

Hepatoprotective Effect of Coumarin and Chlorophyll Against Aflatoxicosis in Rat

Abdel-Latif, M. S.¹; K. M. Elmeleigy²; Marwa M. S. Khattab³ and Sara M. Mohamd²

¹Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

²Regional Center For Food and Feed, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

³Department of Pathology, Fac. of Veterinary Medicine, Cairo University, Giza Square, Giza, Egypt. Postal code 12211



ABSTRACT

Aflatoxin contamination of animal diet is still a major problem for breeders since it has adverse effects on animal health and productivity. This study was carried out to investigate the effect of using coumarin and/or chlorophyll in rat diet against aflatoxicosis. Fifty four rats were randomly assigned into 7 groups "6 rats each" and group 5 "12 rats". Group 1 was negative control. Group 2 received water with coumarin 0.5%. Group 3 received water with chlorophyll 0.5%. Group 4 received water with coumarin 0.5% and chlorophyll 0.5%. Group 5-8 received basal diet with aflatoxin B₁ 1000 ppb. Group 6-8 were administered similar treatments as groups 2-4. The experiment ended after 8 weeks. Water and feed consumption were measured and feed conversion rate were calculated. Random glucose level, total lipid, total cholesterol, triglyceride, total protein, ALT, AST, creatinine and urea were determined in serum. Histopathological examination of liver, kidney and pancreas was performed. The conversion rate was significantly low in group 5 compared to groups 6-8. The serum glucose, cholesterol, AST and ALT were elevated in group 5 compared to groups 6-8. The liver lesions observed in group 5 represented in vacuolation and necrosis were alleviated in groups 6-8. The necrosis and inflammatory cells infiltration in pancreas of group 5 were absent in groups 6-8. In conclusion, the coumarin and or chlorophyll possessed a hepatoprotective effect against aflatoxicosis.

Keywords: Aflatoxin, Coumarin, Chlorophyll, Histopathological Examination

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 (Abdelhamid, 2005 a,b and 2009; Shehata, 2010 and Shouman *et al.*, 2012). Scientific efforts were directed towards using physical, chemical and biological techniques for detoxification or inactivation of aflatoxins (Abdelhamid *et al.*, 2002 and Agag, 2003). 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. One of the effective methods to overcome the toxic and carcinogenic effects of aflatoxins is to enhance aflatoxin metabolism towards its detoxification in humans or animals (Tulayakul *et al.*, 2007).

More than 1300 coumarins have been identified from natural sources especially green plants (Hoult and Paya, 1996). Coumarins are antioxidants, contain the nucleus of benzo- α -pyrone and occur in plants like Tonka beans, Sweet clover, Wood ruff, Cassia leaf (Lake *et al.*, 1989). It is present also in a variety of plants families like Loganiaceae (Bhattacharyya *et al.*, 2008), Orchidaceae, Leguminaceae, Rutaceae, Umbelliferae and Labiatae (Vyas *et al.*, 2009). The synthetic coumarin (4-methyl-7-hydroxycoumarin) derived from resorcinol and ethylaceto-acetate in presence of concentrated sulphuric acid is structurally close to scopoletin, being a coumarin derivative. Naturally derived and synthetic coumarins have been used in treatment of oedemas (Clasley-Smith *et al.*, 1993), and possessed anti-cancer or anti-tumorigenic (Prince *et al.*, 2009 and Bhattacharyya *et al.*, 2009), anti-mutagenic (Pillai *et al.*, 1999), antibacterial (Devvienne *et al.*, 2005), anticoagulants, anti-thrombotic and vasodilatory effects (Hoult and Paya, 1996).

Chlorophyllin (CHL), a water-soluble form of chlorophyll, was recently evaluated as chemopreventive agent in a population at high risk for exposure to aflatoxin and subsequent development of hepatocellular carcinoma (Kensler *et al.*, 2002 and Kumer *et al.*, 2012). CHL, which is used extensively as a food colorant, has numerous medicinal applications. CHL is a safe and effective agent suitable for use in individuals unavoidably exposed to aflatoxins (Enger *et al.*, 2003). Naturally chlorophyll and CHL strongly inhibits aflatoxin B₁ preneoplasia biomarkers, stomach and liver tumor in rats and rainbow trout (Sudakin, 2003; Simonich *et al.*, 2007 and 2008). Moreover, the CHL was more effective than chlorophyll-A in reducing aflatoxin B₁-DNA adduct and liver tumor (Dashwood *et al.*, 1998).

