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Effect β glucan extracted from *Candida albicans* on pathological changes produced by *Penicillium chrysogenum* infection in mice

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Abstract

This research aims to evaluate the effect of β glucan extracted from *Candida albicans* on the pathological effect of *P. chrysogenum* isolated from subclinical bovine mastitis from Abu Ghraib area in Baghdad by California mastitis test in winter 2020 on some internal organs such as (bone, brain, lung and intestine) by intramammary injection for two weeks then the beta-glucan extracted from the yeast *Candida albicans* was used for treatment of *P. chrysogenum* infection at two concentrations 50 and 100 mg/kg by two methods of injection (intramammary and intraperitoneal). The present study findings demonstrated different degrees of inflammation in these organs especially in the bone and brain, in addition, treatment with beta-glucan extracted from *C. albicans* showed a strong recovery response at a 50 mg/kg concentration by intraperitoneal injection in all organs from a 100 mg/kg concentration intraperitoneal injection was better than intramammary injection. Additionally, the results showed that the concentration of IgG was determined in serum samples of mice infected with *P. chrysogenum* using radial immunodiffusion plate, which showed different diameters of precipitation rings in the gel of plate with an increase in IgG concentration in all mice compared to the normal value of the concentration of IgG.

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Introduction

Candida albicans is classified as an opportunistic fungus (1,2) because it poses major medical complications, particularly for immunocompromised patients, such as those who are undergoing corticosteroid treatment, chemotherapy for cancer, or receiving organ transplants (3). There are three parts to the *C. albicans* cell wall, which is predominantly made up of polysaccharides (4). The mannoprotein barrier (mannan), which serves as a filter for high-molecular-weight molecules, is formed by protein coupled to mannose polysaccharides in the outer surface layer. The glucose polysaccharide β-(1,3)-glucan and β-(1,6)-glucan, which is crucial for cross-linking other elements of the wall, make up the majority of the inner layer. Cell walls of many different organisms, including yeast, bacteria, fungi, algae, and plants, contain the biopolymer β -D- glucan. A major component of the cell wall in *C. albicans*, accounting for 60% of the dry mass of the cell wall, is β glucan. Due to its immunostimulatory properties, yeast β glucan has been shown to be advantageous for both human and animal health systems. Because of this, it is a biological response modifier (BRM) and can be used in functional foods and medications (5). It is well recognized that β glucan can modify cellular processes, which in turn affect immunological responses (6). Since it is generally recognized to increase pro-inflammatory reactions, researchers have used β glucan as a cancer adjuvant therapy or as a treatment for infectious diseases (7) *Penicillium notatum*, also called *P. chrysogenum*, can be found in large numbers in the environment. It can be found on wood, decomposing plants, or the soil (8). It is well-known for producing a number of significant Beta-lactam antibiotics, including penicillin.

The primary goal of the current investigation is to assess the effectiveness of this β glucan on the histopathological changes in mice infected with *P. chrysogenum* due to the paucity or rarity of studies for employing it against *P. chrysogenum* infection in mice.

Materials and methods

Ethical approve

the name of scientific or institutional board that give the ethical approve to conduct this scientific work is College of Veterinary Medicine, University of Baghdad in 14/6/2021

Fungal isolates

Candida albicans was received from the Department of Microbiology at the University of Baghdad's College of Veterinary Medicine. *Candida albicans* were cultured on Sabouraud Dextrose Agar (Himedia-India) at 37°C for 48 hours. then, diagnose both macro- and microscopically in accordance with Kidd *et al.* (9). *P. chrysogenum* was isolated from milk samples of Bovine subclinical mastitis that determined by CMT in winter of 2020 from Abu-Ghraib region in Baghdad province according to Saadoon (10). This isolate was grown on Sabouraud dextrose agar (Himedia - India) with 0.05 mg/ml of chloramphenicol and incubated at 25°C for 4 to 7 days before being identified macroscopically and microscopically in accordance with Washinton *et al.* (11). Spore suspension was prepared for this fungus according to Van der velden *et al.* (12). Throughout the investigation, standard β glucan (1, 3-glucan) from *Euglena gracilis* was employed (β glucan derived from *Euglena gracilis* which is one type of algae used as standard for β glucan), which was obtained from Sigma company (Germany origin).

