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COMPARATIVE STUDY OF A NEW LECTIN EXTRACTED FROM ROOTS OF PLANTS: *CYPERUS ROTUNDUS, PISTACIA LENTISCUS AND RUTA GRAVEOLENS*

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ABSTRACT

A lectin present in Root of plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* were extracted by soluble proteins (crude extract) in phosphate buffer (0.1M, pH 7.2). The lectin of the plants agglutinated specifically rabbit erythrocytes. The hemagglutinating activity assay showed that the lectin of plants were not specific for blood human group. Extracted lectin of *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* showed thermo stability in more than 100°C. However, the lectin of *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* were stable in the PH ranged between 2 to12, 5to 12 and 3to 12 respectively and were showen to be inhibited by the glycoproteins fetuin, BSA, casein and ovalbumin and by the sugars D-glucosamine and methyl-mannopyranoside. Immunomodulatory activity of extracted lectins from rhizome plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* were 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 root plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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 rhizome plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* exhibited significantly phagocytic index

dose-dependent against control group, indicating stimulation of the reticulo-endothelial system. Present study thus reveals that extracted lectins from rhizome plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* marine holds promise as immunomodulatory agent, which act by stimulating dose dependent phagocytic function.

KEYWORDS: Immunomodulatory, Carbon Clearance rate, *Cyperus Rotundus, Pistacia Lentiscus, Ruta Graveolens*

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] Lectins are heterogeneous proteins of non-immune origin and with at least one non-catalytic domain. Lectins are able to specifically recognise carbohydrates.[5] These molecules could reversibly bind to carbohydrates without altering their covalent structure.[6] Lectins have been extensively distributed in nature. Their presence has been noted in plants, viruses, bacteria, invertebrates, and vertebrates.[5] These molecules may have several functions in living organisms but the entomotoxic properties of plant lectins are important in pest control strategies.[7] In fact, the majority of plant lectins bind to O- and N-glycans of animal glycoconjugates. This means that lectins are supposed to play a part in plant defense against plant-eating (phytophagous) invertebrates or higher vertebrates.[8] Certain plant tissues, such as seeds, bark, and bulbs, contain high lectin concentrations which might indicate that lectins play a role as storage proteins.[7] Lectins extracted from different plant sources exhibit a considerable degree of structural similarity but also considerable differences in their carbohydratebinding specificities.[5] Decades of research have led to the classification of plant lectins into twelve lectin families.[9] Several phenomena induce expression of lectin including: salt stress, pathogen infection, jasmonic acid treatment, and insect herbivory.[7] In

the present work we describe the extracted of a new lectin from the roots plants of *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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 roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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 roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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.[10] 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 roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens.*

Extracted Lectin from Roots Plants *Cyperus Rotondus, Pistacia Lentiscus and Ruta Graveolens* by Sephadex G75

Supernatant sample of roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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)11. 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.

PH Test

The buffers used to study the stability of roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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 roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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.[12] 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 roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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.[13]

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

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

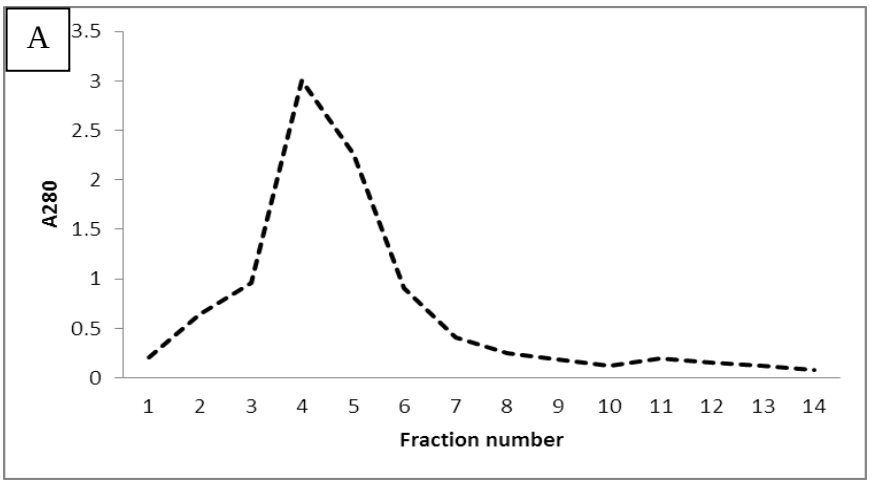
Statistical Analysis

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 roots plants *Cyperus Rotondus, Pistacia Lentiscus and Ruta Graveolens* by sephadex G75

