International Journal of Medical Toxicology & Legal Medicine **Volume 28, No. 1, 2025**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V28I5/018

Study extraction and antifungal effect of oat Beta-glucan against Aspergillusflavus in mice

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Received: 11.09.2024 Revised: 10.10.2024 Accepted: 14.11.2024

ABSTRACT
Objective: In Iraq, may be no research or information about the use of β-glucan extracted from oat as an antifungal against Aspergillusflavus. Therefore, the aim of this study is to evaluate the effect of oat β-glucan against A. flavusin mice.
Materials and Methods: Fifty nasal swabs were collected from dogs (stray and veterinary clinics) suffering from respiratory. Forty of albino mice used for the experiment and divided into four groups, each group contained 10 mice. Group1 was a control (un-treated), group2 control positive for Aspergillus flavusat dose (1 $\times 10^{6}$) spore/ mlintraperitonealI/P for 2 weeks, in group3, the mice treated with oat β-glucan in concentration 500µg in 100 µl per mouse for ten days and group4 was served as a control oat β-glucanintraperitoneal injection in same dosage of group 3;at the end of experimenthematology and histopathology examination.
Results: The results of isolation were reported a high percentage of Aspergillusspp. at 42%, which included (A. flavus andA. niger at 18% respectively, and A. terreus at 6%). The results of oat β-glucan extraction by hot water method were 3 grams from 100grams of oat. The HPLC analysis revealed its structural similarity to the standard of oat β-glucan. The hematological study shows alteration in blood parameters after infection by A. flavus and treated by oat β-glucancause significantly increase ($P \leq 0.01$)in blood parameters(WBCs, neutrophil, lymphocyte and monocytes)and significant decrease ($P \leq 0.05$)in RBCsand Hb. Histopathological study findings demonstrated different degrees of inflammation in three organs (lung, liver and kidney) in second group included presence of hyphae, necrosis, amyloid deposition and degeneration while in third group that included treatment by oat β-glucan was better therapy.
Conclusion: The present study was concluded that the A.flavus causes high pathological lesions and the oat β-glucanhas therapeutics activity against A.flavusdepending on the hematological and histopathological findings.

Keywords: β-glucan, Aspergillusflavus, lung, liver, kidney.

INTRODUCTION

Cereal β-glucan (1 →3) (1→4)-β-d-glucans are defined as fibrous structures found in the aleurone, Cellwalls of cereals sub-aleurone and endosperm. In general, the composition of b-glucaninoats is 3–7% (Karimet al., 2024). According to its well-known beneficial qualities, β-glucan is a polysaccharide that is crucial, particularly in pharmaceutical and nutritious food items (Zhonget al., 2023). β-glucanenhance simmunity through a number of mechanisms, it bindstoleukocytes (phagocytes and macrophages)at specific receptorsites and activates their function and fighting , β-glucanalso has efficacy as an effective immune stimulant in a varietyof infection anti-toxic substance (Alaubydiet al., 2011). Aspergillus spp. is one of the three fungal genera;the other two are Fusarium spp. and Penicilliumspp., being the most significant in the deterioration of foods and in the creation of mycotoxins (Taniwakiet al., 2018). Fungi infection called opportunistic pathogens (AL-Tameemi and Khalaf, 2013). The most opportunistic pathogen is A.flavus, which infect humans and other animal species both superficially and invasively (Nargesiet al., 2021).
Aspergillusflavus isolates which are considered on conidial shape through microscopic examination and mycelia colour through cultural properties. presence of bright yellow-orange pigments indicated the presence of aflatoxins(Abdulateefet al., 2014). In order to infect the host, hazardous virulence factors are required for Aspergillus strains. Aspergillus species produce virulence factors that facilitate the development of an infection and may subsequently lead to invasive or widespread infections. In recent years, the significance of Aspergillus infections has increased (Sales et al., 2013).

MATERIALS AND METHODS

Ethical approval

Ethical approval was granted through the local committee of animal care and use at the College of Veterinary Medicine, University of Baghdad No. P. G. 1225 in 2024\6\26.

Source of fungus

Aspergillusflavuswas isolated from respiratory infection of dogs in Baghdad province (Al-Rusafa and Al Karkh)by cotton swabs. This fungus wascultured on Sabouraud Dextrose Agar (Himedia-India), incubated at 25°C for 4 - 7 days then, diagnoses both macroscopically and microscopically. Spore suspension was prepared for this fungus. Throughout the investigation, standard β glucan was obtained from Sigma Company (Germany origin).