Candida albicans cell wall β glucan extraction

After being produced in accordance with Pengkumsri *et al.* (13), the β glucan isolated from the cell wall of *Candida albicans* was evaluated using High Performance Liquid Chromatography (HPLC) in accordance with Salim (14).

Experimental design

Baghdad, Iraq's National Center for Drug Control and Research, provided forty albino white female mice in the lactating stage, each weighing25± 3gm body weight and being eight weeks old. These animals were split into 3 groups: first group include ten mice serves as negative group, second group (positive group) include ten mice infected with 0.1ml of *P. chrysogenum* intramammary at $1x10^6$, third group include 20 mice infected with *P. chrysogenum* and treated with 0.1 ml of *C. albicans* β glucan. This group was divided into two subgroups. Subgroup one has ten mice injected intramammary with different 2 concentration of *C. albicans* β glucan including group a has five mice injected 0.1ml of *C. albicans* β glucan in concentration 50mg/kg. Group b has five mice injected 0.1ml of *C. albicans* β glucan in concentration 100 mg/kg.

Subgroup two has ten mice injected intraperitoneally, with different 2 concentration of *C. albicans* β glucan including: Group a has five mice injected 0.1ml of *C. albicans* β glucan in concentration 50mg/kg. Group b has five mice injected 0.1ml of *C. albicans* β glucan in concentration 100mg/kg. The period for treatment of third group about two weeks according to Baran *et al.* (15).

Collection of blood samples for IgG detection by RID

Blood samples were collected from infected mice with *P. chrysogenum* to determine the IgG concentration by a radial immunodiffusion test (16). The concentration of IgG was measured by the procedure of manufacture (17) by filling the wells of the plate with 5µl of the serum then close the plate and placed it in a moist chamber for 72hr.after that the precipitating ring diameter had been measured using ruler and compared with the precipitating ring diameter of manufacture company.

Histopathological study

One cm^3 of the lung, intestine, brain and bone of each animal from groups were collected, fixed, and dipped in 10% neutral formalin buffer solution. then this formalin solution was replaced after 24hrs. till the preparations of histological sections. Tissues processed with ethanol alcohol and cleared by clearing solution and embedded with paraffin wax. Several tissue samples were cut into histopathological sections and stained with the Hematoxylin-Eosin (H&E) stain (18).

Results

Histopathological changes of bone infected with *P. chrysogenum* (positive control group)

Histopathological examinations of bone infected with *P. chrysogenum* shows congestion of blood vessels with cells infiltration as shown in figure 1.

Histopathological changes of infected bone treated with *Candida albicans* β glucan extracted

In concentration 50 and 100 mg/kg (intramammary injection) showed normal tissue include osteocytes and osteoblast. In concentration 50 and 100 mg/kg (intraperitoneal injection) showed normal tissue include osteocytes and osteoblast.

Histopathological changes of brain infected with *P. chrysogenum* (positive control group)

Histopathological examinations of brain infected with *P. chrysogenum* shows perineural edema and perivascular edema as shown in figure 2, the other section congestion in blood vessels as shown in figure 3.

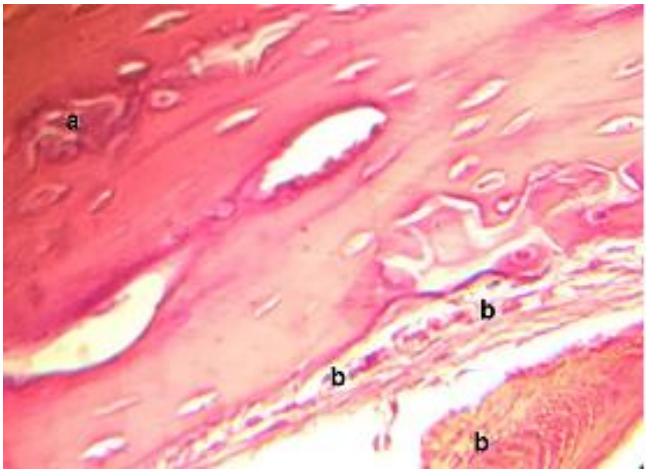

Figure 1: Histopathological section of bone infected with *P. chrysogenum* shows congestion of blood vessels (a) with infiltration of cells (b) (H&E stain, x400).

