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REDUCING DIETARY AFLATOXIN USING EGYPTIAN TAFLA IN GUINEA PIGS

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ABSTRACT

Effect of Egyptian Tafla on reducing the toxicity of aflatoxin was examined by adding different levels of tafla to an aflatoxin – contaminated diet. Thirty growing male guinea pigs (average 133 gm). Were divided into 5 groups (6 in each) equal in number and average body weight. The 1st group fed uncontaminated diet (control), 2nd group fed contaminated diet with aflatoxins B_1, B_2, G_1 and G_2 mixture (equals to 420 ppb aflatoxin B_1). The 3rd, 4th and 5th groups fed aflatoxins contaminated diet supplied with 0.5, 1 or 2% tafla respectively. The following measurement were recorded, daily feed intake, daily body weight gain, feed efficiency, aflatoxin intake (ppb) some blood parameters, internal organs (liver, lung, kidney, heart and spleen) were clinically examined weighted and samples for histopthological studies were taken.

The obtained results revealed that comparing to the uncontaminated (control) diet, feeding aflatoxins B_1, B_2, G_1 and G_2 mixture contaminated diet decreased live body weight, body gain, feed intake and feed efficiency. The weight of internal organs as % of body weight increased by aflatoxins. The blood parameters showed that aflatoxins decreased serum total protein, ablumin and globulin, meanwhile an increase in serum total lipids, total cholesterol, creatinine, urea – N, uric acid and AST,ALT and alkaline phosphatase activities. The differences were significant ($P<0.05$) except in serum albumin, and urea - N.

Addition of 0.5,1 or 2% tafla to the aflatoxins diet improve body weight, daily feed intake blood parameters measured, histological lesions and prolonged the survival period. The best level of tafla was 0.5%.

Keywords: Aflatoxins - Guinea Pigs – Tafla – Blood - Histoology .

INTRODUCTION

Acute aflatoxicosis causes hepatitis, hemorrhage, immune suppression, genetic damage (carcinogenicity, teratogenicity and mutagenicity) and death. Growth impairment and lowering of reproductive performance are the most sensitive clinical signs of chronic aflatoxicosis. Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins. (Abdel hamid *et al.*,1986,1992a,b; Abdelhamid, 1993 and Abdelhamid and Mahomoud 1996). These techniques have not been used on a commercial scale due to high costs, the need for special facilities, losses of important nutrients and the questionable safety of chemical degradation products of aflatoxins.

Many adsorbents as activated charcoal, bentonite, zeolite aluminosilicates and yeast cell wall were tested for binding of several mycotoxins both *in vitro* and *in vivo* the degree of adsorption in *in vitro*

depends on the physical structure of the adsorbent, i.e. the total charge and distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbate molecules (the mycotoxins) such as polarity , solubility, size, shape and in case of ionized compounds, their charge distruibution (Alexander *et al.*, 2001).

It reduce the bioavailability of aflatoxins and their hazardous effects in some animal species (Philips *et al.*, 1990; Harvey *et al.*, 1993; schell *et al.*, 1993 and Schiedeler, 1993;Shehata 2002 ; Shehata *et al.*,2003 ; Nowar, *et al.*,2001; Abd El-Baki *et al.*, 2002 ;Abdel hamid *et al.*, 2005 and Abdel hamid *et al.*, 2002). Generally, the major advantages of adsorbent include low cost, safety and easy addition to animal feed.

This study was carried out to evaluate the alleviation ability of tafla for the toxic effect of aflatoxins B_1, B_2, G_1, G_2 mixture (equals to 420 ppb B_1) growing male guinea pigs in diet.

MATERIALS AND METHODS

For producing aflatoxin the strain of *Aspergillus flavus* NRRL 3357 was grown in synthetic media, yeast extract — sucrose broth (YES) containing 2% yeast extract and 20% sucrose. The substrate was dispensed in conical flask. The flasks were then autoclaved for *15* minutes at 121 C°, then coold and inoculated with spore suspension and incubated for *9* days at *25 — 29 C°*. Aflatoxin concentrations determined using A.O.A.C (1984) and Shih and Marth (1969). The media was found to contain a mixture of aflatoxins B_1, B_2, G_1, and G_2.

Thirty growing male guinea pigs (average weight 133 gm) were randomly assigned to one of five dietary groups (6 animals in each). The 1st group fed uncontaminated diet (control), 2nd group fed diet contaminated with 420 ppb aflatoxin /kg diet, the 3rd , 4th and 5th fed aflatoxin contaminated diet with 0.5 , 1 and 2% tafla, respectively. 1 and 2%. Tafla used in study contained (%) : 50.05 SiO_2; 20.26 AlO_3; 9.74 Fe_2O_3; 2.02 CaO, 1.95 MgO; 2.19 Na_2O; 1.05 K_2O and 12.74 others.

