Research Article

Immunomodulatory Activity of Lectins Extracted from *Illicium Verum*.

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ABSTRACT

Immunomodulatory activity of extracted lectins from *Illicium Verum* 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 *Illicium Verum* 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 *Illicium Verum* exhibited significantly phagocytic index dose-dependent against control group, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that extracted lectins from *Illicium Verum* holds promise as immunomodulatory agent, which act by stimulating dose dependent phagocytic function.

Keywords: lectins extraction, immuno-stimulation, Carbon Clearance rate, *Illicium Verum*.

INTRODUCTION

Lectins constitute a group of proteins or glycoproteins of non-immune origin, which bind reversibly to carbohydrates and usually agglutinate cells or precipitate polysaccharides and glycol conjugates[1]. The lectins were redefined by Peumans & Van Damme (1995)[2] as proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono or oligosaccharide. However, according to Cummings (1997)[3], antibodies and proteins with enzymatic activity related to carbohydrates cannot be considered as lectins. As a consequence of their chemical properties, they have become a useful tool in several fields of biological research (immunology, cell biology, membrane structure, cancer research and genetic engineering). Lectins are present in a wide range of organisms from bacteria to animals, being present in all classes and families, although not in all the kinds and species[4]. Many flowering plants from diverse taxonomic groups accumulate large quantities of so-called vegetative storage proteins (VSPs) in various vegetative storage organs. These VSPs play a primary role in nitrogen accumulation, storage, and distribution in biennial and perennial plants, and, accordingly, are believed to contribute to the survival of the plant in its natural environment[5]. Moreover, some VSPs with a particular enzymatic or other biological activity act as a specific defense proteins against herbivorous animals or phytophagous invertebrates, for example, and hence may play a dual storage/defense role[6,7]. The concept of functional "vegetative" homologs of the classic seed storage proteins was originally developed for two proteins, called VSP_α and VSP_β, that accumulate in large quantities in soybean (*Glycine max*) leaves, seed pods, and hypocotyls forced to act as a nitrogen sink[8,9]. However, it is evident that the term VSP also applies to the previously identified major tuber proteins from potato (*Solanum tuberosum*) patatin,[10] and sweet potato *Ipomoea batatas*; sporamin[11], as well as to all other abundant proteins found in various vegetative storage organs like bulbs, tubers, and rhizomes.

In the present study, we investigated the immunomodulatory effect of an extract of lectins derived from *Illicium Verum* using phagocytic activity by carbon clearance test in vivo experimental model mice.

MATERIALS AND METHODS

The lectins extracted from *Illicium Verum* used in this work originated from Algerian.

Preparation of extracts

Seeds of *Illicium Verum* were grounder 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 24h at 4°C.

The mixture was then centrifuged at 6000 rpm for 30 min, the remaining debris was removed by passing the supernatant through filter paper[12]. 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.

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[13]. 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 *Illicium Verum* at dose of: 10, 30 and 50mg/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 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 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[14]:

$$K = \frac{lnOD1 - lnOD2}{t2 - t1},$$

$$t_{1/2} = \frac{0.963}{k}$$

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 student *t* 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

Effects of lectins extracted from *Illicium Verum* on phagocytic activity

Significant increase in phagocytic activity was observed in treated group dose -dependent were compared with control (Figure 1).

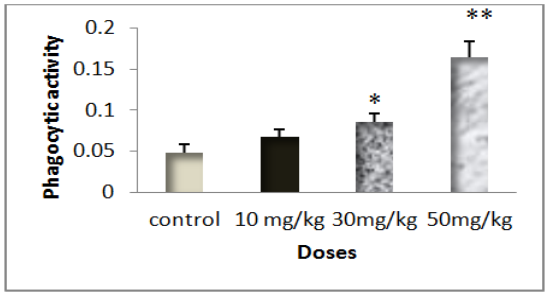

Figure 1: Effect of lectins extracted from *Illicium Verum* on phagocytic activity

Effects of lectins extracted from *Illicium Verum* on half-time $t_{1/2}$ of carbon in blood

Figure 2 show a significant decrease in half-time of carbon in blood dose-dependent in treated group were compared with control.

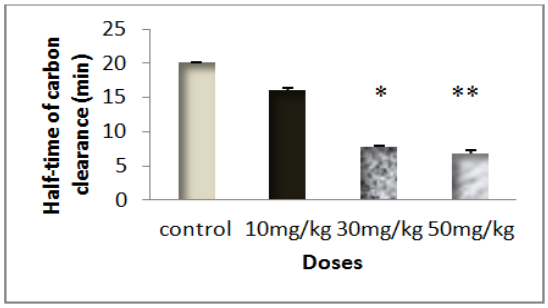

Figure 2: Effect of lectins extracted from *Illicium Verum* on half-life $t_{1/2}$ of carbon in blood

DISCUSSION

The reticulo-endothelial system (R.E.S) consist of the spleen, thymus and other lymphoid tissues, together with cells lining the sinuses of the spleen, bone marrow, and lymph nodes and capillary enthelium of the liver (kuppfers cells), 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. The RES is the best defined functionally by its ability to scavenge debris or other foreign matter and forms first line of defense. The rate of removal of carbon particles, by the sessile intravascular phagocytes in the liver and spleen, from the blood stream is a measure of reticulo-endothelial phagocytic activity. In the present study, carbon clearance test, extracted lectin from *Illicium Verum* treated groups, exhibited significantly high phagocytic index. This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the extracted lectin from *Illicium Verum* 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 test. Necib in the work performed in mice treated by *Argania spinosa* indicate that treatment of mice with this plants exhibited significantly high phagocytic index[15,16].

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