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Effects of aflatoxins on the growth and development of secondary hydatid cysts originating from sheep and their treatment with Mycofix in mice

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Abstract

This study included 24 BALB/c albino mice, which were used to investigate the aggravating toxic effect of aflatoxins and their association with increased severity of hydatid cysts in mice, as well as to record the modifying and reducing effects of the use of the adsorbent Mycofix 0.3+.
The mice were divided into three groups (8 mice per group) as follows:
1. Group One: Positive control, in which 0.2 ml of medium containing protoscoleses was injected intraperitoneally at a dose of 2,000 units of protoscoleses.
2. Group Two: Eight mice were fed the same diet as the control animals, supplemented daily with 1 ppm aflatoxins.
3. Group three: Eight mice were fed the same diet as Group Two, with the addition of the adsorbent Mycofix +0.3.
At the end of the experiment, after 40 days, the results revealed that compared with the control treatment, both aflatoxins and the injection of parasites significantly increased the number of hydatid cysts with diameters of 0.3, 1.6, and 1.5 mm. Additionally, in the third group treated with aflatoxin and Mycofix, only 6 cysts (0.2 mm) were observed, whereas in the second group, 25 cysts (0.2 mm in diameter) were observed, and in the control group, 23 cysts (0.2 mm in diameter) were found. The distribution of hydatid cysts in various organs was significantly lower in the groups fed Aflatoxin and the control group than in the group that received Aflatoxin and Mycofix in the diet. We conclude that compared with control conditions, feed contamination with aflatoxins plays an important role in the spread and severity of hydatid cysts, both in terms of number and distribution. This was significantly reduced in the Mycofix- and aflatoxin-treated groups.

Keywords:
Echinococcus granulosus, Hydatid cysts, Aflatoxins, Mycofix

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Introduction

Echinococcus granulosus is a tapeworm that lives in the small intestine of dogs, and during the hydatid cyst stage, it is found in the liver, lungs, spleen, and body cavity of intermediate hosts (Vuitton & Gottstein, 2010).

Hydatid disease is a common cyclozoonotic disease that represents a widespread global health problem (Ito et al., 1999). It affects both humans and their pets, causing significant health and economic burdens in endemic areas (McManus & Smyth, 1986; Spotin et al., 2016). There is alarming evidence that echinococcosis also imposes significant costs on human and animal disease control and treatment (Díaz

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et al., 2011).
Secondary hydatid disease can be established by injecting protoscoleces of Echinococcus granulosus into the intraperitoneal cavity of mice, which is considered a useful method for studying the host–parasite relationship (Liu et al., 1992).

After the eggs of echinococcaceae are swallowed and the hexacanth embryo settles in various organs of the human body and various hosts, it becomes a hydatid cyst in the organ in which it settles (Freeman, 1973). The growth of these cysts causes pressure symptoms on the surrounding organs; these symptoms depend on the size and location of the cyst. The gravity of the disease is attributed to the inability to detect it in the early stages, as it does not show any symptoms until the size of the cyst increases to the point that leads to pressure on the surrounding tissues. The cysts that grow inside the brain, heart, and bones are rather small (less than 2 cm in diameter); however, despite the size of these cysts, clinical symptoms appear (Kammerer & Schantz, 1993). In contrast, the hydatid cysts in the liver and lungs, which are the two most common sites for the growth of larvae (hydatid cysts), appear when the cysts grow to a large size, and the growth of the diameter of the hydatid cyst increases by approximately 1 cm every year, reaching 20 cm and containing several litres of fluid in places such as the abdominal cavity above the intestine (Ammann & Eckert, 1996); this fluid is called hydatid fluid and contains up to one million protoscoleses.

Dogs are unlikely to ingest human hydatid cysts; thus, when infection occurs in humans, the life cycle and infection cycle of the worm terminate, which is known as the "dead end" (Zeghir-Bouteldja et al., 2009). The specific host (dogs) becomes infected by eating meat or organs containing cysts, such as the livers of infected sheep, which are not properly disposed of.

