Original Article

The in Vitro Effect of Berberine Sulfate and Berberine Chloride on the Growth and Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus*

Mohammad Sadegh Moradi[1], Samin Kamkar[2], Aghil Sharifzadeh[2*], Jalal Hassan[3], Hojjatollah Shokri[4], Javad Abbasi[1]

1. Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Division of Toxicology, Department of Comparative Bioscience, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran.

Use your device to scan and read the article online

How to Cite This Article Moradi, M. S., Kamkar, S., Sharifzadeh, A., Hassan, J., Shokri, H., & Abbasi, J. (2024). The in Vitro Effect of Berberine Sulfate and Berberine Chloride on the Growth and Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Iranian Journal of Veterinary Medicine*, *18*(2), 223-232. http://dx.doi.org/10.32598/ijvm.18.2.1005399

http://dx.doi.org/10.32598/ijvm.18.2.1005399

ABSTRACT

Background: Aflatoxins are harmful mycotoxins that can contaminate animal and human food products. To prevent toxigenic fungi growth and aflatoxin production, researchers have long investigated plant compounds as potential inhibitory agents.

Objectives: This study aimed to evaluate the in vitro effect of berberine sulfate and berberine chloride on the growth and aflatoxin production of *Aspergillus flavus* and *Aspergillus parasiticus*.

Methods: The antifungal activity of berberine salts was determined according to the Clinical and Laboratory Standards Institute (CLSI) document M38-A3. The aflatoxin levels were measured using high-performance liquid chromatography (HPLC) method.

Results: The berberine sulfate and berberine chloride showed inhibitory effects against both *Aspergillus* species, with minimum inhibitory concentration (MIC) ranging from 125 to 500 µg/mL. Berberine sulfate at 2000 µg/mL and berberine chloride at 1000 µg/mL completely inhibited the mycelial growth of *A. flavus*, while berberine chloride at 1000 µg/mL also completely inhibited the mycelial growth of *A. parasiticus*. Berberine sulfate at 2000 µg/mL reduced the mycelial growth of *A. parasiticus* by 96.7%.

Conclusion: Berberine salts significantly decreased the total aflatoxin production of *Aspergillus* species at MIC/2 and MIC/4 concentrations ($P<0.05$). The results suggest that berberine salts could be used as potential antifungal and anti-aflatoxigenic agents against toxigenic *Aspergillus* isolates.

Keywords: Aflatoxins, *Aspergillus flavus*, *Aspergillus parasiticus*, Berberine, Mycelial growth

Article info:
Received: 18 Sep 2023
Accepted: 23 Nov 2023
Publish: 01 Apr 2024

*** Corresponding Author:**
Aghil Sharifzadeh, Associate Professor.
Address: *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (913) 1315376*
E-mail: *asharifzadeh@ut.ac.ir*

Introduction

In recent decades, the global trade of plant products such as grains, flours, and oilseeds has been raised significantly; however, the contamination of these products with various chemical compounds, especially mycotoxins, has become an essential global concern (Moretti et al., 2017; Santos Pereira, 2019). By growing on food products, fungi not only decrease the nutritional value of these products but also severely affect their quality by excreting mycotoxins (Vieira, 2003).

Contamination of food and its essential components with mycotoxins may occur before harvest in the field due to the growth of pathogenic fungi on the plant or during the processing and storage of products due to the growth of saprophytic fungi (Gruber-Dorninger et al., 2019). Mycotoxins are secondary metabolites produced by many fungi. A group of mycotoxins is called aflatoxins. They are mainly produced by different species of the genus *Aspergillus*, in particular *Aspergillus flavus* and *Aspergillus parasiticus*, after harvest, during storage and processing (Nakavuma et al., 2020; Khorrami et al., 2022). So far, more than 20 metabolites of aflatoxins have been identified, but only 4 metabolites, B1, B2, G1, and G2, can poison humans and animals (Santos Pereira et al., 2019). The long list of harmful effects of these toxins on humans and animals include carcinogenesis, mutagenicity, weakening of the immune system, and liver and kidney poisoning (Nakavuma et al., 2020; Jard et al., 2011; Monson et al., 2015; Al-Mudallal, 2023; Mokhtari Hooyeh et al., 2022). The prevention of food contamination with aflatoxins is primarily based on confining these products from fungal spores and then controlling storage conditions such as temperature, humidity, and antifungal gas compounds. Another approach proposed the use of additives that prevent the mycelium growth of toxin-producing fungi and inhibit or reduce their production of aflatoxins (Gruber- Dorninger et al., 2019; Patil et al., 2014; Kadium et al., 2023). In recent years, the use of chemical fungicides has faced restrictions due to the health risks for humans and animals and the emergence of resistance to them. Therefore, using plant compounds with antifungal properties and preventing aflatoxin production has received much attention (Hu et al., 2017; Hasankhani et al., 2023).

