Anti-fungal Activity of Lectin Isolated from Whole Body of *Fasciola gigantica* (Cobbold, 1856)

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Abstract

Lectin with molecular weight of 71 Kilo dalton was isolated from whole body of adult worm of *Fasciola gigantica* parasite and purification by using sephadex G-200. This lectin is protein in nature (3.6 gm. / ml and 0.329 gm. / ml before and after purification, respectively), agglutinate human erythrocytes (1: 5120 and 1: 1280 before and after purification, respectively) and T lymphocytes mitogen by using one week age of chicken as model. Antifungal activity of this material was tested by using four pathogenic fungi (*Alternaria solani*, *Aspergillus flavus*, *Fusarium globosum* and *Penicillium exsporum*) and by using three methods (pouring one milliliter on PDA, method of holes and the mixing method). From the results of present study show the better inhibition rat of lectin against fungi were founded in *F. globosum* (57.8%, 55.6% and 41.1%, respectively in three methods) and the lower rate of inhibition was founded in *A. solani* in mixing method.

The results of current study will provide ability of this worm to use the lectin to defense against any threat.

Key words: Lectin, *Fasciola gigantica*, Antifungal.

الخلاصة

تم في الدراسة الحالية عزل لكتين ذو وزن جزيئي 71 كليو دالتون وتنقيته باستعمال مادة Sephadex G-200 من كامل جسم طفيلي المتورقة الكبدية العملاقة *F. gigantica*. وجد ان اللكتين المعزول ذو طبيعة بروتينية (3.6 و 0.329 غم / مل قبل التنقية وبعدها، على التوالي) وملزن لكريات الدم الحمر للانسان بمعيار 1:5120 و 1:1280 قبل التنقية وبعدها، على التوالي ومشطر للخلايا اللمفية التائية في الجسم الحي من خلال استعمال أفراخ دجاج بعمر اسبوع. اختبرت الفعالية المضادة لنمو الفطريات للكتين المعزول وذلك باستعمال أربعة انواع من الفطريات الممرضة وهي: (*Alternaria solani*, *Aspergillus flavus*, *Fusarium globosum* و *Penicillium exsporum*) باستعمال ثلاث طرائق هي نشر واحد مليليتر من اللكتين على وسط البطاطا دكتروز اكار وطريقة عمل حفر في الوسط وطريقة مزج اللكتين مع الوسط. وجد من نتائج الدراسة الحالية ان اللكتين المعزول له افضل قدرة تثبيطية ضد الفطر *F. globosum* بنسب 57.8% و 55.6% و 41.1%، بالثلاث طرائق، على التوالي أما اقل قدرة تثبيطية فكان ضد *A. flavus* بطريقة المزج اللكتين مع الوسط.

نستنتج من نتائج الدراسة الحالية ان لهذه الديدان القدرة على استعمال هذا اللكتين ضد أي تهديد يواجهها.

الكلمات المفتاحية: اللكتين ، المتورقة الكبدية العملاقة، الفعالية المضادة للفطريات.

Introduction

Lectin defined as carbohydrate binding proteins of non- immune origin that agglutinate cells or precipitate polysaccharides or glycoconjugates (Liener *et al*., 1986). Lectins appeared unique immune activities because they have very selective binding with carbohydrates and can use them in more than 18 biological fields (Sharon & Lis, 2004). According to the natural origin, lectins can be classification into five groups: animal lectins, plant lectins, fungal lectins, bacterial lectins and viral lectins (krengel & Imberty, 2007). Animal lectins are isolated from different animals species involved parasitic worms and used them in different biological fields (Ghosh *et al*., 2005; Farahnak *et al*., 2010; Shnawa *et al*., 2010; Al- Morshidy, 2012).