Contamination of feed with aflatoxins may also contribute to the increased virulence of these parasites. Aflatoxins are produced as secondary metabolites of certain strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Yabe & Hamasaki, 1993). These fungi spread through the air and soil, contaminating the atmosphere of storage facilities and growing on many agricultural crops, such as wheat, rice, barley, corn, tofu, and pistachios. They were discovered by Sargeant et al. in plant produce that was contaminated with molds (Sargeant et al., 1963).

Aflatoxin is considered one of the most dangerous naturally occurring mycotoxins. It is considered an immunosuppressant, a neurotoxin (Moss & Smith, 1985), a carcinogen, and a mutagen (Yabe et al., 1998). It is considered a cause of liver and kidney cancer and has been linked to tumors in body tissues such as the gall bladder and colon, where it affects cell walls and inhibits DNA (Yabe & Hamasaki, 1993).

Recently, there has been increased interest in the use of adsorbents to remove aflatoxins from feed when the adsorbent Mycofix 0.3+ is used. This salt is a stimulating and activating agent for the selective absorption of some mycotoxins without affecting other substances and is added to feed. This study aimed to identify the effects of aflatoxins and to elucidate the relationships and extent of the threat posed by aflatoxins in animal feed in terms of increasing the rate and morbidity of hydatid parasites. They cooperate with the parasite to exacerbate its impact on animal health and performance. It also aimed to record the role of the adsorbent Mycofix in reducing the severity of infection and morbidity of the parasite in the body organs of mice.

Materials and Methods

Isolation of protoscicolices
Livers infected with hydatid cysts were obtained from the Mosul slaughterhouse. Protoscoleses were diagnosed and isolated in the Parasitology Laboratory, Department of Life Sciences, University of Mosul, Iraq.
The outer surface of the hydatid cyst was sterilized using 70% ethanol, after which the contents of the cyst, including the fluid and the protoscoleses, were withdrawn using a syringe under sterile conditions and placed in 50 ml falcon tubes. The protoscoleses were washed by adding sterile saline (PBS) and left until the protoscoleses settled. It was then resuspended five times for 20 minutes until it finally settled. Thus, we washed the protoscoleses and removed the capitate that died as a result of repeated washing.

The vitality of the protoscoleses was then examined using 1% eosin staining (Daryani et al., 2007) under a microscope. The protoscoleses that absorb the red dye are considered dead, the protoscoleses that do not absorb the dye and remain green are considered alive, and their vitality is measured by the following equation (Esfandiari et al., 2010):

$$viability\ of\ protoscoleces\ = \frac{\mathrm{N}o.of\ viable\ proto.}{\mathrm{T}otal\ of\ No.of\ proto.}\ x100$$

The second step involved injecting the protoscoleses into the experimental mice, where approximately 2,000 heads were given intraperitoneally at a dose of 0.2 ml.

Preparation of Aflatoxin

Aflatoxins were prepared using a modified preparation method according to Shotwell et al. (1966) and a modification of West et al. (1973). Rice was inoculated with *Aspergillus parasiticus* NRRL 2999, which was acquired from the College of Agriculture and Forestry, University of Mosul. The rice was fermented and ground, and the aflatoxin content was measured by spectrophotometry (Nabney & Nesbitt, 1965; Wiseman et al., 1967).

Rice powder was added to the rats' diet to achieve the recommended concentration of 1 ppm. The rats in the aflatoxin-treated group were fed a daily diet of rice powder containing aflatoxin.

Mycofix 0.3+ Adsorbent

Mycofix 0.3+ adsorbent manufactured by the Austrian company Biomin was used at a concentration of 100 mg/0.5 to remove aflatoxins.

Experimental Design

In this study, 3–4-week-old male Swiss albino BALB/c mice obtained from the College of Medicine, University of Mosul, were used. The mice were raised in a laboratory environment with continuous access to water and food under standard temperature, humidity, and light conditions. In this study, we divided 24 male mice into three groups of eight mice each to determine the aggravating effect of aflatoxins on hydatid cysts, after which we used an adsorbent.

1. Eight positive control mice were injected with protoscoleses only.
2. Eight mice were given a feed containing 1 ppm AF and injected with protoscoleses as well.
3. Eight mice were given a feed containing 1 ppm AF and 0.3+ Mycofix (0.5 mg) and injected with protoscoleses.