It was found that in elution fraction of roots plants *Cyperus Rotundus, Pistacia Lentiscus* presented on pick but elution fraction of *Ruta Graveolens* presented two pick can be explained by the presence probably two types of lectin in this extract (Fig.1).



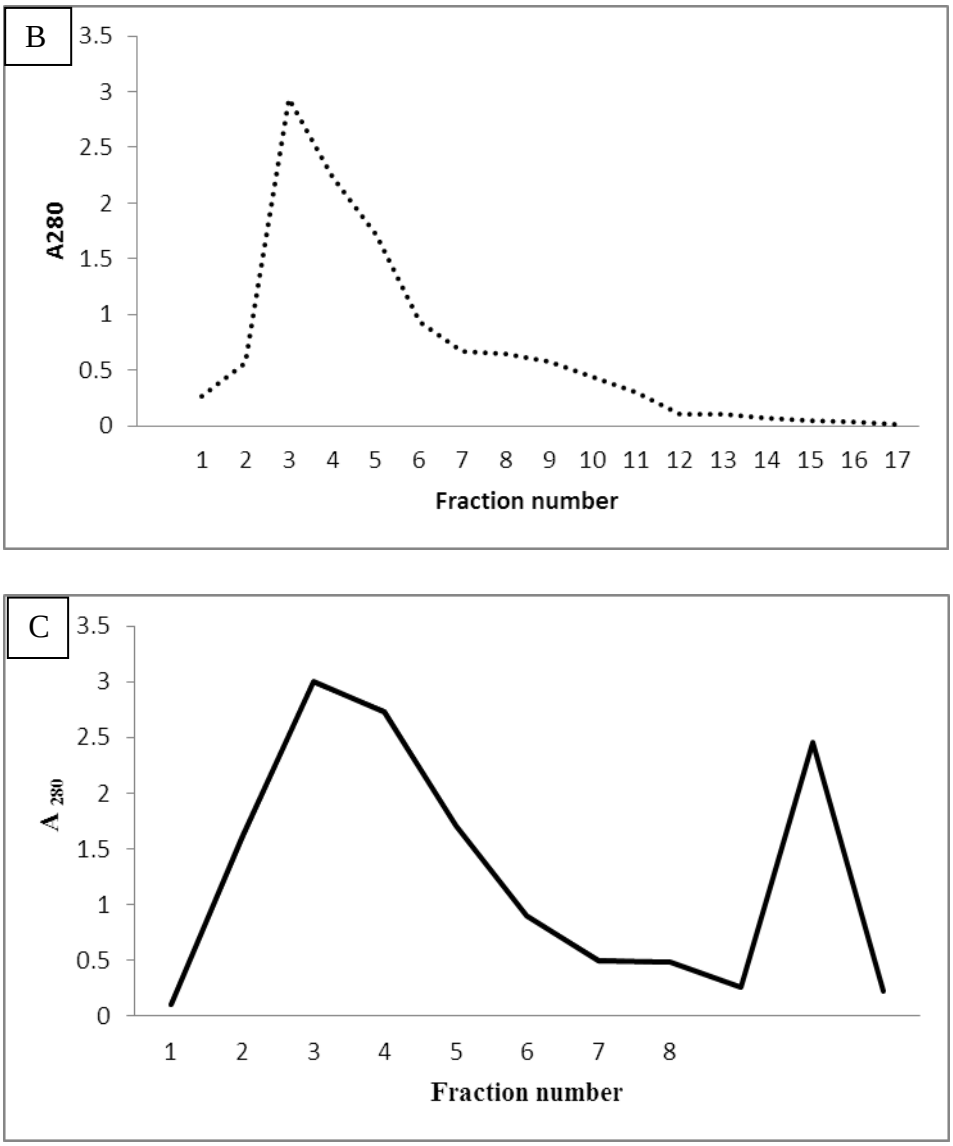


Figure 1: Extracted lectin from the roots plants Cyperus Rotundus (A), Pistacia Lentiscus (B) and Ruta Graveolens(C) by sephadex G75.

