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IMMUNOMODULATORY ACTIVITY OF LECTIN EXTRACTED FROM THE RED MARINE ALGA *PTEROCLADIELLA CAPILLACEA*

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ABSTRACT

A lectin present in the marine red alga *Pterocladiella capillacea* was extracted by soluble proteins (crude extract) in phosphate buffer (0.1M, pH 7.2). The lectin agglutinated specifically rabbit erythrocytes. The hemagglutinating activity assay showed that the lectin was not dependent on divalent cations and was shown an agglutination to B blood human group. Extracted lectin of *Pterocladiella capillacea showed* thermo stability in more than 100°C. However, the lectin was stable in the PH ranged between 2 to12 and was showen to be inhibited by the glycoproteins fetuin, BSA and ovalbumin and by the sugare raffnose. Immunomodulatory activity of extracted lectins from marine red alga *Pterocladiella capillacea* 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 marine red alga *Pterocladiella capillacea* at dose of: 25, 50 and 100 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 marine red alga *Pterocladiella capillacea* exhibited significantly phagocytic index dose-dependent against control group, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that extracted lectins from marine red alga *Pterocladiella capillacea* holds promise as immunomodulatory agent, which act by stimulating dose dependent phagocytic function.

KEYWORDS: Immunomodulatory, Carbon Clearance rate, *Pterocladiella capillacea*

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 glycoconjugates.[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 can not 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] The first report on the occurrence of lectins in marine algae is relatively recent.[5] Although several studies on lectins from marine algae have been reported, the number of these proteins purified and characterised is still considered small. These studies include the green algae *Codium tomentosus* (Huds.) Stackhouse,[6] *Ulva lactuca* L.,[7] and *Caulerpa cupressoides* (Vahl) C. Agardh (Benevides *et al.* 2001); the brown algae *Fucus vesiculosus* L.,[8] *Dictyota dichotoma* (Hudson) Lamouroux[9] and the red algae *Plumaria elegans* (Bonnem.) Schmitz and *Ptilota serrata* Kützing,[10] *Solieria filiformis* (Kützing) Gabrielson[11] and *Enantiocladia duperreyi* (C. Agardh) Falkenberg.[12] Algal lectins differ from higher plant lectins in a variety of properties. In general, algal lectins have lower molecular masses then most higher plant lectins and have no affinity for simple sugars but are more specific for complex oligosaccharides, often glycoproteins. Furthermore, most of marine algal lectins do not require divalent cations for their biological activity.[13] They occur mainly in monomeric forms and have a high proportion of acidic amino acids, with isoeletric points from 4 to 6.[14] Compared to plant lectins, there are only a few reports on the use of marine algal lectins. For example, lectin extracts from some marine algae have shown to agglutinate strongly mouse FM3A tumor cells in lower concentrations than those required for lectins from land plants.[15,16,17] Dalton *et al.* (1995)18 found that the pre-purified lectin of some marine algae exhibited high mitogenic activity for human lynphocytes. Furthermore, Griffin *et al.* (1995)[19] demonstrated the use of *Codium fragile* (Suringar) Hariot lectin conjugated to collodial gold as a new histochemical reagent. Following our continuous investigation of marine algal lectins, in the present work we describe the extracted of a new lectin from the marine red alga *Pterocladiella capillacea* collected from Algeria and investigated the

immunomodulatory effect by using phagocytic activity by carbon clearance test in vivo experimental model mice.

MATERIALS AND METHODS

The lectins extracted from *Pterocladiella capillacea* used in this work originated from Algerian. Human blood group A, B and O erythrocytes were collected from healthy donors. Rabbit was obtained by venous puncture of healthy animals.

Preparation of Extracts

Seeds of *Pterocladiella capillacea* 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.[20] 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 25, 50 and 100 mg/Kg body weight for determination of phagocytic activity.

Preparation of Sephadex G75

4g of dephadex G75 was suspended in 100ml of phosphate buffer (0.1M, PH: 7.2). The mixture was then incubated for 48h at room temperature. Finally it was packed into a 12x1.2 column for used for extracted lectin of *Pterocladiella capillacea.*

Extracted Lectin from *Pterocladiella Capillacea* by Sephadex G75

Supernatant sample of *Pterocladiella capillacea* were loaded into sephadex G75 column equilibrated with phosphate buffer (0.1M, PH7.2). The absorbance at 280 nm was used to estimate protein content in column eluates.