Extraction of β-glucan from oat

This extraction producedaccording to the (Ahmad et al., 2009) that include the following steps:

1. A sample of 100 grams of oats was soaked in 300 milliliters of 80% ethyl alcohol for 6 hours. Water was then added to this mixture in a 1:10 (w/v) ratio and stirred on a heated magnetic stirrer at 55°C for 90 minutes.
2. Mixture was centrifuged at 5000 rpm for 20 minutes at 40°C, and supernatant was collected.
3. The pH was adjusted to 8.5 using 20% sodium bicarbonate (Na_2CO_3), followed by stirring with a magnetic stirrer at 55°C for 30 minutes.
4. After centrifuging the mixture at 5000g for 20 minutes at 40°C, precipitate was discarded. The supernatant's pH was lowered to 4 with 2 M citric acid, centrifuged again, and then subjected to centrifugation at 5000 g for 25 minutes. The supernatant was mixed with 80% ethyl alcohol 1:1 ratio at 20 minutes and centrifuged at 4000 g at 4°C for 25 minutes.
5. Finally, β-glucan was dried in a petri dish in an air oven at 55–60°C for 24 hours.

The crude β-glucan yield percentage was calculated using the formula:

$$\text{β-glucan yield (\%)} = \frac{\text{Weight of crude β-glucan (g)}}{\text{Weight of sample (g)}} \times 100$$

The β-glucan extraction process is depicted in Figure 1.

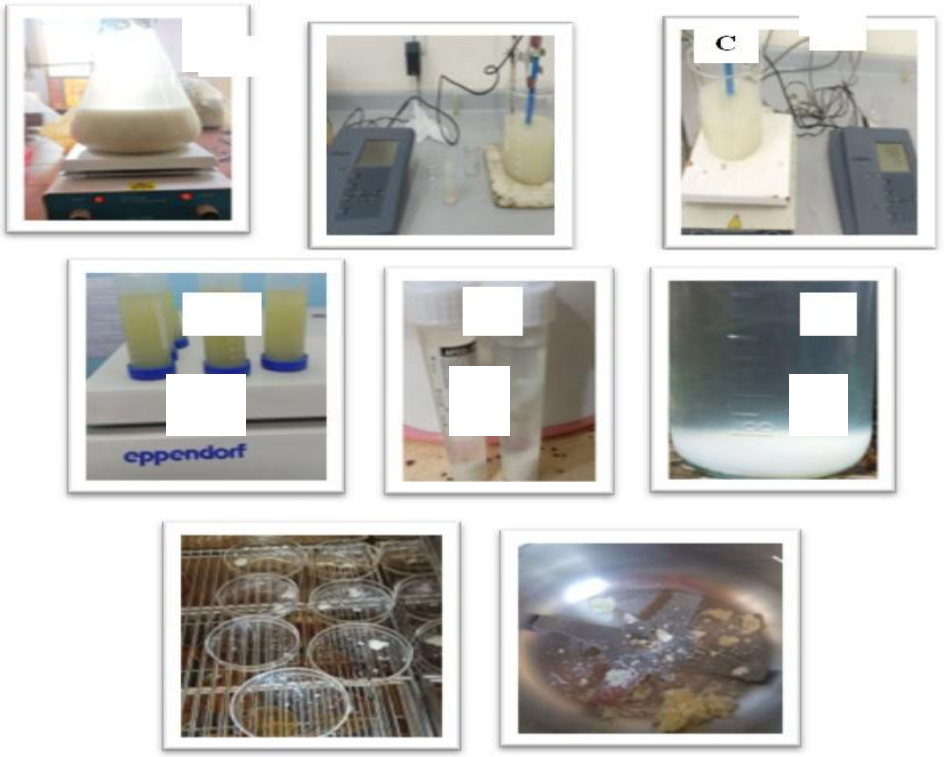

Figure 1. Main steps of oat β-glucan extration A- oat and ethyl alcoholmixedinhot magnetic stirrer B- The pH was adjusted to 8.5 C- supernatant was adjusted to pH 4 D-centrifugal at 5000 g for 25 min E- supernatant suspentionF-Put β-glucan in petri dish G-dried in an air oven H-grinding with electric machine

Evaluation of oat β-glucan efficacy in vivo

A forty albino whitemice were used in the study were purchase from national center for drug control and research, at the age of 10-12 weeks with maintained on a standard laboratory diet, water and temperature-controlled at the animal house laboratory in Veterinary medicine college/ university of Baghdad. These animals were split into 4 groups:First Group(n=10)Control group untreated, Second Group(n=10) Control group (+ve) infected with0. 5ml containing (1×10^{6}) sporeofA. flavusintraperitoneal for 2 weeks, third Group(n=10)infected with A. flavus treated by oat β-glucanintraperitoneal(in concentration 500µg in 100µl per mouse) daily for 10 days (Udeaniet al., 2013), Fourth group (n=10)injected oat β-glucanintraperitoneal (in concentration 500µg in 100 µl per mouse) daily for 10 days (Yun et al., 2003).

Collection of blood samples

Blood samples were collected after completing the experiment from all groups, all mice were anesthetized with chlorophorom about 1ml of blood was collected directly from the heart through cardiac puncture by using a sterile syringe. The blood was collected in sterile test tube with anticoagulant (EDTA K3) for demonstration of RBCs, Hb, WBCs counts, neutrophils, lymphocytes and monocytes(Coles, 1986; Mohammad et al., 2022).