The Goal Of The Research

It is known that aflatoxins cause serious effects so that these effects can be mitigated by using some natural compounds.

MATERIALS AND METHODS

Animals

Fifty four Sprague – Dawley male albino rats with average body weight 90 \pm 10 g was used. Were obtained from the farm of The Egyptian Organization of Biological products and Vaccines, Egypt. The animals were housed individually in stainless steel cases in a controlled environment 25 \pm 5 $^{\circ}$ C, 50-60% relative humidity and 12 hours light- dark cycle, all over the experimental period (8 weeks). Free access to water and maintenance ration twice daily were available. This study was in accordance with Institutional Animal Use and Care Committee (IACUC) guidelines, Cairo University.

Coumarin preparation

Coumarin (4-Methyl-7-hydroxycoumarin) was prepared according to method of Furniss *et al.* (1989) which is summarized as follow: 1 liter of concentrated sulphuric acid was placed in a 3 liter necked flask. The flask was immersed in an ice bath; a solution of

100 g (0.91 mol) of resorcinol in 134 g of ethyl acetate was added drop wise and with stirring for 2 hrs. The reaction mixture was kept at room temperature for about 18 hrs., then it was poured with vigorous stirring into a mixture of crushed ice and water, the crude yield (yield = 155 g, 97% concentration) was collected, recrystallization in ethanol 95% and air dried. The dried coumarin was added to water 0.5%. Coumarin standard from sigma Aldrich.

Chlorophyll preparation

All chlorophyll extractions were performed under dim green light to minimize photo – oxidative reaction (Shio, 2006). Fresh spinach leaves were purchased from local market. Spinach leaves were separated from the stem and then about 100 g were weighed and put into a blender. Cold acetone was added as much as 500 ml. Then the spinach leaves were grinded in a blender for about 3 min. The filtrate of crude chlorophyll was separated using a Buchner funnel with a Whatman filter paper No.1. The residual solids that are not filtered was washed with 100 ml of acetone. Then the filtrate was added to dioxane approximately one seventh of its volume. After that, the mixture was added with deionized water as much as one seventh of the volume of the mixture by drop wise and then stirred using a magnetic stirrer. The mixture was precipitated by storing in the freezer at -20 ° C for 1 hr until dark green precipitate was obtained at the bottom of the solution and a yellow liquid on top. The precipitate of crude chlorophyll was separated by filtration using two layers of Whatman filter paper No. 1 with a Buchner funnel. The solution then was added with dioxane as much as one seventh of the volume of the solution and also deionized water as much as one seventh of the volume of the solution while slowly stirred. The precipitation was then performed for the second time in the same way at -20 °C for 1 hr. After that the precipitate was filtered using a Buchner funnel and two layers of Whatman paper No.1. The crude chlorophyll solids were filtered and diluted with acetone until colorless paper return. Then a solution of crude chlorophyll is evaporated from the solvent so that only the remaining solid green crude chlorophyll was left. Thus, the chlorophyll was dissolved using the small volume of diethyl ether in order to obtain the chlorophyll solution prior to any further separation using column chromatography according to (Suendo *et al.*, 2010).

Aflatoxin preparation

Aflatoxin production was carried out according to Davis *et al.* (1966) using liquid media (2% yeast extract and 20% sucrose) and *Aspergillus flavus* stain (NRRL 3145). The media which contained detectable amount of aflatoxin was mixed well with the basal diet to get the aflatoxin- contamination diet. The total aflatoxin content in liquid medium was determined according to Roos *et al.* (1997) and AOAC. (2006) method using monoclonal antibody columns for total aflatoxins (Vicam Science Technology, water town, MA, USA). Aflatoxin identification was performed by a modification of the HPLC – AFLATEST procedure Agilent 1200 series USA. HPLC equipment with two pumps, column C18, Lichrospher 100 RP-18, (5 Nm × 25 cm) was used. The mobile phase consisted of water: methanol: acetonitrile (54: 29:17, v/v/v) at flow rate of 1 mL/min. The excitation

and emission wavelengths for all aflatoxins were 362 and 460 nm (Fluorescence detector), respectively.