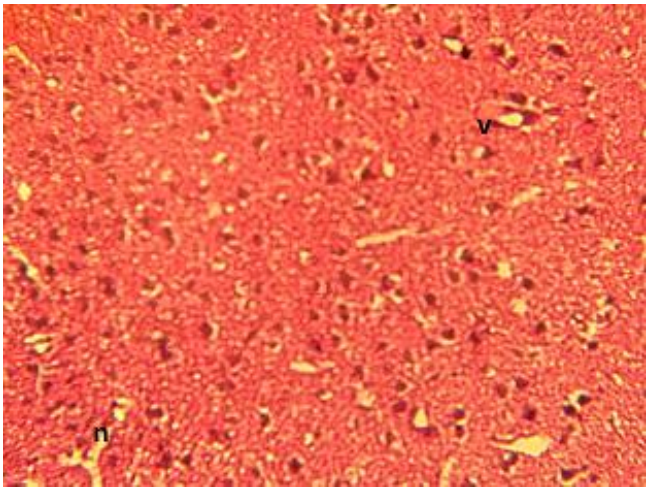

Figure 2: Histopathological section of brain infected with *P. chrysogenum* shows perineural edema (n) and perivascular edema (v) (H&E stain, x100).

Histopathological changes of infected brain treated with *Candida albicans* β glucan extracted

In concentration 50 and 100 mg/kg (intramammary injection) showed normal tissue include pyramidal and granule (stellate) cells. In concentration 50 and 100 mg/kg (intraperitoneal injection) showed normal tissue include pyramidal and granule (stellate) cells.

Histopathological changes of lung infected with *P. chrysogenum* (positive control group)

Histopathological examination of lung infected with *P. chrysogenum* intramammary showed fibrin networks deposition and proliferation of alveolar macrophages in the alveolar spaces as shown in figure 4.

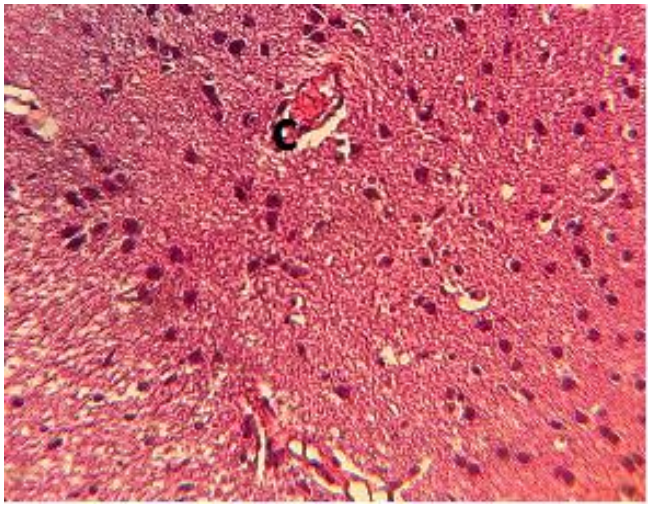

Figure 3: Histopathological section of brain infected with *P. chrysogenum* shows congestion of blood vessels (c) (H&E stain, x400).