Histopathological study

One cm3 of the lung, kidney and liver of each animal from groups were taken fixed 10% neutralformalin buffersolution, and then this formalin solution was replaced after 24hrsuntil the preparations of histological sections (Luna, 1968; Gharban et al., 2023).

Statistical Analysis

TheChi-square test was utilized to determine the effect of various variables on parameters to compare means significantly and the least significant difference (LSD)at $p \leq 0.05$ and $p \leq 0.01$ probability (Al-Abedi et al., 2020; AL-Shaeli et al., 2022).

RESULTS AND DISCUSSION

Extraction of β-Glucan from oat

In this study, extraction of β-glucan from oats was done through hot-water extraction. Initially, inactivation of natural β-glucanase was performed in hot ethanol followed by the extraction in hot water. Moreover, a slightly alkaline solution was used for dissolving acid to separate proteins and fibers, while ethanol precipitation allowed getting the granules of β-glucan. This particular method of extraction has been chosen because it provides the yield of the product in high amount without significant use of chemicals, being thus efficient and ecological (Maheshwariet al., 2017).

By this method, the dry weight of β-glucanobtained from 100gm. of oat was 3 gmas shown in Table 1, another results revealed that the morphological features of β-glucan extracted from oat characterized by crystal creamy in color as shown in Figure 2. This study agree within the range reported in previous studies (Peterson, 2002; Butt, 2008), indicating consistency and reliability in the extraction technique. The creamy, crystalline morphology of the extracted β-glucan suggests a high-quality product with preserved structural integrity, which is essential for potential applications in food and health industries. The choice of hot-water extraction, along with ethanol and minimal chemical intervention, further emphasizes this method's effectiveness in producing a high- quality oat β-glucan product.

Table 1. Percentage of β_glucan extracted from oat.

Method	Type plant	Oat weight	Weight of β-glucan extracted (gm)	Percentage of Extraction (%)
Hot water extraction	Oat	100	3	3

Figure 3. Shows powder of β-glucan extracted from oat

High Performance Liquid Chromatography (HPLC)analysis ofβ–glucan

The HPLC technique was utilized to assess the quality and purity of extracted β-glucan using hot water extraction procedures. The HPLC analysis of isolated β-glucan from oat revealed its structural similarity to the standard. The analysis revealed ismajorpeak 3.703 of liquid samples of β-glucan extracted from oat as shown inTable 2 and Figure 3which representing the purity of the extracted β-glucan from oat.

Table 2. The results of sequences of eluted material of β-glucan detected by HPLC

Sequences	Subject	Retention timeArea		Concentration
1	Standard of β-glucan	3.633	867383	40mg/ml
2	Sampleof β-glucan extracted from oat	3.703	913990	40mg/ml

Evalation of β-glucan efficacy in vivo

The important clinical signes that appeared on mice after one weak of infection this fungusat dosage 0. 5ml containing (1×10^6) spore intraperitonealincludeddecrease food intake, weakness, weight loss , swellingofabdominal and signs of inflammation as shown in Figure 5A.

Cinical signs of mice treated with oat β-glucan

Mice treated with oat β-glucan in concentration 500µg in 100 µl per mouse dailyintreaperitonieal for 10 days after infection byA. flavusshowed reduce in the severity of signs as shown in Figure 5-B.

Figure 5. Miceinfected byA. flavus(A), mice treated by oat β- glucan(B)

Hematological study

The Blood samples were taken from all groups in the end of experiment by employment tubes containing an anticoagulant agent. The results of current study about hematological tests as shown in Table 3which appeared highly significant differences (($P\leq0.01$) between the groups (infection, control β- glucan and treatment) comparative with control group included increase in WBC, lymphocyte, Monocyte and neutrophil count, this increasing observed in the infection group was due to the innate immune system, which serves as the first line of defense against metabolically active fungi and conidia.

Key innate immune cells involved in defense against aspergillosis include macrophages, neutrophils, and monocytes. In contrast, the increase in the other groups (control, β-glucan, and treatment) was attributed to β-glucans, which are used in medicine to stimulate the immune system (Margalitet al., 2015).

The other parameters of this study (RBC and Hb) showed significant decrease ($P\leq0.05$) between the groups while no significant differences in PCV count comparative with control group as shown in Table 4.

This result was contract with (Mansooret al., 2018) who suggested decrease in blood parameters when infected by A. flavus(decreased in RBCsand Hb). The most significant virulence factor in Aspergillus species is the phospholipase enzyme, which the pathogenic fungi utilize to cause anemia as seen by decreases in Hb%, and RBC counts (Li et al., 2012), also arachidonic acid is produced by pathogenic fungus when they penetrate, destabilize, and break down the membrane phospholipids that surround red blood cells. Additionally, phospholipase hydrolase red blood cells to release phosphatidylserine (PS) and produce lysophosphatidic acid (LPA), the latter of which causes in the passage of substances through a blood cell membrane and produces swollen before exploding (Neidlingeret al., 2006).

