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Histopathologic Studies on Liver in Ducklings Administered Aflatoxin G_1 Produced by Korean Industrial Strain of *Aspergillus flavus*

Hwa Joong Yoon, D.V.M., M.S., Ph.D.

Department of Veterinary Medicine, College of Animal Husbandry, Kon-Kuk University

Introduction

A new disease called 'Turkey X disease' has been described since the widespread outbreaks of deaths in turkey poults in 1960.[5] Since the toxic aflatoxins B and G were isolated from the mold *Aspergillus flavus* and their structural formulas were determined, the investigations of the toxins have been studied by many researchers. The determination of the long-term effects of a single oral sub-lethal dose of crystaline aflatoxin (40 per cent aflatoxin B_1 and 60 per cent aflatoxin G_1) to rats have been studied.[7] The histological changes in the liver produced by a single dose of aflatoxin(80 per cent B_1 and 20 per cent G_1) were contrasted with the lesions produced in day-old ducklings by other well-studied liver poisons.[6] Purification and toxicity of aflatoxin G_1 have been examined by Lijinsky and Butler.[12] Carnaghan et al.[8] estimated the LD_{50} value for aflatoxin G_1 to be approximately 39.2 μg per 50 grams duckling when they used dimethylformamide as the solvent.

It is the purpose of this report to determine the toxicity of aflatoxin G_1 produced by Korean Industrial Strain of the *Aspergillus flavus* as the result of histopathologic studies on the liver of the ducklings which were administered the aflatoxin G_1.

Materials and Methods

The day-old Korean native breed ducklings(average weight 47 grams) were used as the experimental animals. During the course of this investigation, the ducklings were housed in a standard chick battery brooder at temperatures of 80~95 degrees F. with sufficient food and water supplied ad libitum. The *Aspergillus flavus*(Strain A-124) was isolated from the fermented soybean mass and then cultured. The aflatoxin that we used in this experiment was produced from the *Aspergillus flavus* by the researchers in the Institute of Applied Microbiology, Kon-Kuk University according to Pons and Goldblatt method[18]. The crystalline aflatoxin G_1 dissolved in propylene glycol was administered by intubation. In order to study the acute lesions by the dose of the toxin and the duration by a single oral intubation, we designed 3 groups: 75 μg, 50 μg and 25 μg of the aflatoxin. There were 5 ducklings in each group, and the survivors were decapitated on the 3rd and 7th day of the experiment. Comparisons of the toxic effects of the aflatoxin G_1 (Korean aflatoxin, aflatoxin G_1, was produced by Strain A.-124 that was isolated from the fermented soybean mass and then cultured in the Institute of Applied Microbiology, Kon-Kuk University) produced by Korean Industrial Strain of the Aspergillus sp. with those of standard aflatoxin G_1(Standard aflatoxin G_1 was produced in Makor Chemicals Ltd. in Jerusalem, Israel.) were studied in this experiment.

Liver tissue obtained from the distal parts of the left lobe immediately after decapitation was cut

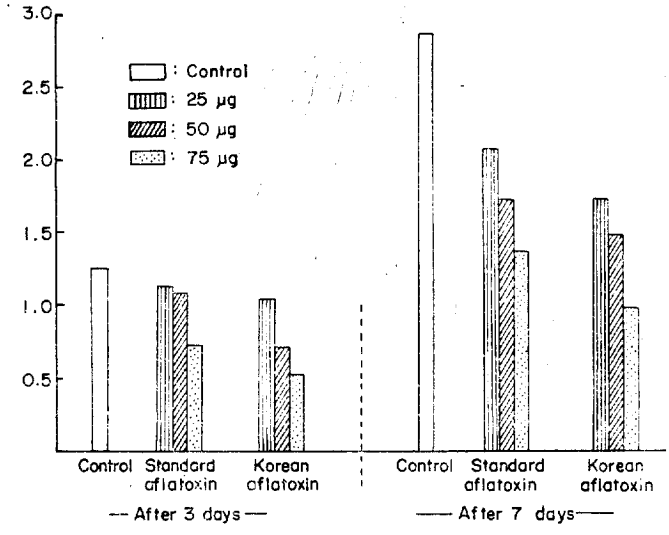

Fig. 1. Comparison of growth ratio after administration two aflatoxin preparations.

