Research Article

# Immunomodulatory Activity of Lectins Extracted from *Terfezia bouderei*.

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**ABSTRACT**

The first part of the present work dealt with the extraction and assay of lectin obtained from white truffles *Terfezia Bouderei*. The major technique employed was chromatography on dextran gel G-75, the activity of lectin is inhibited by mannose, each preparation was checked of lectin activity, the later was measured by the amount of agglutination produced using a buffered and stabilized suspension (3%) rabbit red blood cells. The second part of the study included the immuno-stimulation activity of phagocytes. Immunomodulatory activity of extracted lectins from *Terfezia Bouderei* was evaluated on phagocytic activity by carbon clearance test. Adult Albinos Wistar mice randomly divided into four groups, were the first was served as a control, while the remaining groups respectively treated with extracted lectins from *Terfezia Bouderei* at dose of: 10, 30 and 50 mg/kg by intra-peritoneal injection (IP). Change in phagocytic activity was determined after 48 h injection of carbon ink suspension. In carbone clearance test, extracted lectins from *Terfezia Bouderei* exhibited significantly phagocytic index dose-dependent against control group, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that extracted lectins from *Terfezia Bouderei* holds promise as immunomodulatory agent, which act by stimulating dose dependent phagocytic function.

**Keywords:** lectins extraction, agglutination, immuno-stimulation, *Terfezia Bouderei*.

## INTRODUCTION

The immune system plays a vital role in the natural defense, he has the ability to recognize foreign agents in the body, involving two types of defenses: non- specific, natural or innate defenses and adaptive defenses or adaptive. The innate defense is immediate, she is the first line of defense of the body. The penetration of pathogenic agents in the blood stream causes the activation of phagocytic cells, including macrophages, leads to ingestion and digestion of foreign agent and the production of cytokines which in turn contribute to the inflammatory reaction[1-3]. The adaptive or specific response is the second line defense; it involves the B and T lymphocytes on their surface receptors for antigens[4]. The modulation of the immune-system has become an important aspect of anti-infectious research. Indeed, the excessive activation can lead to adverse consequences for the host.

The interest shown these last few years for mushroom lectins is mainly motivated by the discovery that lectins have valuable pharmacological properties such as the stimulation the immuno-system, antiviral and anticancer[5-8].

In the present study, we investigated the immunomodulatory effect of an extract of lectins derived from an edible mushroom, white truffle (Algerian sahara) using phagocytic activity by carbon clearance test *in vivo* experimental model mice.

## MATERIALS AND METHODS

The lectins extracted from *Terfezia Bouderei* used in this work originated from Algerian sahara.

### Preparation of Extracts

Seeds of *Terfezia Bouderei* were grounded to be a fine powder using blender to top speed. The dry powder was incubated in phosphate buffer (0.1M, pH 7.2) for approximately 12h at 4 °C. The mixture was then centrifuged at 12000xg for 20 min, the supernatant was applied to a gel chromatography on dextran G-75. Following that, the fractions contained lectins were dialyzed against distilled water and then lyophilized; the lyophilized extracts were dissolved in 0.9% NaCl and injected interperitoneally into mice at concentrations of 10, 30 and 50 mg/Kg body weight for determination of phagocytic activity.

### Assay for Hemagglutinating Activity

Agglutination activity was measured in micro-titer plates using serial two fold dilutions of lectins. Each well contained 50µl of rabbit red blood cells (3%) and 50µl of extracted lectins at room temperature the results were read after one hour.

### Phagocytic Activity

Animals *Albinos Wistar* mice were housed under hygienic conditions in the departmental animal house. Animals were housed under standard conditions of temperature (21 ± 1 °C), and up to 12h 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 activity index was determined as per the method reported by Cheng[9]. Phagocytic activity of reticulo-endothelial system was assayed by carbon clearance test. Phagocytic index was calculated as a rate

of carbon elimination of reticulo-endothelial system by clearance test. In this test four groups of animals were used.

Group I was kept as a control, while animals of treatment group: II, III and VI were administrated extracted lectins from *Terfezia Bouderei* at dose of: 10, 30 and 50 mg/kg by interperitoneally injection respectively. After 48 h, phagocytic activity was determined.

Mice were injected with Carbon ink suspension at a dose 0.1 ml/100g via tail vein, the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3% gelatine solution 4 ml.

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 (4 ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer.

The phagocytic activity is expressed by the phagocytic index K which measures all the reticulo-endothelial system function in the contact with the circulating blood.

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[10]:

$$K = \frac{lnOD1 - lnOD2}{t2 - t1}, \quad t_{1/2} = \frac{0.963}{k}$$

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

### Statistical Analysis

The data were subjected to Anova test for comparison between groups. The values are expressed as Mean ± SEM. Significance level was set at P<0.05, P<0.01, P<0.001.

## RESULTS

Agglutination activity against rabbit a red blood cell (3%) was carried out and assessed visually (Figure 1) and (Table1).

The activity was recorded against rabbit blood cells. The inhibitory effect of various carbohydrates on the agglutinating activity of the crude extract was then investigated; the mannose inhibited the agglutination activity. We attempted to purify the lectin using mannose sepharose 4B followed by further purification using FPLC.

