A novel lectin from Freshwater Snail (Lymnaea auricularia; L., 1758): Extraction, Characterization, partial purification and his possible role in host- parasite interaction

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ABSTRACT:

The present study was aimed to extract, characterize and partially purify the lectin from the freshwater snail *Lymnaea auricularia*, which is intermediate host for giant hepatic worm *Fasciolagigantica*. The lectin was extracted and concentrated in the crude (65.5 mg/ml). The extracted lectin was then characterized by using two approaches, Firstly, the lectin was able to agglutinate the erythrocyte(2%) from human blood group type o (titer 1:2560). The second approach was made through the testing the ability of lectin to mitogen the chicken's lymphocytes, chicken of one week age and observed the scar is formed after 24 hours of injection. Three steps achieved the purification of lectin. The first step involves the precipitation of lectin by ammonium sulfate in which the protein content of precipitated lectin was 48.4 mg/ml and the titer of agglutination test of human erythrocytes (2%) was 1: 2560.The second step of lectin purification was performed through ion exchange by useDEAE-cellulose column and then the absorbance measured at 280 nm for each washed fractions. After this step, the protein content of the lectin was 27.9 mg/ml and the titer of the agglutination test of erythrocytes cells (2%) was 1: 2560.

Finally, Gel filtration chromatography using Sephadex G-200 column was applied and the fractions was collected and measured at 280 nm. Our result showed the protein content of the lectin is 22.7 mg/ml and the titer of haemagglutination capacity (2%) was 1: 2560. In additions, molecular weight of lectin after partial purification using polyacrylamide gel electrophoresis technique was 35 KD. In addition, partially purified lectin had the ability to precipitate the following sugars: maltose, xylose, glucose, galactose, sucrose, fructose and arabinose.

Keywords: lectin, freshwater snail, partial purification,*Lymnaea auricularia*

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INTRODUCTION:

Lectins are carbohydrate-binding protein that have many different application in biology field such as anti-bacterial, anti-viral activity,pro-healing malignant tumers, inmmunomodulatery, apoptosis modulating activity and tumor recognitions (1&2). Lectins its widly found in living organisms such as plants (3), fungi (4), invertebrates animals 5) including parasites (6) and animals (7). Some type of lectins have mitogenic and cytotoxic functions. Such properties make lectins as tools for polysaccharides isolation and characterazation(8). Furthermore, lectins act as immunomodulations agent in host especially those found in tegument of the helminths (9).In additions, protozoal parasite such as *Entamoeba histolytica*, lectin is linked with some sugars on the surface of host intestintal epithelial cells . This sugar- lectin molecule have crucial effects on intistinal host as well as host-parasite interactions (10).Therefure, the main functions of lectins depends on abilities to interact with carbohydrate on cell

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surface.Specific binding of polysacchride always are depend on type, molecular size,metal ion and amino acid sequencing(11).Thus, the dramatic biological differences are depends on study of characterizations, isolations and purifications of lectins. Numerous lectins had been recognized in different plant and animals, but few studies characterized the snails lectins and according our knewelege, there is no previously study for isolations and characterizations of Freshwater snail *Lymnaea auricularia* lectins. Freshwater snail *Lymnaea auricularia* is intermediated host of helminths parasite (giant hepatic worm,*Fasciola gigantica*), adult form of this worms found in domesticated animal such as sheeps and goats(12&13).

MATERIALS AND METHODS:

Snail's collection: Freshwater snails were collecting from some small rivers around Hilla city, Babylon province, Iraq. Freshwater snails were classified according to (14).

Lectin Extraction: *L.auricularia* snails were washed three times with 0.15 M phosphate buffer saline (PBS) pH 7.2 to remove any wasted materials. The shell of snail was removed and then snail was homogenized with 10 ml of PBS in a glass homogenizer .Crude lectin were centrifugated at 10000 g for 30 minutes at 4 C° and supernatants were then stored at -20 C° until used (6).

Lectin Characterization:
Protein estimation: Estimation of crud and purified lectin was performed using (15) method. Lectin was characterized by using three approaches as described below:

1-Direct haemagglutination test: The first approach was based on the plate direct haemagglutination test. In the first times, human erythrocytes (2%) from different types of blood groups were used . Then, only blood group giving the highest agglutination titer was used for the haemagglutination test. (16).

2-Lectin mitogenicity *In vivo*: The second approach was achieved through using sixty chickens with one week age. The chickens were randomly divided into two groups. First group (N=3) was subcutaneously injected with 0.1 ml of crud lectin, while the second group was subcutaneously injected with 0.1 ml of normal saline as a control group (17).

