# Protective Effect of Cholestyramine and Oxihumate against some Mycotoxins in Broiler Chickens

### By

Hend Atwa Azazy Selium BV.Sc., Zagazig University (2003) M.V.Sc., Zagazig University (2016)

### Under supervision of

#### Dr.

Abd El-Moneim Ahmed Ali

Emeritus Prof. of Pathology Faculty of Veterinary Medicine Zagazig University

#### Dr.

Mohamed M.M. Metwally

Professor of Pathology Faculty of Veterinary Medicine Zagazig University

#### Dr.

Mahmoud Fawzy M. Fahmy

Emeritus Prof. of Pathology Faculty of Veterinary Medicine Zagazig University

#### Dr.

**Rehab El-Said M. Mowafy** 

Chief Researcher of Pathology Animal Health Research Institute, Zagazig Branch

## A Thesis

Submitted to Zagazig University for the Degree of Ph.D of Veterinary Medical Sciences (Pathology) Department of Pathology 2020

-

# CONTENTS

	Page
Introduction	1
Review of literature	5
Material and methods	41
Results	49
Discussion	112
Summary	133
References	137
Vita	165
Arabic Summary	

## **List of Abbreviations**

<u>Abbr.</u>	Description
AFB1	Aflatoxin B1
ΟΤΑ	Ochratoxin A
СН	Cholestyramine drug.
СҮР	Cytochrome P450 enzymes
B.wt	Body weight.
AOAC	Association Official Analytical Chemists
MP	malt peptone
HPLC	High performance liquid chromatography
FAO	Food and agriculture organization
IARC	International Agency for Research on Cancer.
CAST	Council for Agricultural Science and Technology.
EFSA	European Food Safety Authority.
JECFA	Joint FAO/WHO Expert Committee on Food Additives.
Fig	Figure
ppm	Part per million
PPb	Part per billion
gp	group
IHC	Immunohistochemical

## **List of Tables**

Tab.	<u>Title</u>	Page
1	Chicken groups, number of chicks/group, treatment, and schedule of sacrificed on 36 day	48
2	The number, percent and time of death of morbid and dead chickens	62
3	Lesion score of experimental chickens during the whole experiment	63
4	AFB1 and OTA residues in different tissues (mean $\pm$ SD)	70

# **List of Figures**

Fig.	<u>Title</u>	Page
1	Liver (gp. 1) showing severe congestion of hepatic blood	
	vessel (arrow) and hepatic sinusoids (arrow head) (H&E	
	X 300).	71
2	Liver (gp. 1) showing periductal focal necrosis (arrows)	
	with vascular congestion (arrowhead) (H&E x 1200)	71
3	Liver (gp. 1) showing fibroblastic proliferation in the	
	portal area (arrow) with cholestasis (star) and mild	
	congestion (arrowhead) (H&Ex1200)	72
4	Liver (gp.1) showing focal area of leukocytic cell	
	aggregation (arrow) with scattered pyknosis (arrowhead)	
	(H&E x 1200)	72
5	Liver (gp.1) showing perivascular fibroblastic	
	proliferation (arrows) (H&E x 300)	73
6	Liver (gp.1) showing necrotic area replaced with RBCs	
	(arrows) (H&E x 1200)	73
7	Liver (gp.1) showing cholestasis (arrowhead) (H&E x	
	1200)	74
8	Liver (gp.1) showing subcapsular edema (arrows) and	
	numerous pyknotic cells (arrowhead) (H&E x300)	74
9	High power of the previous figure to show subcapsular	
	edema (arrows) and pyknotic cells (arrowhead) (H&E x	
	1200)	75
10	Kidney (gp.1) showing focal leukocytic cell aggregation	
	(thin arrow) and focal hemorrhage (arrow head) (H&E x	
	300)	75
11	Kidney (gp.1) showing interstitial hemorrhage (arrow)	
	and degenerated renal tubule (arrowhead) (H&E x 1200)	76
12	Kidney (gp.1) showing interstitial mononuclear cell	
	aggregation (arrows) and vascular congestion	
	(arrowhead) (H&E x 1200).	76