Plants have a wide range of herbal compounds with therapeutic and biological properties. These compounds are mainly classified as alkaloids, flavonoids, tannins, terpenoids, and steroids and have been widely used as medicine and additives by humans throughout history (Savoia, 2012). Berberine, a naturally occurring benzylisoquinoline alkaloid, is found in the roots, rhizomes, and stem bark of natural herbs, such as Berberis aquifolium, *Berberis vulgaris*, and *B. aristata* (Ghavipanje et al., 2022). Berberine has been used for more than 3000 years in the traditional medicine of Iran and China as a herbal compound with many therapeutic properties against Alzheimer disease, Parkinson disease, cancer, obesity, and diabetes. Also, this composition has antiviral, bacterial, and fungal properties (Arayne et al., 2007). Berberine and its derivatives have inhibitory effects on the growth and production of toxins by fungi, and so far, this effect has been identified in *Candida*, *Fusarium*, *Penicillium*, and *Aspergillus* species (Da Silva et al., 2016; Ismail et al., 2020; El-Zahar et al., 2022). Recently, various studies have been conducted to evaluate this isoquinoline alkaloid as a natural preservative with significant antioxidant and antimicrobial properties (Geerlofs et al., 2019; Malekinezhad et al., 2021). So far, a few studies have investigated the effect of berberine on the growth and mycotoxin production by fungi. Therefore, this study aimed to evaluate the effect of berberine sulfate and berberine chloride on the growth and aflatoxin production by *A. flavus* and *A. parasiticus*.

Materials and Methods

Fungal strains

A frozen stock of *A. flavus* (ATCC 28539) and *A. parasiticus* (ATCC 15517) was obtained from the fungal collection of the Department of Mycology, Faculty of Veterinary Medicine, University of Tehran, Iran.

Berberine salts

Berberine chloride and berberine sulfate were purchased from Sigma company (Sigma-Aldrich, St. Louis, MO, USA).

Preparation of *Aspergillus* suspensions

A. flavus and *A. parasiticus* were subcultured in potato dextrose agar (PDA) (Merck Co., Germany) at 28°C for 5 days. Then, 10 mL of PST solution (physiological salt solution containing 0.01% Tween 80) was poured on the surface of the colonies and gently scraped with a U-shaped glass rod. The resulting suspension was kept at room temperature without movement for 15 minutes to precipitate possible hyphae fragments. Then, the number of conidia present in 1 mL of the suspension was counted using a hemocytometer slide. The final concentration of the suspension was 2×10^6 conidia/mL.

Microdilution broth assay

The minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) values of berberine salts were evaluated based on the Clinical and Laboratory Standards Institute (CLSI) document M38-A2 with some modifications (CLSI, 2008). The RPMI (Roswell Park Memorial Institute) 1640 medium containing 3-(N-morpholino) propane sulfonic acid (MOPS) buffer was prepared according to CLSI standard instructions, and its pH was set to 7. Finally, the medium was sterilized using a 0.22-µ syringe filter. At first, two-fold serial dilutions of berberine sulfate and berberine chloride were prepared in RPMI 1640 medium in rows of 96 cell culture plates. Each well in the row contained 100 µL of different dilutions of berberine salts ranging from 2000 to 15.6 µ/mL. Then, 100 µL of fungal suspension with a concentration of 0.4-5×10^4 conidia/mL was inoculated into each well, and the plates were incubated for 48 h at 28˚C. For each experiment, a positive control without berberine containing fungi and a negative control without berberine and fungi were considered. All tests were performed in triplicate. The MIC was defined as the lowest concentration of completely inhibiting the growth of fungi. The MFC of berberine salts was determined by culturing from the MIC well and subsequent wells in PDA for 7 days at 28˚C. MFC was estimated at concentrations in which either no fungi or less than three colonies were grown (CLSI, 2008).

Effect of berberine salts on the radial growth of *A. flavus* and *A. parasiticus*

The effect of berberine salts on the radial growth was measured through culture in a solid medium. Briefly, PDA plates containing 125, 250, 500, 1000, and 2000 µg/mL of berberine salts were prepared, and a sterile 5-mm blank disk was placed in the center of each plate. Ten microliters of *Aspergillus* suspensions containing 2×10^6 conidia/mL were inoculated into the disks. A plate without berberine was selected as a control for each species. The plates were incubated at 28˚C, and the mean diameter of the colonies was measured after the incubation period. The antifungal effect was calculated as the percentage of radial growth inhibition according to the Equation 1:

1. $$(\%) = \frac{D_c - D_s}{D_c} \times 100$$

, Where D_c represents the fungal colony diameter in the control plate, and DS represents the fungal colony diameter in the treated plates.

The effect of berberine salts on aflatoxin production by *A. flavus* and *A. parasiticus*

Berberine sulfate and berberine chloride at concentrations of MIC/2 and MIC/4 were added to 50 mL of flasks containing yeast extract broth (YEB) (Merck Co., Germany). Then, the flasks were inoculated with a 1.5×10^6 conidia/mL concentration. The flasks were kept for 10 days in an incubator with a temperature of 28˚C and a rotation of 100 rpm. Also, flasks containing YEB without fungal inoculation were considered negative control, and those containing YEB without berberine were regarded as positive control.

Aflatoxin production assay

For evaluating aflatoxin formation, berberine sulfate and berberine chloride at concentrations of MIC/2 and MIC/4 were used. Spore suspension (1.5×10^6 conidia/mL) was added to 50 mL of flasks containing YEB containing different concentrations of berberine sulfate and berberine chloride. The flasks were kept for 10 days in an incubator with a temperature of 28˚C and a rotation of 100 rpm. After incubation, the mycelia were dried to a constant weight at 80˚C, and the weight of dried matter was estimated. Determination of aflatoxins B1, B2, G1, and G2 was performed by immunoaffinity column extraction using RP-HPLC (reversed-phase high-performance liquid chromatography) according to the Association of Official Agricultural Chemists (AOAC). Briefly, the filtrated content of each flask was mixed with 150 mL MeOH: H_2O (80:20) and 2.5 g NaCl, followed by vortexing for 3 min. Sixty-five microliters of phosphate buffer solution (PBS) was added to 10 mL of this mixture, shaken vigorously, and passed through a glass fiber filter. Seventy milliliters of solution were transferred onto an immunoaffinity column (Puri-Fast AFLA IAC, Libios, France) at a 3 mL/min flow rate. The column was then washed with 15 mL PBS and dried by gently passing air through it. The aflatoxins were eluted with 500 and 750 µL methanol at 1 min intervals. The elution diluted with 1750 µL H_2O, and the aliquot of 200 µL was injected into an HPLC system equipped with a separator module (2695, Waters, USA), a Nova-Pak LC-18 column, and a fluorescence detector (474, Waters, USA). Aflatoxins were derivatized by KB cell post-column derivatization system (Libios, Chemin de Plagne 69210 Bully, France) in a H_2O–MeCN–MeOH mobile phase containing HNO_3 and KBr at a flow rate of 1 mL/min and detected at an excitation wavelength of 365 nm and an emission wavelength of 435 nm. Aflatoxins were quantified using the peak height by Millenium 32 v 4.0 software (Waters, USA). Aflatoxin standards were purchased from Sigma