The result of blood cells parameter in group control β-glucan and treatment, the RBC count decreased Hemoglobin and RBC amounts that contact with (Shafiq and Al-Joofy, 2010); which also reported high leucocytes values using β-glucansupplemented to red snapper Lutjanusguttatus, and also a decrease in Hb and RBC values after the first treatment week; cause of the decrease may be over experimental time which also reported decrease in Hb and RBC values after the treatment agree with (Hussainet al., 2020). In general, a lower hematocrit percentage may indicate that they are susceptible to stress caused by experimental management or the pathogenic load found in the culture environment (Parady, 2018).

Table 3. Comparison between difference groups in White Blood Cell parameters

Parameters	Control	Infection	Control β-glucan	Treatment	LSD value
WBCs	1. 46 ±0. 03 b	4. 48 ±1. 15 b	4. 35 ±0. 62 b	12. 78 ±5. 14 a	4. 175 **
Lymph	0. 792 ±0. 02 b	1. 215 ±0. 29 b	1. 835 ±0. 19 b	3. 74 ±0. 82 a	1. 379 **
Monocyte	0. 175 ±0. 008 b	0. 717 ±0. 22 ab	0. 467 ±0. 04 b	1. 407 ±0. 39 a	0. 703 **
Neutrophil	0. 472 ±0. 009 b	2. 54 ±0. 65 b	2. 003 ±0. 365 b	7. 43 ±4. 29 a	3. 714 **
Means having with the different letters in same row differed significantly. * (P≤0. 05), ** (P≤0. 01).					

The other parameters of this study (RBS and HB) showed significant decrease (P≤0. 05) between the groups while no significant differences in PCV count comparative with control group as shown in Table 4.

Table 4. Comparison between difference groups in Red blood Cell parameters

Parameters	Control	Infection	Control β-glucan	Treatment	LSD value
RBC	10. 08 ±0. 01 a	9. 01 ±0. 31 ab	7. 92 ±0. 08 b	9. 10 ±0. 95 ab	1. 548 *
Hb	14. 27 ±0. 04 a	13. 22 ±0. 29 b	11. 67 ±0. 19 c	11. 15 ±0. 40 c	0. 831 **
PCV	42. 15 ±0. 03	39. 00 ±5. 09	43. 67 ±2. 24	40. 20 ±2. 95	9. 712 NS
Means having with the different letters in same row differed significantly. * (P≤0.05), ** (P≤0.01).					

Histopathological changes

Histopathological changes of lung

Histopathological changes of lung infected with A. flavus (positive control group)

Histopathological examination of lung infected with A. flavusshowed abundant hyphae in pulmonary parenchyma with sever inflammatory infiltration and necrosisas shown in Figure6. Other section showedbronchiectasisas shown in Figure 7.

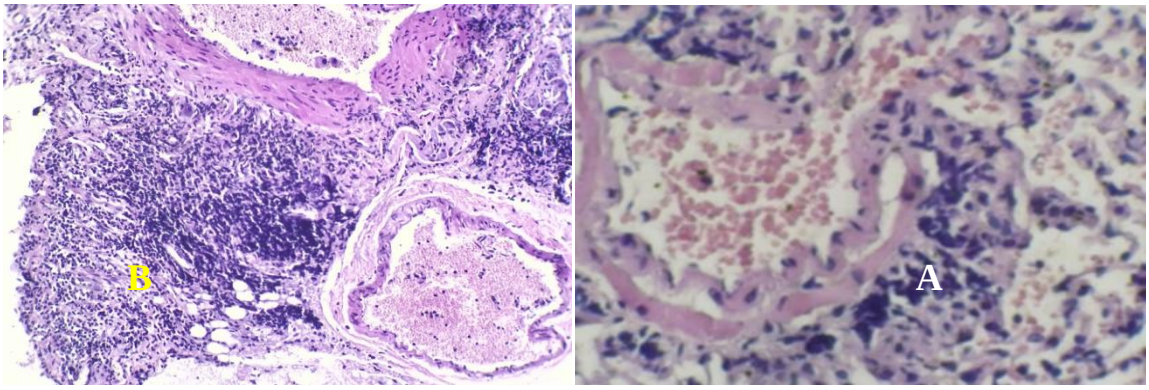

Figure 6. Cross section of Lung infected shows massive infiltration of MNCs(A) with neutrophils accompanied abundant hyphaein pulmonaryparenchyma(B) 40X (H and E) Stain

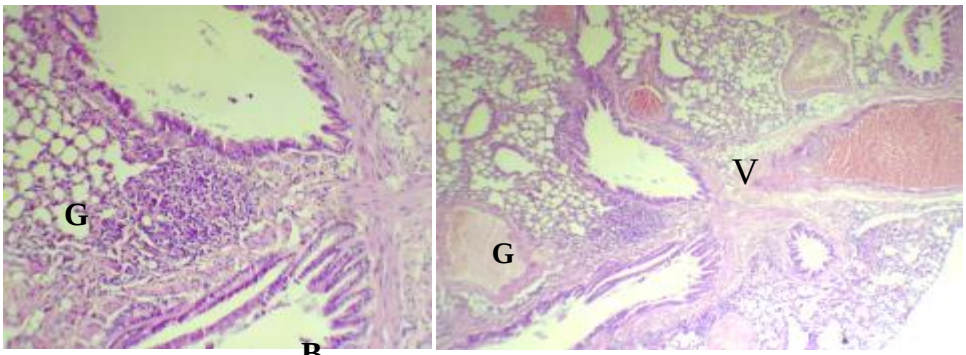

Figure 7. Histopathological section of Lung infected in control positive group shows presence of granuloma recognized near bronchial tissue accompanied(G)with marked pulmonary vessels congestion(V) bronchiectasis(B)(H and E Stain 10X)