Fasciola gigantica is considering one of important parasitic worms because it is caused lethal disease in veterinary animals and human called fasciolosis (Spithill *et al*., 1999). These worm belong to phylum Platyhelminthes, class trematoda, order digenea (Soulsby, 1982). The life cycle of these worm is very complex and consist of eight phases (egg, miracidium, sporocyst, redium, cercarium, metacercarium, juvenile and adult) , these phases is passed among two different hosts (final host from vertebrates and intermediate host is sails belong to *Lymnaea* spp. class of gasropoda, phylum of mollusca) and two phases is free living (Bowman & Lynn, 1999). These phases are different in shaped and chemical compounds such as proteins, carbohydrates, lipids and glycoprotein (lectins). In additionally, the adult worm has tegument consist of four layers (Sobhon *et al*., 2000). These layers are included different materials such as enzymes, proteins and lectin (Sobhon *et al*., 1998; Al- Morshidy, 2012).

Antifungal activity of different materials such as lectins are acted on damage of pathogenic fungi, These lectins are produced by animals (Lee *et al*., 1995; Melo *et al*., 2000; Wang & Ng, 2002; Banerjee *et al*., 2004), plants (Joshi *et al*., 1998; Ciopraga *et al*., 1999; Wang & Bunkers, 2000), and fungi (Grenier *et al*., 2000; Lam & Ng, 2001). Cytotoxic effects of lectins may be worked on fungi growth inhibition by different ways: by binding to hyphas resulting in poor absorption of nutrients as well as by interference on spore germination process (Lis & Sharon, 1981) or by binding to chitin of fungi cell wall and stopped synthesis of chitin (Selitrennikoff, 2001).

This study aimed to isolation, purification of lectin from *F. gigantica* parasite and studies the antifungal activity of this material on different species of fungi.

Materials and Methods

A- Isolation, characterization and purification of lectin

1- Isolation of lectin from whole body of worms:

Live *F. gigantica* worms were collected from natural liver infected of cows in Hilla slaughter house. Worms were washed three times by 0.15 M phosphate buffer saline (PBS) pH 7.2 to remove any wasted materials. Each worm was homogenized with 10 ml of PBS in a glass homogenizer. Crud materials were collected into centrifuge tube and centrifuged at 10000 g for 30 minutes at 4 Cº and supernatants were stored at -20 Cº (Farahnak *et al*., 2010).

2- Lectin purification:

Lectin was purified by using Sephadex- G 200. The later was preparated by suspension the sephadex G-200 with 0.15 M PBS and degassing the air by vacuum pump. The gel was putted in glass tube (1.25 * 50 cm). The lectin was eluted with 0.15 M PBS pH= 7.2 and each 5ml of elution was collected in test tubes with a fraction collector. Each five milliliter from eluted solution was monitored using UV to measurement of absorbance at 280 nm by UV spectrophotometer (Whitaker, 1972).

Gel electrophoresis

The purity and molecular weight of purified lectin was determined by sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE 12.5%). The electrophoresis was carried at 100 volts for four hours. Lectin bands were visualized by staining with Coomassie brilliant Blue R-280 (Laemmli, 1970).

3- Lectin characterization

Lectin characterization by three testes before and after lectin purification:

- Protein estimation: protein amount was estimated by Biuret method Ross (1985).
- Direct haemagglutination test: plate direct haemagglutination test was preparated by used human erythrocytes according to Burell (1979).
- Lectin mitogenecity *In vivo*: Sixty chicken of one week age were divided into two groups, first group (contain three bird) were injected intradermal by 0.1 ml from lectin, second group were injected by 0.1 ml of normal saline as control group (Shnawa *et al*., 2010).

B-Antifungal activity

1- Potato dextrose agar (PDA)

The culture was prepared by dissolving 40 gm. from (PDA) powder in amount of distilled water and completes the volume to 1000 ml, then it sterilized in autoclave at 121C^{o} and 15pound /in^{2} pressure for 20 second. This medium was used for determination of optimum culture media for inhibition process.

