Toxic Effects of Aflatoxin B1 on Duodenum Tissue

Neda Aboutalebi

Department of Science, Islamic Azad University, Science and Research Branch, Urmia, Iran
Email: nedaaboutalebi@yahoo.com

Abstract: Aflatoxins are toxic metabolites produced by specific strains of *Aspergillus flavus* and *Aspergillus parasiticus*. The main goal of this investigation was studying the toxic effect of aflatoxin B1 on duodenum tissue which has a critical role in digestion and absorption of foods. Histopathologial examinations revealed that aflatoxin has less toxic effects on duodenum tissue at lower dosages; however with increasing the aflatoxin concentration severe degenerative alterations in intestinal villi were observed which demonstrated that hyperplasia was occurred in villi and mucosa. Villi lengths were shorter in treatment groups compared to control which reveal that aflatoxin decreases the surface area in villi for nutrition's absorption and therefore causes dyspepsia. At the end it can be concluded that aflatoxin has severe toxic effects on different parts of intestinal tract and must be considered as one of the important toxins in food industries.
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Introduction

Aflatoxins are toxic metabolites produced by specific strains of *Aspergillus flavus* and *Aspergillus parasiticus*. It is demonstrated that five major forms of aflatoxins: B_1, G_1, B_2, G_2, and M_1 have cytotoxic effects (Tomkova et al., 2001; Venturini et al., 1996). Aflatoxins bearing carcinogenic activity and they are mainly hepatocarcinogen (Reddy et al., 2005; Speijers and Speijers, 2004).

Among these aflatoxins, aflatoxin B_1 is more toxic and metabolized to its active form mainly in the liver, so Its absorption in the small intestine is a very fast process without any evident changes(Dugyala and Sharma, 1996). It is the most prevalent carcinogenic of the aflatoxins and has been classified as a class I human carcinogen by the International Agency for Research on Cancer (Cancer., 1993). The carcinogenic activity of AFB_1 related to metabolically activated reactive intermediates that covalent binds to hepatocellular DNA, which causes mutations in the host genome (Jaeschke et al., 2002).

Contamination of food stuffs to AFB1 is a global problem. Razaghi *et al* have shown that the soil of corn stead in Mazandaran and Semnan was contaminated by aflatoxins (Razzaghi-Abyaneh et al., 2006). Furthermore, these toxins can be transmitted to raw products and meat by contaminated nourishment. The main aim of this study was to investigate the toxic effects of AFB1 on duodenum in digestion tract which plays an important role in digesting and absorbing of foods.

Material and Methods

Animals

Male ICR mice aged 8 weeks and weighting about 20 g, were purchased from animal house of veterinary department of Urmia University. An ambient condition with temperature of 22 ± 2◦C, relative humidity of 60 ± 10%, and photoperiod of 12 h was maintained throughout the study. Commercial pellet diet and distilled water were provided for mice ad libitum. All the animals were quarantined and acclimated in the controlled environment for seven days prior to the study. Forty mice were divided into four groups with ten mice in each group. . All animal experiments are performed in compliance with the local ethics committee.

Aflatoxin administration

Aflatoxin B1 from Aspergillus flavus (Sigma, Germany) was used. .Each rats in test group1, 2 and 3, received 100µg/kg, 350µg/kg, and 700µg/kg AFB1 respectively through gavage for duration of 35 days.

Histopathological examination

For conventional histology, doudenum tissues were taken immediately after the sacrifice of the animals, fixed in 10% formaldehyde, embedded in paraffin, cut into 5-7 µm thick sections, and stained with hematoxylin and eosin.

Statistical analysis

Results were analyzed by one-way analysis of variance (ANOVA) using SPSS 15.0. (IBM Co. USA). A statistical significant difference for all tests was considered to be $p<0.05$.

Results

Histopathological observations

Histopathological observations in test group one which received 100µg/kg aflatoxin revealed no significant alterations. However in test group two

which received 350µg/kg aflatoxin severe degenerative changes in intestinal villi were observed as pathological lesion. In some parts of these villi severe hypoplasia and epithelial cells atrophy were seen. Slight pathological alterations were observed in Lieberkuhn's glands regions and no changes were seen in Brunner's glands region under sub mucosa layer. Congestion under sub mucosa layer was only noticeable finding in this region. Severe degenerative alterations in mucosa layer were detected in test group three which obtained 700µg/kg aflatoxin. Necrosis was occurred in some parts of villi and hyperplasia was seen in these regions. Slight pathological changes were detected in Lieberkuhn's glands and no significant alterations were observed in Brunner's glands and smooth muscle regions.

Histomorphometric observations

Measurement of villi length revealed that there are significant differences ($p<0.05$) between control and treatment groups. There are also significant differences between test group one and other groups, however statistical analysis revealed no significant differences ($p<0.05$) among test group two and three.

Diameter measurements of muscular part in test group one, two, three and control group were 0.031, 0.033, 0.034 and 0.052 micrometer respectively which declare significant differences ($p<0.05$) between control and treatment groups.

According to our measurements, crypt depth in test group one, two, three and control group were 0.144, 0.136, 0.122 and 0.228 micrometer respectively which present significant differences ($p<0.05$) between control and treatment groups.

Epithelium length in test group one, two, three and control group were 0.027, 0.028, 0.029 and 0.034 micrometer respectively. Statistical analysis revealed that there are significant differences ($p<0.05$) between control and treatment groups.

Measurement of sub mucosa layer demonstrated that there are significant differences ($p<0.05$) between control and test group two and three. There are also significant differences ($p<0.05$) between test group two and three.

No significant differences were observed in Goblet cells analysis among control and treatment group.

