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Protective Effect of Nanoparticle Oxides against Aflatoxicosis in Rats

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Abstract

This study was conducted to determine the efficacy of nano-composite Magnesium Oxide and Silicon Oxide ($MgO\text{-}SiO_2$) to reduce the toxic effects of aflatoxin B1 (AFB1) in adult male rats for 8 weeks. Animals were randomly divided into a control and three experimental groups (25 each). The first experimental group received feed contained 200 ppb (0.2 mg/kg) AFB1. The second group received feed contained 200ppb AFB1/kg and 0.5 g/kg nano-composite of $MgO\text{-}SiO_2$. Rats of the third experimental group received 0.5 g/kg nano-composite of $MgO\text{-}SiO_2$. Result showed that AFB1 markedly reduced body weight gain and food and water consumption. Postmortem examination reveled that AFB1 intake also induced pathologic changes in the liver and spleen. Activity of lymphocyte transformation and serum lysozymes activity showed significant changes. On the other hand, the deleterious effects of AFB1 were alleviated in the group that received feed contain 200ppb of AFB1 and 0.5g/kg $MgO\text{-}SiO_2$. These findings suggested that $MgO\text{-}SiO_2$ has high affinity to adsorb AFB1 and can effectively modulate its toxicity in rats and may offer a novel approach to the preventive management of aflatoxicosis in animals.

Key words: Nanoparticle Oxides(MgO-SiO2) , Aflatoxicosis, Rats, pathology , lymphocyte transformation ,serum lysozymes
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Introduction

Fungi produce a variety of secondary metabolites which known as mycotoxins. Mycotoxins at high concentrations directly affect specific organs including liver, kidney, oral and gastric mucosa, brain, or reproductive tract. In these cases, the signs of disease often are clearly apparent and attributable to impairment of specific tissues. At low concentrations, the effects of mycotoxins may be subtle. Thereby they reduce the growth rate of young animals or interfere with mechanisms of resistance and immune function making the animals more susceptible to infections. These effects on immunity are difficult to recognize because the signs of disease are associated with the infection rather than with mycotoxin that predisposed the animal to infection. Approximately 400 mycotoxins have been isolated and many are immunosuppressive. The feed-borne immunosuppressive mycotoxins include aflatoxins, fumonisins, ochratoxin and patulin (Pier et al., 1980; Harvey et al., 1991 and Cotty and Garcia, 2007).

Aflatoxin has clear negative effects on all aspects of the immune system of poultry, swine and ruminants. In fact, aflatoxin was transmitted via the egg in poultry and from the sow to the piglet reducing immune function of the offspring. Aflatoxin causes failure in the acquired immunity system of lambs by decreasing antibody production Aflatoxin in lambs reduced bacteriostatic activity and cellular immunity (Fernandez et al., 1997 and 2000). Aflatoxin suppressed lymphocyte response to mitogens and lymphocyte response of *Mycobacterium bovis*-infected animals to specific antigen (Brown et al., 1981).

Nanotechnology is a powerful new technology for taking apart and reconstructing

nature at the atomic and molecular level. Nanotechnology embodies the dream that scientists can remake the world from the atom up, using atomic level manipulation to transform and construct a wide range of new materials, devices, living organisms and technological systems. Broadly, nanotechnology promises several applications including disease diagnostics therapeutics and environmental remediation **(Michalet et al., 2005)**. In the food sector, nano-materials have seen the greatest expansion on a commercial level. Although the number of foods that contain nano-materials is still small, it appears set to change over the next few years as the technology develops. Nano-materials are already used to lower levels of fat and sugar without altering taste, or to improve packaging to keep food fresher for longer. They are also being used to increase the bioavailablity of nutrients in food supplements **(Liu and Zhao, 2007)**.

Nano-composite $MgO-SiO_2$, *in vitro* system, showed an effective adsorbing activity for aflatoxin B1 in wheat flour, and the amount of reduction is related to aflatoxin concentration **(Moghaddam et al., 2014)**. No work has been reported, in vivo system, on the role of $MgO-SiO_2$ in nano particles form as a binder for aflatoxins or other mycotoxins to decrease their toxic effects on animals.

Therefore, this work aims to study the potential effects of these formulations to relive the toxic effects of aflatoxin on rats hopping for its applications industrially and medically to remove aflatoxin from contaminated rations. In addition, this work employs to prove that these nanoparticles have no any negative harmful effects on animals.

Material and Methods

Materials

Animals: One hundred adult male Sprague Dawley rats were used in this study. The animals were kept under observation for a week before the start of the experiment at suitable laboratory conditions in the normal day light and fed on mash ration for broiler and tap water *ad libitum*. All animals received humane care in compliance with the guidelines for the care and use of laboratory animals as approved by the Research Ethics Committee for Animal Use.