3-Specificity of the lectin to carbohydrates:
Vikram *et al*. (18) method was used to confirms ability of purified lectin for precipitation of sugars, where seven different types of sugars are used in current study; these were glucose, sucrose, fructose, maltose, arabinose, galactose, and xylose.

Lectin Partial Purfuction:
Parial purification of lectin was avhived throgh three consecutive steps.
1-Concentrate of crud lectin by ammonium sulphate:
Ammonium sulphate in different saturation percentage (20, 30, 40, 50, 60, 70, 80 and 90%) was used for concentrated of crud lectin. Then, under cool conditions (4 C°) these lectin separated and precipitated by centrifugation at 8000 rpm for 30 minutes in phosphate buffer solution (19). Protein concentration and direct haemagglutination used as tool for specific lectins selections.

2-Ion exchange chromatography by using DEAE- cellulose:
After the first step, lectin solutions (3 ml) was loaded into an ion exchange column .The column was washed with 0.1 M of acetate buffer prepared at flow rate 30 ml/ hours and eluted with gradient (1M- 0.1 M) of Naclsolution.

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One hundred Fractions (3 ml/ tube) were collected, after that, each fraction was measured at 280 nm (3). Finally, protein concentration and direct haemagglutination were established for each fractions that showed absorbent peaks.

3-Lectin separation through sephadex G-200:

The lectin solution separated from ion exchange chromatography was then added gently on the surface of gel (Sephadex G-200), elution was achieved by using lectin fractions were measured at 280 nm. Then collected the activation parts and measured the activity, volume and protein concentration, divided in vials and stored in freeze condition for another steps(20).

Polyacrylamide gel electrophoresis and molecular mass of partial purified lectin:

A gel formed by cross-linked polymerization of two organic monomers, acrylamide and crosslinking agents, N, N, N, N- methylene bis acrylamide was showing as suitable medium for electrophoresis (21). The solubility denaturing agents such as sodium dodecyl sulphate (SDS) are widely used in the separation of protein by gel electrophoresis (22). SDS has high affinity for proteins and promotes protein denaturation. The proteins are separated by SDS page migrate according to their molecular weight regardless of their charges. Polyacryalmide gel (PAG) composed of different pore size by alteration the concentration of cross linking agents. Electrophoresis by (PAG) depends on both molecular weight and electrophoretic mobility (23).

RESULTS:

1- Blood groups specificity:
Blood group specificity of crude lectin showed the ability to agglutinate human erythrocytes (2%) for blood group O (Titer 1:2560). Furthermore, this ability was lower than for other blood groups A, B and AB were 1:1280, 1:160 and 1:20, respectively.
2-Lectinmitogenicity*In vivo*:
The results showed that the isolated crude lectin had the lymphocytes mitogen ability by observing scar formed in the chicken that injected with 0.1 ml of crude lectin after 24 hours.

3-Purification of Crude Lectin:
Three steps were used to partial purify the lectin; these steps include precipitate of crude lectin with ammonium sulphate, ion exchange by using DEAE- cellulose and gel filtration by using Sephadex G-200.
3-1: Precipitation with ammonium sulfate:
The ammonium sulfate was used in different saturation ratios (20, 30, 40, 50, 60, 70, 80, and 90%). Current study showed that 60% ratio has been considered the best one for precipitate crude lectin because at which the best agglutination titer (1:2560) andbest protein content (48.4 mg/ml) was reached.
3-2: Ion exchange chromatography:
As shown in Figure 2 four protein peaks was founded from one hundred fractions by using ion exchanger DEAE-cellulose. Protein content were estimated by spectrophotometric method at wavelength 280nm.In current study we evaluated each one of this peaks for estimations blood agglutination titer. The best blood agglutination was found in one of these peaks at which blood agglutination titer is1:2560 and protein content 27.9 mg/mal.