2- The source of fungi isolates

The fungi which include *Aspergillus flavus, Penicillium exsporum, Alternaria solani* and *Fusarium globosum* were obtained from the unit of isolation and classification of fungi isolates /Department of Biology /College of Science /University of Babylon.

3- Assay of antifungal activity

A- Pouring one milliliter on PDA

The fungi isolated were cultured in Potato dextrose agar (PDA) medium for five days. Then, previously prepared one milliliter portion from lectin extract was spread on sterilized melted PDA medium was poured (about 20 ml/plate,100 µg/ml) in sterilized petri dishes. The plate should be moved gently on the table in a figure –of – eight motions to effect proper dispersion, at the center of each plate, five days old fungal mycelia block (four mm in diameter) was inoculated and incubated at 25 C^{o}. (PDA) medium in petri dish. Antifungal activity was evaluated by measuring the zone of inhibition in millimeters. All experiments were done in triplicates. When the mycelia colony of the control had grown to almost fill the plate, the area of the mycelia colony was measured, and the inhibition of fungal growth in the other plates was determined by calculating the percentage reduction in the area of the mycelia colony as follows:

% Inhibition = (C-T/C) × 100

Where, C = diameter of the fungal colony in the control petri dish

T = diameter of the fungal colony in the treated petri dish (Wang & Ng, 2002).

B- Method of holes

Antifungal bioassays were performed on petri dish plates contain 20 ml of standard PDA agar .After the mycelia colonies were developed, five wells of 0.5 centimeter in diameter were made one centimeter from the rim. The suitably diluted lectin (100µg/ ml) were (10 ml) to add to the wells and the plates were incubated at 25

Cº for 5days. Growth inhibition zone were observed (Wang & Ng, 2002). The percentage inhibition of radial mycelia growth of the test fungus was calculated.

C- The mixing method

The *In vitro* antifungal activity of the purified lectin was determined by the poisoned food technique (Miah *et al*., 1990). PDA medium was used for the culture of fungi. A required amount of PDA was taken in conical flasks separately and was sterilized by autoclave (121Cº, 15 p) for 15 minutes. Purified lectin (solution) was mixed with sterilized melted PDA medium to have 100 µg/ml PDA and this was poured (about 20 ml/plate) in sterilized petri dishes. At the center of each plate, five days old fungal mycelia block (4 mm in diameter) was inoculated and incubated at 25 Cº. A control set was also maintained in each experiment. Linear mycelia growth of fungus was measured after 3-5 days of incubation in triplicate. The average of two measurements was taken as mycelia colony diameter of the fungus in millimeter.

Results and Discussion

F. gigantica lectin (FGL) is protein in nature, agglutinated erythrocytes of human blood and mitogenic of T lymphocytes in chicken (Table 1). These characters are main characters of lectin (Sharon & Lis, 2004). According to the definition of lectin, lectin is protein or glycoprotein of non-immune origin which specifically bind or cross- link carbohydrate (Pusztai, 2003). The simply definition of agglutination is interaction between a lectin with cells and it is occur when a lectin molecules form many cross-bridges between cells, These process affected by different conditions such as: 1- lectin properties (number of sugar binding sites and size of lectin molecules) 2- properties of cell surface such as proximities of receptor sites or membrane liquidity, 3- external conditions such as temperature, cell concentration…etc. (Wang & Ng, 2002). Stimulating of lymphocytes to dividing is called mitogenic property. This character is founded in many type of lectin, in addition to, mitogenic lectin stimulates about 80% of the susceptible cell but the any antigen stimulates less than 0.1 of the total T lymphocyte (Reynoso- Camacho *et al*. 2003). The different values of parameters in before and after purification of lectin maybe belongs to found some materials removal in purification.

Table (1): Characters of lectin before and after purification.