Aflatoxin B1: It was prepared by the Mycology Laboratory, Animal Health Research Institute, Dokki, Cairo, Egypt **(Saher et al., 2011)**. It was added to the diet at concentration of 200 ppb (0.2 mg/kg); the selected concentration is nearly double the maximum permissible level for monogastric animals **(FAO, 1997 and FAO/WHO 2004)**. The ration was analyzed before adding the selected dose of aflatoxin B1 for the presence of aflatoxins and after its addition to ensure proper mixing.

Nano-composite $MgO-SiO_2$: Nanoparticles of Magnesium Oxide (MgO) and Silicon Dioxide (SiO_2) were purchased from Nano-Tech, Dreamland, 6th October, Giza, Egypt. They were characterized by transmission electron microscopy (TEM) and were mixed in a ratio of 40/60 according to **(Moghaddam et al., 2014)**. Magnesium oxide nanocrystallites (100nm) were synthesized by precipitation method by using polyvinyl pyrrolidone as capping agent via sol gel method as reported by **Mehta et al., (2012)**. Silica Nanoparticles (20nm) were prepared as spherical monodisperse and uniform- size using ultra sonication of tetraethyl orthosilicate by sol–gel process. Tetraethyl orthosilicate was hydrolyzed in a low molar-mass alcohol containing ammonia as a catalyst **(Tadanaga et al., 2013)**.

Chemicals: All chemicals and reagents used in this study were analytically pure and were purchased from Sigma-Aldrich Co. (St. Louis, USA), Aldrich (Germany) and El-Nasr Co. (Cairo, Egypt).

Experimental Design and Methods:

Animals were randomly classified into a control and three experimental groups (25 each); treated for 8 weeks. The first experimental group received feed contained 200 ppb AFB1. The second group received feed contained 200ppb AFB1/kg and 0.5 g/kg nano-composite of $MgO-SiO_2$. Rats of the third experimental group received 0.5 g/kg nano-composite of $MgO-SiO_2$. All groups were daily observed during the whole length of the experiment for apparent clinical signs and symptoms. Food and water

consumption were measured daily for two weeks (n= 14). The animals in each group received a known amount of the diet; at the next day the remaining feed was reweighed then the consumed amount was calculated by difference, divided on the number of animals per each group and expressed as gm or ml per animal per day.

Every two weeks, ten animals from each group were weighted then blood samples were collected from the inner census of the eye. Blood samples were used to estimate the activity of lymphocytes transformation and to measure serum lysozymes activity.

Lymphocyte transformation was performed by a colorimetric method for the determination of viable cell density. Lymphocytes can enter mitosis when they are activated by a mitogen as phytohemagglutinin that encourages cells to commence cell division. Dimethythiazol-diphenyl tetrazolium bromide dye (MTT) is a yellow dye, which is reduced into purple crystals by the activity of mitochondrial succinate dehydrogenase enzyme in viable cells reflecting the number of the viable cells **(Rai-Elbalhaa et al., 1985).**

Lysozyme activity in the serum was assayed through hydrolyzing the cell wall of *Micrococcus lysodeikticus* as a substrate in agarose gel suspension making a clear zone ring of lysis. At the end of 18 hours incubation period, the diameters of the clear zone rings were measured to the nearest 0.1 mm with an enlarger-viewer (Kalesttad Laboratories., Inc., and Austin, TX). The lysozyme activity in the sample was determined from a plotted standard curve against the corresponding clear zone ring diameter on the linear axis **(Schultz, 1987).**

The obtained values were calculated as means and standard errors (S.E) of the mean. The standard errors were used to measure the degree of dispersion of the values around their mean. Comparisons between different groups were carried out by one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test, Graph pad software Instat (version 2) at $P \leq 0.05$.

Results

1- Clinical Signs and Symptoms:

Rats of the control and Nano-composite of MgO-SiO_2 groups did not have clinical signs of disease or abnormal behavior, whereas AFB1treated group were lethargic with no mortality.

2- Body Weight:

Control group and that received 0.5g/kg nano-composite of $MgO.SiO_2$ showed gradual increase in their body weight by the time without significant difference between them Table (1). Body weight of the group received feed contain 200 ppb AFB1 showed significant decrease when it compared with control group started at the 2nd week of the experiment and persisted among the experimental time. Body weight of the group received feed contain AFB1 (200ppb) and 0.5g/kg nano-composite of MgO-SiO_2 showed non- significant difference than that of the control and than that of the AFB1 treated group at the 2nd and 4th weeks. On other hand, at the 6th and 8th weeks, body weight showed non-significant decrease from the control group.