Hemagglutinin Assay

The experiment was performed in microtiter plates, according to Correia and Coelho (1995)21. 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.

Inhibition Tests

Inhibition tests were carried out using stock solutions (in 0.9% NaCl) of sugars and glycoproteins. A two-fold dilution series was prepared for each substance in 0.9% NaCl with a final volume of 50 µL. Aliquots of the diluted lectin were added to each tube of the diluted inhibitor series. The mixture was incubated at room temperature for 1 h, before the addition of the erythrocytes suspension (50µL). The hemagglutination inhibition activity was recorded as the highest sugar dilution which inhibited the agglutinating activity.

Metal Ions Test

To evaluate the effect of metal ions and EDTA on hemagglutinating activity, serial aliquots of two-fold dilutions of lectin solution were previously dialysed against 5 mM EDTA. The material was used for hemagglutination assays in the absence and presence of either 5 mM CaCl2 or MnCl2. The hemagglutinating activity was measured by addition of rabbit erythrocytes.

PH test

The buffers used to study the stability of *P. capillacea* lectin under different conditions of pH were phosphate buffer (0.1M) at different PH (1 to 12).

Heat Stability Test

The heat stability of the hemagglutinating activity of *Pterocladiella capillacea* lectin was determined by incubation of aliquots of lectin solution at different temperatures (40, 60, 80 or 100°C) for 1h and the remaining hemagglutinating activity determined.

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 acess 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 *et al*., 2005[22]. 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. GroupI was kept as a control, while animals of treatment group: II, III and VI were administrated extracted lectins from *Pterocladiella capillacea* at dose of: 25, 50and 100mg/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.[23]

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

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 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

Extracted Lectin from *Pterocladiella Capillacea* by Sephadex G75

It was found that in elution fraction of *Pterocladiella capillacea* presented on pick (Fig.1).

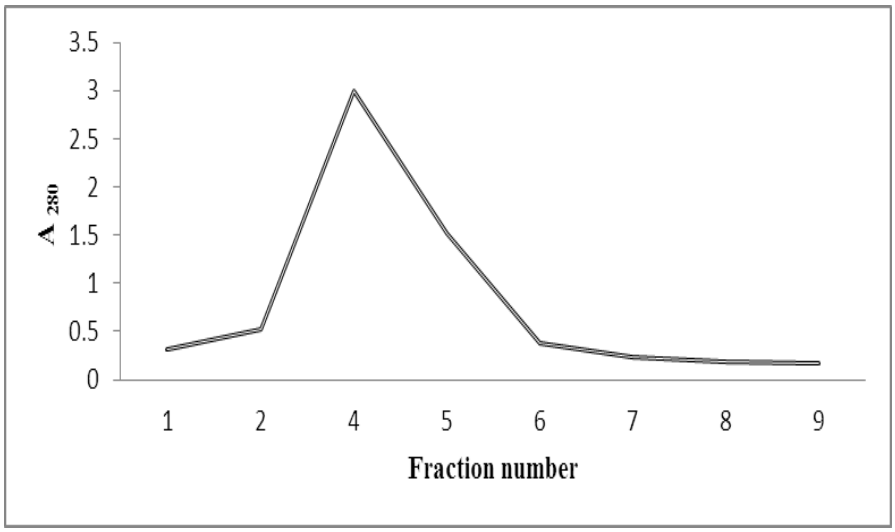

Figure 1: Extracted lectin from the marine alga pterocladiella capillacea by sephadex G75.

Hemagglutinin Assay

The extracted lectin from *Pterocladiella capillacea* showed a highly agglutination when addition of rabbit erythrocytes suspension (Fig. 2).

