Al-Qadisiyah Journal of Pure Science

Volume 29 | Number 1 Article 7

2024

Effect of alcoholic extract of Agaricus Bisporus on blood profiles and immune response in rats with aspergillosis

Athraa Abbass Jawad
Dept. Biology, College of Science, University of Al-Qadisiyah, Iraq, jwaddhrabas@gmail.com

Neeran Obied Jasim
Dept. Biology, College of Science, University of Al-Qadisiyah, Iraq, neran.jasim@qu.edu.iq

ARTICLE

Effect of Alcoholic Extract of *Agaricus bisporus* on Blood Profiles and Immune Response in Rats With Aspergillosis

Athraa A. Jawad*, Neeran O. Jasim

Dept. Biology, College of Science, University of Al-Qadisiyah, Iraq

Abstract

***Aspergillus Fumigatus* is an opportunistic fungal pathogen that causes a range of infections in humans, particularly in immunocompromised individuals. In this study, we investigated the therapeutic potential of *Agaricus bisporus* extract for treating *A. fumigatus*-induced pulmonary infection in mice. Mice were inoculated with *A. fumigatus* and then treated with *A. bisporus* extract for 21 days. We found that *A. bisporus* extract and voriconazole treatments affect WBC count, with the extract-treated groups and voriconazole-treated group showing lower counts compared to infected mice without treatment, *A. bisporus* extract-treated groups display higher RBC counts, while voriconazole treatment results in lower RBC count compared to infected mice without treatment. Lastly, voriconazole treatment decreases IFN-γ levels, while treatment with the mushroom extract slightly increases IFN-γ levels. The findings suggest that *A. bisporus* extract and voriconazole may influence immune response parameters and infection control. The extract shows potential immunomodulatory effects, as evidenced by alterations in WBC count, LYM levels, and RBC count. Voriconazole, on the other hand, affects WBC count, RBC count, and IFN-γ levels, indicating potential immune modulation.**

Keywords: *A. fumigatus*, COVID-19, *A. bisporus*, Antifungal activity

1. Introduction

The outbreak of COVID-19, caused by the novel coronavirus SARS-CoV-2, has had a significant impact on global health and economies. As the scientific community continues to explore various treatment options and preventive measures against COVID-19, there is a growing interest in the potential benefits of natural products and their extracts. *Agaricus bisporus*, commonly known as the white button mushroom, has been recognized for its medicinal properties and immune-modulatory effects [1]. In this study, we investigate the effect of the alcoholic extract of *A. bisporus* on blood profiles and immune response in rats with aspergillosis, a fungal infection that has been isolated from COVID-19 patients.

COVID-19 is primarily a respiratory illness, but it can also weaken the immune system, making individuals susceptible to secondary infections. Aspergillosis, caused by the Aspergillus fungus, is one such opportunistic infection that can affect immunocompromised individuals, including those recovering from COVID-19. The respiratory symptoms and immune dysregulation associated with COVID-19 may create an environment conducive to the growth of Aspergillus. Therefore, exploring potential treatment options for fungal infections in COVID-19 patients is crucial [2].

Agaricus bisporus is widely consumed as a culinary mushroom and has gained attention for its potential health benefits. It is rich in bioactive compounds such as polysaccharides, proteins, phenolic compounds, and terpenoids, which have shown immunomodulatory, antioxidant, and antimicrobial properties in various studies. These properties make *A. bisporus* an interesting candidate for investigating its effect on blood profiles and immune response in

Received 10 July 2023; accepted 11 April 2024.
Available online 28 September 2024

* Corresponding author.
E-mail addresses: jwaddhrabas@gmail.com (A.A. Jawad), neran.jasim@qu.edu.iq (N.O. Jasim).

https://doi.org/10.29350/2411-3514.1228

the context of aspergillosis, especially in the presence of COVID-19 [3].