Hemagglutinin Assay

The extracted lectin from the roots plants *Cyperus Rotundus* (A), *Pistacia Lentiscus* (B) *and Ruta Graveolens*(C) showed a highly agglutination when addition of rabbit erythrocytes suspension (Fig. 2).

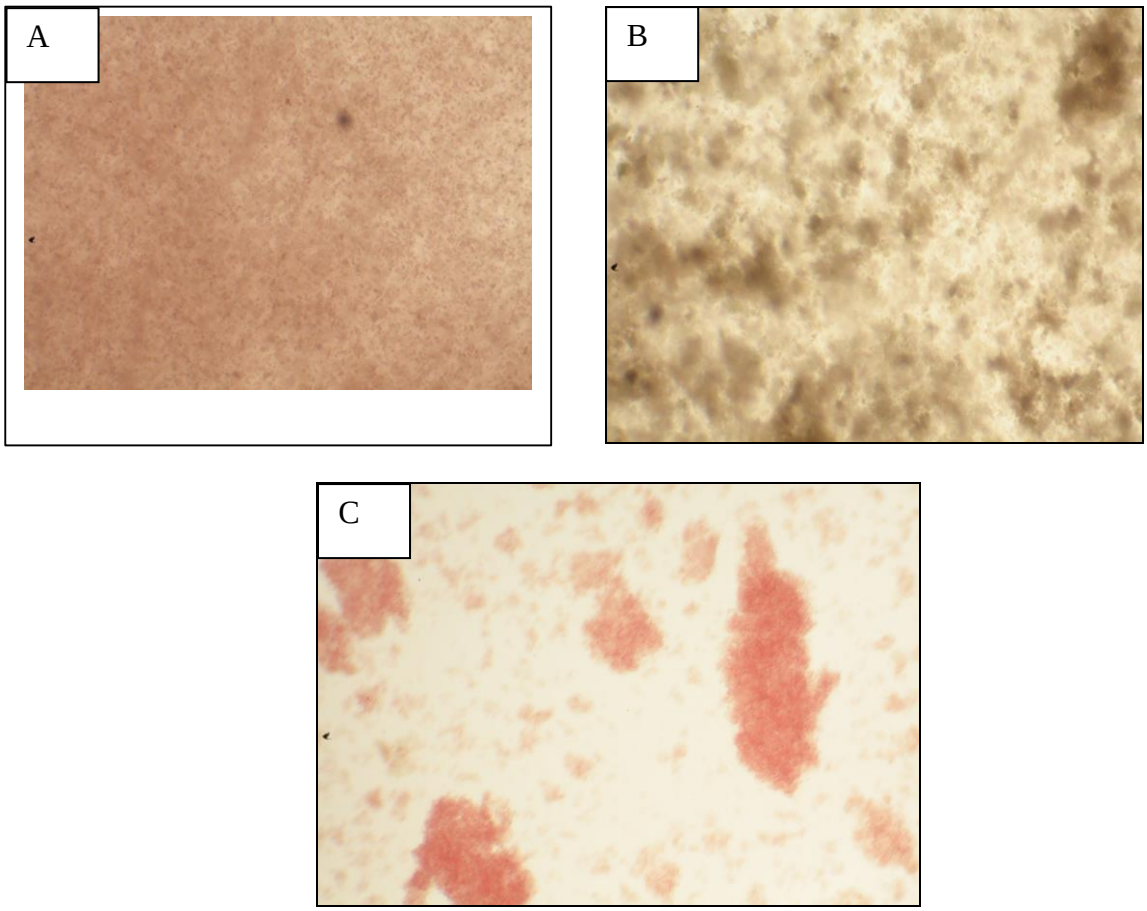


Figure 2: hemagglutinin of lectin extracted from the roots plants Cyperus Rotundus (A), Pistacia Lentiscus (B) and Ruta Graveolens(C) with suspension of rabbit erythrocytes GX40.