Table 1. Comparison of Growth Ratio after Administration of Two Aflatoxin Preparations

Dosage of Aflatoxin	After 3 days		After 7 days	
	Standard Aflatoxin	Korean Aflatoxin	Standard Aflatoxin	Korean Aflatoxin
25 μg	1.62	1.54	2.56	2.23
50 μg	1.58	1.21	2.22	1.96
75 μg	1.21	1.02	1.85	1.47
Control		1.75		3.36

Table 2. Histopathologic Findings of Livers That were Administered Standard Aflatoxin

Dosage of Aflatoxin / Duckling No. / Tissue Changes	75 μg					50 μg					25 μg				
	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Hemorrhages	++	+	—	—	—	+	±	—	—	—	—	—	—	—	—
Necrosis	±	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fat Change	++	++	+	+	+	+	+	±	—	—	+	+	±	—	—
Bile Duct Cell Proliferation	—	—	++	+	+	—	—	+	±	—	—	—	+	±	±
Regeneration of Hepatic Cells	—	—	+	+	+	—	—	+	±	—	—	—	±	±	—

Degree of histopathologic changes: — Within normal limits, ± Minimal in degree, + Slight in degree, ++ Moderate in degree, *Dead duckling

and processed in a routine manner for paraffin sections. The sections of the livers were stained with Mayer's hematoxylin and eosin, and frozen sections were prepared to stain Oil Red O. for the accumulation of large quantities of lipid. All sections were scored using an arbitrary system whereby the degrees of the lesions in the microscopic appearances were classified by the following five criteria: —:

Table 3. Histopathologic Findings of Livers That were Administered Korean Aflatoxin

Dosage of Aflatoxin / Duckling No. / Tissue Changes	75 μg					50 μg					25 μg				
	1*	2*	3	4	5	6	7	8	9	10	11	12	13	14	15
Hemorrhage	##	##	+	—	—	+	+	—	—	—	—	—	—	—	—
Necrosis	±	±	±	—	—	—	—	±	—	—	—	—	—	—	—
Fat Change	##	##	+	+	+	+	+	+	±	±	+	+	±	±	±
Bile Duct Cell Proliferation	±	±	##	+	±	—	—	+	—	—	—	—	+	±	
Regeneration of Hepatic Cells	±	±	+	+	±	—	—	+	±	±	—	—	±	—	—

Degree of histopathologic changes: — Within normal limits, ± Minimal in degree, + Slight in degree, ## Moderate in degree, ### Marked in degree, *Dead duckling.

within normal limits, ±: minimal, +: slight, ##: moderate, and ###: marked in degree.

The formula of growth ratio is as follows: increased body weight/initial body weight.

Results

The typical clinical symptoms of toxicity were not shown in the ducklings of this experiment, except 2 ducklings of 75 μg treated with Korean aflatoxin died and 1 duckling of 75 μg treated with standard aflatoxin died. Body weights of the ducklings were decreased more than that of the control. The retardation of the growth by aflatoxin G_1 is illustrated in Fig. 1 and Table 1. The dead ducklings developed ataxia followed by convulsions shortly before death and died in the form of opisthotonus. Besides the dark red petechial hemorrhages on the livers of dead ducklings, most of the livers were normal-fresh in color.

After exposure to 75μg of aflatoxin, 2 ducklings were killed with Korean aflatoxin and 1 duckling was killed with standard aflatoxin. Marked hemorrhages and fatty changes were observed in the livers (Fig. 2 and 3), but only fatty changes were shown in the livers of the ducklings that were decapitated on the 3rd day. Slight fatty changes, moderate bile duct cell proliferation (Fig. 5) and slight regeneration of the hepatic cells(Fig. 7) were observed in the livers of the ducklings that were decapitated on the 7th day. Moderate hemorrhage and slight proliferation of bile duct cells were produced with Korean aflatoxin (Fig. 4).

In the livers of a few ducklings that were administered 50μg aflatoxin G_1, slight hemorrhages, slight fatty changes, slight bile duct cell proliferation and slight regeneration of hepatic cells were revealed. In the livers that were exposed to 25 μg aflatoxin G_1, the appearances of the livers were demonstrated to be similar to those of the normal livers except slight fatty changes in some cases.

Discussion

When the growth ratio was compared with that of the control, the growth depression was conspicuous in all of the experimental ducklings. Butler[6] showed that the clinical symptoms with the two toxins were similar in their experiments. However, the toxicity of the aflatoxin G_1 is less than that of the aflatoxin B_1 in this experiment.