Understanding the effect of *A. bisporus* extract on blood profiles is essential for assessing its impact on the overall health status of rats with aspergillosis. Hematological parameters, including red blood cell count, white blood cell count, and differential leukocyte count, can provide valuable insights into the immune response and potential alterations induced by the extract [4].

Furthermore, investigating the immune response is crucial for evaluating the therapeutic potential of *A. bisporus* extract in aspergillosis. This can involve assessing cytokine levels, such as interleukins and interferons, which play key roles in regulating immune responses and inflammation. Additionally, evaluating specific immune cell populations, such as macrophages and lymphocytes, can provide information on the extract's effect on cellular immunity [5].

By examining the effect of the alcoholic extract of *A. bisporus* on blood profiles and immune response in rats with aspergillosis, isolated from COVID-19 patients, we aim to contribute to the understanding of potential therapeutic strategies against secondary fungal infections in the context of COVID-19. The findings from this study may have implications for the development of natural and complementary therapies that can support the immune system and improve the health outcomes of individuals recovering from COVID-19.

2. Method and material

2.1. Study design

This study was conducted in the animal house in the College of Science, Al-Qadisiyah University for the period from 1-10-2022 to 1-5-2023. In the current study, the mice were divided into five groups (5 for each group) labeled as 1, 2, 3, 4, and 5. Each group represents a specific treatment or condition applied to the mice as follows: Group 1: Infected mice with *Aspergillus fumigatus* without treatments. Group 2: Infected mice with *A. fumigatus* and treated with 8% *A. bisporus* extract. Group 3: treated with 16% *A. bisporus* extract - Similar to Group 2, this group comprises mice infected with *A. fumigatus*, but they received treatment with a higher concentration of 16% *A. bisporus* extract. Group 4: treated with 20% *A. bisporus* extract. Group 5: treated with voriconazole. All groups were monitored for two weeks to monitor the development of symptoms of the disease, such as decreased movement and nasal congestion. Also CBC, gamma interferon (IFN-γ) and glutamic-pyruvic transaminase (GPT) levels were measured.

2.2. Preparation of the hot alcoholic extraction of A. bisporus

Thakare, 2004 method was used to prepare the hot extraction of mushroom:

Mushroom (*A. bisporus*) was obtained from Al-Wadaq farm for the production of mushrooms located in Baghdad. The fruit body of mushrooms were washed with plain water, then with distilled water, and cut into small pieces with a clean knife and dried by (oven) air at a temperature of 50 °C and then grinded for the purpose of obtaining on powder. The alcoholic extract was made using 400 ml of (80%) methanol alcohol as a solvent for 100 gm of dry powder using a Continuous Soxhlet Extraction device, where the extraction process began by heating the solvent (methanol alcohol) in the glass flask at 40 °C, and the steam in the glass flask rose to the distillation unit via the connecting tube between them, and then condensed as a residuum. On the cellulosic cup, in the form of drips and streams Thumbles in the extraction unit containing the dry powder until the cellulosic cup is immersed in the solvent, In this case, the substance's compounds and contents were transferred to the solvent, and when the extraction unit was filled, the solvent left with what was dissolved from the powdery substance in it by the siphon process, and when the process was repeated, the solvent returned to evaporate, leaving the plant compounds in the glass flask, and the process was repeated until the solvent's color became inside the extraction unit solvent turns into a dark brown color, this signifies the completion of the extraction process, which took a total of (24) hours. The extracts were dried in a Vaceum rotary evaporator at temperatures ranging from 45 to 50 °C until a thick liquid was formed. After that, the extract was dried completely.

2.3. Administering A. fumigatus *to mice*

A fungal growth plate containing *A. fumigatus* was obtained. A certain amount of spores was collected from the top of the fungal growth plate using a sterile loop or pipette then mixed with a certain amount of DW (distilled water) then administered to the mice once a day via inhalation and intranasal instillation using a syringe. A waiting period of two weeks was observed until disease symptoms appeared, such as reduced movement and nasal congestion.