Histopathological changes of infected lung that treated with oat β-glucan
Histopathological examination of infected lung treated by oat β-glucanshowedbronchiolar epithalial hyperplasia with prominant catarrhal exudate and difference interstial inflammatory infiltration as shown in Figure 8.

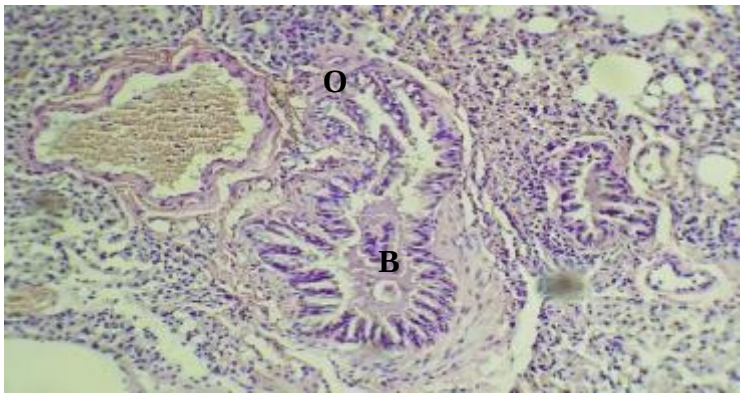

Figure 8. Histopathological sectionof infected Lung treated with oat β-glucan shows bronchiolar epithalial hyperplasia (B)with prominant catarrhal exudate(O) and difference interstial inflammatory infiltration. (H and E Stain 10X).

Histopathological changes oflung(control β-glucan)
Histopathological examination of lung treated by oat β-glucan only without infection with A. flavus, showed leukocytic infiltration and Minimal thickening of interalveolar tissue as shown in Figure9.

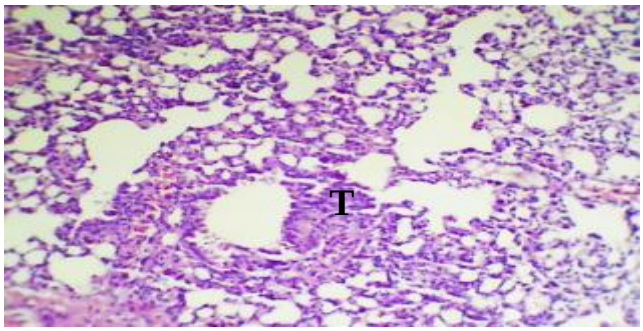

Figure 9. Histopathological section of Lung in group (control β-glucan) showsMinimal thickening of interalveolar tissue withleukocytic infiltration(T). (H and E Stain 10X)

Histopathological changes of liver
Histopathological changes of liver infected with A. flavus (positive control group)
Histopathological examination of liver infected with A. flavusshowed hydropic swelling of hepatocytes, scattered apoptotic hepatocytes as shown in Figure10, other section showeddegenerative finding of hepatic cordswith nuclear pyknosis and scattered apoptotic hepatocyte mainly recognized portal region with evidence of portal inflammatory infiltration particularly around dilated and congested portal vein and other section showedgranulomatous lesion composed mainly of MNC_S and PMN_Sother section evidence of focal and diffuse amyloid like substance deposition as shown in Figure 11, 12.

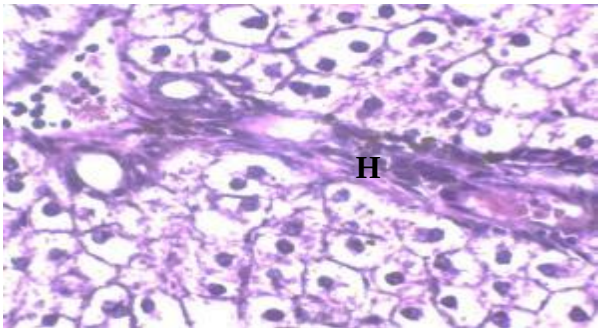

Figure10. Histopathological section of Liver infected group showed massive hydropic swelling of hepatocytes with slight portal inflammatory cell infiltration (H) (H and E Stain 40X)