(St. Louis, MO, USA). The Equation 2 calculated the inhibition percentage of aflatoxin production:

$$2.\ \text{Inhibition of aflatoxin production}\ (\%)=\frac{A_c-A_s}{A_c}\times 100$$

, where Ac is the amount of aflatoxin in the control sample, and As is the amount of aflatoxin in the treated sample (Hassan et al., 2015).

Statistical analysis

The quantitative data of fungal growth and HPLC analyses were subjected to variance (one-way ANOVA) in the Tukey range (SPSS software, version 16). The differences with P<0.05 were considered significant.

Results

MIC and MFC

Based on the broth microdilution method, berberine sulfate revealed MIC values of 250 and 500 µg/mL for *A. flavus* and *A. parasiticus*, respectively (Table 1). Berberine chloride exhibited stronger activity than berberine sulfate, with MIC values of 125 and 250 µg/mL against *A. flavus* and *A. parasiticus*, respectively. Subcultures of these treated inoculums were negative, confirming MFC against *A. flavus* and *A. parasiticus* at 500 to 2000 µg/mL (Table 1).

The effect of berberine sulfate and chloride on the growth of *A. parasiticus* and *A. flavus*

As demonstrated in Table 2 and Figure 1, all concentrations of berberine sulfate and berberine chloride exhibited significant growth inhibition of *A. flavus* and *A. parasiticus* compared to the control group, suggesting a dose-dependent pattern (P<0.05). Berberine sulfate (2000 µg/mL) and berberine chloride (1000 µg/mL) exhibited a 100% growth inhibition of mycelia production by *A. flavus*. In addition, berberine sulfate at a concentration of 2000 µg/mL and berberine chloride at a concentration of 1000 µg/mL inhibited the growth of mycelia production by *A. parasiticus* by 96.7% and 100%, respectively (Table 2).

Table 1. Anti-*aspergillus* susceptibility of berberine sulfate and berberine chloride based on microdilution broth method

Test Compound	MIC (µg/mL)		MFC (µg/mL)	
	A. flavus	*A. parasiticus*	*A. flavus*	*A. parasiticus*
Berberine sulfate	250	500	1000	2000
Berberine chloride	125	250	500	1000

Abbreviations: MIC: Minimum inhibitory concentration; MFC: Minimum fungicidal concentration.

Table 2. The effects of berberine sulfate and berberine chloride on the radial growth of *A. flavus* and *A. parasiticus*

Berberine Concentration (µg/mL)	Mean±SD							
	A. flavus				*A. parasiticus*			
	Berberine Sulfate		Berberine Chloride		Berberine Sulfate		Berberine Chloride	
	Colony Diameter (mm)	Growth Inhibition (%)	Colony Diameter (mm)	Growth Inhibition (%)	Colony Diameter (mm)	Growth Inhibition (%)	Colony Diameter (mm)	Growth Inhibition (%)
0	41±1	0	41±1	0	36.8±1.4	0	36.8±1.4	0
125	29.2±0.8	28.8	18.8±0.84	54.1	29.4±0.5	20.1	22.2±1.8	39.7
250	14±1.2	65.9	10.8±1.1	73.7	24.6±0.9	33.2	16.8±1.1	54.3
500	10±2	75.6	1.2±1.1	97.1	16±1.4	56.5	6±1.4	83.7
1000	1.6±1.7	96.1	0	100	7.2±1.8	80.4	0	100
2000	0	100	0	100	1.2±1.8	96.7	0	100