Substances	Direct haemagglutination test (titer)	Mutagen test	Protein concentration gm./ml
Crud lectin	1:5120	+	3.6 gm./ml
Purified lectin	1:1280	+	0.329 gm./ml

The extract of whole worm of *F. gigantica* has a single polypeptide chain by Gel filtration chromatography using sephadex G-200 column equilibrated with phosphate buffer slain pH 7.2 (Figure 1). Many researches are used sephadex G-200 for purification different lectin from different origin because this materials have different properties such as stability against pressure because have epichorohydrin (Kuku & Oladiran, 2004 & Sharma *et al*., 2009).

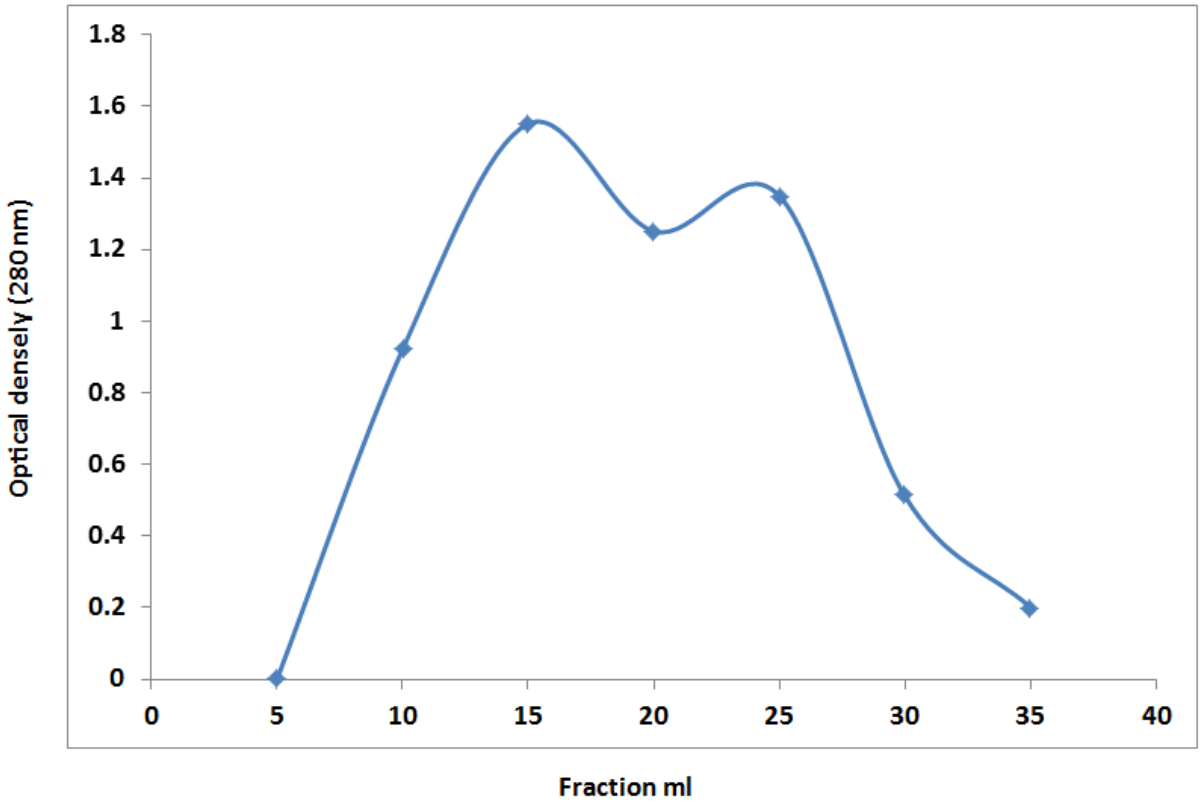

Figure (1): Gel filtration chromatography for purification of FGL by using sephadex G-200 column equilibrated with phosphate buffer slain pH 7.2, flow rate 20 ml / hrs. and 5 ml fraction.