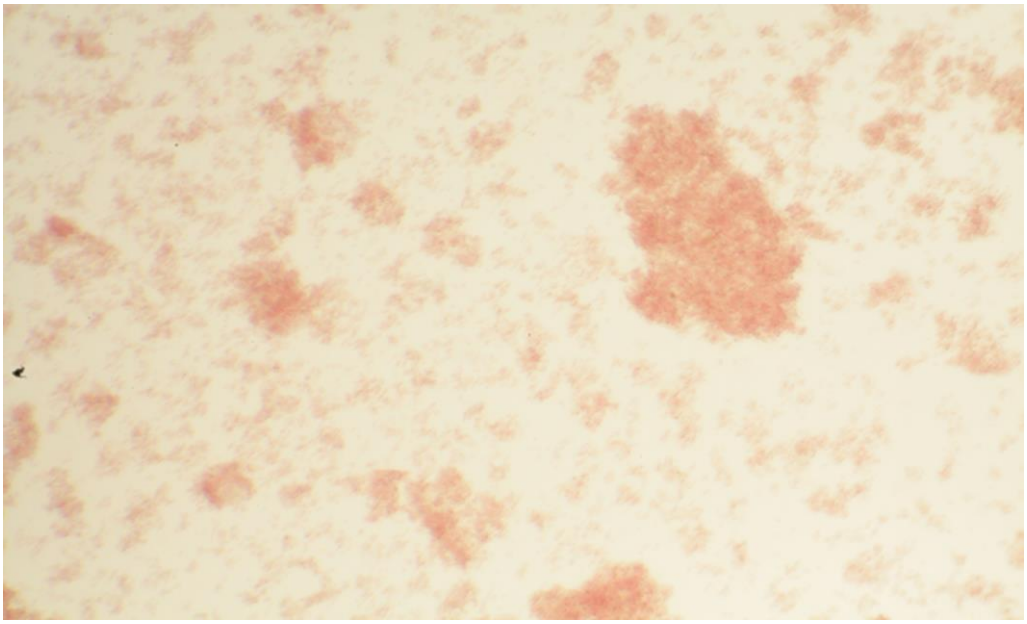

Figure 2: hemagglutinin of lectin extracted from marine red alga pterocladiella capillacea with suspension of rabbit erythrocytes GX40.

Inhibition Tests

The results of sugar inhibition tests using a large number of simple sugars and glycoproteins for *Pterocladiella capillacea* lectin are presented in table1 and 2.

The extracted lectin from *Pterocladiella capillacea* did not show any inhibition by all simple sugars tested, only with raffnose at 200mM concentration. of the glycoproteins tested, only fetuin, BSA and ovalbumin were inhibitory requiring the same concentration.

Table1: Inhibition of the heamagglutinating activity of the lectin extracted from the red alga Pterocladiella capillacea by glycoproteins.

Glycoproteins	Hemagglutinating activity
Fetuin	+
Insuline	-
Casein	-
BSA	+
Ovalbumin	+

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Table2: Inhibition of the heamagglutinating activity of the lectin extracted from the red alga Pterocladiella capillacea by Sugars.

Sugars	Hemagglutinating activity
Glucose	-
Galactose	-
Lactose	-
Mannose	-
D-glucosamise	-
Xylose	-
Galactopyranose	-
Manitol	-
Maltose	-
Melibiose	-
inositol	-
Fucose	-
Raffnose	+
Arabinose	-
Fructose	-
N-acétyl-glusamine	-
Sorbose	-
Saccharose	-
Methyl-fucopyranoside	-
Methyl-mannopyranoside	-
N-acétyl-galactosamine	-
Sorbitol	-
Methyl-B-L-fucopyranoside	-
Xylitol	-
Cellulose	-
Rhamnose	-

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Effect of Metal ions on Heamagglutinating Activity of Extracted Lectin from Marine Red Alga *Pterocladiella Capillacea*

The hemagglutinating activity of the extracted lectin was not affected by the presence of 5mM EDTA, showing that the lectin is not a metallic protein (Table 3).

Table3: effect of metal ions on heamagglutinating activity of extracted lectin from Pterocladiella capillacea.

Metal ions (5mM)	EDTA	MnCl2	MgCl2	CaCl2
Heamagglutanating activity	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Effect of PH on Heamagglutinating Activity of Extracted Lectin from Marine Red Alga *Pterocladiella capillacea*

The extracted lectin was stable in the PH 2to12 retaining 50% of its hemagglutinating activity at PH 1 (Table 4).