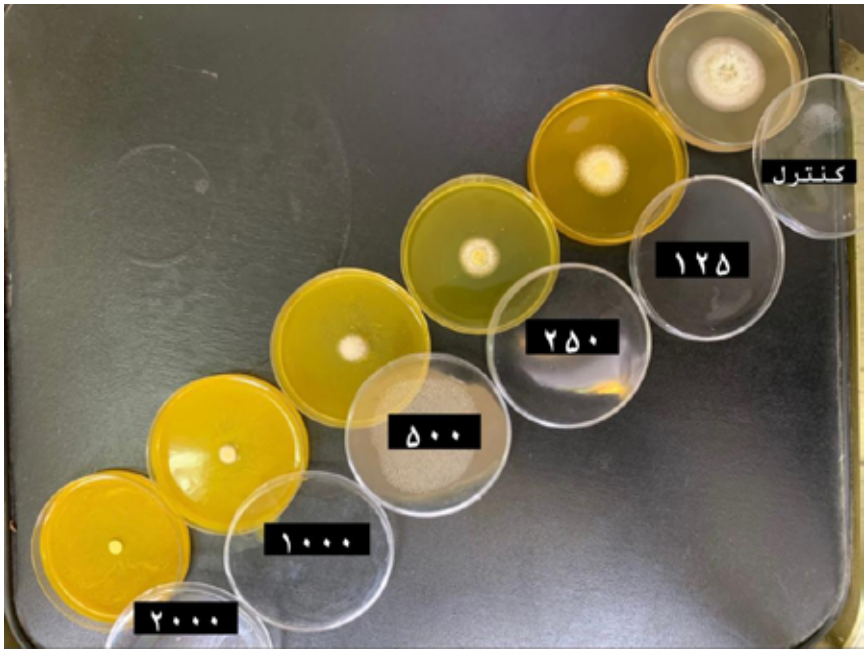

Figure 1. The effect of berberine chloride on the growth of *A. flavus* colonies after 7 days

Table 3. The Mean±SD of aflatoxin concentration by *A. flavus* treated with berberine sulfate and chloride

Test	Concentration	Mean±SD				
		Aflatoxin (µg/L)				
		B1	B2	G1	G2	Total
Control		538.38±2.58	41.13±0.49	211.98±4.37	2.86±0.08	794.34±2.2
Berberine sulfate	MIC/2	173.95±4.17	19.75.2±1.2	41.63±1.67	1.19±0.07	236.52±3.63
	MIC/4	436.37±2.61	34.39±1.86	98.51±1.26	2.1±0.22	571.38±2.9
Berberine chloride	MIC/2	24.48±0.63	0.8±0.8	0	0	25.28±1.43
	MIC/4	170.05±6.51	15.7±1.06	16.8±0.99	0	202.55±8.56

Table 4. The Mean±SD of aflatoxin concentration by *A. parasiticus* treated with berberine sulfate and chloride

Test	Concentration	Mean±SD				
		Aflatoxin (µg/L)				
		B1	B2	G1	G2	Total
Control		1642.25±9.50	112.74±4.72	980.68±7.07	25.53±3.96	2761.20±17.60
Berberine sulfate	MIC/2	542.33±4.84	28.07.2±2.37	348.79±4.26	79.7±1.20	926.98±12.67
	MIC/4	909.21±12.09	39.55±2.97	748.26±12.27	12.27±0.88	1709.28±65.16
Berberine chloride	MIC/2	24.48±0.63	0.8±0.8	0	10.5±0.42	51.65±15.3
	MIC/4	170.05±6.51	15.7±1.06	291.36±29.5	18.08±0.17	1151.86±18.5

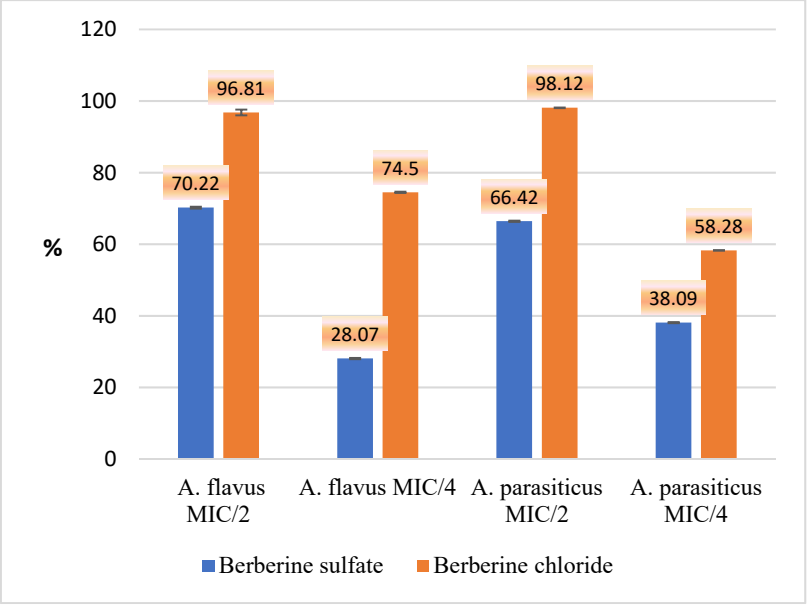

Figure 2. Comparing the effect of different concentrations of berberine sulfate and berberine chloride on the total aflatoxin production by *A. flavus* and *A. parasiticus*

The effect of berberine chloride and berberine sulfate on aflatoxin production

In our study, when the certain concentrations (MIC/2 and MIC/4) of berberine sulfate and berberine chloride were added to the cultures, significant reductions in aflatoxins production were observed by *A. flavus* and *A. parasiticus* compared to control (P<0.05) (Table 3, Table 4, and Figure 2). As shown in Table 3, berberine chloride exhibited a higher inhibitory effect on aflatoxin production than berberine sulfate by *A. flavus* (P<0.05). Berberine chloride caused significant reductions down to 100% for aflatoxin G1 and aflatoxin G2 by *A. flavus*. According to Table 4, aflatoxin production by *A. parasiticus* treated with berberine sulfate and berberine chloride at MIC/2 concentration was significantly lower than MIC/4 concentration (P<0.05). Also, berberine chloride exhibited a higher inhibitory effect on aflatoxin production than berberine sulfate by *A. parasiticus* (Figure 2). Berberine chloride caused a significant 100% reduction for aflatoxin G1 by *A. parasiticus* (Table 4). At MIC/2 concentration, berberine chloride decreased aflatoxin production by *A. flavus* and *A. parasiticus* by 96.81% and 98.12%, respectively, 100% for aflatoxin B2, 98.9% for aflatoxin G1, 100% for aflatoxin G2 and 97.5% for total aflatoxin (P<0.05) (Figure 2).