Animal of each group were housed in similar metallic cage under same managerial, hygienic and environmental conditions. All dietary treatments were offered to animal *ad libitum* and drinking waters was available all time.

On day 17 of feeding period, three animals from each group were slaughtered and internal organs (liver, heart, lungs, spleen and kidneys) were removed from the body and subjected to the clinical and histopathological examination, according to Bancroft *et al.*,(1990). Also, blood sample were taken. Serum total protein, albumin, total lipids, total cholesterol, aspertate amino transferase (AST), alanine amino transferase (ALT), urea — N, uric acid and creatinine were determined by using commercial kits purchased from Egyptian — American Company for Laboratory Services, Egypt.

The data of all parameters were statistically analyzed as completely randomized design by using analysis of variance according to Snedecor and Cochran (1982). Significant differences among treatment means were statistically tested by using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Guinea pigs performance :

Data presented in table (1) showed that aflatoxins reduced daily body gain, feed intake, feed conversion and survival period. Addition of tafla at all levels impoved these parameters. Generally, the best level was 0.5%. These results were in agreement with those obtained by Nowar *et al., 2001;* Shehata 2002 and Abd El-Baki *et al* (2002) on rabbit, Shehata *et al., (2003)* and Abdel hamid *et al* (2004b) on Tilapia fish, Schell *et al.* (1993) and Harvey *et al. (*1993) on growing pigs and Abdel hamid *et al (*2005) on rats.

The results of tafla may be due to binding of aflatoxins and increase its excreation in feces, therefore reduce the bad effect of aflatoxin on animal performance and health (Smith, 1982; Shehat, 2002 and Abdel hamid, *et al.,* 2005).

Blood serum analysis :

Table (2) shows some serum components, of guinea pigs fed aflatoxins B_1, B_2, G_1 & G_2 mixture (equals to 420 ppb as B_1) diet alone or with different level of tafla. Comparing to the control diet, feeding aflatoxin diet significantly ($P<0.05$) decreased serum total protein, albumin and globulin and increased serum total lipids, total cholesterol, creatinine, urea - N, uric acid and AST,ALT, alkaline phosphatase and acid phosphatase activities.

Addition of tafla especially the 0.5 % level to the aflatoxins diets improve serum components. However, the differences than the control animals were still significant ($P<0.05$) in serum total lipids, creatinine, uric acid and alkaline and acid phosphatase activities.

These results agreed with those of Abdel hamid *et al.* (2005) on rats, Nowar *et al* (1996); Abdel Baki *et al* (2002) and Shehata (2002) on growing male NZW rabbits, Hoda El — Zahar *et al.* (1996) on mature rabbits, Schell *et al* (1993) and Harvey *et al* (1993) on growing pigs.

Internal organs :

Animals fed aflatoxins diet alone or with tafla were lower in absolute weight of internal organs (liver lungs, kidneys, heart and spleen) than the control group (Table 3). The relative weight of internal organs showed an opposite trend being significantly higher in animal fed aflatoxin diet alone or with Tafla than those fed the control diet. These results agreed with those of Nowar *et al,* 1996 and (2000).

Decreasing of serum protein by aflatoxin may be attributed to degeneration of endoplasmic reticulum and inhibition of protein synthesis (Srivastava, 1984), while increasing of AST, ALT and alkaline phosphatase may be due to hepatocellular necrosis (Harvey *et al.* 1995, Zaky *et al* 2000). The increase in creatinine may be due to the inhibition of the excretory function of kidney as result of increasing its liver synthesis form ammonia.

Table (1): Effect of aflatoxins B_1,B_2,G_1 & G_2 mixture (equals to 420 ppb as B1) and addition of tafla on performance of guinea pigs.

Items		Control	Aflatoxins			
			0% tafla	0.5% tafla	1% tafla	2% tafla
Initial body weight	(gm).	133.3±16.7	136.7±13.4	133.3±16.7	130±17.3	133.3±10.2
Finial body weight	(gm).	261.7±1.7	127.5±10.1	150±00	135±0.0	130±0.0
Surivival period	(day).	30	19	29	25	22
Daily body weight gain	(gm).	4.3	-0.48	0.6	0.2	-0.2
Daily Feed intake	(gm).	26.7	11.3	15.03	13.1	13.1
Daily Aflatoxins intake µg/animal		-	4.8	6.3	5.5	5.5
Daily Aflatoxins intake as %of LD_{50}		-	2.4	2.6	2.7	2.7
Feed Efficiency		0.2	-0.04	0.04	0.02	-0.01

Table (2): Effect of feeding aflatoxins B_1,B_2,G_1 & G_2 mixture (equals to 420 ppb as B1) contaminated diet alone ore with different levels Egyptian Tafla (0.5, 1 & 2%) on the blood serum analysis of guinea pigs.