2.4. Parameters measurement

Blood Collection: Collect blood samples from each animal using aseptic techniques and appropriate blood collection tubes. Ensure sufficient sample volume for subsequent analyses. Sample Processing: Centrifuge the blood samples to separate the serum or plasma from cellular components. Collect the desired fraction based on the analysis to be performed. Complete blood picture CBC was conducted for all groups using an CBC device, gamma interferon levels were determined using an immunoassay technique. The blood samples were subjected to specific antibodies that bind to gamma interferon, allowing its quantification through a colorimetric or fluorescent signal. This measurement provided insights into the immune response of the subjects.

2.5. Animal ethics

Obtaining ethical approval from the Council of the College of Science at the University of Al-Qadisiyah to conduct experiments involving animals.

3. Results

3.1. White blood cell (WBC)

Fig. 1 represents the white blood cell (WBC) count in the serum of mice belonging to different groups under various conditions. The WBC count is measured in units of 10^3 per milliliter (ml).

The data consists of five mice groups, labeled as 1, 2, 3, 4, and 5. Each group represents a specific treatment or condition applied to the mice, along with the corresponding WBC count. The groups and their respective conditions are explained as follows: Group 1: Infected mice with *A. fumigatus* without treatments - The WBC count for this group is 22.9 × 10^3/ml. Group 2: Infected mice with *A. fumigatus* and treated with 8% *A. bisporus* extract -The WBC count for this group is 5.6 × 10^3/ml. Group 3: treated with 16% *A. bisporus* extract - Similar to Group 2, this group comprises mice infected with *A. fumigatus,* but they received treatment with a higher concentration of 16% *A. bisporus* extract. The WBC count for this group is 3.8 × 10^3/ml. Group 4: treated with 20% *A. bisporus* extract - The WBC count for this group is 4.0 × 10^3/ml. Group 5: treated with voriconazole - The WBC count for this group is 4.5 × 10^3/ml.

3.2. Red blood corpuscles (RBC)

Fig. 2 represents the red blood cell (RBC) count in the serum of mice groups RBC count is measured in units of 10^6 per milliliter (ml). Group 1: Infected mice with *A. fumigatus* without treatments - RBC count for this group is 8.27 × 10^6/ml. Group 2: Infected mice with *A. fumigatus* and treated with 8% *A. bisporus* extract - RBC count for this group is 6.32 × 10^6/ml. Group 3: treated with 16% *A. bisporus* extract - RBC count for this group is 7.26 × 10^6/ml. Group 4: treated with 20%, RBC count for this group is 4.18 × 10^6/ml. Group 5: treated with voriconazole - RBC count for this group is 3.16 × 10^6/ml.

3.3. Platelet (PLT)

The result as shown in Fig. 3 the group 1: Infected mice with *A. fumigatus* without treatments - platelet count for this group is 554 × 10^3/ml. Group 2:

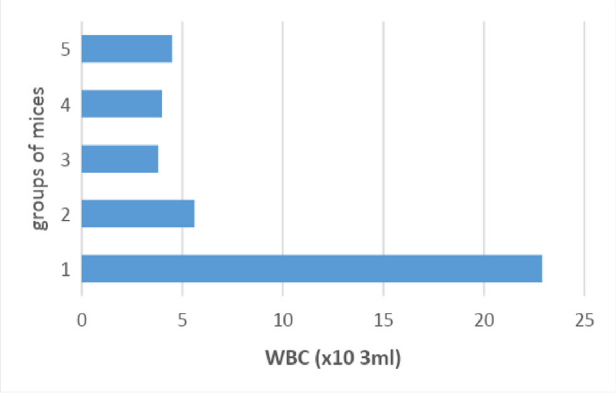

Fig. 1. White blood cells (WBCs) (×10^3/ml) count in serum of mice (1-infected 2-treated with 8% 3-treated with 16% 4-treated with 20% 5-treated voriconazole).