Discussion

In the present study, we showed a new biological activity for berberine as an inhibitor of aflatoxins B1, B2, G1, and G2 by *A. flavus* and *A. parasiticus*, besides its ability for strong fungal growth inhibition. MIC and MFC techniques assessed the fungistatic and fungicidal properties of berberine sulfate and berberine chloride. Several studies have been carried out on the chemical composition of *B. vulgaris* and have shown that the most important constituents of this plant are isoquinoline alkaloids such as berberine (Tabeshpour et al., 2017). In the case of the antifungal effects of berberine, few studies approved the high potential of berberine against some pathogenic fungal strains (Da Silva et al., 2016; Mahmoudvand et al., 2014). According to Ghareeb et al. (2013) study, a 62% berberine ethanolic extract from dried *B. vulgaris* roots displayed antifungal activity against five fungal infections at dosages ranging from 1:1 to 1:8 (*Penicillium verrucosum*, *Fusarium proliferatum*, *A. parasiticus*, *A. niger*, and *A. flavus*) (Ghareeb et al., 2013). In a study by El-Zaher (2022), the MIC values of *B. vulgaris* leaf and root extracts for *A. flavus* were 70 and 90 µg/mL, respectively, while these values for *A. parasiticus* were found to be 85 and 100 µg/mL (El-Zahar et al., 2022). Lei et al. (2011) showed that the MIC range of berberine over the 42 strains of *Aspergillus* spp. was 4–256 µg/mL. The growing rate of *Trichophyton mentagrophytes* treated with berberine hydrochloride was significantly lower than those obtained in untreated control, demonstrating

that berberine hydrochloride was fungicidal (Xiao et al., 2019). Additionally, a few studies have found that *B. vulgaris* and its major component, berberine, have antifungal action against *Candida* spp. In a study conducted by Da Silva et al. (2016), fluconazole-resistant *Candida* and *Cryptococcus neoformans* strains showed berberine MICs equal to 8 µg/mL and 16 µg/mL, respectively (Da Silva et al., 2016). Cytometric analysis showed that treatment with berberine caused alterations to the integrity of the plasma and mitochondrial membranes and DNA damage, which led to cell death, probably by apoptosis (Da Silva et al., 2016). Li et al. (2013) demonstrated that berberine has a strong antifungal effect on *Candida albicans*, causing cell cycle arrest and DNA damage. Other studies have also suggested that berberine can bind to DNA, affecting DNA replication, transcription, and cell cycle (Bhadra & Kumal, 2011).

In this study, berberine salts inhibited the radial growth of *A. flavus* and *A. parasiticus* mycelium (Table 2). El-Zahar et al. (2022) showed that *B. vulgaris* root extract inhibited the mycelial growth of *P. verrucosum*, *F. proliferatum*, *A. ochraceous*, *A. niger*, and *A. flavus*. For *P. verrucosum* and *A. ochraceous*, the maximum inhibition zones ranged from 1.7 to 2.35 cm at the 100 µL concentration. In a study by Lei et al. (2011), *Aspergillus* treated with berberine exhibited smaller colony size, slower mycelial growth, and reduced conidia. These cultures also lost conidial pigment such that the conidial surface observed was white rather than green-gray (Lei et al., 2011). These results demonstrated that berberine can restrain *Aspergillus* growth, development, and conidial pigmentation. Some studies demonstrated that berberine significantly inhibits gene expression in the *Aspergillus* ergosterol biosynthesis pathway and that berberine is significantly more effective than azoles at inhibiting expression of the *Erg5*, *Cyp51A*, *Cyp51B*, and *IMP* genes, which are related to pigment production in *Aspergillus conidia*. The *IMP* gene is closely associated with cell wall biosynthesis, and by inhibiting its expression, berberine may thus inhibit the biosynthesis of fungal cell walls and cause growth and developmental aberrations in *Aspergillus* (Ouyang et al., 2010). Da Silva et al. (2016) demonstrated that the berberine concentration necessary to inhibit both planktonic cells and preformed biofilm cells is similar. This finding indicated that berberine may reduce planktonic cell growth and inhibit cell viability in preformed biofilms at concentrations of 8 µg/mL and 37.5 µg/mL, respectively.

Up to now, there has been no research on aflatoxin inhibition by berberine, but a few investigations reported the effect of *B. vulgaris* on aflatoxin production. For example, Ghareeb et al. (2013) reported that ethanolic extract of *B. vulgaris* could inhibit the production of 44% and 98.3% of aflatoxin B1 and 67.2 and 89% of aflatoxin B2 at concentrations of 0.01% to 0.1%, respectively. Safari et al. (2020) exhibited that the inhibition of aflatoxin B1 production by *A. flavus* in *B. vulgaris* extract (6 mg/mL) was significant. Their findings demonstrated a highly significant correlation between the gene expression and the aflatoxin B1 biosynthesis, such that certain doses of the extract reduced or blocked the expression of the *aflR*, *aflM*, and *aflP* and consequently reduced the synthesis of aflatoxin B1. Interestingly, compared to the regulatory gene (*aflR*), the downregulation of expression in the structural genes (*aflM* and *aflP*) was more consistent and correlated with the inhibition of aflatoxin B1 production. In another study by Tintu et al. (2012), α-amylase inhibitors, such as berberine, can control the growth of *A. flavus* and the production of aflatoxins. Malekivezhad et al. (2021) showed that the addition of different levels of berberine to chickens challenged with aflatoxin reduced the negative effect of this toxin on broiler feed intake. Also, supplementation of aflatoxin B1-contaminated diets with berberine improved growth performance and reduced vascular congestion, inflammatory cell infiltration into the liver portal space, and hepatocyte apoptosis. Furthermore, it protects against toxin-induced damage to the ileal epithelium. These findings suggested that berberine could be a useful dietary strategy to prevent the effects of aflatoxicosis in animals and humans.