Items		Control	Aflatoxins			
			0%clay	0.5%clay	1%clay	2%clay
		A	b	B	b	b
Total protein	(g/dl)	5.2±0.2	4.4±0.2	5.1±0.1	4.9±0.1	4.9±0.1
		a	a	A	a	a
Albumin	(g/dl)	2.7±0.1	2.3±0.1	2.7±0.1	2.5±0.1	2.6±0.1
		a	b	Ab	ab	ab
Globulin	(g/dl)	2.5±0.1	2.1±0.1	2.3±0.03	2.3±0.1	2.3±0.03
		c	a	b	b	a
Total lipids	(mg/dl)	301±5.8	344.3±2.4	323.7±3.7	323.7±3.7	345.3±5.2
		d	a	d	c	b
Total cholesterol	(mg/dl)	92.7±2.2	130.3±0.7	100±1	109.7±5.8	120±1
		d	a	cd	bc	ab
GOT	(IU/L)	35.3±0.9	67.7±0.9	43±2	52.7±5.4	64±3.1
		b	a	b	b	a
GPT	(IU/L)	21±0.6	32.7±0.3	18.7±0.9	22±2.5	30±0.6
		d	a	c	bc	ab
Alkaline phosphatase	(IU/L)	40.3±0.3	64.3±2.2	50.3±1.7	53.3±3.9	58.3±1.8
		d	a	b	b	a
Acid phosphates	(IU/L)	12±0.6	21.7±0.3	١٨,٣±0.7	١٧±1.2	22±0.6
		d	a	c	bc	ab
Creatinine	(mg/dl)	0.9±0.1	2.4±0.1	1.6±0.2	1.8±0.2	2.1±0.3
		a	a	a	a	a
Urea – N	(mg/dl)	2.9±0.03	3±0.1	3±0.1	3±0.1	3±0.1
		c	a	b	ab	a
Uric acid	(mg/dl)	4.1±0.03	5.1±0.1	4.6±0.2	4.9±0.2	5.1±0.1

Means in the same row bearing different superscripts differ significantly ($P<0.05$).

Table (3): Effect of feeding aflatoxins B_1,B_2,G_1 & G_2 mixture (equals to 420 ppb as B1) and addition of tafla on the internal organs weight of guinea pigs.

Items	Control	Aflatoxins			
		0% tafla	0.5% tafla	1% tafla	2% tafla
Liver	c	a	b	b	a
(%).	4.2±0.1	5.7±0.1	5.1±0.3	5.1±0.2	5.8±0.2
Lungs	c	ab	a	a	a
(%).	1.2±0.1	2.2±0.2	1.8±0.1	2.3±0.1	2.4±0.1
Kidney	b	a	a	a	a
(%).	1.1±0.1	2.2±0.3	1.8±0.1	1.9±0.1	1.9±0.1
Heart	b	a	a	a	a
(%).	0.5±0.01	0.9±0.1	0.8±0.03	0.8±0.1	0.2±0.01
Spleen	b	a	a	a	a
(%).	0.1±0.01	0.2±0.01	0.2±0.01	0.2±0.01	0.2±0.01

Means in the same row bearing different superscripts differ significantly (P<0.05).

Pathological examination:

1- The kidneys:

Grossly, the kidneys are slightly enlarged, grey in colour and slightly firm in consistency. Microscopically the kidneys revealed that their glomeruli; shirnked with small intrarenal empty spaces. The renal tubules are suffered from various degenerative changes while appeared as cloudy swelling and vacuolation in other renal tubules. The renal blood vessels are also congested.

2- The liver:

Grossly, the liver appeared enlarged and congested. The cut surface appeared also redish in color and freely blood oozed. Microscopically, the hepatic blood vessels are severely enlarged. Some hepatic cells suffered from variable degenerative changes and other, hepatic cells are reversed. The histopathological aleration observed in sections of kidney and livers from guinea pigs fed on aflatoxin are in agreement with those found by Nowar *et al.*,(2001), Soliman *et al* (2001) Vinita *et al* (2003) and Abdelhamid *et al* (2004a).

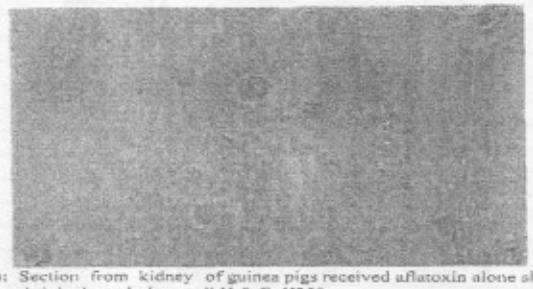

Fig. (1): Section from kidney of guinea pigs received aflatoxin alone showed shrinked renal glomeruli H & E X250.