Inhibition Tests

The results of sugar inhibition tests using a large number of simple sugars and glycoproteins for the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* lectin are presented in table1 and 2.

The extracted lectin from the roots plants *Pistacia Lentiscus* did not show any inhibition by all simple sugars and glycoproteins tested. *Cyperus Rotundus* presented inhibition with sugars only with D-glucosamine and glycoproteins with fetuin and casein but *Ruta Graveolens* presented inhibition by some sugars D-glucosamine, arabinose and methyl-mannopyranoside 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 roots plants Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens by glycoproteins.

Glycoproteins	*Cyperus Rotundus*	*Pistacia Lentiscus*	*Ruta Graveolens*
Fetuin	+	-	+
Insuline	-	-	-
Casein	+	-	+
BSA	-	-	+
Ovalbumin	-	-	+

+: Inhibition of the heamagglutinating activity.

-: non inhibitory.

Table2: Inhibition of the heamagglutinating activity of the lectin extracted from the roots plants Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens by Sugars.

Sugars	*Cyperus Rotundus*	*Pistacia Lentiscus*	*Ruta Graveolens*
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 PH on Heamagglutinating Activity of Extracted Lectin from the Roots Plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens*

The extracted lectin from the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* were stable in the PH 2to12, 5 to 12 and 3 to 12 respectively (Table 3).

Table 3: effect of PH on heamagglutinating activity of extracted lectin from the roots plants Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens.

PH	1	2	3	4	5	6	7	8	9	10	11	12
Cyperus Rotundus	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Pistacia Lentiscus	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
Ruta Graveolens	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Effect of Heat on Heamagglutinating Activity of Extracted Lectin from the Roots Plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens*

In addition, the hemagglutinating activity of extracted lectin from the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* when submitted to heat treatment was stable until 100°C during 1h (Table 4).

Table 4: effect of Heat on heamagglutinating activity of extracted lectin from the roots plants Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens.

Heat	40°C	60°C	80°C	100°C
Cyperus Rotundus	+++	+++	+++	+++
Pistacia Lentiscus	+++	+++	+++	+++
Ruta Graveolens	+++	+++	+++	+++

+++: highest heamagglutinating activity.

Blood Human Test (ABO)

Extracted lectin from the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* not presented any specific agglutination to blood human group (Table 5).

Table 5: effect of suspension erythrocyte human on heamagglutinating activity of extracted lectin from the roots plants Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens.

Blood human	A	B	O
Cyperus Rotundus	+++	+++	+++
Pistacia Lentiscus	+++	+++	+++
Ruta Graveolens	+++	+++	+++

+++: highest heamagglutinating activity

---: non heamagglutinating activity

Effects of Lectins Extracted from the Roots Plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* on Phagocytic Activity

Significant increase in phagocytic activity was observed in treated groups with lectins extracted from the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* respectively dose -dependent were compared with control (Figure 3).

Effects of Lectins Extracted from the Roots Plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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 with lectins extracted from the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* respectively were compared with control.