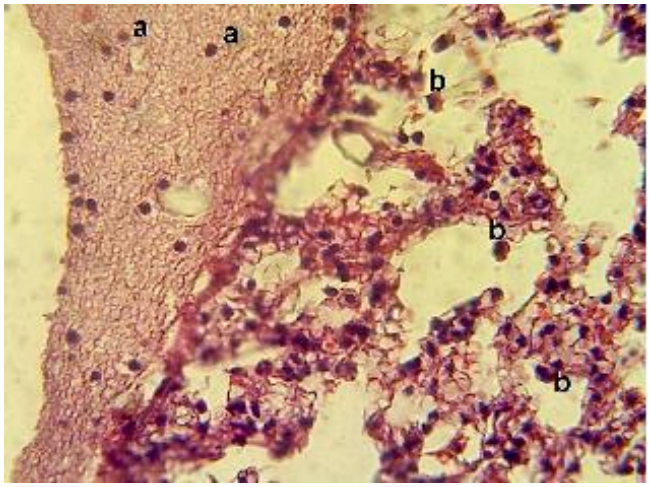

Figure 4: Histopathological section of lung infected with *P. chrysogenum* shows fibrin networks deposition (a) and proliferation of alveolar macrophages in the alveolar spaces(b) (H&E stain 400X).

Histopathological changes of infected lung treated with *Candida albicans* β glucan extracted

In concentration 50 and 100 mg/kg (intramammary injection) showed aggregation of mononuclear cells around congested blood vessels as shown in figure 5. In concentration 50 and 100 mg/kg (intraperitoneal injection), the concentration 50 mg/kg showed inflammatory cells in congested blood vessels as shown in figure 6 While the concentration 100 mg/kg demonstrated a thickening of the inter alveolar septa brought on by the invasion of mononuclear cells, as depicted in figure 7.

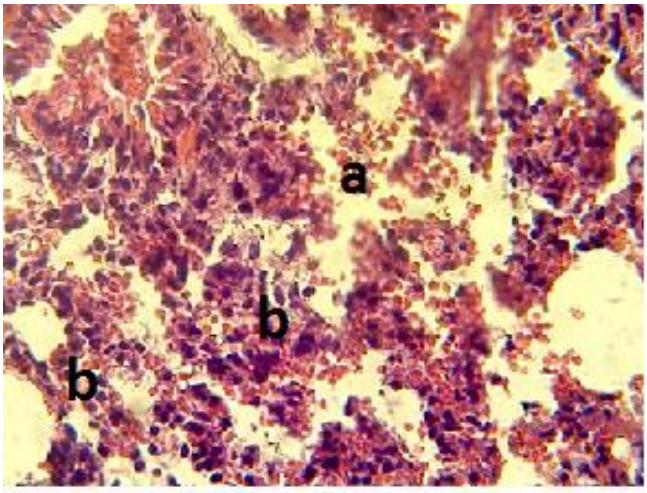

Figure 5: Histopathological section of infected lung treated with β glucan extracted from *C. albicans* intramammary injection in concentration 50 mg/kg shows RBCs (a) and inflammatory cells in the alveolar spaces (b) (H&E stain 400X).

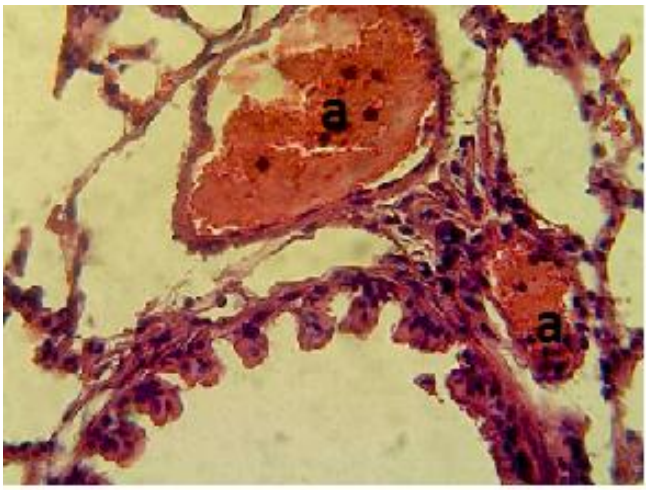

Figure 6: Histopathological section of infected lung treated with β glucan extracted from *C. albicans* intraperitoneal injection in concentration 50 mg/kg shows inflammatory cells in congested blood vessels (a) (H&E stain 400X).