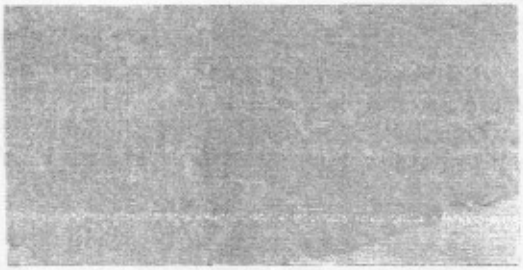

Fig. (2): Section from kidney of guinea pigs received aflatoxin with Tafla showed variable degenerative changes H & E X 100.

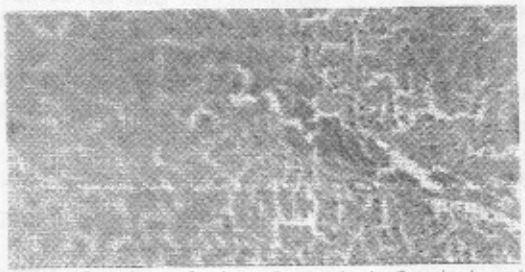

Fig. (3): Section from liver of guinea pigs received aflatoxin showed sever congested hepatic B. Ves. H & E X 250.

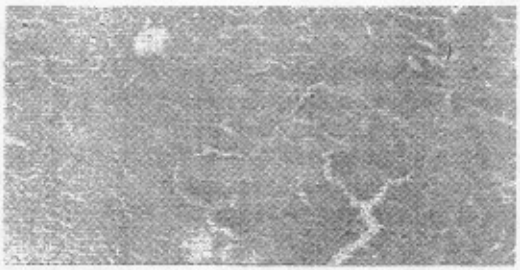

Fig. (4): Section from liver of guinea pigs received aflatoxin with Tafla showed necrosis of the hepatic cells H & E X 1000.

CONCLUSION AND PRACTICAL APPLICATION

The data from the present study demonstrate that adding tafla (specially 0.5%) to a diet contaminated with aflatoxins B1,B2,G1 & G2 mixture (equal to 420 ppb aflatoxin B1) may provide a safe and practical method to alleviation aflatoxin effect in guinea pigs diet.

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تقليل سمية العلائق الملوثة بالأفلاتكوسين باستخدام الطفلة المصرية في خنازير غنيا

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تأثير الطفلة المصرية في تقليل سمية الأفلاتوكسين تم فحصه بإضافة مستويات مختلفة من الطفلة للأعلاف الملوثة بالأفلاتوكسين، ثلاثين ذكر نامي من خنازير غنيا (متوسط الوزن ١٣٣ جرام) تم تقسيمها إلى ٥ مجموعات (٦ حيوانات في كل واحدة) متساوية في العدد ومتوسط الوزن. المجموعة الأولى تغذت على عليقة غير ملوثة (كنترول) والمجموعة الثانية تغذت على عليقة بالأفلاتوكسين (G_1,G_2,B_2,B_1) يعادل ٤٢٠ جزء في البليون أفلاتوكسين B_1 المجموعة الثالثة والرابعة والخامسة تغذى على العليقة الملوثة بالأفلاتوكسين مضاف إليها ٠,٥ ، ٠,١ و٢% طفلة على التوالي. يتم أخذ القياسات التالية وكمية العليقة المأخوذة يوميا والزيادة اليومية في الوزن وكفاءة تحويل الغذاء وكمية الأفلاتوكسين المأخوذة يوميا وبعض قياسات الدم والأعضاء الداخلية (كبد– رئة – الكلية – القلب– الطحال) تم فحصها ظاهريا ووزنها وتم أخذ عينات للفحص الهستوباثولوجي.

النتائج المتحصل عليها كشفت على أن المقارنة بمجموعة الكنترول والتي تناول عليقة ملوثة بمخلوط الأفلاتوكسين يعادل ٤٢٠ جزء في البليون أفلاتوكسينB_1. نقص في وزن الجسم الحي وعائد وزن الجسم والغذاء المتناول وكفاءة تحويل الغذاء. وزن الأعضاء الداخلية كنسبة مئوية من وزن الجسم يزيد عند التغذية على الأفلاتوكسين. قياسات الدم أوضحت أن الأفلاتوكسين قليل بروتينات السيرم الكلية، الكوليسترول الكلي، الكرياتين، اليوريا – نتروجين وحامض اليوريك ونشاط إنزيم الفوسفاتيز القاعدي الاختلافات تكون معنوية ما عدا البيومين السيرم ويوريا – نتروجين.

٠,٥ ، ٠,١ و٢% طفلة للعلائق الملوثة بالأفلاتوكسين يحسن وزن الجسم والغذاء المأخوذ يوميا والأضرار في قياسات الدم والهستوباثولوجي ويطيل فترة الحياة. المستوى الأفضل من الطفلة يكون ٠,٥%.

قام بتحكيم البحث

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