Conclusion

In summary, our findings indicated the potential of berberine as a natural inhibitor of the growth and aflatoxin production by *A. flavus* and *A. parasiticus*, the well-known agents of food-borne aflatoxicosis.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This work was supported by the Research Council of the University of Tehran.

Authors' contributions

Project administration: Mohammad Sadegh Moradi and Samin Kamkar; Formal analysis: Jalal Hassan; Funding, supervision, and writing: Aghil Sharifzadeh and Javad Abbasi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

References

Al-Mudallal, N. H. (2023). The expression of MMP1 and MMP7 in mice liver after exposure to aflatoxin B1 using immunohistochemistry technique. *Archives of Razi Institute, 78*(1), 63-72. [DOI:10.22092/ari.2022.358774.2306]

Arayne, M. S., Sultana, N., & Bahadur, S. S. (2007). The berberis story: Berberis vulgaris in therapeutics. *Pakistan Journal of Pharmaceutical Sciences, 20*(1), 83-92. [PMID]

Bhadra, K., & Kumar, G. S. (2011). Therapeutic potential of nucleic acid-binding isoquinoline alkaloids: Binding aspects and implications for drug design. *Medicinal Research Reviews, 31*(6), 821-862. [DOI:10.1002/med.20202] [PMID]

Clinical and Laboratory Standards Institute (CLSI). (2008). *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved standard*. Wayne, PA: Clinical and Laboratory Standards Institute. [Link]

da Silva, A. R., de Andrade Neto, J. B., da Silva, C. R., Campos, R.deS., Costa Silva, R. A., & Freitas, D. D., et al. (2016). Berberine antifungal activity in fluconazole-resistant pathogenic yeasts: Action mechanism evaluated by flow cytometry and biofilm growth inhibition in Candida spp. *Antimicrobial Agents and Chemotherapy, 60*(6), 3551-3557. [DOI:10.1128%2FAAC.01846-15] [PMID]

El-Zahar, K. M., Al-Jamaan, M. E., Al-Mutairi, F. R., Al-Hudiab, A. M., Al-Einzi, M. S., & Mohamed, A. A. (2022). Antioxidant, antibacterial, and antifungal activities of the ethanolic extract obtained from berberis vulgaris roots and leaves. *Molecules, 27*(18), 6114. [DOI:10.3390/molecules27186114] [PMID]

Geerlofs, L., He, Z., Xiao, S., & Xiao, Z. (2019). Efficacy of berberine as a preservative against mold and yeast in poultry feed. *Approaches in Poultry, Dairy & Veterinary Sciences, 7*(2). [DOI:10.31031/APDV.2019.07.000659]

Ghareeb, D. A., Abd El-Wahab, A. E., Sarhan, E. E., Abu-Serie, M. M., & El Demellawy, M. A. (2013). Biological assessment of Berberis vulgaris and its active constituent, berberine: Antibacterial, antifungal and anti-hepatitis C virus (HCV) effect. *Journal of Medicinal Plants Research, 7*(21), 1529-1536. [Link]

Ghavipanje, N., Fathi Nasri, M. H., & Vargas-Bello-Pérez, E. (2023). An insight into the potential of berberine in animal nutrition: Current knowledge and future perspectives. *Journal of Animal Physiology and Animal Nutrition, 107*(3), 808–829. [DOI:10.1111/jpn.13769] [PMID]

Gruber-Dorninger, C., Jenkins, T., & Schatzmayr, G. (2019). Global mycotoxin occurrence in feed: A ten-year survey. *Toxins, 11*(7), 375. [DOI:10.3390/toxins11070375] [PMID]

Hasankhani, T., Nikaein, D., Khosravi, A., Rahmati-Holasoo, H., & Hasankhany, M. (2023). The effect of echinacea purpurea l. (eastern purple coneflower) essential oil on hematological parameters and gut microbial population of zebrafish (danio rerio) with aflatoxicosis. *Iranian Journal of Veterinary Medicine, 17*(2), 173-182. [DOI:10.32598/IJVM.17.2.1005271]

Hassan, J., Shams, G. R., & Meighani, H. (2015). Application of low density miniaturized dispersive liquid-liquid extraction method for determination of formaldehyde in aqueous samples (water, fruit juice and streptococcus vaccine) by HPLC-UV. J*ournal of Analytical Chemistry, 70,* 1495-1500. [DOI:10.7868/S0044450215120099]

Hu, Y., Zhang, J., Kong, W., Zhao, G., & Yang, M. (2017). Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (Curcuma longa L.) on Aspergillus flavus. *Food Chemistry, 220,* 1-8. [DOI:10.1016/j.foodchem.2016.09.179] [PMID]

Ismail, N., Ghareeb, D., El-Sohaimy, S., EL-Demellawy, M., & El-Saied, M. (2020). Evaluation of the anti-Fusarium effect of Cinnamoum zeilanicum, Berberise vulgaris and Caluna vulgaris ethanolic extracts. *International Journal of Cancer and Biomedical Research, 4*(2), 143-150. [DOI:10.21608/jcbr.2020.30493.1039]