Histopathological changes of intestine infected with *P. chrysogenum* (positive control group)

Histopathological examinations of intestine infected with *P. chrysogenum* shows sever inflammatory cells infiltration between mucosal glands as shown in figure 8.

Histopathological changes of infected intestine treated with *Candida albicans* β glucan extracted

In concentration 50 and 100 mg/kg (intramammary injection), the concentration 50 mg/kg showed mononuclear cells infiltration between mucosal glands and cellular debris in the lumen of these glands as shown in figure 9, while the concentration 100 mg/kg showed no clear lesions. In concentration 50 and 100 mg/kg (intraperitoneal injection), the concentration 50 mg/kg showed few inflammatory cells infiltration between mucosal cells as shown in figure 10. While in concentration 100 mg/kg showed sever inflammatory cells particularly neutrophils infiltration between mucosal glands as shown in figure 11.

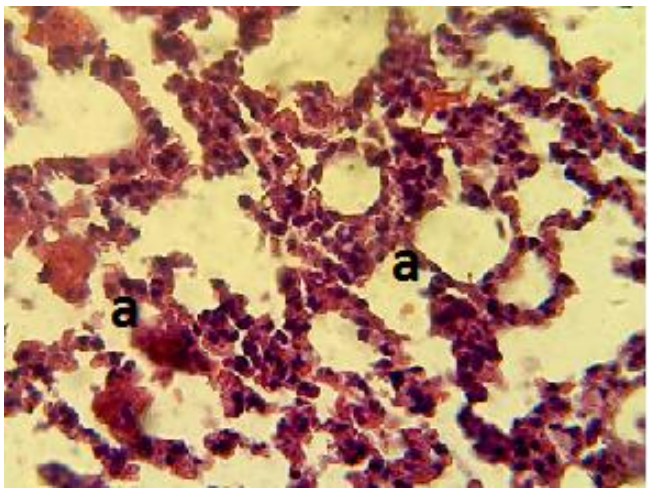

Figure 7: Histopathological section of infected lung treated with β glucan extracted from *C. albicans* intraperitoneal injection in concentration 100 mg/kg shows thickening of the inter alveolar septa brought on by the invasion of mononuclear cells (a) (H&E stain 400X).

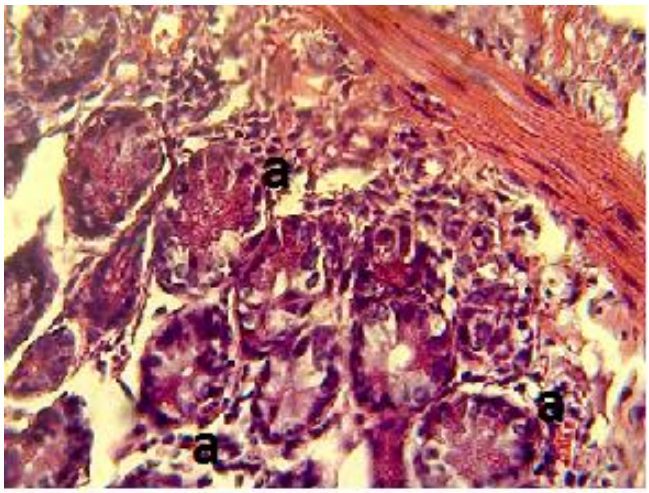

Figure 8: Histopathological Section of intestine infected with *P. chrysogenum* shows severe inflammatory cells infiltration between mucosal glands (a) (H&E stain 400X).