3- Food Consumption:

Food consumption of the control and the groups treated with AFB1 and/or MgO-SiO_2 showed non-significant difference during whole time of the experiment (Table 2). While the group that received AFB1 (200ppb) showed significant decrease in food consumption when it compared with those of control and other experimental groups at the 6th and 8th weeks.

4- Water Consumption:

Result of the water consumption of the control and experimental groups showed insignificant difference from each other except the group that received feed contain AFB1 (200ppb) as it showed significant decrease at the 6th and 8th weeks when it compared with control and the other groups (Table 3).

5- Post Mortem Finding:

On postmortem examination, the internal organs of control group and that received feed contain 0.5g/kg Nano-composite of $MgO-SiO_2$ showed no abnormal postmortem change when it compared with AFB1 group. There were gastritis and enlarged stomach with undigested food inside it in most cases of AFB1treated group. In addition, congested hepatomegaly and liver tumor with swollen gall bladder and splenomegaly were found in some rats. Meanwhile, group that received feed contain AFB1 and nanocomposite $MgO-SiO_2$ showed nearly normal appearance in postmortem except in one animal it showed tumor in its liver.

6- Lymphocyte Transformation Activity:

Lymphocyte transformation activity of the group received AFB1 (200ppb) showed a significant decrease than those of control and the other experimental groups (Table 4). This decrease was propagated by time until the last week. Lymphocyte transformation activity of the group received AFB1 and nano-composite $MgO-SiO_2$ showed inconstant significant decrease than control and the group received nano-composite $MgO-SiO_2$ but still significantly, higher than AFB1 treated group. In addition, it showed no difference than the control at the 4th week.

7- Serum Lysozyme Activity:

Serum lysozyme activity of control and Nano-composite $MgO-SiO_2$ groups showed significant higher activity than the group received AFB1 (200ppb) among the experimental time (table 5). While the group received AFB1 and 0.5g/kg Nano-composite $MgO-SiO_2$ showed significant increase than AFB1 group during the whole experiment except at the 2nd week. In the meantime lysozyme activity of this group was insignificant than control group at the 4th and 6th weeks.

Table (1): Mean values and ± S.E of the body weight (gm) in control and experimental groups received feed contain 200ppb AFB1 and/or 0.5 g/kg nano-composite of $MgO-SiO_2$.

Groups / Time	Control	AFB1	AFB1 $MgO-SiO_2$	$MgO-SiO_2$
2nd week	190 ± 4.4 a*	159 ± 5.5 b *	177 ± 6.5 ab	188 ± 3.8 a
4th week	226 ± 8.8 a	192 ± 7.4 b	201 ± 8.5 ab	217 ± 6.4 a
6th week	244.25 ± 11 a	175.5 ± 9.2 b	231.7 ±9.2 a	234.75 ± 8.7 a
8th week	262.5 ± 13.2 a	111 ± 11.5 b	258.8 ± 12.1 a	268 ± 12.1 a

*: Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n=14)

Table (2): Mean values and ± S.E of the food consumption (g/day/animal) in control and experimental groups received feed contain 200ppb AFB1 and/or 0.5 g/kg nano-composite of $MgO-SiO_2$.

Groups / Time	Control	AFB1	AFB1 $MgO-SiO_2$	$MgO-SiO_2$
2nd week	35.8 ± 3.6 a	30 ± 3.3 a*	35.8 ± 3.2 a	35.2 ± 3.1 a
4th week	41.9 ± 4.5	32.7 ± 3.8	40.6 ± 4	45.7 ± 5.8

	a	b	a	a
6th week	50 ± 6.2 a	25.5 ± 2.3 b*	45.8 ± 6.5 a	53.9 ± 5.5 a
8th week	55.2 ± 4.8 a	20 ± 2.5 b	50.9 ± 5.9 a	55.5 ± 5.5 a

*: Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n=14).

Table (3): Mean values and ± S.E of the water consumption (ml/day/animal) in control and experimental groups received feed contain 200 ppb AFB1 and/or 0.5 g/kg nano-composite of $MgO\text{-}SiO_2$.

