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$MgO-SiO_2$ Nanocomposite Can Adsorb Aflatoxin in Wheat Flour

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Abstract: Aflatoxin is a fungal toxin that causes liver tumor and hepatitis, and is produced mostly by *Aspergillus flavus*on food and culture media. In this *in-vitro* lab trial study we used nanocomposite magnesium oxide-silicon dioxide ($MgO-SiO_2$) for aflatoxin adsorption in wheat flour samples. This study showed that nanocomposite $MgO-SiO_2$ was an effective adsorbing agent for aflatoxin, and the amount of reduction is related to concentration of aflatoxin, incubation times, concentrations of nanocomposite and temperature. The maximum reduction was 88% at aflatoxin concentration of 1,000 ppb, after 1.5 h of incubation with 0.4 g/mL of nanocomposite at 25 °C.

Key words: Aflatoxin, adsorption, nanocomposite, magnesium oxide, silicon dioxide.

1. Introduction

Many fungi in the nature may easily grow on the foodstuff which issued by humans or animals [1]. Some of these fungi can produce toxins, hence causing mycotoxicosis, the consequences of which range from headache and nausea to hepatitis (inflammation of liver), hepatic cirrhosis (liver failure), cancer, and even death [2]. Estimates made by Food and Agriculture Organization show contamination of 25% of cereals with mycotoxins each year. This huge amount shows its importance and need for global solutions [3].

Aflatoxin is a lethal poison mainly produced by *A. flavus*and *A. parasiticus* on food or culture media. It may induce liver failure and malignant tumor (hepatocellular carcinoma) [4]. Four main types of this toxin are B1, B2, G1, and G2. Aflatoxin B1 has the most toxicity and carcinogenicity [5], and is rapidly absorbed in the gastrointestinal tract [6]. Through the blood circulation, it is distributed in the body, including milk, in which it is known as aflatoxin M1, and can harm the babies of humans and other animal species [7].

The growth of *A. flavus*and aflatoxin production depends on temperature and humidity. When the storage conditions in cereal pools (as in silo) are not standard, this toxin will be accumulated on them [8]. The economic effects of this contamination on the agriculture sector is enormous, because it results in reduced nutritional value of foodstuff, decreased meat production from animals, and toxicity in users of dairy products. Since people use large amounts of milk or its derivatives each day, the adverse consequences of aflatoxin are of utmost importance from a medical point of view [9, 10].

The Food and Drug Administration has established specific guidelines on acceptable levels of aflatoxins in human and animal food, and has established action levels that allow for the removal of lots that violate it. The action level for human food is 20 ppb total

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aflatoxins, with the exception of milk which has an action level of 0.5 ppb for aflatoxin M [10].

The preventive measures include providing suitable piling conditions for cereals, use of chemicals (like ammonia) to fight against fungi, and boiling the milk. However, despite all of them, there is still some aflatoxins in dairy products [11]. Aflatoxin-adsorbing agents which bind and prevent its uptake by gastrointestinal cells are apparently useful for decreasing blood exposure to this toxin. Adsorbent agents for aflatoxin include aluminosilicates, activated charcoal, cell walls of fungi, bacteria and polymers [12]. Also, some microorganisms are capable of changing aflatoxin to some harmless compounds by special enzymes, collectively known as mycotoxin biotrans formers [13, 14].

There have been few studies using nanoparticles for adsorption of aflatoxin. The purpose of this *in-vitro* laboratory trial research was to evaluate the capacity of nanocomposite MgO-SiO_2 at different concentration of aflatoxin, nanocomposite, incubation times and temperature for adsorbing aflatoxin in contaminated wheat flour samples. The potential applications of such composites, which found in future studies to be hygienic and devoid of bioenvironmental hazards, may include its routine use in storage pools of wheat and flour.

2. Material and Methods

2.1 Preparation of Aflatoxin

The fungus *A. flavus*was isolated from decaying bread, and inoculated on potato dextrose agar medium (Merck, Germany) at room temperature for one week. After microscopic confirmation of the proper fungal genus and species, then chloroform (Merck, Germany) was used for extraction of the produced aflatoxin. Determination of the presence of aflatoxin was done by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). Ten titers (5-1,000 ppb) of the aflatoxin extract were prepared by 95% methanol.

2.2 TLC and HPLC Test

TLC: 10 microliters of the extract was placed on silica gel plates, and after drying was transferred to the TLC tank containing 50 mL of chloroform/methanol with the ratio of 98/2. After passage of the solution on the plate, it was allowed to dry, and was studied under ultraviolet light at 365 nm. A blue fluorescence was proof for presence of aflatoxin.