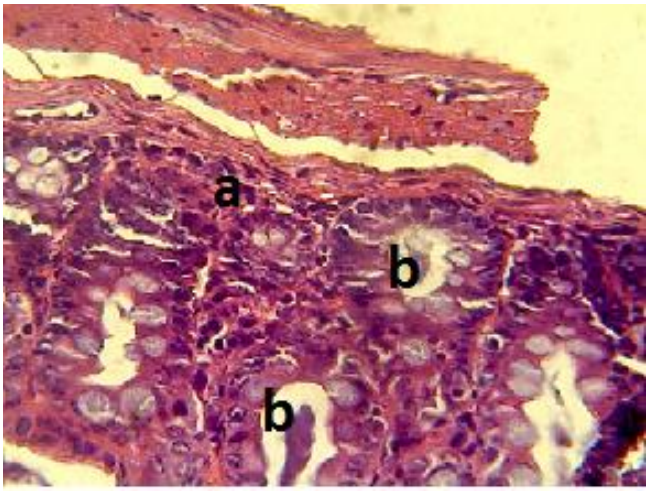

Figure 9: Histopathological section of infected intestine treated with β glucan extracted from *C. albicans* intramammary injection in concentration 50 mg/kg shows mononuclear cells infiltration between mucosal glands (a) and cellular debris in the lumen of these glands (b) (H&E stain 400X).

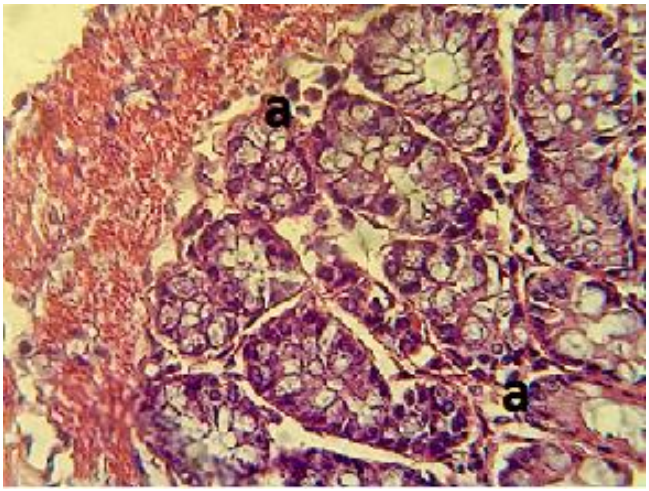

Figure 10: Histopathological section of infected intestine treated with β glucan extracted from *C. albicans* intraperitoneal injection in concentration 50 mg/kg shows few inflammatory cells infiltration between mucosal cells (a) (H&E stain 400X).

Immunological study

The results of determination of IgG concentration in serum samples of mice infected with *P. chrysogenum* appeared that all mice's serum IgG levels were rising. Comparative with normal value of IgG concentration 800-1800mg/dl via measure the diameter of precipitate rings of IgG on the plates as shown in figure 12 and table 1.

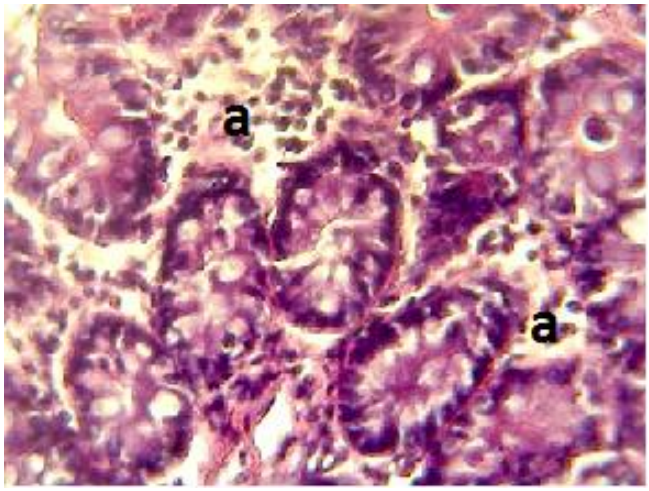

Figure 11: Histopathological section of infected intestine treated with β glucan extracted from *C. albicans* intraperitoneal injection in concentration 100 mg/kg shows severe inflammatory cells particularly neutrophils infiltration between mucosal glands (a) (H&E stain 400X).