The figure (2) shows the molecular weight of isolated lectin from whole *F. gigantica* under denaturing conditions is 70KDa. The researches deals with molecular mass of lectin isolated from *Fasciola* spp. are very few. Ghosh *et al*. (2005) isolated lectin from adult worm of this parasite with molecular weight 27 KDa and use it in diagnostic of fasciolosis in cows, while Farahnak *et al*. (2010) isolated lectin from surface area of *F. hepatica* with molecular weight 50 KDa. Shnawa *et al*. (2010) isolated surface and whole lectin from *F. hepatica* without detection molecular weight and Al- Morshidy (2012) isolated surface lectin from *F. gigantica* without detection molecular weight.

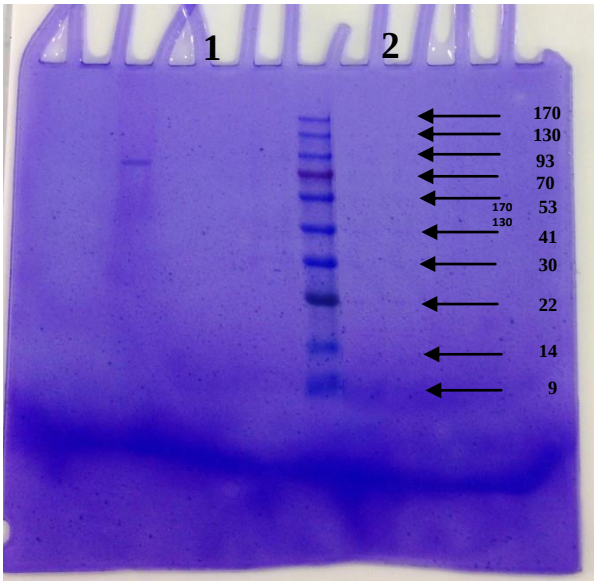

Figure (2): Electrophoresis shows the lectin purified from *F. gigantica* under denaturing conditions, since 1: represents the purified Lectin and 2: protein ladder.

The inhibition effect of isolated lectin on different species of Fungi growth show in table (2). The better inhibition rates of lectin are founded in *Fusarium globosum* growth by three methods: pouring one milliliter on PDA media, the method of holes and the mixing method (57.8%, 55.6%, and 41.1%, respectively). The next better result was founded in *Alternaria solani* by pouring one milliliter on PDA and the method of holes methods (54.4% and 50%, respectively), but in *Penicillium exsporum*, the better result was founded in the pouring one milliliter on PDA method (41.1%) while, the weak inhibition rate is founded on *Aspergillus flavus* by three methods especially in the mixing method (13.3%, 5.6% and 2.2%, respectively). Most antifungal lectin is plant origin because the plant used lectin as defense methods against microorganisms (Hamid *et al*., 2013) but antifungal lectin also isolated from animals (Lee *et al*., 1995 & Wang & Ng, 2002) and from fungi (Grenier *et al*., 2000 ; Lam & Ng, 2001). The inhibition effect of lectin on fungi may be occurring in two main pathways: 1- lectin caused poor absorption of nutrients or interference on spore germination when binding to hyphas (Lis & Sharon, 1981). 2- Chitin is polysaccharide and it is most compounds of fungi cell wall and when binding with lectin it caused impairment of syntheses and or deposition of chitin in fungi cell wall (Selitrennikoff, 2001).

Table (2): The effect of lectin extract on fungi growth.

		A		B		C	
	Control cm	**Diameter of growth (cm)**	**Inhibition rate %**	**Diameter of growth (cm)**	**Inhibition rate %**	**Diameter of growth (cm)**	**Inhibition rate %**
Alternaria solani	8.7	4.1	54.4	4.5	50	6.9	23.3
Aspergillus flavus	9	7.8	13.3	8.5	5.6	8.8	2.2
Fusarium globosum	9	3.8	57.8	4	55.6	5.3	41.1
Penicillium exsporum	8.8	5.3	41.1	6.1	32.2	6.1	32.2
A= **Pouring one milliliter on PDA** B= **Method of holes** C= **The mixing method**							

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