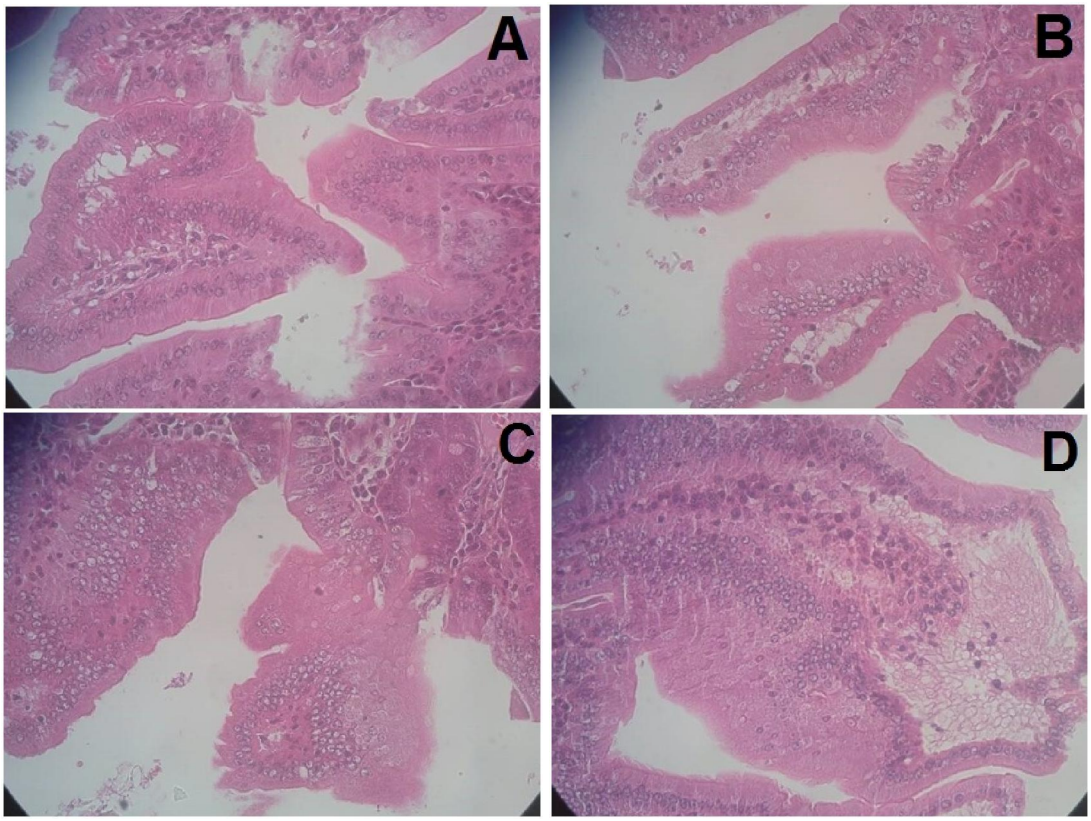

Figure1. Histopathological observations in test groupn one, two and three which received 100µg/kg, 350µg/kg, and 700µg/kg AFB1 respectively through gavage for duration of 35 days. (A) Decrease of duodenum villi lengths and degenerative changes in epithelium cells. (B) Degenerative changes in cylindrical cells of villi. (C) Decrease of duodenum villi lengths and hypo plastic changes in this region. (D) Hypoplasia in duodenum tissue

Table 1. Histomorphometric observations of duodenum tissue

Groups	Vili lengths	Diameter of muscular part	Crypt depths	Epithelium lengths	Sub mucosa layer	Goblet cella
Test group 1 (100µg/kg)	(0.356±0.081)[b]	(0.031±0.0109)[a]	(0.144±0.021)a	(0.027±0.007)a	(0.22±0.082)a	(0.83±0.977)a
Test group 2 (350µg/kg)	(0.265±0.067)[a]	(0.033±0.0107)[a]	(0.136±0.033)a	(0.028±0.028)a	(0.107±0.034)c	(0.77±0.831)a
Test group 3 (700µg/kg)	(0.296±0.096)[a]	(0.034±0.0128)[a]	(0.122±0.028)a	(0.029±0.006)a	(0.188±0.0008)b	(1.15±1.176)a
Control	(0.426±0.085)[c]	(0.052±0.0146)[b]	(0.228±0.092)b	(0.034±0.007)a	(0.244±0.076)a	(1.08±1.124)a

Discussion

Mycotoxins are absorbed by passive diffusion through the small intestine at a very high rate. The data show that almost complete aflatoxin absorption could take place in the intestinal tract (Ramos AJ and E., 1996). Aflatoxin B1 is more toxic than other types of aflatoxins. Aflatoxin can disrupt intestine tissue including duodenum. It can also be classified as carcinogens(Jaeschke et al., 2002).

The main goal of this investigation was studying the toxic effect of aflatoxin B1 on duodenum tissue which has a critical role in digestion and absorption of foods. Histopathologial examinations revealed that aflatoxin has less toxic effects on duodenum tissue at lower dosages; however with increasing the aflatoxin concentration severe degenerative alterations in intestinal villi were observed which demonstrated that hyperplasia was occurred in villi and mucosa. Villi lengths were shorter in treatment groups compared to control which reveal that aflatoxin decreases the surface area in villi for nutrition's absorption and therefore causes dyspepsia.

In histology, Lieberkuhn' s glands are glands found in the epithelial lining of the small intestine and colon. These glands secrete various enzymes, including sucrase and maltase, along with enteropeptidase. Lieberkuhn" s glands damage was proved in this investigation which more reveals this fact that various enzymes secretion would decrease with increasing the aflatoxin concentration.

At the end it can be concluded that aflatoxin has severe toxic effects on different parts of intestinal tract and must be considered as one of the important toxins in food industries.

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