Jard, G., Liboz, T., Mathieu, F., Guyonvarc'h, A., & Lebrihi, A. (2011). Review of mycotoxin reduction in food and feed: From prevention in the field to detoxification by adsorption or transformation. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 28*(11), 1590-1609. [DOI:10.1080/19440049.2011.595377] [PMID]

Kadium, S. W., Semysim, A. A., & Sahib, R. A. (2023). Antifungal activity of phenols compound separated from quercus infectoria and citrullus colocynthis against toxic fungi. *Archives of Razi Institute, 78*(1), 297-303. [DOI:10.22092/ari.2022.358960.2347]

Khorrami, R., Pooyanmehr, M., Soroor, M. E., & Gholami, S. (2022). Evaluation of some aflatoxins in feed ingredients of livestock and poultry by HPLC Method, a local study in Kermanshah Province. *Iranian Journal of Veterinary Medicine, 16*(3), 298-310. [DOI:10.22059/ijvm.2022.329690.1005192]

Lei, G., Dan, H., Jinhua, L., Wei, Y., Song, G., & Li, W. (2011). Berberine and itraconazole are not synergistic in vitro against Aspergillus fumigatus isolated from clinical patients. *Molecules, 16*(11), 9218-9233. [DOI:10.3390/molecules16119218] [PMID]

Li, D. D., Xu, Y., Zhang, D. Z., Quan, H., Mylonakis, E., & Hu, D. D., et al. (2013). Fluconazole assists berberine to kill fluconazole-resistant Candida albicans. *Antimicrobial Agents and Chemotherapy, 57*(12), 6016-6027. [DOI:10.1128/aac.00499-13] [PMID]

Mahmoudvand, H., Ayatollahi Mousavi, S. A., Sepahvand, A., Sharififar, F., Ezatpour, B., & Gorohi, F., et al. (2014). Antifungal, antileishmanial, and cytotoxicity activities of various extracts of Berberis vulgaris (Berberidaceae) and its active principle berberine. *International Scholarly Research Notices, 2014,* 602436. [DOI:10.1155/2014/602436] [PMID]

Malekinezhad, P., Ellestad, L. E., Afzali, N., Farhangfar, S. H., Omidi, A., & Mohammadi, A. (2021). Evaluation of berberine efficacy in reducing the effects of aflatoxin B1 and ochratoxin A added to male broiler rations. *Poultry Science, 100*(2), 797-809. [DOI:10.1016/j.psj.2020.10.040] [PMID]

Mokhtari Hooyeh, M., Aminianfar, H., Sharifzadeh, A., Lalehpoor, M., & Samiee, N. (2022). An incidence of aflatoxicosis in hand-fed ewe lambs exhibiting icterus subsequent to hepatic failure and hemoglobinuria. *Iranian Journal of Veterinary Medicine,* 1-11. [DOI:10.22059/ijvm.2022.343852.1005279]

Monson, M. S., Coulombe, R. A., & Reed, K. M. (2015). Aflatoxicosis: Lessons from toxicity and responses to aflatoxin B1 in poultry. *Agriculture, 5*(3), 742-777. [DOI:10.3390/agriculture5030742]

Moretti, A., Logrieco, A. F., & Susca, A. (2017). Mycotoxins: An underhand food problem. In: A. Moretti, & A. Susca (Eds.), *Mycotoxigenic Fungi. Methods in Molecular Biology, vol 1542.* New York: Humana Press. [Link]

Nakavuma, J. L., Kirabo, A., Bogere, P., Nabulime, M. M., Kaaya, A. N., & Gnonlonfin, B. (2020). Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. *International Journal of Food Contamination, 7*(1), 1-10. [DOI:10.1186/s40550-020-00079-2]

Ouyang, H., Luo, Y., Zhang, L., Li, Y., & Jin, C. (2010). Proteome analysis of Aspergillus fumigatus total membrane proteins identifies proteins associated with the glycoconjugates and cell wall biosynthesis using 2D LC-MS/MS. *Molecular Biotechnology, 44*(3), 177–189. [DOI:10.1007/s12033-009-9224-2] [PMID]

Patil, R. D., Sharma, R., & Asrani, R. K. (2014). Mycotoxicosis and its control in poultry: A review. *Journal of Poultry Science and Technology, 2*(1), 1-10. [Link]

Safari, N., Mirabzadeh Ardakani, M., Hemmati, R., Parroni, A., Beccaccioli, M., & Reverberi, M. (2020). The potential of plant-based bioactive compounds on inhibition of aflatoxin B1 biosynthesis and down-regulation of aflR, aflM and aflP genes. *Antibiotics, 9*(11), 728. [DOI:10.3390/antibiotics9110728] [PMID]

Santos Pereira, C., C Cunha, S., & Fernandes, J. O. (2019). Prevalent mycotoxins in animal feed: Occurrence and analytical methods. *Toxins, 11*(5), 290. [DOI:10.3390%2Ftoxins11050290] [PMID]

Savoia, D. (2012). Plant-derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiology, 7*(8), 979–990. [DOI:10.2217/fmb.12.68] [PMID]

Tabeshpour, J., Imenshahidi, M., & Hosseinzadeh, H. (2017). A review of the effects of Berberis vulgaris and its major component, berberine, in metabolic syndrome. *Iranian Journal of Basic Medical Sciences, 20*(5), 557-568. [DOI:10.22038%2FIJBMS.2017.8682]

Tintu, I., Dileep, K. V., Augustine, A., & Sadasivan, C. (2012). An isoquinoline alkaloid, berberine, can inhibit fungal alpha amylase: Enzyme kinetic and molecular modeling studies. *Chemical Biology & Drug Design, 80*(4), 554-560. [DOI:10.1111/j.1747-0285.2012.01426.x] [PMID]