HPLC: 1 mL of the HPLC-grade 40% methanol was added, vortexed, and the contents were passed through 0.45 micrometer filters to yield a clear solution. A 100 microliter extract from each tube was injected to the HPLC system (model Waters 695, USA) which was equipped with a fluorescent detector (excitation wavelength = 360 nm, emission wavelength = 420 nm). The speed of the moving phase was 1 mL/min, at 100 µA flow intensity. All samples passed the columns in the HPLC system, and the curves for each specimen were plotted. The area under each curve was calculated to determine the amount of aflatoxin, using the standard curve for comparison.

2.3 Preparation of the Nanocomposite

Nanoparticles of magnesium oxide (MgO) with average size of 150 nm and also silicon dioxide (SiO_2) with average size of 50 nm were purchased from Lolitech, Germany, and were mixed to the ratio of 40/60. 100 grams of this nanocomposite was added to distilled water to make 1000 mL of solution (0.1 g/mL).

2.4 Aflatoxin Adsorption Test

Ten titers (5-1,000 ppb) of the aflatoxin extract were prepared by 95% methanol, and 1 mL from each dilution was added to 1 gram of wheat flour obtained from the main silo in Yazd, Iran.

In the next step, 1 mL of nanocomposite was mixed with 1 gram of contaminated wheat flour. After 30 minutes incubation time at room temperature, we dried

it, and non-adsorbed aflatoxin was quantified according to the above-mentioned HPLC protocol.

2.5 The Effect of Different Incubation Times, Concentrations of Nanoparticles and Temperatures on Aflatoxin Adsorption

(1) In order to evaluate the incubation times, 0.5 h, 1 h, 1.5 h, 2 h and 2.5 h of incubation were studied. The other parameters for this test were nanocomposite concentration = 0.1 g/mL, temperature = 25 °C.

(2) For evaluating the concentrations of nanocomposite, 0.1 g/mL, 0.2 g/mL, 0.3 g/mL, 0.4 g/mL and 0.5 g/mL were studied. The other parameters for this test were incubation times = 1.5 h, temperature = 25 °C.

(3) In order to evaluate the different temperatures, 25, 37, 50, 60 and 7 0 °C were studied. The other parameters included: incubation times = 1.5 h, nanocomposite concentration = 0.4 g/mL.

For this evaluation only 1,000 ppb of aflatoxin was tested.

2.6 Measurement of Aflatoxin Reduction and Statistical Test

The formula of aflatoxin reduction was:

Reduction% = [aflatoxin concentration before adding nanocomposite]-[aflatoxin concentration after adding nanocomposite and passing incubation time] / [aflatoxin concentration before adding nanocomposite] × 100.

On the other hand, forevaluating significance of difference, paired *t*-test was carried out on the control group and different test groups. $P < 0.05$ was considered a significant difference.

3. Results and Discussion

This study showed that the MgO-SiO_2 nanocomposite was efficiently capable of adsorbing aflatoxin and reducing its amount in wheat flour. However, the adsorbing capacity depends on and was roughly inversely proportional to the amount of aflatoxin, i.e., 100% removal for 5-20 ppb, about 95% removal for 40-100 ppb, and about 80% removal for 200-1,000 ppb (Fig. 1).

The paired t-test showed that there was a meaningful difference between the control group and various concentrations of aflatoxin after 30 minutes incubation (p-value = 0.001).

Incubation for different times showed that after 1.5 h and nanocomposite concentration of 0.1 g/mL and temperature at 25 °C, 83% reduction is achieved, and after this point there would be no more decrease (Fig. 2). Statistical paired t-test showed that there was a significant difference between the control group and all of the time groups (p-value = 0.003).

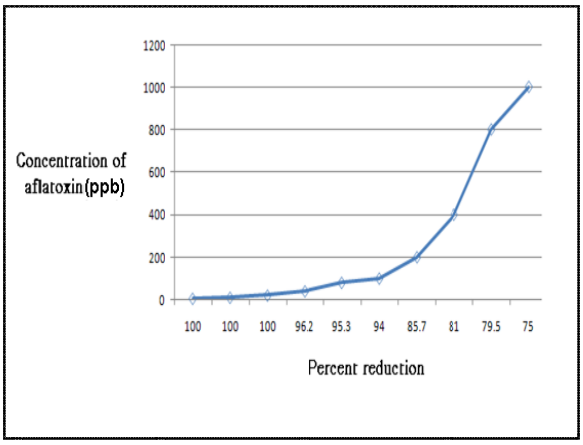

Fig. 1 Percent reduction of aflatoxin versus different concentrations of aflatoxin.
Incubation times: 30 min, nanocomposite concentration: 0.1 g/mL, temperature: 25 °C.