Time \ Groups	Control	AFB1	AFB1 $MgO\text{-}SiO_2$	$MgO\text{-}SiO_2$
2nd week	16.8 ± 1.6 a*	16.1 ± 1.3 a	17.8 ± 2.2 a	17.2 ± 1.1 a
4th week	18.9 ± 1.5 a	14.7 ± 1.8 a	18.6 ± 2.04 a	19.7 ± 1.8 a
6th week	19.1 ± 1.2 a	14.5 ± 1.3 b*	19.8 ± 1.5 a	19.9 ± 1.5 a
8th week	19.2 ± 1.8 a	13 ± 1.5 b	19.9 ± 2.9 a	19.5 ± 1.5 a

*:Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n=14).

Table (4): Mean values and ± S.E of the lymphocyte transformation (expressed as OD) in control and experimental groups received feed contain 200ppb AFB1 and/or 0.5 g/kg nano-composite of $MgO\text{-}SiO_2$

Time \ Groups	Control	AFB1	AFB1 $MgO\text{-}SiO_2$	$MgO\text{-}SiO_2$
2nd week	1.22 ± 0.1 a*	0.46 ± 0.04 b*	0.90 ± 0.05 c	1.1 ± 0.05 a
4th week	1.49 ± 0.13 a	0.33 ± 0.02 b	1.01 ± 0.13 a	1.47 ± 0.18 a
6th week	1.76 ± 0.17 a	0.20 ± 0.01 b	1.06 ± 0.18 c	1.65 ± 0.17 a
8th week	2.12 ± 0.17 a	0.18 ± 0.01 b	1.12 ± 0.17 c	1.87 ± 0.17 a

*: Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n = 10).

Table (5): Mean values and ± S.E of serum lysozyme activity (u/ml) in control and experimental groups received feed contain 200ppb AFB1 and/or 0.5 g/kg nano-composite $MgO\text{-}SiO_2$.

Time \ Groups	Control	AFB1	AFB1 $MgO\text{-}SiO_2$	$MgO\text{-}SiO_2$
2nd week	204.5 ± 7.6	126.6 ± 10.4	197.2 ± 6.97	200.0 ± 6.97

	a*	b*	b	a
4th week	226.5 ± 6.7 a	120.9 ± 5.8 b	222.1 ± 10.4 a	235.3 ± 7.9 a
6th week	248.5 ± 5.8 a	107.3 ± 5.9 b	224.2 ± 13.7 a	241.9 ± 6.8 a
8th week	257.3 ± 3.8 a	67.6 ± 8.1 b	228.7 ± 8.1 c	249.6 ± 5.3 ac

*: Values have different scripts at the same row are significantly different at $P \leq 0.05$ (n = 10).

Discussion

Based on the present results, MgO-SiO_2 in nano particles form offered significant benefit to combat AFB1. It prevented the absorption of AFB1 from the gastrointestinal tract and hence it increased the performance parameters as recorded in weight gain and food and water consumption. In addition, it lowered the toxic effect on the liver, spleen, and gastrointestinal tract as showed on postmortem inspection. The adverse effect of AFB1 on growth performance could be related to the recorded gastritis and associated decrease in the food consumption. Poor growth has been reported to the decrease protein and energy utilization probably because of deterioration of the digestive and metabolic efficiency. Body weight gain may be decreased in association with prolonged poor nutrition, decreased hepatic synthesis or increased loss from the gastrointestinal or urinary systems (**Verma et al., 2002**).

In this study, a pronounced hepatomegaly and splenomegaly with hepatic tumors were observed in rats treated with AFB1. These changes in the livers are comparable to those reported in the literature on avian aflatoxicosis (**Denli et al., 2005**). Liver is considered the target organ for AFB1 where most aflatoxins are bioactivated to the reactive 8,9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures, inducing cancers and increasing liver weight (**Miazzo et al., 2005 and Pasha et al., 2007**). Significant increases in the relative spleen weight of broilers exposed to aflatoxin-contaminated diets have also been reported by **Bailey et al. (2006) and Shi et al. (2006)**.

In a series of experiments, MgO or SiO were found to have an effective adsorbing agent for aflatoxin by apparently dissolving AFB1 in their pores due to their large surface area. Silicon dioxide nanoparticles are the basis for a great deal of biomedical research due to their stability, low toxicity and ability to be functionalized with a range of molecules and polymers. Silica nanoparticles have attracted significant interest because of their unique properties amenable for in vivo applications such as hydrophilic surface, excellent biocompatibility, ease of large-scale synthesis, and low cost of its nanoparticles production (**Yiming et al., 2011; Li and Jianjun, 2013 and Hui et al., 2015**).

In the current study, cellular immune response as estimated by the activity of lymphocyte transformation and lysozyme of the group received AFB1 were severely decreased when it compared with the group supplemented with nano-composite MgO-SiO_2. This finding suggested that nanoparticles of MgO-SiO_2 indirectly alleviated these decreases by preventing AFB1 to induce its effect on immune system.