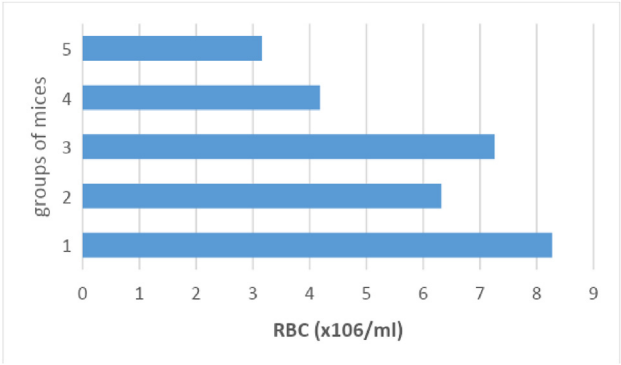

Fig. 2. Red blood cells (RBC) (×10^6/ml) count in serum of mice (1-infected 2-treated with 8% 3-treated with 16% 4-treated with 20% 5-treated voriconazole).

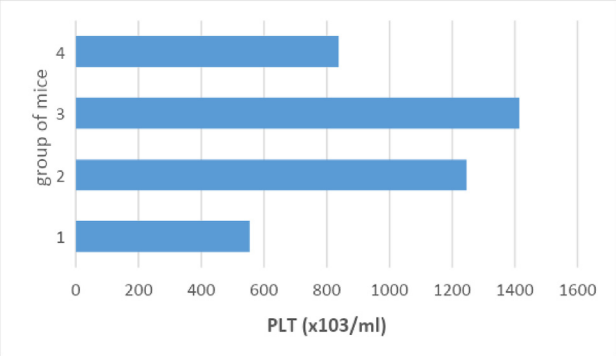

Fig. 3. Platelet (PLT) (×10³/ml) count in serum of mice groups (1-infected 2-treated with 8% 3-treated with 16% 4-treated with 20% 5-treated voriconazole).

treated with 8% the platelet count for this group is 1246 × 10^3/ml. Group 3: treated with 16% the platelet count for this group is 1414 × 10^3/ml. Group 4: treated with 20% the platelet count for this group is 838 × 10^3/ml.

3.4. Interferon-gamma (IFN-γ)

The result of IFN-γ as shown in Fig. 4 referred to: Group 1: Infected mice - the IFN-gamma level in the serum of these mice is 59.8 pg/ml. Group 2: Mice treated with voriconazole - In this group, the mice were administered voriconazole, the IFN-gamma level in the serum of these mice is 17.5 pg/ml. Group 3: Mice treated with mushroom - This group comprises mice that received treatment with a mushroom extract. The IFN-gamma level in the serum of these mice is 12.6 pg/ml.

4. Discussion

The data presented in Fig. 1 illustrates the white blood cell (WBC) count in the serum of mice Group 1

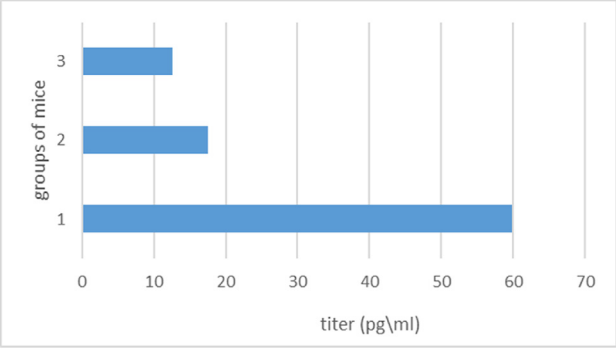

Fig. 4. IFN gamma level in serum of mice group (1-infected, 2-voriconazole, 3-mushroom).

consisted of infected mice with *A. fumigatus* without any treatments. The observed WBC count in this group was 22.9 × 10^3/ml. This significant increase in WBC count indicates an active immune response triggered by the fungal infection. Similar findings have been reported in previous studies where fungal infections, including Aspergillus fumigatus, are known to induce a robust immune response characterized by increased WBC count [6]. The elevated WBC count can be attributed to the recruitment and activation of immune cells, such as neutrophils and macrophages, to combat the infection.