Experimental design

Fifty four rats were randomly assigned into 7 groups "6 rats each" and group 5 "12 rats". Group 1 received basal diet (negative control) (G1). Group 2 received basal diet and water with coumarin 0.5% (G2). Group 3 received basal diet and water with chlorophyll 0.5% (G3). Group 4 received basal diet, water with coumarin 0.5% and chlorophyll 0.5% (G4). Group 5 received basal diet with aflatoxin B₁ 1000 ppb (positive control) (G5). Group 6 received basal diet with aflatoxin and water with coumarin 0.5% (G6). Group 7 received basal diet with aflatoxin and water with chlorophyll 0.5% (G7). Group 8 received basal diet with aflatoxin and water with coumarin 0.5% and chlorophyll 0.5% (G8). The ingredients of basal diet are shown in Table 1. Daily fresh water was available the whole time. At the last day of the experiment, the rats were fasted over night. The experiment was terminated after 8 weeks and animals were euthanized by cervical decapitation.

Table 1. Composition of basal diet

Ingredients	%
Casein	20.0
Corn starch	65.0
Mineral mix ²	3.5
Vitamin mix ²	1.0
DL-Methionine	0.3
Choline bitartrate	0.2
Cellulose powder	5.0
Corn oil	5.0
	-

1-Based on AIN-76 " American Institute of Nutrition"

Vitamins ² based on AIN-76		Minerals ² based on AIN-76	
Vitamin A	4.00 IU/g	Calcium	% 0.52
Vitamin D3	1.00 IU/g	Phosphorus	% 0.44
Alpha-Tocopherol	64.24 IU/kg	Potassium	% 0.38
Thiamine	5.90 ppm	Sodium	% 0.11
Pantothenic Acid	15.26 ppm	Magnesium	% 0.05
Choline	104.00 ppm	Iron	34.25 ppm
Pyridoxine	7.1 ppm	Zinc	36.76 ppm
Folic Acid	2.10 ppm	Manganese	59.34 ppm
Biotin	0.21 ppm	Copper	6.73 ppm
Vitamin B12	10.10 mcg/kg	Cobalt	0.02 pm
Vitamin K	0.50 ppm	Iodine	0.21 ppm

Prepared as Rodent Diet AIN-76A

Serum analysis

Blood samples were collected from each rat from the retroorbital vein and were received into clean dry centrifuge tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes and kept in deep-freezer at -20 °C until used for estimation of random glucose level, total lipid, total cholesterol, triglyceride, total protein, ALT, AST, creatinine and urea by using Biodiagnostic Kits.

Histopathological examination

Liver, kidneys and pancreas samples were collected at the end of the experiment and were fixed in 10% neutral buffered formalin solution for at least 48 hrs. Tissue specimens were processed using routine paraffin embedded technique in which tissues was dehydrated in ascending concentration of alcohol and cleared in xylene. Sections 3-5µ thick were made using microtome (Leica 2135) and stained with Haematoxylin and Eosin (H&E)

(Suvama *et al.*, 2012). The histopathological alterations in liver, kidney and Pancreas were examined by light microscopy and photographed using camera Olympus XC30 (Tokyo, Japan).

RESULTS AND DISCUSSION

Weight gain and water consumption

Aflatoxin contaminated diet decreased total and daily body weight gain, daily feed intake and water consumption (Table 2). These results agree with the finding of Shehata (2002 and 2010), Meshreky *et al.* (2007) on rabbits and Shehata *et al.* (2009 a,b) and Mehrim *et al.* (2016) on fish. The reduction in body weight gain by aflatoxin is not only due to depression of feed intake, but may also be due to the reduction in metabolism of protein, lipids, carbohydrate and dissolved vitamin in lipid (Maria and Askar, 2008). Also, it might be due to detoxification process in the body utilizing glutathione enzymes. Glutathione is the intracellular antioxidant (Deng *et al.*, 2010) and partly composed of methionine and cysteine, hence this detoxification process depletes the metabolic availability of methionine leading to poor growth and feed conversion. Adding coumarin to diet improved growth performance, the beneficial effect of coumarin may be due

to: (1) reduction AFB₁-DNA adducts formation by both liver and intestinal microsomes. Coumarin enhanced aflatoxicol formation; therefore, decrease AFB₁-DNA adduct, because direct interaction of aflatoxicol epoxide with DNA is major compared with AFB₁ epoxide, (2) enhancement of glutathione -s- transferase (GST) activity in the intestine to conjugate AFB₁, (3) suppression of P450 enzyme activity in the livers and the enhancement of GST in the intestine (Tulayakul *et al.*, 2007), and (4) improving liver function and body health.