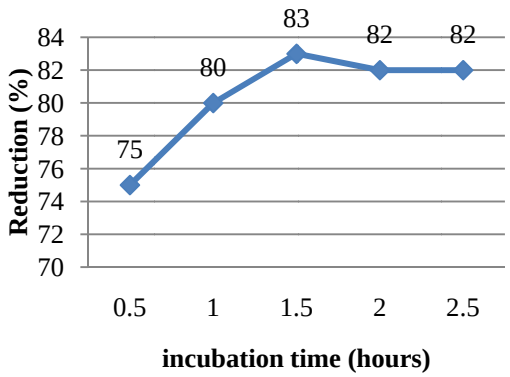

Fig. 2 Percent reduction of aflatoxin versus different incubation times.
Nanocomposite concentration: 0.1 g/mL, temperature: 25 °C.

Comparison of the different concentrations of nanocomposite showed 88% reduction after 1.5 h of incubation with 0.4 g/mL of nanocomposite at 25 °C, and it showed saturation above this point (Fig. 3). Paired *t*-test showed that there is a significant difference between the control group and 0.1 g/mL, 0.2 g/mL and 0.3 g/mL groups (p-value = 0.003).

Different temperatures (25 °C, 37 °C, 50 °C, 60 °C and 70 °C) were also studied and showed that increased temperature results in decreased reduction (Fig. 4). Again, paired *t*-test showed that there is a significant difference between the control group and all groups (*p*-value = 0.004).

This study showed that the adsorption reached to saturation after incubation for 1.5 h and 0.4 g/mL of nanocomposite. The reason of decreased adsorption in longer incubation times may be related to saturation of nanoparticles with aflatoxin. On the other hand, decreased adsorption in higher temperatures may be related to release of aflatoxin due to Brownian motion. Competition between aflatoxin and other materials present in wheat flour which cause non-specific binding may be the reason for adsorption curve to reach a plateau with concentrations of nanocomposite greater than 0.4 g/mL.

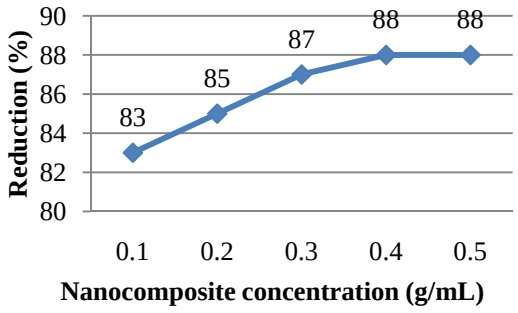

Fig. 3 Percent reduction of aflatoxin versus different nanocomposite concentrations.

Incubation times: 1.5 h, temperature: 25°C.

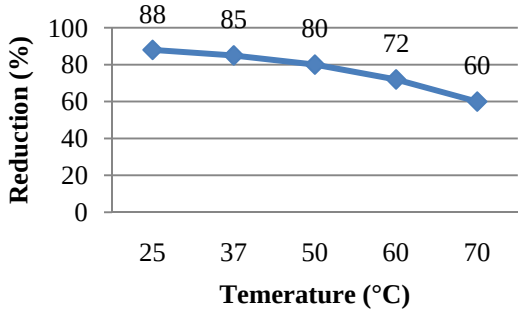

Fig. 4 Percent reduction of aflatoxin versus different temperatures.

Incubation times: 1.5 h, nanocomposite concentration: 0.4 g/mL.

One of the applications of nanoparticles has been used for adsorbing various compounds, because of their high surface areas [15]. The adsorption characteristics of the nanocomposite MgO-SiO_2 depend on the concentration of aflatoxin in the specimens, incubation times, concentrations of nanocomposite and temperature. Since the surface-to-volume ratio in nanoparticles determines the adsorption capacity, it seems possible to achieve higher capacities by decreasing their size.

There has been no previous report on usage of MgO-SiO_2 nanoparticles for adsorption of aflatoxin, but montmorillonite nanocomposite (MMN) was applied. Findings suggested that MMN nanocomposite can effectively reduce the toxicity of aflatoxin and be a potential ameliorator of aflatoxicosis in broiler chicks [15]. The current study depicted that liquid-phase nanocomposite also has high potency for adsorption of aflatoxin. Because of high efficacy, it may be that MgO-SiO_2 will become industrially and medically of routine use for removal of aflatoxin in stockpiled wheat and flour. Obviously, other mycotoxins may be treated in the same way, and other cereals could be managed in a similar manner, hence increasing food safety in the world.

The most important aspect of usage of nanocomposites for foodstuff is their safety and biocompatibility, since they become attached to food, and can be released or absorbed in the intestines. So, future research is needed to assess the potential adverse effects of these nanocomposites on the alimentary tract, blood cells, and other tissues.

3. Conclusions

In this *in-vitro* lab trial study we used nanocomposite MgO-SiO_2 for aflatoxin adsorption in wheat flour. It showed that nanocomposite MgO-SiO_2 was an effective adsorbing agent for aflatoxin, and the amount of reduction is related to concentration of aflatoxin in the specimens, incubation time, concentrations of nanocomposite and temperature.

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