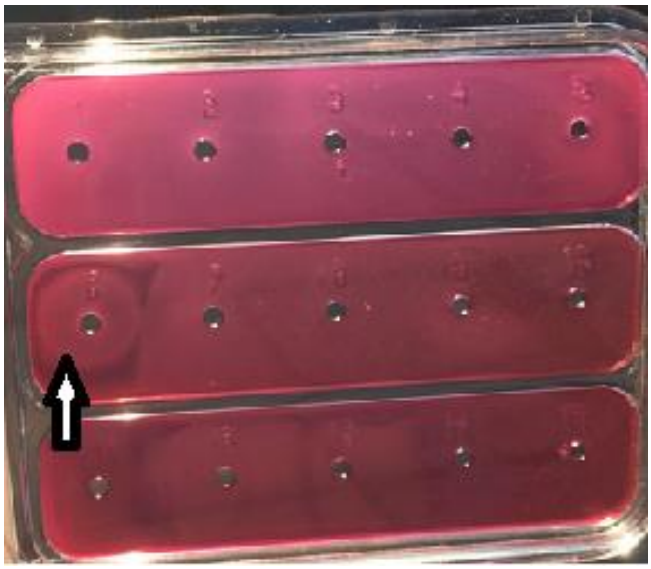

Figure 12: concentration of IgG through the precipitation rings in the gel plate for mice serum samples.

Table 1: Diameters of precipitation rings with concentration of IgG level for mice serum samples

No. of mice	Classification (ring diameter)	Diameter of precipitation ring (mm)	Concentration of IgG
10	6	11.1	3285.4
	3	11	3223.4
	1	10	2634.6

Note: Normal value of IgG concentration 800-1800 mg/dl.

Discussion

One of the most prevalent ambient mesophilic genera in nature is *Penicillium*. The spores of *P. chrysogenum* are widely dispersed in the environment and can be found in household dust, wet spaces, and decaying bread, fruit, vegetables, and other foods. It is possible to classify *P. chrysogenum* as an allergen because it causes skin reactivity and colonizes the airways of people who have respiratory allergies. Despite having a low pathogenicity, it has been described as a human pathogen due to its thermotolerant nature. Cases of skin infections, esophagitis, keratitis, endophthalmitis, pneumonia, endocarditis, infections of the central nervous system, and even very rare cases of disseminated infection in immunocompromised patients have also been reported Aviles-Robles *et al.* (19).

The present study was seemed two types of injection, intramammary and intraperitoneal, the reason behind adopting of intramammary injection (for infection and treatment) because the fungus is isolated from milk samples from cattle infected with bovine mastitis while intraperitoneal is perfect compared with intramammary as well as, technique is rapid, simple to learn, barely stressful to animals, and may be useful for substances that are difficult to dissolve (20). Another study Vetvicka and Vetvickova (21) showed that intraperitoneal injection was more profound effect than oral administration in mice to avoid gastric acidity. Additionally, this technique usually absorbs drugs one- half to one-fourth as quickly as the intravenous approach (22). Very few studies about the *C. albicans* β glucan extraction, as well as the result of HPLC analysis of the *C. albicans* β glucan extraction established the structural likeness with the standard of β glucan (23).

In this study, the internal organs of mice (bone, brain, lung and intestine) were infected with *P. chrysogenum* in different degrees of infection, in bone, the blood vessels appeared congested with infiltration of cells due to infection by this fungus, there are no studies about histopathological effects of *P. chrysogenum* on the bone also the other infected organ in this study brain that showed perineural edema and perivascular edema with congestion in other section, this result agreed with Lyratzopulos *et al.* (24); Kantarcioglu *et al.* (25); Noritomi *et al.* (26) who investigate the effect of *P. chrysogenum* on brain and this research considered the first for reach the infection of *P. chrysogenum* to bone and brain by intramammary injection. On the other hand, several of studies indicated infection of lung by *P. chrysogenum* (27,28).