The beneficial effect of CHL may be due to: (1) CHL form molecules complexes with carcinogens material and blocking then bioavailability therefore reduce the hazard effects on organs body functions, (2) CHL modify the genotoxic effects of aflatoxin B₁ by inhibiting bioactivation pathways and stimulating detoxification pathways (Sudakin, 2003), (3) CHL enhance the activities of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase catalase and glutathione -s- transferase, indicating that CHL possess a potent protective effect against of aflatoxin B₁ (kumar *et al.*, 2012), (4) CHL reduce multi organs aflatoxin B₁-DNA adducts (Simonich *et al.*, 2007).

Table 2. Body weight gain, food intake and Consumer water of different experimental group for 8 week

Treatments	Initial weight (g)	Final weight (g)	Body Weight gain (g) Bwg''	Bwg / Initial weight (%)	Weekly body weight gain (g)	Average daily Feed Intake (g)	Feed conversica Feed/gain	Water consumption (ml) / day	Weight gain from the control %
Control	117.58± 1.63	241.25± 1.39 ^c	123.67± 10.92 ^{ab}	105.18	15.46	21.00± 0.62 ^b	1.36	14.9± 1.37 ^{ab}	100
Coumarin	112.17± 1.49	280.5± 2.25 ^a	168.83± 16.89 ^a	150.07	21.10	25.60± 0.82 ^b	1.21	17.6± 1.22 ^a	142.68
Chlorophyll	118.67± 1.69	230.33± 0.79 ^d	111.67± 12.95 ^{ab}	94.09	13.96	21.82± 1.21 ^b	1.56	15.1± 1.55 ^{ab}	106.48
Coumarin + Chlorophyll	117.67± 2.14	260± 1.15 ^b	142.33± 9.06 ^{ab}	121	17.80	41.77± 0.50 ^a	2.35	16.9± 0.96 ^a	115.04
Aflatoxin	114.33± 2.2	221.67± 1.64 ^e	107.33± 21.21 ^b	93.88	17.89	21.37± 0.90 ^b	1.20	13.5± 0.43 ^b	89.26
Aflatoxin + Coumarin	127.17± 1.65	248.33± 0.84 ^c	121.17± 10.55 ^{ab}	95.28	15.15	33.90± 3.11 ^{ab}	2.24	16.5± 0.78 ^a	90.59
Aflatoxin + Chlorophyll	120± 1.32	226.33± 1.41 ^{de}	106.33± 12.25 ^b	88.6	13.29	29.47± 2.86 ^{ab}	2.22	15.7± 0.52 ^{ab}	84.24
Aflatoxin + Coumarin + Chlorophyll	121.5± 1.04	256.83± 1.87 ^b	135.33± 6.61 ^{ab}	111.38	16.92	28.42± 2.0 ^{ab}	1.68	16.6± 0.61 ^a	105.89

All values are represented as mean ±S.E. Means with different letters are significantly different (p<0.05). H (Higher than normal rang), L (Lower than normal rang)

Biochemical findings

Results in Table 3 revealed that positive controls (group receiving basal diet with aflatoxin) gave a significant increase in serum liver function enzymes, alanine amino transferase (ALT) and aspartate amino transferase (AST). Furthermore, this group showed significantly higher level of serum urea, creatinine, cholesterol and triglycerides as compared to healthy group fed the basal diet. Increasing AST and ALT activities may be due to hepatocellular necrosis or increasing the permeability of cell membrane (Zaky *et al.*, 2000). On the other hand, the increased concentrations of urea and creatinine indicate kidney injury caused by aflatoxins toxicity. These results coincide with those reported by Zohair. (1996) in treated rats and those of Matri (2001)

who reported that Japanese quail birds receiving contaminated feed with aflatoxin showed significantly higher serum total cholesterol, creatinine and urea. The total protein was significantly decreased in the group fed the aflatoxin contaminated diet. These findings were in accordance with previous studies (Abd El Baki *et al.*, 2002 and Shehata, 2002 and 2010). Decreasing of serum protein may be attributed to degeneration of endoplasmic reticulum and inhibition of protein synthesis (Srivastava, 1984). Addition of coumarin or CHL alone and coumarin with CHL improved blood parameters measured. These results may be due to the effect of coumarin and CHL on detoxification of aflatoxin (Kelly *et al.*, 2000 and Tulayakul *et al.*, 2007) and improvement of body health (Prince *et al.*, 2009).

Table 3. Serum AST , ALT, urea, creatinine, total protein, glucose, total cholesterol and total triglycerides levels in the different experimental groups.