Table 4: effect of PH on heamagglutinating activity of extracted lectin from Pterocladiella capillacea.

PH	1	2	3	4	5	6	7	8	9	10	11	12
Heamagglutinating activity	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Effect of Heat on Heamagglutinating Activity of Extracted Lectin from Marine Red Alga *Pterocladiella capillacea*

In addition, the hemagglutinating activity of extracted lectin from *Pterocladiella capillacea* when submitted to heat treatment was stable until 100°C during 1h (Table 5).

Table 4: effect of Heat on heamagglutinating activity of extracted lectin from Pterocladiella capillacea.

Heat	40°C	60°C	80°C	100°C
Heamagglutinating activity	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Blood Human Test (ABO)

Extracted lectin from *Pterocladiella capillacea* presented a spécifique highly agglutination to B blood human group (Table 5).

Table 5: effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from Pterocladiella capillacea.

Blood human	A	B	O
heamagglutinating activity	---	+++	---

+++: highest heamagglutinating activity

---: non heamagglutinating activity.

Effects of Lectins Extracted from *Pterocladiella Capillacea* on Phagocytic Activity

Significant increase in phagocytic activity was observed in treated group dose -dependent were compared with control (Figure 3).

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

Figure 4 show a significant decrease in half-time of carbon in blood dose-dependent in treated group were compared with control.

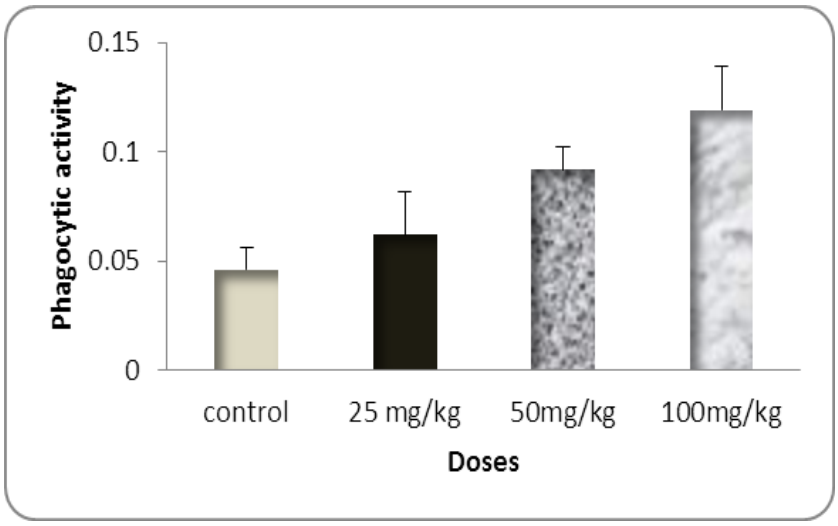

Figure 3: Effect of lectins extracted from marine red alga Pterocladiella capillacea on phagocytic activity.

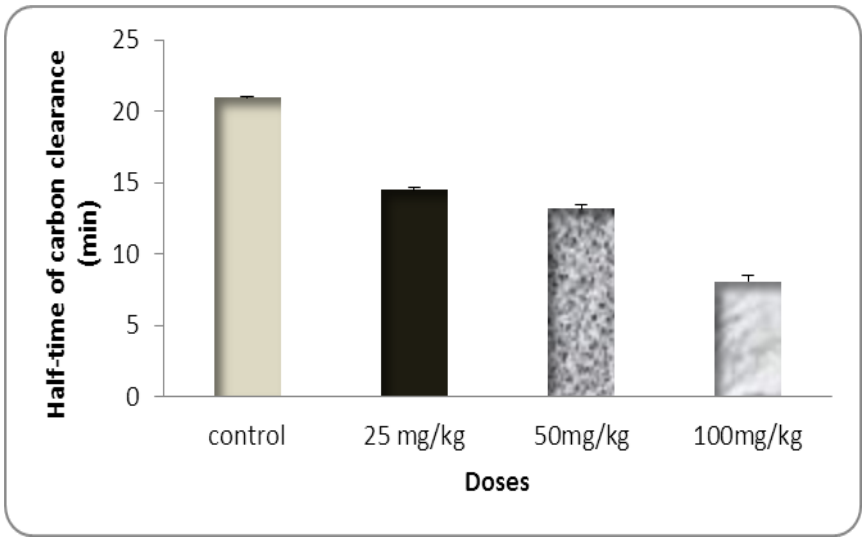

Figure 4: Effect of lectins extracted from extracted lectin from marine red alga Pterocladiella capillacea on half -life t1/2 of carbon in blood.