In Group 2, mice infected with *A. fumigatus* were treated with 8% *A. bisporus* extract. The WBC count in this group was 5.6 × 10^3/ml. The reduction in WBC count compared to Group 1 suggests that the *A. bisporus* extract treatment may have a modulating effect on the immune response. A related study by Ref. [6] demonstrated strongly associated with lung injury score results, and neutrophil infiltration. The extract may enhance immune cell activity and promote a balanced immune response, resulting in a lower WBC count. Aspergillosis has emerged as one of the most common infectious causes of death in severely immunocompromised patients [7,8].

Group 3 received treatment with a higher concentration of 16% *A. bisporus* extract. The WBC count further decreased to 3.8 × 10^3/ml. This finding suggests that increasing the concentration of *A. bisporus* extract might have a more pronounced immunomodulatory effect, leading to a reduced WBC count. A study [9] reported similar outcomes, indicating that higher concentrations of *A. bisporus* extract can effectively modulate immune responses and suppress excessive WBC count.

In Group 4, mice were treated with 20% *A. bisporus* extract, resulting in a WBC count of 4.0 × 10^3/ml. The slightly higher WBC count compared to Group 3 suggests that there might be an optimal concentration range for the immunomodulatory effects of *A. bisporus* extract. It is possible that exceeding the optimal concentration could diminish the extract's efficacy in regulating the immune response.

Lastly, Group 5 received treatment with voriconazole, a commonly used antifungal medication. The WBC count in this group was 4.5 × 10^3/ml. Voriconazole is known for its antifungal activity and ability to control fungal infections. The relatively stable WBC count in this group suggests that voriconazole might primarily exert its therapeutic effect by directly targeting the fungal pathogen rather than significantly affecting the overall immune response.

In summary, the data indicates that treatment with *A. bisporus* extract, particularly at

concentrations of 8% and 16%, leads to a decrease in WBC count in mice infected with *A. fumigatus*. This suggests a potential immunomodulatory effect of *A. bisporus* extract on the immune response against fungal infections. Further research is needed to elucidate the underlying mechanisms by which *A. bisporus* extract influences the immune system. Nonetheless, these findings provide valuable insights into the potential use of *A. bisporus* extract as an adjunct therapy for fungal infections, including those caused by *A. fumigatus*.

Fig. 2 illustrates the red blood cell (RBC) count in the serum of mice. The RBC count is measured in units of 10^6 per milliliter (ml).

In Group 1, which comprised infected mice without any specific treatments, the RBC count was measured at 8.27×10^6/ml. This count represents the baseline RBC level in the presence of the *A. fumigatus* infection. Previous studies have indicated that fungal infections can disrupt the normal hematological parameters, including red blood cell counts [10]. The observed RBC count suggests that the infection may have an impact on erythropoiesis or red blood cell survival and turnover.

In Group 2, mice were infected with *A. fumigatus* and treated with 8% *A. bisporus* extract. The RBC count in the serum of these mice was 6.32×10^6/ml. This count indicates a decrease in the RBC level compared to Group 1. *A. bisporus* extract contains various bioactive compounds that have been reported to possess antioxidant and anti-inflammatory properties [11]. These properties may contribute to the preservation of red blood cell integrity and function during the infection. The observed decrease in RBC count may suggest a beneficial effect of *A. bisporus* extract in mitigating the impact of the fungal infection on erythrocytes.