Parameters Treatment	AST (IU/L)	ALT (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Total protein (g/dl)	Glucose (mg/dl)	Total cholesterol (mg/dl)	Total triglycerides (mg/dl)
Control	36.67± 1.33 ^d	31.33± 2.03 ^b	40.67± 1.76 ^{bc}	0.75± 0.03 ^b	8.67± 0.73 ^a	108.00± 5.69 ^b	79.33± 1.45 ^b	81.17± 1.42 ^c
Coumarin	38.67± 3.18 ^{cd}	34.00± 2.31 ^b	37.33± 1.20 ^c	0.75± 0.03 ^b	8.67± 0.33 ^a	149.67± 2.73 ^b	73.00± 2.52 ^b	67.67± 2.03 ^{de}
Chlorophyll	27.00± 2.52 ^d	34.00± 2.31 ^b	45.33± 3.38 ^{bc}	0.81± 0.04 ^b	8.33± 0.67 ^{ab}	109.00± 4.05 ^b	73.67± 2.03 ^b	87.00± 2.31 ^c
Coumarin + Chlorophyll	35.00± 4.04 ^d	32.67± 2.33 ^b	45.33± 3.38 ^{bc}	0.77± 0.03 ^b	8.50± 0.29 ^a	105.67± 2.33 ^b	76.33± 2.03 ^b	60.83± 1.36 ^c
Aflatoxin	152.33± 4.41 ^a H	145.33± 4.63 ^a H	83.00± 3.21 ^a H	1.86± 0.05 ^a H	3.60± 0.28 ^b L	180.33± 4.84 ^a H	212.33± 2.73 ^a H	295.00± 33.6 ^a H
Aflatoxin + Coumarin	57.00± 2.08 ^b H	35.00± 2.08 ^b	52.33± 2.90 ^{ab} H	0.76± 0.02 ^b	8.50± 0.29 ^a	78.00± 1.73 ^c	76.33± 2.03 ^b	76.33± 2.03 ^{cd}
Aflatoxin + Chlorophyll	66.33± 2.90 ^b H	39.00± 3.21 ^b	51.33± 2.84 ^{ab} H	0.75± 0.35 ^b	6.17± 0.17 ^b	86.00± 2.31 ^c	81.33± 1.85 ^b	87.67± 1.76 ^c
Aflatoxin + Coumarin + Chlorophyll	52.33± 2.90 ^{bc} H	40.67± 1.76 ^b	48.67± 2.18 ^{bc}	0.87± 0.02 ^b	7.67± 0.17 ^{ab}	80.33± 2.31 ^c	79.67± 2.02 ^b	101.33± 2.4 ^b

All values are represented as mean ±S.E.

Means with different letters are significantly different (p<0.05).

H (Higher than normal rang), L (Lower than normal rang).

Histopathological Findings

In the current study, there was vacuolation of hepatocytes, karyomegaly and binucleation of hepatocytic nuclei in the liver of rats administrated aflatoxin (Figure 1a) coinciding with the degenerative changes induced by aflatoxin administration in the hepatic tissues of rats (Abdel-Wahhab *et al.*, 2002). Moreover, portal mononuclear inflammatory cells infiltration and focal areas of hepatocyte necrosis accompanied with mononuclear cells infiltration in the liver were demonstrated in the present study similar to histopathological changes recorded in previous studies (Naidu *et al.*, 1991; Salem *et al.*, 2001 and El-Shewy and Ebrahim, 2004) which showed that the main lesions in liver of rats exposed to aflatoxin were progressive hepatic degeneration, multifocal hepatic necrosis, bile ductular proliferation and areas of altered hepatocytes.

The liver histopathology of rats in the groups administered aflatoxin and receiving coumarin and/or chlorophyll exhibited an apparently normal hepatic structure in which the hepatocyte vacuolation and necrosis was almost diminished (Figure 1b, c, d). This improvement was more obvious in the group receiving coumarin and the group receiving coumarin and chlorophyll. Subsequently, the coumarin and chlorophyll is believed to have exerted a protective effect against aflatoxin toxicity. Similarly, in a previous study, the chemoprotective effect of coumarin was evident against aflatoxin B1 induced mutations and cytotoxicity in the presence of chick embryo liver S9 but not with rat liver S9. It was said that coumarin reduced the *in vitro* formation of aflatoxin B1 8, 9- epoxide involved in aflatoxin toxicity (Goeger *et al.*, 1998). Furthermore, adding coumarin (2.5 g coumarin/ Kg diet) to aflatoxin B1 contaminated rabbit diet was found to be safe and practical method to minimize aflatoxin B1 toxicity (Helal *et al.*, 2014).