The experiment lasted for 40 days. After the experimental period, the mice were anaesthetized and sacrificed. Samples of the liver, spleen, intestines, and other internal organs were collected to determine the number, diameter, and distribution of hydatid cysts in the various organs.

Analysis of results

The results were obtained using analysis of variance according to the method of Bruning and Kintz (1997). Duncan's multiple range test was used at a significance level of $P<0.5$ to test the significance of differences between the treatment rates in the experiment, and a t test was also performed.

Results

Table (1) shows a significant increase in the number of hydatid cysts in the group that consumed a diet containing aflatoxins, with 79 cysts, representing 39.3% of the total. The third group, which was treated with aflatoxins and the adsorbent Mycofix, had 3 cysts, representing 17.4% of the total.

Adding the adsorbent to the group that consumed aflatoxins injected with the protoscoleses had a positive effect on counteracting the negative effects of aflatoxins, reducing the hydatid cyst counts compared with those of the control and the second groups.

As shown in Table (2), the number and size of hydatid cysts in the third group were lower than those in the control group and the group in which only aflatoxins were added to the feed: (21) cysts in the liver, (0) cysts in the spleen, and (14) cysts in the intestine, representing 23.8%, 55%, and 13%, respectively.

Discussion

Hydatid disease does not cause acute symptoms and is discovered incidentally during an X-ray or ultrasound, leading to stable, lifelong immunity for the infected individual. According to a study by Li et al. (2012), this is due to proteins that signal defense strategies and manipulate the cellular mechanisms of the intestinal epithelium to facilitate the entry and reproduction of the parasite. The intestinal epithelium is characterized as a selective, permeable barrier regulated by binding proteins (junctions) and a primary site of infection for a number of intestinal pathogens, including viruses, parasites, and bacteria.

In cases where the immune system is severely damaged or profoundly impaired, as is the case with exposure to aflatoxin AFB1, a mycotoxin that suppresses cellular immunity. In the current study, compared with mice fed an uncontaminated diet, the mice fed a diet contaminated with AFB1 developed more numerous, larger, and more dispersed hydatid cysts. The results also demonstrated that the use of the modified Mycofix adsorbent had a protective effect, as compared with the other two groups, the group fed a diet containing the Mycofix adsorbent had lower numbers and diameters of hydatid cysts.

AFB1 has been associated with immunosuppressive effects due to its direct inhibition of protein production, including that associated with specific functions such as IgG and IgA, as well as inhibition of macrophage migration, interference with the hemolytic activity of the complement system, decreased lymphocyte count, and impaired cytokine production by lymphocytes (Giambrone et al., 1978). Mycotoxins can reduce mucus production and damage tissue junctions, which can negatively impact intestinal integrity and the intestinal barrier, which acts as a physical barrier to prevent the passage of toxins and infection (Aghwan, 2024).

In addition, mycotoxins limit the proliferation and resistance of intestinal cells, reducing the ability of the intestine to self-repair and regenerate. This leads to increased permeability of the intestinal epithelium to mycotoxins and endotoxins, which enter the bloodstream easily. Mycotoxins increase intestinal sensitivity and reduce the minimum dose required to induce an inflammatory response, which has negative effects on many organs. The Food and Agriculture Organization of the United Nations (FAO) estimates that more than 25% of global food production is contaminated at some point with mycotoxins. Consequently, between 25% and 40% of cereals worldwide may contain one or more mycotoxins (Lawlor & Lynch, 2001). Mycotoxins can be detected in food during various stages of growth in cereals and other vegetables, such as during postharvest, transport, processing, and storage. Among all known mycotoxins, aflatoxins are considered the most dangerous (Coker, 1997). Once AFB1 is ingested by animals through dietary exposure, it is readily absorbed from the gastrointestinal tract into the blood stream because of its high lipid solubility (Leeson, 1995). It is highly toxic to the liver because it can inhibit protein synthesis through the binding of AFB1 products to cellular macromolecules, causing necrosis, degeneration, and inflammation of hepatic tissue (Hultmark, 2003), as the livers of mice exposed to aflatoxin show variable levels of Caspase-3, Bcl-2, and Bax proteins, as well as altered gene expression of these proteins (Wang et al., 2019). Given the potential of parasites to evade host immunity, Ritter et al. reported a decrease in the expression of the antiapoptotic protein Bcl-2 in living echinococcal cysts (Ritter et al., 2010), which coincides with the presence of aflatoxin, which increases immunosuppression, thus increasing the likelihood of the spread of parasitic infection (Amirmajdi et al., 2011).