Vieira, S. L. (2003). Nutritional implications of mould development in feedstuffs and alternatives to reduce the mycotoxin problem in poultry feeds. *World's Poultry Science Journal, 59*(1), 111-122. [DOI:10.1079/WPS20030007]

Xiao, C. W., Liu, Y., Wei, Q., Ji, Q. A., Li, K., & Pan, L. J., et al. (2019). Inhibitory effects of berberine hydrochloride on Trichophyton mentagrophytes and the underlying mechanisms. *Molecules, 24*(4), 742. [DOI:10.3390%2Fmolecules24040742] [PMID]

مطالعه پژوهشی

اثر بربرین سولفات و بربرین کلرید در شرایط برون‌تنی بر رشد و تولید آفلاتوکسین توسط آسپرژیلوس فلاووس و آسپرژیلوس پارازیتیکوس.

محمدصادق مرادی[1]، سمین کامکار[2]، *عقیل شریف‌زاده[2]، جلال حسن[3]، حجت الله شکری[4]، جواد عباسی[1]

۱. گروه بهداشت و تغذیه دام و طیور، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران.
۲. گروه میکروبیولوژی و ایمونولوژی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.
۳. بخش سم‌شناسی، گروه علوم زیستی مقایسه‌ای، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.
۴. گروه پاتوبیولوژی، دانشکده دامپزشکی، دانشگاه تخصصی فناوری‌های نوین آمل، آمل، ایران.

Use your device to scan and read the article online

How to Cite This Article Moradi, M. S., Kamkar, S., Sharifzadeh, A., Hassan, J., Shokri, H., & Abbasi, J. (2024). The in Vitro Effect of Berberine Sulfate and Berberine Chloride on the Growth and Aflatoxin Production by *Aspergillus flavus* and *Aspergillus parasiticus*. *Iranian Journal of Veterinary Medicine, 18*(2), 223-232. http://dx.doi.org/10.32598/ijvm.18.2.1005399

http://dx.doi.org/10.32598/ijvm.18.2.1005399

چکیده

زمینه مطالعه: آفلاتوکسین‌ها، سموم قارچی مضری هستند که می‌توانند خوراک دام و محصولات غذایی را آلوده کنند. ترکیبات گیاهی به‌عنوان عوامل بالقوه برای مهار رشد و تولید آفلاتوکسین توسط قارچ‌های توکسین‌زا مورد بررسی قرار گرفته‌اند.

هدف: این مطالعه باهدف بررسی اثر بربرین سولفات و بربرین کلرید در شرایط آزمایشگاهی بر رشد و تولید آفلاتوکسین در آسپرژیلوس فلاووس و آ. پارازیتیکوس انجام شد.

روش کار: فعالیت ضدقارچی نمک‌های بربرین براساس سند M38-A3 مؤسسه استانداردهای بالینی و آزمایشگاهی (CLSI) تعیین گردید. سطح آفلاتوکسین با استفاده از روش کروماتوگرافی مایع با کارایی بالا (HPLC) اندازه‌گیری شد.

نتایج: حداقل غلظت بازدارندگی بربرین سولفات و بربرین کلرید علیه آسپرژیلوس فلاووس به‌ترتیب ۲۵۰ و ۱۲۵ میکروگرم بر میلی‌لیتر بود. این مقادیر برای آسپرژیلوس پارازیتیکوس به‌ترتیب ۵۰۰ و ۲۵۰ میکروگرم بر میلی‌لیتر محاسبه شد. بربرین سولفات با غلظت ۲۰۰۰ میکروگرم بر میلی‌لیتر و بربرین کلرید با غلظت ۱۰۰۰ میکروگرم در میلی‌لیتر منجر به مهار کامل رشد میسلیوم آسپرژیلوس فلاووس شد. علاوه‌براین، بربرین سولفات با غلظت ۲۰۰۰ میکروگرم در میلی‌لیتر باعث کاهش ۹۶/۷ درصدی رشد میسلیوم آسپرژیلوس پارازیتیکوس شد، درحالی‌که کلرید بربرین با غلظت ۱۰۰۰ میکروگرم در لیتر به مهار ۱۰۰ درصدی رشد میسلیوم منجر شد.

نتیجه‌گیری نهایی: نمک‌های بربرین تولید آفلاتوکسین کل توسط هر دو گونه آسپرژیلوس را در غلظت‌های MIC/4 و MIC/2 به‌طور معنی‌داری کاهش دادند ($P<0/05$). نتایج نشان می‌دهد که نمک‌های بربرین می‌توانند به‌عنوان عوامل ضدقارچی و ضدآفلاتوکسیژنیک بالقوه در برابر جدایه‌های سمی آسپرژیلوس استفاده شوند.

کلیدواژه‌ها: آفلاتوکسین‌ها، آسپرژیلوس فلاووس، آسپرژیلوس پارازیتیکوس، بربرین، رشد میسلیوم.

تاریخ دریافت: ۲۷ شهریور ۱۴۰۲
تاریخ پذیرش: ۲ آذر ۱۴۰۲
تاریخ انتشار: ۱۳ فروردین ۱۴۰۳

* **نویسنده مسئول:**
دکتر عقیل شریف‌زاده
نشانی: تهران، دانشگاه تهران، دانشکده دامپزشکی، گروه میکروبیولوژی و ایمونولوژی.
تلفن: ۱۳۱۵۳۷۶ (۹۱۳) ۹۸+
رایانامه: asharifzadeh@ut.ac.ir