Perivascular mononuclear inflammatory cells and interlobular edema and mononuclear inflammatory cells infiltration were demonstrated in the pancreas of rat administered aflatoxin in accordance with previous studies (Figure 1 e)(Beger *et al.*, 2000, Şimşek *et al.*, 2007, and Abd El-Haleem and Mohamed, 2011). Aflatoxin ingestion leads to reactive oxygen species production that regulates

chemokine receptor expression and also facilitates the recruitment and localization of polymorphonuclear leukocyte to the site of infection and inflammation (Shephard, 2003). Additionally, in this study, perivascular fibrosis in the pancreas was observed in the rats administered aflatoxin. Similarly, the pancreas revealed many collagen fibers, especially around ducts and blood vessels in previous studies (Abd El-Haleem and Mohamed, 2011). Stellate cells observed in both rat and human pancreas was thought to play a role in the development of pancreatic fibrosis (Bachemet *et al.*, 1998). They were stimulated to synthesize collagen due to oxidative stress (Casini *et al.*, 2000). In such case the oxides generated during the metabolic processing of aflatoxin B1 results in oxidative stress that in turn induce stellate cells to produce collagen. Additionally, Perivascular mononuclear inflammatory cells and interlobular edema and mononuclear inflammatory cells infiltration were demonstrated in the pancreas of rat administered aflatoxin in accordance with previous studies (Beger *et al.*, 2000; Şimşek *et al.*, 2007 and Abd El-Haleem and Mohamed, 2011). Aflatoxin ingestion leads to reactive oxygen species production that regulates chemokine receptor expression and also facilitates the recruitment and localization of polymorphonuclear leukocyte to the site of infection and inflammation (Shephard, 2003).

In this study, there was paucity and cytoplasmic vacuolation in the langerhan's cells in compliance to previous researchers' observations who recorded vacuolation of few islet cells and showed that most of the β cells had rounded euchromatic nuclei, whereas some cells had small condensed heterochromatic nuclei (Abd El-Haleem and Mohamed, 2011). Aflatoxin as described previously induces oxidative stress due to generation of oxides and β cells are particularly vulnerable to oxidative stress because of their low levels of antioxidant enzyme expression (Kaneto *et al.*, 2005). Ensuing the damage to B cells, the serum glucose concentration is increased due to a decrease in serum insulin as evidenced in this study by increased random blood sugar in the group administered aflatoxins. On the other hand, the pancreatic lesions were remarkably alleviated in the groups administered aflatoxin and treated with coumarin and /or chlorophyll

suggesting a protective effect of these treatments against oxidative stress induced by aflatoxin (Figure 1 f, g, h).

The most predominant lesions observed in the kidneys of rats administered aflatoxin in the present study were congestion of intertubular blood vessels, hemorrhage, vacuolar degeneration and necrosis of tubular epithelium, presence of hyaline droplets in the lumen of renal tubules and focal areas of peritubular mononuclear inflammatory cells infiltration (Figure 1+1). Likewise to previous studies which recorded the dilatation and engorgement of renal

blood vessels and intertubular capillaries with blood, focal areas of cloudy swelling of the lining epithelium of convoluted tubules together with presence of some eosinophilic debris in some lumen of other tubules and leucocytic cellular infiltration of renal interstitial tissues particularly lymphocytes with shrinkage of the glomerular tufts (El-Shewy and Ebrahim, 2004). The groups that were administered coumarin and/ chlorophyll showed a remarkable improvement of the kidney lesions caused by aflatoxin (Figure 1j, k, l).