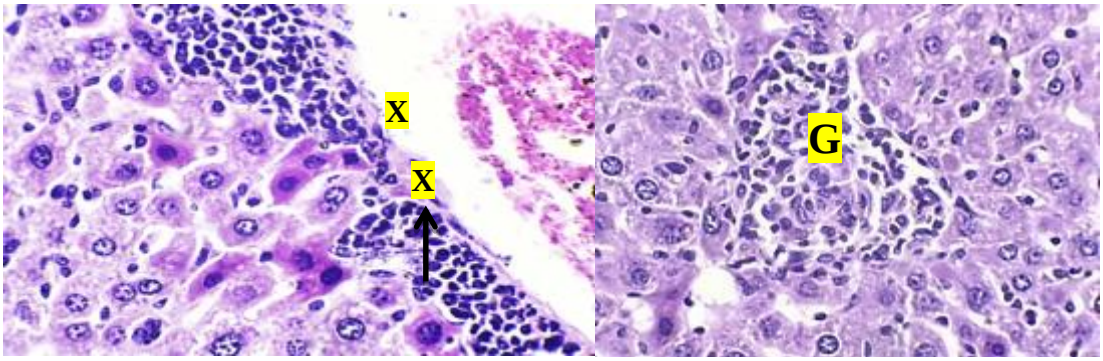

Figure 11. Histopathological section of Liver infected group perivascular MNCS cuffing (X) with scattered apoptotichepatocytes H and E 40X (arrow) granulomatous lesion composed mainly of MNC_S and PMN_S (G). Hand E Stain 40X

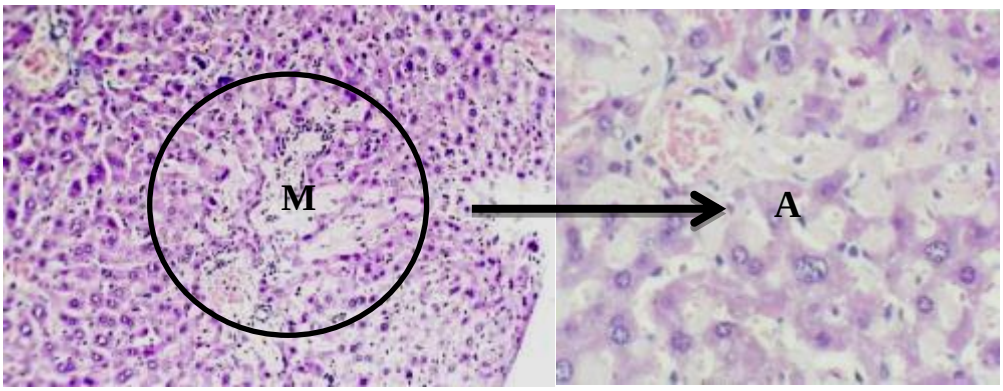

Figure 12. Histopathological section of Liver infected group show evidence of focal and diffuse amyloid like substance deposition (A) with mild sinusoidal MNC_S infiltration (H and E Stain 10X)

Histopathological changes of infected liver that treated with oat β-glucan

Liver section of treated group showed multiple and perivascular MNC_S infiltrationsurrounded by scattered apoptotic hepatocytes together with prominent proliferation of kupffer cells as shown in Figure 13.

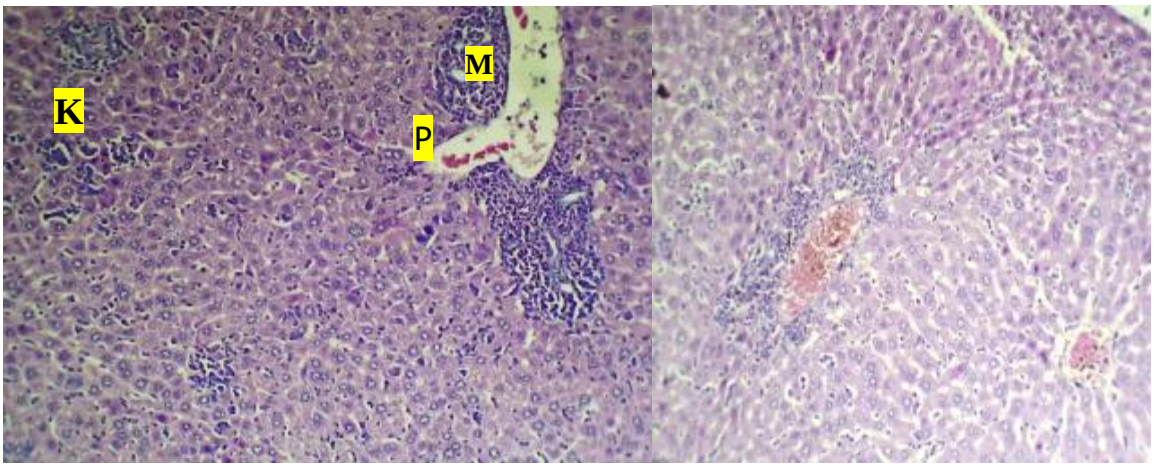

Figure 13. Histopathological section of Liver treated group showed multiple and perivascular MNC_S infiltration (M) surrounded by scattered apoptotic hepatocytes (P) together with prominent proliferation of kpuffer cells (K) (H and E Stain 40X).