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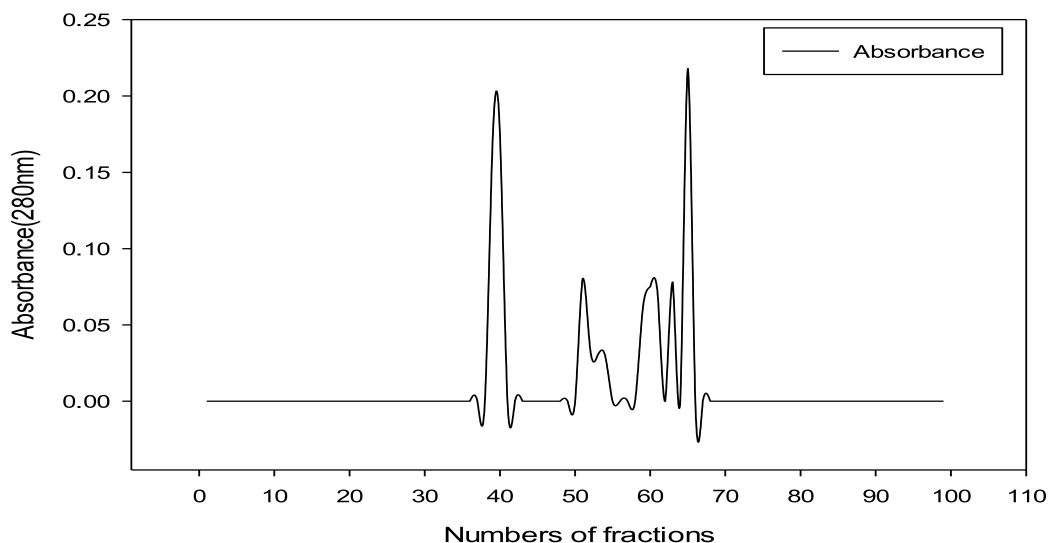

Figure 2: - Ion exchange chromatography using DEAE-cellulose column (2.8×18) cm with phosphate (0.1M, pH 8), flow rate 30ml/hr and fraction volume 3ml for purification lectin from *L. auricularia*.

2-3: Gel filtration chromatography:

The lectin solution obtained from ion exchange chromatography passed through Sephadex G-200 column (1.5*85) cm that equilibrated with acetate buffer (0.1M, pH8), the fraction collected and measured at 280nm absorbency. Result showed just one peak at 0.25 as shown in figure 3.This peak gave agglutination titer 1:2560 and protein content 22.7 mg/mal.

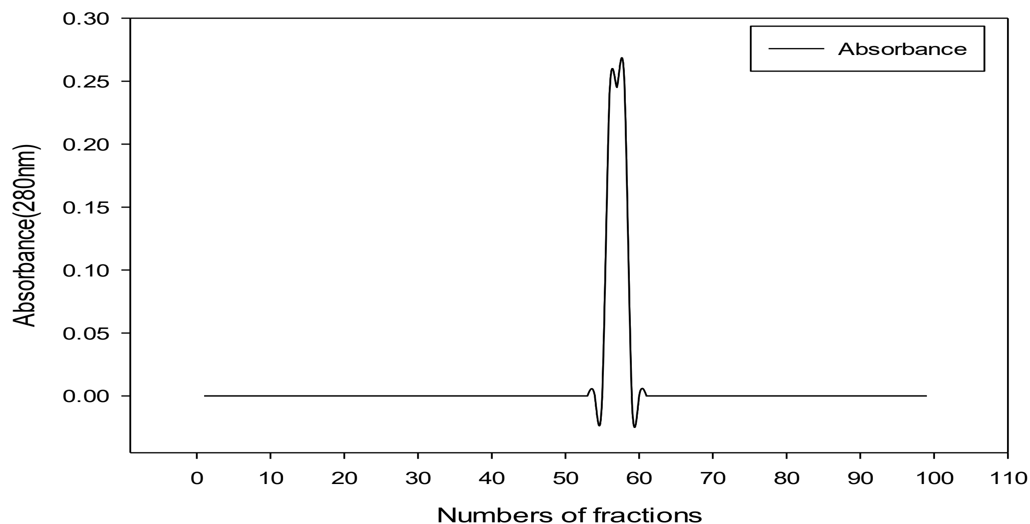

Figure 3:- Gel filtration uses Sephadex G-200 column (1.5×85 cm) cm with phosphate buffer (0.1M, pH 8), flow rate 30ml/hr. and fraction volume 3ml for purification lectin from *L. auricularia*.

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3- Estimation of Molecular Weight:

In order to calculate the molecular weight of the lectin by electroplating method, the relative movement of the lectin and the standard on the gel calculated. This calculation based on the equation of the relative motion calculation and the standard curve showing the relationship between the molecular weight logarithm and the relative movement of the standard in a polyacrylamide gel with the presence of denaturing conditions (SDS , 2-mercaptoethanol). It isfound that the molecular weight of the *L. auricularia* lectin is 35 KDa (figure 4).