The results of infected lung by this fungus revealed infiltration of inflammatory cells mainly neutrophils which agreed with Hoselton *et al.* (29) who suggested that inoculation of mice by *P. chrysogenum* produced higher neutrophilic cells. Traynor and Huffnagle (30) reported that tissue phagocytes play a crucial role in host defense against fungal infection, which may explain why more neutrophils were produced in this study as a kind of host protection. However, despite the macrophages' incredible ability to destroy fungal conidia, they are not always successful. By preventing the fungus' germination and growth, this defense mechanism significantly diminishes the infected fungi's pathogenicity (31). The result of infected intestine with *P. chrysogenum* similar to findings given by Mccormick *et al.* (32) when inoculated this fungus to mice subcutaneously. The results of treatment of these organs (bone, brain, lung and intestine) with β glucan extracted from *candida albicans* were appeared great recovery response at 50 mg/kg than 100 mg/kg these results contradicted with Jameel (33).

About the results of immunological study, the presence of precipitating ring which indicate that specific antigen (serum of mice) reacted with the antibodies on the plate of agar gel and found precipitation ring with different diameter and this finding was in line with Homburger-Robles and Singh (34).

Conclusion

Despite of low pathogenicity of *P. chrysogenum*, it is clear that this fungus has a significant part in causing a diversity of histopathological effects in various organs. Furthermore, the current study showed the greater efficacy of β glucan extracted from *C. albicans* with concentration of 50 mg/kg by intraperitoneal injection in mice to treat bone, brain, lung and intestine infected by *P. chrysogenum* than intramammary injection and this is considering first research specify the bone infected by *P. chrysogenum* in Iraq. In addition, this study showed that all mice infected by *P. chrysogenum* appeared highest concentration of IgG when using a radial immunodiffusion plate.

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Conflict of interest

There is no conflict of interest

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تأثير البيتا كلوكان المستخلص من خميرة المبيضات البيض على التغيرات المرضية التي يحدثها فطر بنيسيليوم كريسوجينوم في الفئران

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الخلاصة

هدف هذا البحث إلى تقييم تأثير بيتا كلوكان المستخلص من خميرة المبيضات البيض على التأثير المرضي لفطر بنيسيليوم كريسوجينوم المعزول من التهاب الضرع البقري (تحت السريري) من منطقة أبو غريب في بغداد بواسطة اختبار كاليفورنيا لالتهاب الضرع في شتاء ٢٠٢٠على بعض الأعضاء الداخلية مثل العظام والدماغ والرئة والأمعاء

عن طريق الحقن في الثدي لمدة أسبوعين ثم تم استخدام بيتا كلوكان المستخلص من خميرة المبيضات البيض لعلاج عدوى بنيسيليوم كريسوجينوم بتركيزين ٥٠ و ١٠٠مجم / كغم بطريقتين من الحقن داخل الثدي وداخل الصفاق. أظهرت نتائج الدراسة الحالية درجات مختلفة من الالتهاب في هذه الأعضاء خاصة في العظام والدماغ، بالإضافة إلى أن العلاج باستخدام بيتا كلوكان المستخرج من المبيضات البيض أظهر استجابة قوية للشفاء عند تركيز ٥٠ مجم / كجم عن طريق الحقن داخل الصفاق في جميع الأعضاء. من تركيز ١٠٠ غم/ كجم وكان الحقن داخل الصفاق أفضل من الحقن داخل الثدي. بالإضافة إلى ذلك، أوضحت النتائج أنه تم تحديد تركيز الكلوبيولين المناعي ج في عينات مصل الفئران المصابة بفطر بنيسيليوم كريسوجينوم باستخدام لوحة الانتشار المناعي الشعاعي، والتي أظهرت أقطارا مختلفة من حلقات الترسيب في جل الصفيحة مع زيادة تركيز الكلوبيولين المناعي ج في جميع الفئران مقارنةً بالمستوى الطبيعي. لقيمة تركيز الكلوبيولين المناعي ج.