Histopathological changes ofliver that treated with oat β-glucan (control β-glucan)

Histopathological examination of kidney treated by oat β-glucan showed no clear pathological alteration in liver section with few binucleated hepatocytestogether with prominent kupffer cells as shown in Figure 14.

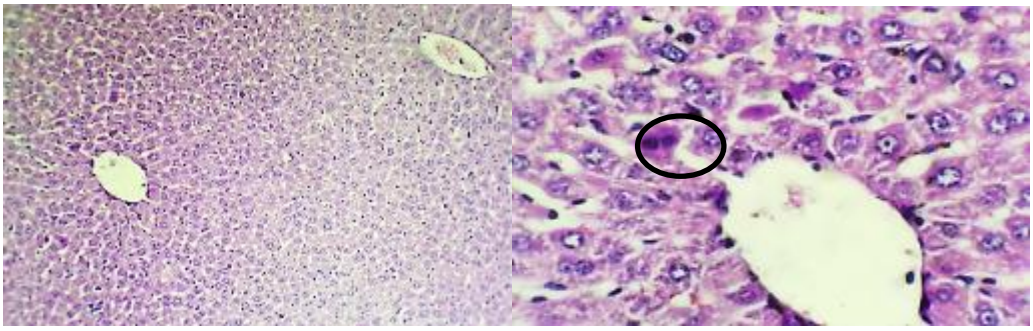

Figure 14. Histopathological section of Liver control β-glucan group showed no clear pathological alteration in liver section with few nucleated hepatocytes together with prominent kupffer cells(H and E Stain 40X)

Histopathological changes of kidney

Histopathological changes of kidney infected with A. flavus (positive control group)

Renal tubulesshowed majorty of various form of cystic dilationwith nucleur pyknosis of some tubles and diffuse intrestial MNC_Sinfiltration accompanied with sever atrophy of glomerularetuftas shown in Figure 15 and in other section cellular swelling with sloughed epithelial and blood vessel congestion as shown in Figure 16.

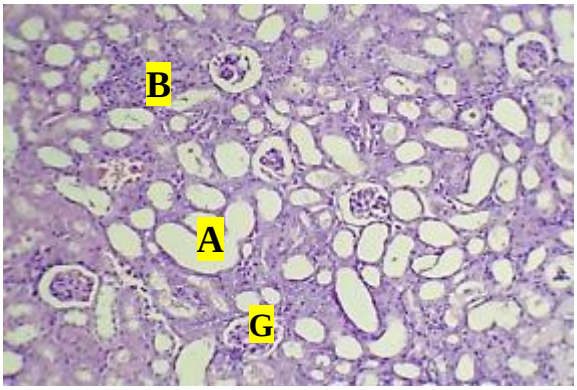

Figure 15. Histopathological section of infected kidney showed majorty of renal tubulesvarious form of cystic dilation (A)with nucleur pyknosis of some tubles and diffuse intrestial MNC_Sinfiltration (B) accompanied with sever atrophy of glomerularetuft(G) (H and E Stain 40X).

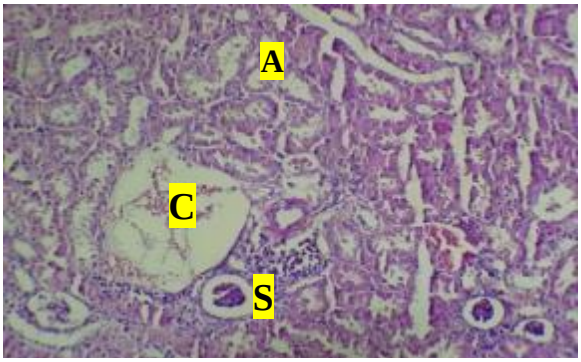

Figure 16. Histopathological section of infected kidney showed moderate cellular swelling (A) with sloughed epithelial (S) and blood vessel congestion (C). (H and E Stain 10X).

Histopathological changes of infected kidney that treated with oat β-glucan

Showed marked perivascular MNC_S aggregation with slight cellular swelling of adjacenttubules as shown in Figure17.

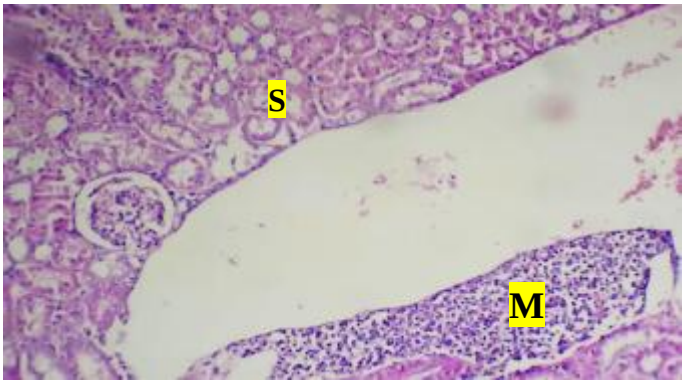

Figure 17. Histopathologicalsection of treated kidney with β-glucan showed marked perivascular MNC_S aggregation (M) with slight cellular swelling of adjacenttubules (S). (H and E Stain 10X)

Histopathological changes of kidney that treated with oat β-glucan (control β-glucan)
Showed mild to moderate MNC_S infiltration composed mainly of macrophage between tubules as shown in Figure 18.