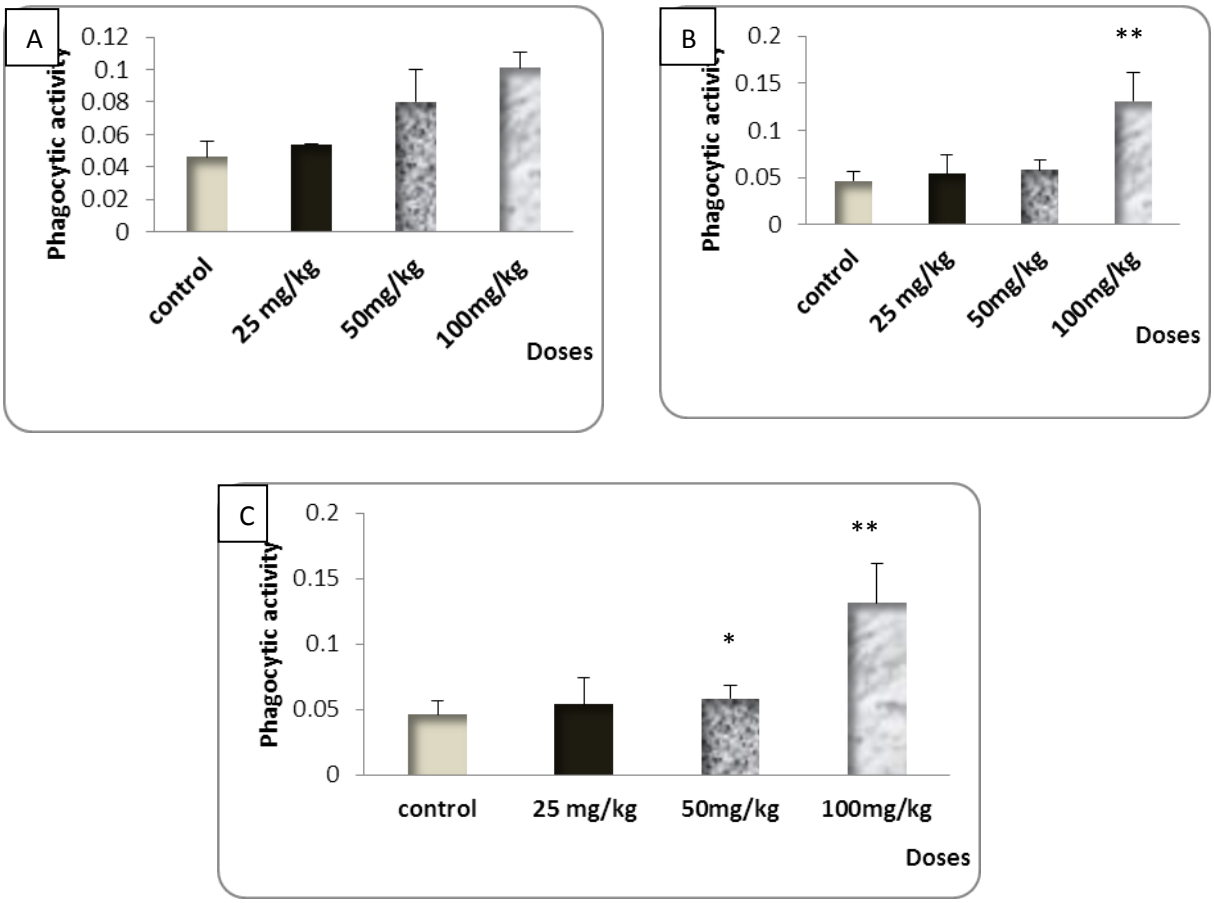


Fig. 3: Effect of lectins extracted from the roots plants *Cyperus Rotundus* (A), *Pistacia Lentiscus*(B) *and Ruta Graveolens*(C) on phagocytic activity.