In a study conducted in Turkey, the risk of aflatoxin exposure was investigated in infants fed both breast milk and formula. Serum levels of AFB1 were significantly higher in the formula-fed group than in the breastfed group (42.8% versus 8.5%, respectively) (Erkekoğlu et al., 2008).

Mycofix contains plant compounds such as hepatoprotective flavonoids and silymarin, which prevent toxins from entering liver cell membranes. It also contains tresenoid compounds that reduce inflammation and protect mucous membranes. Mycofix enhances the natural immune response through its plant components, which compensates for the immunosuppressive effect of AFB1 and promotes the modification of metabolic functions. These plant materials support the synthesis of nucleic acids, in addition to the conversion and breakdown of amino acids, which are important factors in cell proliferation (Omar, 2010; Shareef & Sito, 2018). Feed is an essential factor for the nutrition of animals such as sheep and cows, which rely on human food as a source of meat and are also considered major intermediate hosts for the parasite Echinococcus granulosus. Researchers have expressed interest in the use of adsorbents as holders for mycotoxins. These substances have a

protective effect by reducing or obstructing the absorption of these toxins in the digestive tract and their excretion from the body with waste when animals consume feed containing them. The addition of the activator Mycofix to feed has reduced the effects of aflatoxins (Dale & Wyatt, 1995; Ibrahim et al., 1997).

The adsorbent contains the microorganism BBSH 797, which secretes mycotoxin-inhibiting enzymes. The adsorbent also contains a medicinal herb extract that protects the liver by preventing mycotoxins from entering hepatic cells. The adsorbent also coordinates the immune response and improves metabolic functions by enhancing amino acid metabolism (Shareef & Taher, 2008).

The effect of Mycofix on aflatoxin was also recorded for its functional polar group because the absorption components in Mycofix are stable, and adsorption begins in the oral cavity during saliva secretion and continues in the stomach and intestines; thus, mycotoxins are stable and unable to enter the blood and are subsequently excreted in the stool (Diaz et al., 2005).

Tables

Table 1. Number and percentage of Hydatic cysts in different organs of mice fed with aflatoxin, with aflatoxin and Mycofix, and control +

Hydatid cysts	**Control +**	**Aflatoxin (AF)**	**Mycofix + Aflatoxin**	**Total number of hydatid cysts**
Number	79	7	35	201
Percentage	39.3%	43.28%	17.4%	100%

Table 2. Number and percentage of hydatid cysts in the control and treatment groups (aflatoxin, aflatoxin + mycofix) on the basis of several internal physiological organs.

Organ	**Control +**	**Feeding with AF**	**Feeding with AF + Mycofix**	**Cysts in organs**
Liver	34 (38.63%)	33 (37.5%)	21 (23.86%)	88 (43.78%)
Spleen	5 (83.5%)	1 (16.5%)	0 (0.0%)	6 (2.98%)
Intestine	40 (37.38%)	53 (49.53%)	14 (13.08%)	107 (53.2%)
Total	79 (39.30%)	87 (43.38%)	35 (17.41%)	201

Table 3. Hydatid cyst diameter in the different organs of three groups of mice (Control +, AF-fed, and AF + Mycofix-fed).

Cyst diameter (mm)	**Control +**	**AF**	**AF +Mycofix**	**Total number**
0.10	56 (41.48%)	50 (37.03%)	29 (21.48%)	135 (67.16%)
0.20	23 (42.59%)	25 (46.29%)	6 (11.11%)	54 (26.86%)
0.30	_____	1 (100%)	_____	1 (0.49%)
1.6	_____	5 (100%)	_____	5 (2.48%)
1.5	_____	6 (100%)	_____	6 (2.98%)
	79	87	35	201

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