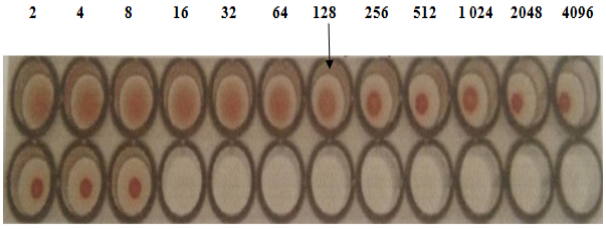


**Figure 1:** Haemagglutination activity of Truffle lectin against rabbit blood cells.

**Table 1:** Haemagglutination Titer and the Concentration of Protein at different dilutions measured at 280 nm.

| Dilution Tubes | Protein Concentrations (mg/ml) |
|---|---|
| 2 | 3,64 |
| 4 | 1,82 |
| 8 | 0,91 |
| 16 | 0,455 |
| 32 | 0,2275 |
| 64 | 0,11375 |
| 128 | 0,056875 |
| 256 | 0,0284375 |
| 512 | 0,01421875 |
| 1024 | 0,00710938 |
| 2048 | 0,00355469 |
| 4096 | 0,00177734 |

### Effects of Lectins extracted from *Terfezia bouderei* on Phagocytic Activity

An increase in phagocytic activity was observed in treated group dose-dependent were compared with control (Figure 2).

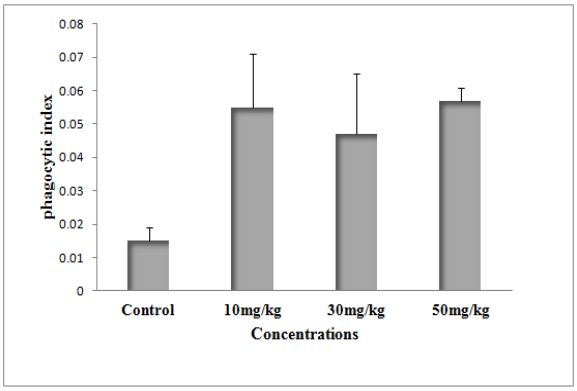


**Figure 2:** Effect of lectins extracted from *Terfezia bouderei* on phagocytic activity

### Effects of Lectins extracted from *Terfezia bouderei* on half-time $t_{1/2}$ of carbon in blood

Figure 3 show decreases in half-time of carbon in blood dose-dependent in treated group were compared with control.

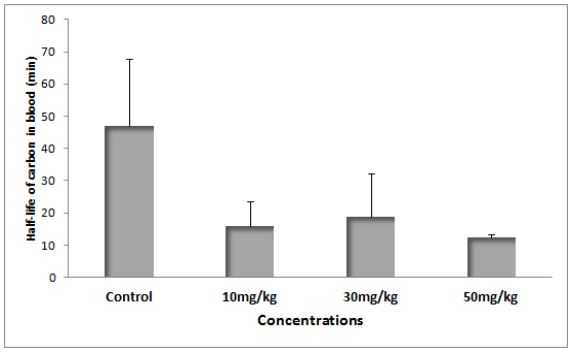


**Figure 3:** Effect of lectins extracted from *Terfezia bouderei* on half -life $t_{1/2}$ of carbon in blood

## DISCUSSION

The lectin has been extracted from white truffles .The crude lectin obtained from the mushroom is a glycoprotein. Agglutination was observed with rabbit red blood cells due to the interaction between lectins and sugars on the surface of the cells.

The agglutination of red blood cells by lectin was inhibited specially in the presence of mannose. The lectin extracted from white truffles is found to stimulate the macrophage. The phagocytic cells may be activated by the interaction of their glycans surface with lectin.

These results show us that the two parameters (K index and clearance rate) are not moving parallel to the lectin injected dose.

At this stage of raisonment, we can say that the intensity of the phagocytic response obtained in mice occurs rapidly just after inoculation by a foreign body; between 5 and 15 minutes after. This stimulation of the phagocytic activity of reticulo-endothelial system could certainly be of two parameters defense specific and non specific.

The specific mechanism is linked to the formation of antibodies against the foreign body and the mechanism of immunity is directly related to the increase in mononuclear phagocytic activity of macrophages, which is agreed with the experiences of several researchers[5,6] which revealed immunomodulatory activity of a new lectin isolated from a fungus volvarrelle volvacea.

Necib[10,11], Aribi[12] and Hou[13] in the work performed in mice treated by *Argania spinosa* and red beans lectins in the presence of polysaccharide from Astragalus mongholicus respectively.

Modulation of the immune system is and will remain an issue in development, and it's clear that a number of plants, fungi molecules such as lectins have important therapeutic properties. A trough search of their mechanism of action and structure, should allow their selection.

In this study we are limited to looking for eventual immunomodulatory effects associated with lectins contained in the Algerian Sahara white truffle.

The choice of this fungus fairly widespread and consumed by southern populations on the one hand, and due to the lack of research in the context of other parts, we steered in that direction what immunomodulation.

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