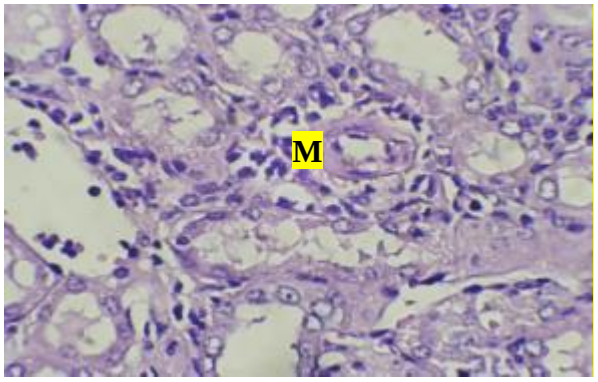

Figure 18. Histological section of kidney control β-glucan showed mild to moderate MNC_S infiltration composed mainly of macrophage between tubules (M) (H and E stain 40X)

The results of the Histopathological study in the second group (control positive group) revealed a unique feature: A. flavus can enter blood vessels and move to different body systems, where it can develop and spread infection. lung infected with A. flavusshowed abundant hyphae in pulmonary parenchyma with sever inflammatory infiltration and necrosis that contact with previous researchers' (Shafiq and Al-Joofy, 2010; Hussainet al., 2020) findings confirmed thatAspergillus causes high degrees alterations in mice lung tissue, including congestion and bleeding of pulmonary blood vessels, thickening of the alveolar wall, and infiltration of inflammatory cells.
Themetabolic effects of A. flavus led to liver congestion, primarily due to aflatoxins (AF), which are secondary metabolites produced by the fungus A. flavus. The presence of amyloid may indicate an innate immune response aimed at eliminating fungal colonization through structural disruption and cytotoxicity (Parady, 2018). Toxins cause congestion and leukocyte infiltrationin liver. Aspergillusmetabolic substance causes necrosis in liver cell (Fadhilet al., 2017).
Compared to the infected group, the third group pathological lesions were less widespread in the organs supplemented with β-glucan. According to this research, β-glucanhavestimulation the innate immune response, which is crucial for triggering the adaptive immune response and affecting the course of an infection. According to Babineauet al. (1994), β-glucan activates receptors that start an innate immune response to infections. Granuloma development results from the activation and aggregation of mature macrophages around invading pathogens, which is mostly dependent on β-glucan. This aligns with findings by (Yadav and Schorey, 2006). β-glucan has the capacity to modify body's natural healing processes by promoting epithelial hyperplasia, inflammatory cell activity, angiogenesis and fibroblast proliferation an showed that β-glucan had the degradation effects on biofilm (Khadam and Salman, 2024). β-glucan bind to various types of cell surface receptors including monocytes, macrophages, neutral killer cells, neutrophils and lymphocyte populations, resulting in activation of lymphocyte, production of inflammatory cytokines and chemokines and microbial killing (Mahdi, 2012)who shown how β-glucan stimulates immunological responses including phagocytosis, which aids in the removal of pathogenic organisms. Macrophages absorb particulate β-glucan, disperse it throughout the body, and break it down to create a soluble, bioactive glucan that stimulates the synthesis of IL-

12(Hong et al., 2004;Alkhalidiet al., 2019). β-glucan could inhibit the growth of Aspergillus flavus and also reduce the toxins produced, namely aflatoxins (AFB1 and AFB2. β-glucan possesses significant immune modulatory properties, capable of potentiating cellular immunity by activating immunecells and promoting cytokine production (Mahmoud and Yassein, 2024).Finally, understanding the host's antifungal immunity mediated by β-glucan has been a significant challenge in biomedical research. Studies using β-glucan in mouse models have greatly advanced our knowledge of its role in fungal disease progression, susceptibility, and resistance over recent decades (Desamero and Chung, 2021).

CONCLUSION

Fungal infections pose a serious health threat, and current options for combating fungi are quite limited. Therefore, the search for new, effective, and economical antifungal solutions is essential. Fungi are a diverse group of organisms, making it challenging to identify a compound with a broad antifungal spectrum that is also safe for humans, animals, and plants. This research concludes that oat β-glucan exhibits antifungal activity, particularly against A.flavus, and can be effectively used as an immunostimulatory agent.

ACKNOWLEDGMENTS

The chief veterinarian of the Baghdad governorate, the medical team, and the veterinary clinic are all appreciated by the authors for their help with sample collection. The authors also appreciate the personnel in the microbiology departments of the University of Baghdad, Iraq's college of veterinary medicine for their assistance and the facilities they provided for the processing of the samples.

Conflict of interest

The authors have declared no conflict of interest.

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