The lesions that were seen in the livers of the ducklings treated with aflatoxin G_1 were similar to those found in the ducklings treated with aflatoxin B_1. An exception noted with aflatoxin G_1 was that did not demonstrate necrosis of the livers. Besides the intensity of the lesions, hemorrhages, fatty changes and proliferation of bile duct cells coincided with those in the experiments of many researchers.[5~8,15,16]

After administration of higher doses (over 50 μg aflatoxin G_1), marked and moderate hemorrhages

were revealed in the liver sections of dead ducklings that were similar to the experiments of Butler[6]. Many types of necrosis were evidenced in the liver sections of the ducklings treated with aflatoxin B_1, but no necrosis was indicated in the livers of the ducklings treated with aflatoxin G_1. The reasons for an absence of necrosis in the livers is a problem for study in the future.

In liver sections of experimental ducklings, minimal, slight and moderate fatty changes were revealed, and this is coincided with the fatty degeneration with bacterial toxins.[20] The vacuoles of the fat present in an earlier stage of the administration were smaller than the later stage. Newberne and Butler[15] observed that the young duckling normally has a significant amount of lipid in its liver, but this is increased when the animal is exposed to aflatoxin.

The duckling is a highly sensitive experimental animal to the acute effects of aflatoxin, and the liver displays typical microscopic appearances to determine the infection of *Aspergillus flavus*. The typical microscopic appearance is the proliferation of bile duct cells.[6] In liver sections of this experiment, the microscopic appearances of the proliferation and mitotic figures of bile ductule cells were slightly demonstrated in the later stage (on 7 th day), and the intensity was less in a lower dosage (25μg) than in a higher dosage. These appearances are similar to the opinions of other researchers.[6,7]

In the sections of the livers on 7th day, the mitoses of the hepatic parenchymal cells were slightly revealed throughout the sections, and this is similar to Butler's experiment.[6] The histological lesions on the liver of ducklings treated with aflatoxin G_1 were shown less severe than those on the liver of ducklings treated with aflatoxin B_1.

Conclusion

The purpose of this report is to determine the toxicity by the comparison of the histopathological lesions induced in ducklings by a single oral administration of aflatoxin G_1 produced by Korean Industrial Strain of the *Aspergillus flavus* and standard aflatoxin G_1.

The basic histopathological lesions associated with administration of the aflatoxins consisted of hemorrhages, fatty changes and proliferation of bile duct cells. Variations occurred in doses of the aflatoxin and the duration of the experiment. Korean aflatoxin G_1 and standard aflatoxin G_1 are similar in the toxicity.

Korean aflatoxin G_1 was demonstrated as less severer in the toxicity of the comparable doses than Korean aflatoxin B_1.

Legens for Figures

Fig. 2. The liver of a duckling 3 days after intubation of 75μg Korean aflatoxin G_1. Marked hemorrhage and fatty changes are revealed throughout the liver. H & E stain. ×150.

Fig. 3. The magnification of Fig. 2. Erythrocytes are extravasated in the sinusoids and many fatty vacuoles are revealed. H & E stain. ×450.

Fig. 4. The liver of a duckling 3 days after intubation of 75μg Korean aflatoxin G_1. Moderate hemorrhage and slight proliferation of bile duct cells are observed in the center of this picture. H & E stain. ×150.

Fig. 5. The liver of a duckling 7 days after intubation of 75μg Korean aflatoxin G_1. Moderate proliferation of bile duct cells is shown in this figure. H & E stain. ×450.

Fig. 6. The liver of duckling 7 days after intubation of 50μg Korean aflatoxin G_1. Slight hemorrhages and bile duct cells are slightly proliferating from the center of this photograph. A few mitotic figures of bile duct cells are revealed. H & E stain. ×450.

Fig. 7. The liver of duckling 7 days after intubation of 50μg Korean aflatoxin G_1. Slight regeneration of the hepatic cells and a mitotic figure in the center of this picture are shown. H & E stain. ×450.

