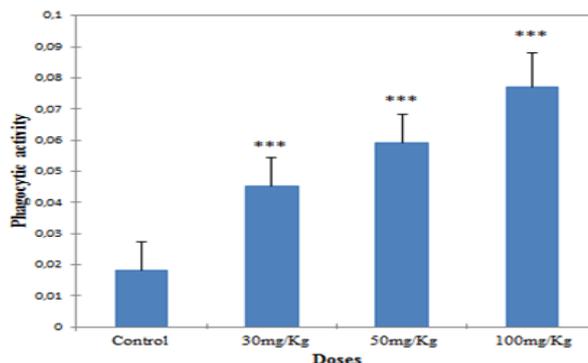
Statistics was applied using XLSTAT 2013 for Windows. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's

comparison test. The values are expressed as mean±SEM and P<0.05 was considered significant.

RESULTS

Effect of intracellular crude extract on the phagocytic activity and the carbon clearance rate is shown in Figure 1. Significant increase in phagocytic activity was observed in treated group dose dependent were compared with control (p<0.001)

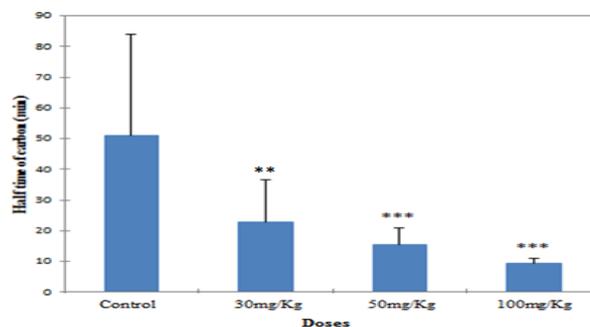
This indicates that crude extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependant manner.



All values are mean±SEM, n=7, **P<0.05, ***P<0.01 when compared to control group

Figure 1: Effect of intracellular crude extracted from *Nocardiosis dassonvillei* Vic8 on phagocytic activity.

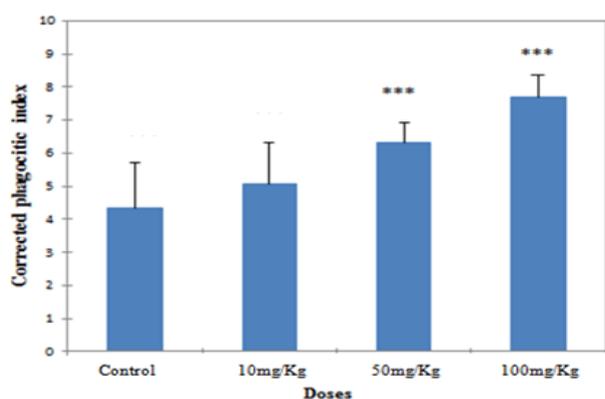
As shown in Figure 2, the half-life of colloidal carbon was highly significantly faster at 48h, after the administration of crude extractions between groups (P= 0,001), and the clearance rate was decreased highly and significantly in groups (GII,GIII and GIV) as compared to the control group (GI) at P<0.05.



All values are mean±SEM, n=7, **P<0.05, ***P<0.01 when compared to control group

Figure 2: Effect of intracellular crude extracted from *Nocardiosis dassonvillei* Vic8 on half time t_{1/2} of carbon in blood.

The results of this study showed in Figure 3, that there is a high significantly difference in the means for the corrected α between groups (GI, GII, GIII and GIV) P<0,0001 and the corrected α was increased highly and significantly in groups (GIII and GIV) as compared with the control group (GI) at <0.001.



All values are mean±SEM, n=7, **P<0.05, ***P<0.01 when compared to control group

Figure 3: Effect of intracellular crude extracted from *Nocardioopsis dassonvillei* Vic8 on corrected phagocytic index.

DISCUSSION

The Reticuloendothelial System (RES) consist of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow and lymphnodes and capillary enthelium of the liver (kuppferscells) and of the adrenal and pituitary glands, these comprise the sessile or fixed macrophage, are transported by the body fluids or wander through the tissues.

In the immune system, phagocytosis a major mechanism by which certain living cells, collectively called as phagocytes, ingest or engulf microorganisms, malignant cells, inorganic particles and tissue debris¹⁴. Assessment of the phagocytic response by the carbon clearance test was aimed to evaluate the effect of intracellular crude extract from Vic8 strain on the reticuloendothelial system (RES), a diffuse system comprising phagocytic cells, including fixed tissue macrophages and mobile macrophages.

Cells of the RES play an important role in the clearance of particles from the blood stream. When colloidal ink containing carbon particles is injected in to the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation¹⁵.

In the present study pre-treatment of animals with *Nocardioopsis dassonvillei* crude extract in various doses showed a remarkable augmentation in the phagocytic function by exhibiting a dose-related increase in the clearance rate of carbon particles by cells of the RES. It may be possible that the crude extract influence the mechanism of phagocytosis, largely distributed monocytes macrophages or R.E.S which result in significant increase in the phagocytic index with carbon clearance test¹⁶.

CONCLUSION

The present study established that *Nocardioopsis dassonvillei* extractions stimulated non specific immune response of the animals by increasing the phagocytic

activity, measured in terms of phagocytic index and this could be attributed to its natural components. This stimulation indicates the presence of active principles in the intracellular of *Nocardioopsis dassonvillei*. The active principles responsible for the stimulatory effect are yet to be identified.

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