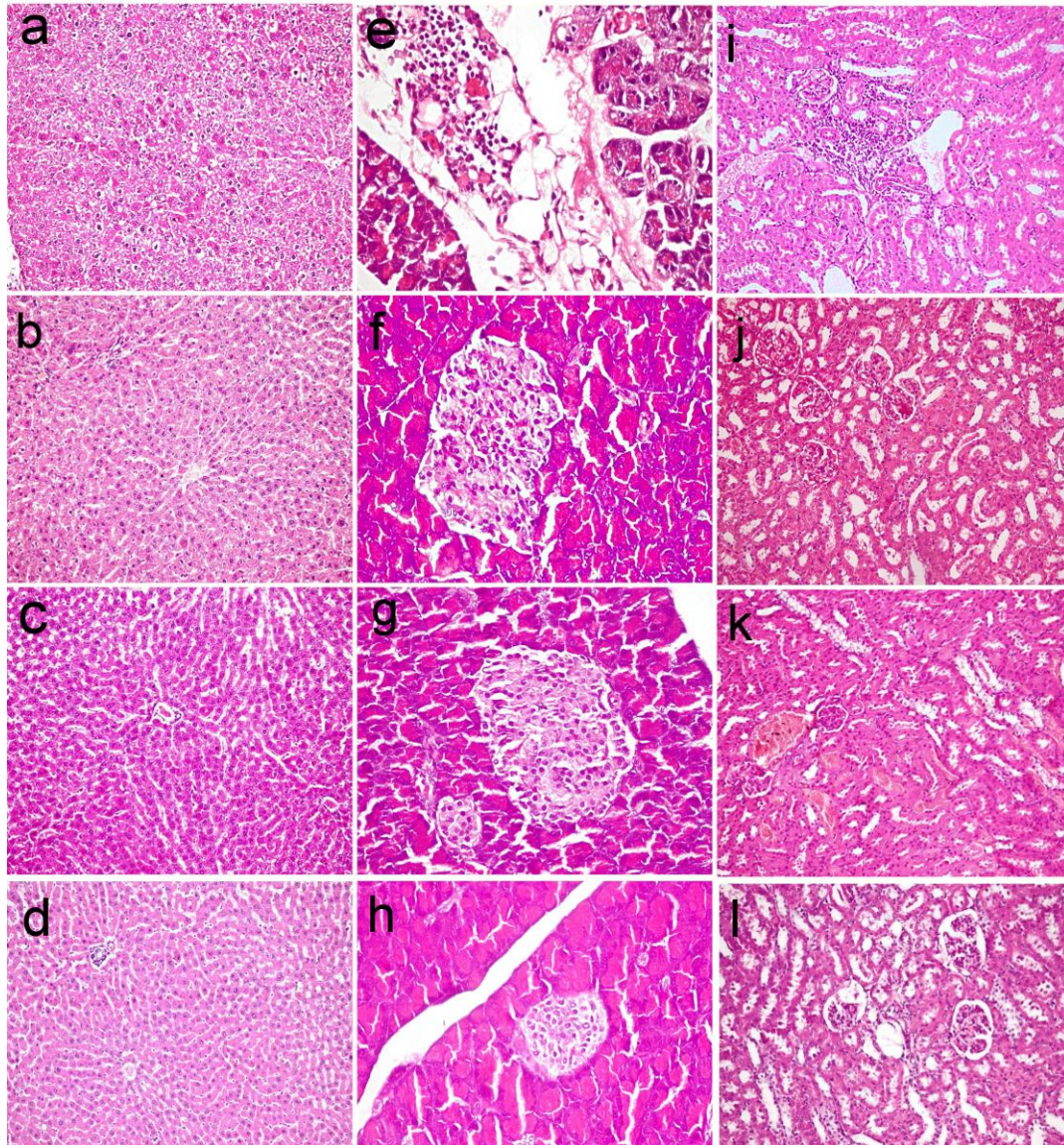


Figure 1. (a-d) Liver, rat. (a) vacuolation and karyomegaly of hepatocytes in G5 receiving aflatoxin 1000 ppb in diet. (b,c,d) apparently normal hepatocytes in G6 receiving aflatoxin in diet and coumarin in water, in G7 receiving aflatoxin in diet and chlorophyll in water and in G8 receiving aflatoxin in diet, coumarin and chlorophyll in water, respectively. (X 200). (e-h) Pancreas, rat. (e) Interlobular edema and mononuclear cells infiltration in G5 receiving aflatoxin in diet. (f, g, h) showing apparently normal pancreatic structure (normal islets of langerhans and normal acini tissues G6 receiving aflatoxin in diet and coumarin in water, in G7 receiving aflatoxin in diet and chlorophyll in water and in G8 receiving aflatoxin in diet, coumarin and chlorophyll in water respectively (X 400). (i-l) Kidney, rat. (i) Focal area of peritubular mononuclear inflammatory cells infiltration in G5 receiving aflatoxin in diet. (j) Apparently normal renal structure with few intratubular casts in G6 receiving aflatoxin in diet and coumarin in water (k) congestion of intertubular blood vessels and slight vacuolation of renal tubular epithelium in G7 receiving aflatoxin in diet and chlorophyll in water. (l) Apparently normal renal structure with vacuolation of few renal tubular epithelium in G8 receiving aflatoxin in diet, coumarin and chlorophyll in water (X 200). Hematoxylin and eosin stain.