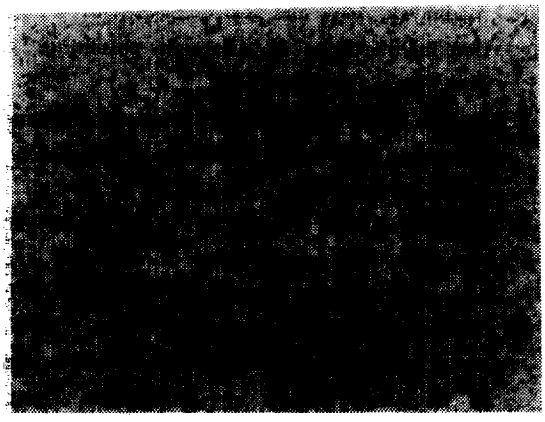

Fig. 2.

Fig. 3.

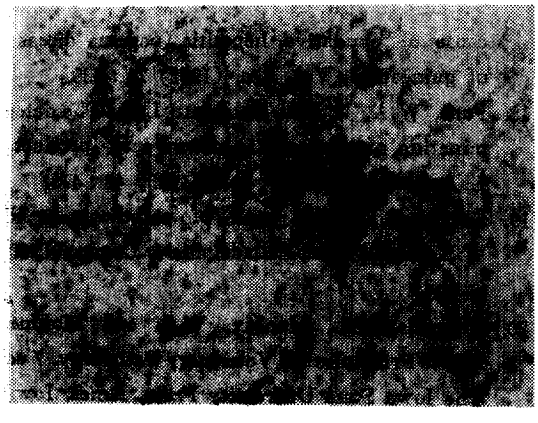

Fig. 4.

Fig. 5.

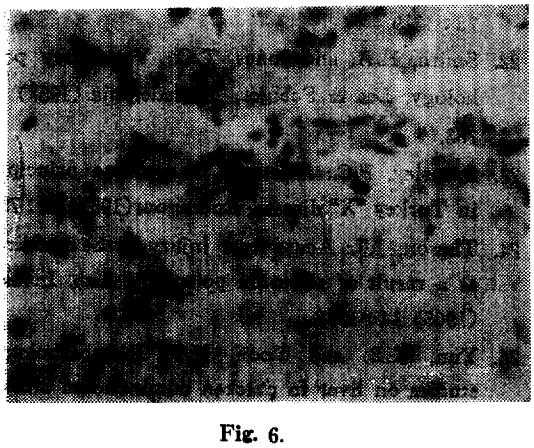

Fig. 6.

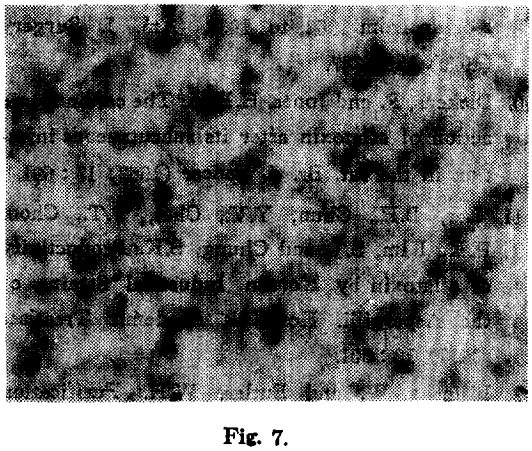

Fig. 7.

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오리 병아리의 肝臟에서 韓國產 Aflatoxin G_1 이 유발시킨 病變에 관한 病理組織學的硏究

윤 화 중

건국대학교 축산대학 수의학과

국 문 초 록

韓國產 aflatoxin G_1 의 毒性을 比較究明하기 위하여, 韓國產 aflatoxin G_1 과 標準 Aflatoxin G_1 을 各各 1匹씩 오리병아리에 經口投與해서 얻은 結果는 다음과 같다.

1. Aflatoxin G_1 의 毒性에 依하여 誘發되서 肝臟에 나타난 病理組織學的 病變은 出血, 脂肪變性 및 輕한 膽管細胞의 增殖等이었다.

2. 出血은 75 μg 의 韓國產 aflatoxin G_1 의 投與로 폐사한 오리에서 가장 현저하였다. 膽管細胞의 增殖은 75 μg 과 50 μg 의 aflatoxin G_1 을 投與한 경우에 輕하게 나타났다.

3. 毒量이 같은 경우에도 Aflatoxin B_1 에 比해 Aflatoxin G_1 의 病變이 훨씬 가볍게 나타났다.

4. 韓國產 aflatoxin G_1 을 투여해서 發生된 病變은 標準 aflatoxin G_1 을 투여해서 發生된 病變과 類似하였으며, 그 程度도 비슷하였다.