DISCUSSION

The high content of water is a peculiar characteristic in the diverse classes of algae studied, probably due to the necessity of a thermal regulator mechanism of these organisms. Among the organic constituents the carbohydrates was the highest one. The results show that this alga species can be regarded as a protein source, comparable to values found to various leguminous seeds and, consequently a lectin source. Cross-linked guar-gum, a galactomannan consisting of chains of (1→4) linked β-D-mannose with α-D-galactose linked (1→6) as single unit side chains, has been used as an efficient, inexpensive and rapid general affinity medium for the purification of lectins from land plants.[24] The utilization of affinity chromatography is also an important tool in the process of purification of algae lectins. Many lectins from these vegetables were isolated by this technique, such *Ptilota filicina* J. Agardh.[24] *Enantiocladia duperreyi*[12] and *Caulerpa cupressoides.*[25] Most of the lectins isolated from marine red algae have low molecular weight. The marine red alga *Hypnea japonica* Tanaka contains four lectins with molecular weights of 4.2-12.0 kDa.[16] The lectin from the red alga *Bryothamnion seaforthii* (Turner) Kützing exhibited a molecular mass of 3.5 kDa,[26] while the lectin from *Bryothamnion triquetrum* (Gmelim) Howe displayed a molecular mass of 4.5 kDa.[27] The hemagglutination inhibition studies carried out with purified *Pterocladiella capillacea* lectin, revealed that the lectin is not inhibited by simple sugars but by glycoproteins. This is in general agreement with those found for the numerous marine algal lectins, such as *Cystoclonium purpureum* (Huds.) Batters, *Solieria chordalis* (C. Agardh) J. Agardh, *Plumaria elegans* and *Ptilota serrate.*[10] *Gracilaria bursa-pastoris* (Gmelin) Silva.[28] *Solieria filiformis*[11] and *Gracilaria verrucosa* (Hudson) Papenfus.[29] Algal lectins are, in general, more specific for complex oligosaccharides often glycoproteins.[13] Therefore, the inhibition of the hemagglutinating activity from the marine algae lectins by glycoproteins was also observed in some marine algal, such as *Agardhiella tenera* Schmitz, *Ulva lactuca,*[7] *Bryothamnion seaforthii* (Turner) Kützing and *B. triquetrum,*[26] and *Amansia multifida* Lamouroux.[30] Like most lectins from marine red algae,[13] the *Pterocladiella capillacea* lectin does not require divalent cations for the maintenance of its biological activity, since the addition of EDTA to the reaction medium did not affect the haemagglutinating activity, suggesting that this lectin is not a metallic protein. However, the lectins from the red algae *Ptilota serrate,*[10] *Ptilota filicina.*[25] *Enantiocladia duperreyi*[12] and from the green algae *Ulva laetevirens* Areschoug and *Ulva lactuca*[7] exhibited dependence of metals such as Ca2+, Mn2+ and Mg2+, as is the case with most plant lectins. In accordance with Rogers & Hori (1993)13 lectins from the red algae do not

require divalent cations for their biological activity. The hemagglutinating activity from the *Pterocladiella capillacea* lectin was not affected by exposure to a temperature of100°C for 1h . The absence of carbohydrate in the structure of the lectin differ of the observed to another lectins from marine algae: *Codium tomentosum* (Huds) Stackhouse, *Bryothamnion seaforthii* and *Bryothamnion triquetrum*,[26] *Solieria filiformis* (Kützing) Gabrielson[11] *Enantiocladia duperreyi*[12] and *Caulerpa cupresssoides.*[25]

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 *Pterocladiella capillacea* treated groups, exhibited significantly high phagocytic index.[31] This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the extracted lectin from *Pterocladiella capillacea* 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.[32]

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