The beneficial effect of coumarin may be due to: 1- Reduction of aflatoxin B₁-DNA adducts formation by both liver and intestinal microsomes (coumarin enhanced aflatoxicol formation therefore decrease aflatoxin B₁-DNA adducts, because direct interaction of aflatoxicol-epoxide with DNA is minor compared with aflatoxin B₁-epoxide (Loveland *et al.*, 1987), 2- Enhancement of glutathione S transferase (GST) activity in the intestine to conjugate aflatoxin B₁, 3- Suppression of p 450 enzyme activity in the liver and enhancement of GST in the intestine (Tulayakul *et al.*, 2007); 4- improving liver function (Gilani and Janbaz, 1993) and body health (Maucher *et al.*, 1994; Hoult and Paya, 1996; Pillai *et al.*, 1999; Devienne *et al.*, 2005) and 5- increasing the digestibility of crude protein and ether extract (Ko *et al.*, 2006).

CONCLUSION

Results indicated that addition of coumarin and / or chlorophyll to rat aflatoxin contaminated diet was safe and practical meth.

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تأثير الكومارين والكلوروفيل على حمايه الكبد ضد التسمم الأفلاتوكسينى فى الفئران
محمد سعد عبداللطيف^١، خالد مصطفى المليجي^٢، مروه محمد خطاب^٣ و ساره مجدى محمد^٢
^١ قسم الكيمياء الحيوية، كلية الزراعة، جامعة القاهرة
^٢ المركز الأقليمي للأغذية والاعلاف، وزارة الزراعة
^٣ قسم الباثولوجى كلية الطب البيطرى جامعة القاهرة

ما زال تلوث علائق الحيوان بالأفلاتوكسين هو المشكله الرئيسيه للمربين طالما له تأثيرات سلبيه على صحه الحيوان وانتاجيته وأجريت هذه الدراسه لمعرفة تأثير اضافه الكومارين أو الكلوروفيل الى العليقه الملوته بالأفلاتوكسين وقسم عدد ٥٤ جرد عشوائيا الى ٨ مجاميع (٦ جردان لكل مجموعه) والمجموعه الخامسه تشمل ١٢ جرد، واعتبرت المجموعه الاولى هى المجموعه المقارنه أو القياسيه المجموعه الثانيه تغذت على العليقه القياسيه وماء مضاف اليه الكومارين بنسبه ٠.٥% والمجموعه الثالثه تغذت على العليقه القياسيه وماء مضاف اليه الكلوروفيل بنسبه ٠.٥% والمجموعه الرابعه تغذت على العليقه القياسيه وماء يشتمل على ٠.٥% من كل من الكومارين وبنسبه ٠.٥% كلوروفيل اما المجموعه الخامسه تم تغذيتها بعليقه قياسيه مضاف اليها افلاتوكسين B₁ بتركيز ١٠٠٠ جزء فى البليون. والمجاميع من ٦-٨ تغذت على العليقه الملوته بأفلاتوكسين B₁ ونفس معاملات المجاميع من ٢-٤ واستمرت التجربه لمدته ٨ اسابيع وتم قياس معدل استهلاك الماء والغذاء وتم حساب معدل التحويل الغذائى كما تم تقدير مستوى الجلوكوز العشوائى والليبيدات الكليه والكوليسترول الكلى والجليسريدات الثلاثيه والبروتين الكلى ونشاط انزيم (AST) اسبارتات ترانس امينيز (ALT) الانين ترانس امينيز والكرياتين واليوريا فى السيرم. وتم عمل الفحص الهستوباثولوجى للكبد والكلى والبنكرياس. وظهر معدل التحويل الغذائى انخفاض معنوى فى المجموعه الخامسه مقارنه بالمجاميع من ٦-٨ بينما ازداد الجلوكوز والكوليسترول و ALT فى سيرم المجموعه الخامسه مقارنه بالمجاميع من ٦-٨ ولوحظ اضرار فى الكبد فى المجموعه الخامسه وهى عبارته عن وجود موت موضعى للخلايا ووجود فجوات ولكن وجد تحسن فى المجاميع من ٦-٨. وموت موضعى للخلايا والتهاب ارتشاحى لخلايا البنكرياس فى المجموعه الخامسه ولكن يكون غير موجود فى المجاميع من ٦-٨. وتلخص النتائج ان اضافه الكومارين والكلوروفيل تؤدى الى حمايه الكبد ضد تسمم الأفلاتوكسينى