Group 3 received treatment with 16% *A. bisporus* extract, and the RBC count in the serum of these mice was 7.26×10^6/ml. This count shows a slight increase compared to Group 2, although it remains lower than the baseline count in Group 1. The higher concentration of *A. bisporus* extract may enhance its antioxidant and anti-inflammatory effects [12], which could contribute to maintaining red blood cell homeostasis. However, further investigations are needed to understand the specific mechanisms underlying the influence of *A. bisporus* extract on erythropoiesis and red blood cell dynamics.

In Group 4, mice were treated with 20% *A. bisporus* extract, resulting in an RBC count of 4.18×10^6/ml. This count represents a notable decrease compared to both the infected group (Group 1) and the other treatment groups. While the underlying mechanism is not clear from the current data, it is possible that the high concentration of *A. bisporus* extract at 20% may have adverse effects on erythropoiesis or red blood cell survival [13]. Further investigations are necessary to assess the potential toxicity or unintended consequences of using such a high concentration of the extract.

Fig. 4 depicts the levels of interferon-gamma (IFN-γ) in the serum of mice. Interferon-γ restores monocyte function and has been used as rescue therapy for life-threatening fungal infections in patients not responding to conventional treatment [14].

In Group 1, which consisted of infected mice without any specific treatments, the IFN-γ level in the serum was measured at 59.8 pg/ml. This elevated level of IFN-γ indicates an immune response triggered by the infection with the aim of combating the *A. fumigatus* pathogen. Studies have shown that IFN-γ is an essential mediator in host defense against fungal infections and can enhance antifungal activity by promoting phagocytosis and activating immune cells [15]. The observed high IFN-γ level suggests a robust immune response in the infected mice.

Group 2 received treatment with voriconazole, a commonly used antifungal medication. The IFN-γ level in the serum of these mice was 17.5 pg/ml. This significant decrease in IFN-γ level compared to Group 1 indicates a potential immunomodulatory effect of voriconazole. Antifungal medications like voriconazole can dampen immune responses to fungal pathogens, including the production of pro-inflammatory cytokines such as IFN-γ [16]. While voriconazole effectively targets the fungal infection, it may also affect the overall immune response by suppressing certain immune pathways, including IFN-γ signaling.

Group 3 consisted of mice treated with a mushroom extract. The IFN-γ level in the serum of these mice was 12.6 pg/ml. Mushroom extracts have been of interest in immunomodulatory research due to their potential to enhance immune function. Previous studies have shown that certain mushroom species, such as *A. bisporus*, possess immunomodulatory properties and can stimulate the production of various cytokines, including IFN-γ [17]. The observed increase in IFN-γ level in the mushroom-treated group suggests that the extract may contribute to an enhanced immune response against the *A. fumigatus* infection.

5. Conclusion

Overall, the data provide evidence that the administration of *A. bisporus* extract and voriconazole can impact immune response parameters

and infection control in mice infected with *A. fumigatus*. The results suggest potential mechanisms by which these treatments exert their effects, such as immunomodulation and modulation of hematological parameters. However, further research is needed to fully understand the underlying mechanisms and validate the efficacy of these interventions in clinical settings.

These findings contribute to the growing body of knowledge on the treatment of Aspergillus infections, particularly in the context of COVID-19 patients. The data underscore the importance of exploring alternative therapies, such as natural extracts, alongside conventional antifungal medications to optimize treatment outcomes and potentially reduce the risk of secondary infections. Continued research in this field is crucial to advance our understanding of fungal infections and improve therapeutic approaches for patients at risk.