Many studies conducted in poultry, pigs, and rats showed that, exposure to aflatoxin in contaminated food results in suppression of the cell-mediated immune responses, thymic aplasia, reduce the function and number of T-lymphocyte, suppress phagocytic activity and reduce complement activity. Impairment of cellular function by aflatoxin seems to be due to its effects on the production of lymphokines and antigen processing by macrophages, as well as a decrease in or lack of the heat-stable serum factors involved in phagocytosis (**Raisuddin et al., 1993; Cusumano et al., 1996; Ottinger et**

al., 2000; Marin et al., 2002 and Theumer et al., 2003).

Lymphocytes are a type of white blood cell responsible for a variety of actions for initiating an immune response when a foreign invader enters the body. These cells are primarily in the lymph nodes and circulate in the blood. The rate of its transformation, in terms of proliferation and enlargement measures the viability of cells and indicates protein synthesis. Lysozyme is an enzyme that hydrolyses the cell wall of some microorganisms by cleaving the sugar backbone of the peptidoglycan component thereby kills the organism. It is found in monocytes and macrophages, neutrophils and glandular cells. Comparison of lysozyme activity reveals evolutionary aspects of the immune system (**Schultz, 1987 and Hinton, 2000**). Magnesium Oxide originally was used as an anti-bacterial agent against commonly found bacteria spores and viruses, scavenge fluoride from drinking water (**Wanchanthuek and Thapol, 2011;**

Venkatesha, 2012 and Parth et al., 2014).

In conclusion, aflatoxin B1 induced toxic changes in rats; $MgO\text{-}SiO_2$ nanoparticles was able to adsorb AFB1 thereby avoid these changes. Results of this study could prove the efficacy of $MgO\text{-}SiO_2$ nanoparticles in preventing aflatoxicosis in animals. In addition, it can provide an effective, safe cheap and accessible source of animal feed additive in Egypt to conventional prevention of mycotoxicosis in animal feed. Further research into the mechanism of action and testing in other animal models is required to determine how the industrial utility of this agent as an anti-mycotoxin can be developed.

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الملخص العربى

أجريت هذه الدراسة لتحديد مدى فعالية أكسيد المغنيسيوم و أكسيد السيليكون متناهي الصغر للحد من الأثار السامة للأفلاتوكسين ب1 فى ذكورالفئران البالغه لمده 8 أسابيع.

قسمت الحيوانات عشوائيا إلى مجموعه ضابطه و ثلاث مجموعات تجريبية (25 لكل منهم). المجموعه الاولى التجريبيه تغذت على عليقه تحتوى على 200 جزء من البليون (0.2 ملجم/كجم) من الافلاتوكسين ب1. المجموعه الثانيه التجريبيه تغذت على عليقه تحتوى على 200 جزء من البليون من سم الافلاتوكسين ب1 و 0.5 جرام/كجم من أكسيد المغنيسيوم و أكسيد السيليكون متناهي الصغر. المجموعه الثالثه التجريبيه تغذت على عليقه تحتوى على 0.5 جرام /كجم من أكسيد المغنيسيوم و أكسيد السيليكون متناهي الصغر فقط. وقد أظهرت النتائج ان الفلاتوكسين ب1 لها تاثير ملحوظ فى انخفاض وزن الجسم و أستهلاك المياة والطعام. و أظهرالتشخيص المرضى بعد الوفاة عن تغيرات مرضيه فى الكبد والطحال . و قد أثرت ايضا على نشاط تحول الخلايا اللمفاويه و نشاط انزيم الليزوزم المصلى. من ناحيه أخرى تم تخفيف الاثار الضاره للافلاتوكسين ب1 فى المجموعه التى تلقت عليقه تحتوى على 200 جزء من البليون من سم الافلاتوكسين ب1 و 0.5 جرام/كجم من الجزيئات متناهيه الصغر لأكسيد المغنيسيوم والسيليكون. ولقد أشارت هذه النتائج أن هذه الجزيئات منتاهيه الصغر لديها قابليه عاليه من امدصاص الافلاتوكسين ب1 و تستطيع ان تعدل على نحو فعال من سميتها فى الفئران و خلاصه الامر نتائج هذه الدراسه أثبتت فعاليه جزيئات أكسيد المغنيسيوم والسيليكون المتناهيه الصغر فى الحد من تسمم الافلاتوكسين فى الحيوانات. لذلك ، فإنه يمكن توفيره كماده فعالة ورخيصة و آمنة فى أضافات الاعلاف الحيوانيه فى مصر للحد من التسمم الفطرى.