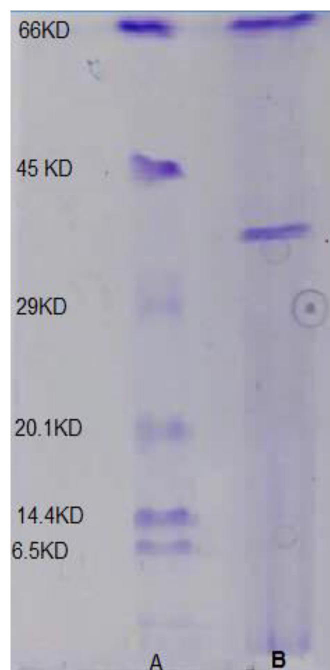

Figure 4: Polyacrylamide gel electrophoresis of the lectin from *L. auricularia* under denaturing conditions, since A=molecular weight marker, B= represents the partial purified lectin produced from gel filtration.

4-The specificity of the partially purified lectin to carbohydrates:

The present study showed that the partially purified lectin has the ability to precipitate sugars maltose, xylose, glucose, galactose, sucrose, fructose and arabinose.

DISCUSSION:

A new lectin from Freshwater Snail (*Lymnaea auricularia*) has been successfully extracted, isolated and partial purified .Besides, extracted lectin was showed strongly agglutinations capacity for blood groups especially O-type. This is may be related the erythrocytes sugary receptors on their own surfaces, which differ from those found in other groups (24).Previous works showed the lectin has ability to precipitate L- fructose on the surface of the erythrocytes of the blood group O (24).In the same line current study, confirm that the partial purified lectin has ability for fructose precipitated. In opposite, (25) found that the isolated lectin from sea hare (*Aplysia kurodai*) agglutinated of human erythrocytes type A (Titer = 2048).

One of the most important characteristics features of lectin its ability to stimulate the mitogen of T and B-lymphocytes. This is can be observed by scar formed at the injection site in animals (1). (17) found parasite (*Fasciola hepatica*) lectin has ability to mitogen the lymphocytes through subcutaneous injection with one-week-old chicken.

The precipitation process by ammonium sulfate mainly also checked in current study. This method based on salting out, which involves the equal of the charges on the surface of the protein molecules by ammonium sulfate ions and the degradation of the surrounding water layer for precipitation. Several factors influence precipitation with ammonium sulphate, the size and shape of the protein molecules and the presence of other compounds that reduce

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its dissolving speed. Precipitation by saline depends on number and distribution of protein charges, non-ionic groups and the distribution and number of hydrophobic groups (26).(27) found the 80% ammonium sulfate is the best ratio to precipitate lectin isolated from fungus *Macrophomina phaseolina*, but (17) found the 40% ammonium sulfate is the best ratio to precipitate lectin isolated from tapeworm *Fasciola hepatica*. After ammonium sulphate precipitation, highest haemagglutination fractions was taken for anther purifications methods.The next steps of purification is ion exchanger DEAE-cellulose, which made by the most systems using the separation of materials (including lectins). Ion exchanger DEAE-cellulose have several advantages, these are high resolving power, high protein-binding capacity, simplicity of separation depends mainly on the difference in the charge and the ion exchanger is a cellulose derivative (26). Because of these characters, many researchers used this ion exchanger to purify lectins from different organisms (2).In same directions, Sephadex G-200 has many advantages included fast run, high recovery separation, simple preparation and its stability for long time permits reusing of the gel many times in protein separation (28, 29,30).For these reasons, this substance used to purify many chemical substances extracted from different biological sources (plant, animal, etc.).For example, (31) used this substance (Sephadex G-200) to purify the isolated lectin from the black sea cucumber (*Holothuria atra*).In addition; present study examined partially purified Lectin by Polyacrylamide gel electrophoresis technique for molecular weight estimation. Previous researcher workshave been showed different molecular weight of lectin for example (8) found the lectin that isolated from the sponge (*Haliclona cratera*) had a molecular weight of 29 KDa. While (32) found that the lectin isolated from the marine bivalve (*Macoma birmanica*) had a molecular weight of 47 KDa. These molecular weight differences depending on the source of the lectin and extraction and purification methods. As well-known methods play an important role in maintaining the chemical composition of the protein and its stability (33).
There are many reasons for study lectin carbohydrate binding specificity, one of this reasons its applications of lectin in cell biology and biochemistry. The reason for the specifications of lectin to sugars due to existence at least two link sites of sugar to one molecule of lectin. This indicates the difference of specialization of lectin for carbohydrates and these acids have a significant role in maintaining the composition of the site of the link (34).

Authors' Contributions

AL-Morshidy K.A.H. designed the study and wrote the manuscript, Al-Hassnawi, A.T.S. helped in collecting the dataand edited the manuscript. Mohsen, L.Y.performed the lab work.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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