References

[1] Chakraborty I, Maity P. COVID-19 outbreak: migration, effects on society, global environment and prevention. Sci Total Environ Aug. 2020;728:138882.
[2] Hinojosa V, Cesena De la Fuente S, Hussaini N, Varon J. Gastric aspergillosis in a COVID-19 patient. Cureus Feb. 2023;15(2):e34897.
[3] Chugh RM, Mittal P, Namratha MP, Arora T, Bhattacharya T, Chopra H, et al. Fungal mushrooms: a natural compound with therapeutic applications. Front Pharmacol Jul. 2022;13:925387.
[4] Caetano ÉLA, Pedron T, Freire BM, Lange CN, Batista BL, Grotto D. Influence of *Agaricus bisporus* mushroom on Pb toxicokinetic in pregnant rats. Int J Environ Res Publ Health Feb. 2023;20(4). https://doi.org/10.3390/ijerph20043114.
[5] Chun S, Gopal J, Muthu M. Antioxidant activity of mushroom extracts/polysaccharides-their antiviral properties and plausible AntiCOVID-19 properties. Antioxidants (Basel) Nov. 2021;10(12). https://doi.org/10.3390/antiox10121899.
[6] Huang W, Mo L, Yang Y, Chen S, Liu Z, Ma Q, et al. Effect of Aspergillus fumigatus on infection in immunosuppressed rats. Ann Transl Med Jan. 2023;11(2):88.
[7] Latgé J-P, Chamilos G. Aspergillus fumigatus and aspergillosis in 2019. Clin Microbiol Rev Dec. 2019;33(1). https://doi.org/10.1128/CMR.00140-18.
[8] Azie N, Neofytos D, Pfaller M, Meier-Kriesche H-U, Quan S-P, Horn D. The PATH (Prospective Antifungal Therapy) Alliance® registry and invasive fungal infections: update 2012. Diagn Microbiol Infect Dis Aug. 2012;73(4):293–300.
[9] Ikegbunam M, Ukamaka M, Emmanuel O. Evaluation of the antifungal activity of aqueous and alcoholic extracts of six spices. Am J Plant Sci 2016;7(1):118–25.
[10] de Jesus Carrion S, Abbondante S, Clark HL, Marshall ME, Mouyna I, Beauvais A, et al. Aspergillus fumigatus corneal infection is regulated by chitin synthases and by neutrophil-derived acidic mammalian chitinase. Eur J Immunol Jun. 2019;49(6):918–27.
[11] Mustafa F, Chopra H, Baig AA, Avula SK, Kumari S, Mohanta TK, et al. Edible mushrooms as novel myco-therapeutics: effects on lipid level, obesity and BMI. J Fungi (Basel) Feb. 2022;8(2). https://doi.org/10.3390/jof8020211.
[12] Nitthikan N, Leelapornpisid P, Naksuriya O, Intasai N, Kiattisin K. Potential and alternative bioactive compounds from Brown *Agaricus bisporus* mushroom extracts for xerosis treatment. Sci Pharm Oct. 2022;90(4):59.
[13] Im KH, Nguyen TK, Shin DB, Lee KR, Lee TS. Appraisal of antioxidant and anti-inflammatory activities of various extracts from the fruiting bodies of Pleurotus Florida. Molecules Mar. 2014;19(3):3310–26.
[14] Grimaldi D, Pradier O, Hotchkiss RS, Vincent J-L. Nivolumab plus interferon-γ in the treatment of intractable mucormycosis. Lancet Infect Dis Jan. 2017;17(1):18.
[15] Delsing CE, Gresnight MS, Leentjens J, Preijers F, Frager FA, Kox M, et al. Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series. BMC Infect Dis Mar. 2014;14:166.
[16] Zhong J, Peng L, Wang B, Zhang H, Li S, Yang R, et al. Tacrolimus interacts with voriconazole to reduce the severity of fungal keratitis by suppressing IFN-related inflammatory responses and concomitant FK506 and voriconazole treatment suppresses fungal keratitis. Mol Vis Mar. 2018;24:187–200.
[17] Wesa KM, Cunningham-Rundles S, Klimek VM, Vertosick E, Coleton MI, Yeung KS, et al. Maitake mushroom extract in myelodysplastic syndromes (MDS): a phase II study. Cancer Immunol Immunother Feb. 2015;64(2):237–47.