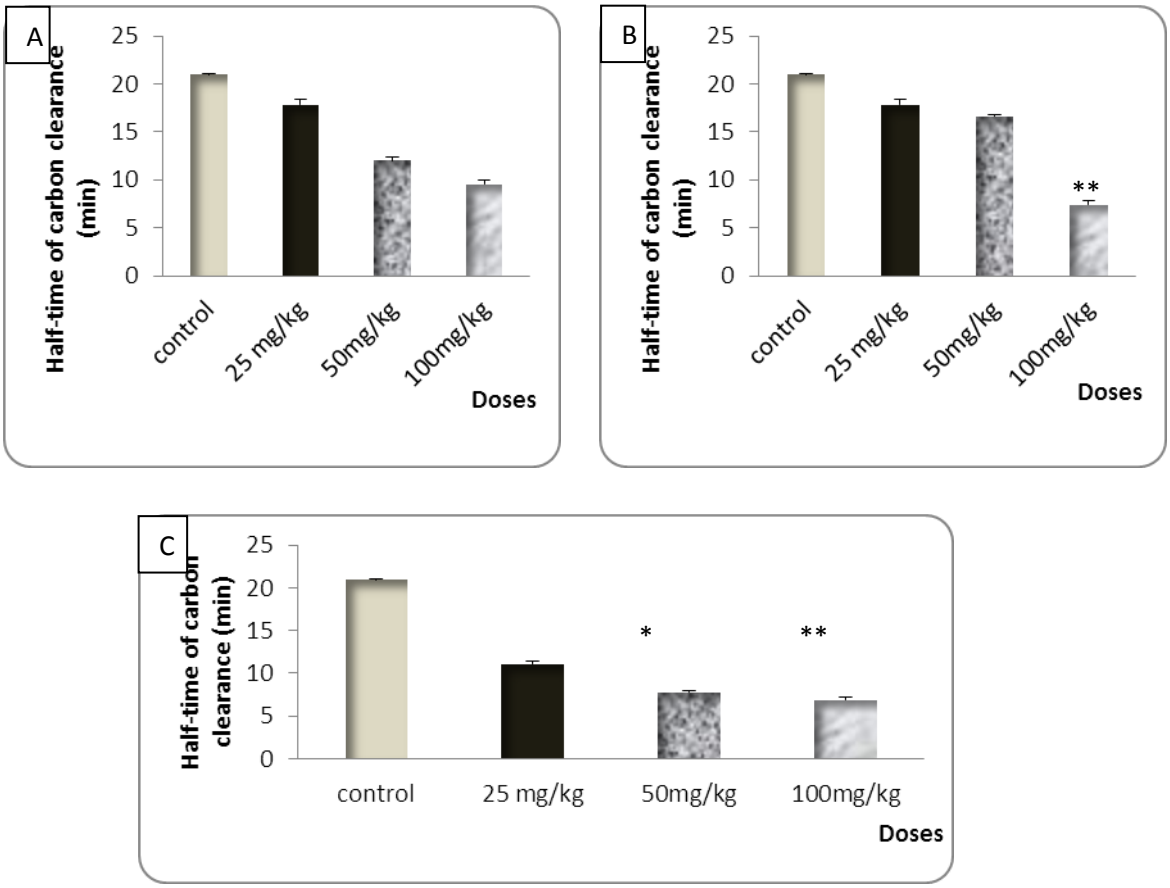


Fig. 4: Effect of lectins extracted from the roots plants *Cyperus Rotundus* (A), *Pistacia Lentiscus*(B) *and Ruta Graveolens*(C) on half -life $t_{1/2}$ of carbon in blood.

DISCUSSION

Cross-linked guar-gum, a galactomannan consisting of chains of (1→4) linked β-D-manose 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.[14] 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,[14] *Enantiocladia duperreyi*[15] and *Caulerpa cupressoides.*[16] The hemagglutination inhibition studies carried out with purified from Roots of plants: *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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,*[17] *Gracilaria bursa-pastoris* (Gmelin) Silva,[18] *Solieria filiformis*[19] and *Gracilaria verrucosa* (Hudson) Papenfus.[20] Roots of plants: *Cyperus Rotundus, Pistacia Lentiscus and*

Ruta Graveolens lectins are in general, more specific for complex oligosaccharides often glycoproteins.[21] Therefore, the inhibition of the hemagglutinating activity from Roots of plants: *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* lectins by glycoproteins was also observed in some marine algal, such as *Agardhiella tenera* Schmitz, *Ulva lactuca,*[22] *Bryothamnion seaforthii* (Turner) Kützing and *B. triquetrum,*[23] and *Amansia multifida* Lamouroux.[24] The hemagglutinating activity from the Roots of plants: *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* 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,*[23] *Solieria filiformis* (Kützing) Gabrielson,[19] *Enantiocladia duperreyi,*[15] and *Caulerpa cupresssoides.*[16]

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 the roots plants *Cyperus Rotundus, Pistacia Lentiscus and Ruta Graveolens* treated groups, exhibited significantly high phagocytic index.[25] This indicates stimulation of the reticulo-endothelial system by drug treatment. It may be possible that the extracted lectin from the roots plants *Cyperus Rotundus , Pistacia Lentiscus and Ruta Graveolens* 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.[26]

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