13	Spleen (gp.1) showing vacuolation of tunica media of	
	splenic blood vessels (arrows) with hypertrophied	
	endothelium (arrowhead) (H&E x 1200)	77
14	Spleen (gp.1) showing perivascular fibrosis (arrows)	
	(H&E x 1200)	77
15	Bursa of Fabricius (gp.1) showing mild depletion of	
	lymphocytes from the center of some lymphoid follicles	
	(arrows) (H&E x 300).	78
16	Bursa of Fabricius (gp.1) showing necrosis (arrows) of	
	some lymphocytes in the center of lymphoid follicle	
	(H&E x1200)	78
17	Intestine (gp.1) showing mucinous degeneration of	
	enterocytes of intestinal villi (arrows) (H &E x 1200)	79
18	Intestine (gp.1) showing peri-glandular leukocytic cell	
	infiltration (arrows). (H&E x 1200)	79
19	Liver (gp.2) showing periportal necrotic area (arrows)	
	(H&E x 300).	80
20	Liver (gp.2) showing numerous pyknotic hepatocytes	
	(arrows) with mild congestion (arrowhead) (H&E x	
	1200).	80
21	Kidney (gp.2) showing glomerular mesangeal cell	
	proliferation (thin arrows) with peritubular congestion	
	(arrowhead) and regenerative renal tubules (thick arrows)	
	( (H&E x 1200).	81
22	Kidney (gp.2) showing mild leukocytic cell infiltration	
	(arrow) with degenerative changes of some renal tubules	
	(arrow head) (H&E x1200).	81
23	Bursa of Fabricius (gp.2) showing vesicle formation	
	(arrows) in the epithelial covering the lymphoid follicles	
	(H&Ex 300).	82
24	Intestine (gp.2) showing complete fusion of some	
	intestinal villi (arrow) with massive mucosal lymphocytic	
	cell infiltration (arrowhead) (H&E x 300).	82

25	Liver (gp.3) showing diffuse congestion of hepatic	
	sinusoids (arrows) with atrophied hepatocytes	
	(arrowhead) (H&E x 1200)	83
26	Liver (gp.3) showing congestion of the portal blood	
	vessels (arrows) (H&E x 300)	83
27	Liver (gp.3) showing focal replacement of hepatic	
	parenchyma with lymphocytes (arrow) (H&E x 300)	84
28	Kidney (gp.3) showing severe congestion (arrow) of renal	
	blood vessels with perivascular fibrosis (arrow head)	
	(H&E x300)	84
29	Kidney (gp.3) showing intertubular extravasated blood	
	(tailed arrows) with regenerative attempts (arrowhead)	
	and mild congestion (arrows) (H&E x 300)	85
30	Spleen (gp.3) showing mild congestion of splenic blood	
	vessels (arrow) (H& E x 300)	85
31	Bursa of Fabricius (gp.3) showing lymphocytic	
	proliferation under the covering epithelium (arrowhead)	
	with vesicle formation (arrows) (H&E x 300)	86
32	Intestine (gp.3) showing normal mucosa and submucosa	
	(H&E x 50)	86
33	Kidney (gp.4) showing congestion of peritubular	
	capillaries and blood vessels (arrows) (H&E x 1200)	87
34	Kidney (gp.4) showing interstitial extravasated $RBC_S$	
	(arrow) with dissociation of some renal tubules	
	epithelium (arrowhead) (H&E x 1200)	87
35	Kidney (gp.4) showing focal interstitial mononuclear cell	
	infiltration (arrow) with inter tubular extravasated	
	erythrocytes (arrowhead) (H&E x 300)	88
36	Kidney (gp.4) showing urates deposition in some renal	
	tubules (arrow) (H&E x 300)	88
37	Kidney (gp. 4) showing focal area of heterophils and	
	lymphocytes infiltration (arrow) (H&Ex 1200)	89

38	Kidney (gp.4) showing focal hemorrhagic area (arrow)	
	and degeneration of renal tubules (arrowhead) (H&E x	
	1200)	89
39	Kidney (gp.4) showing ureter infiltrated by mononucular	
	cells (arrow) with presence of cellular debris in its lumen	
	(arrowhead) associated with epithelial degeneration of the	
	renal tubules (H&E x 300)	90
40	Kidney (gp. 4) showing extravasated erythrocytes (arrow)	
	among the degenerated renal tubules (arrowhead) (H&E x	
	1200)	90
41	Liver (gp. 4) showing dissociation of hepatocytes	
	(arrowhead) with severe congestion of hepatic blood	
	vessels (arrow) (H&E x 300).	91
42	Liver (gp.4) showing focal replacement of the hepatic	
	parenchyma with leukocytic cells mainly lymphocytes	
	(arrow). (H&E x 1200)	91
43	Liver (gp.4) showing perivascular leukocytic cells	
	infiltration (arrow) with mild congestion (arrowhead)	
	(H&Ex1200)	92
44	Liver (gp. 4) showing hypertrophied endothelium (thin	
	arrow), portal fibroblastic proliferation (thick arrow) and	
	cholestasis (arrowhead) (H&E x 1200).	92
45	Spleen (gp. 4) showing depletion of lymphocyte from the	
	white pulp with partial splitting of capsule (arrow) (H&E	
	x 300)	93
46	Spleen (gp.4) showing vacuolation (thin arrows) of tunica	
	media of splenic arteriole with perivascular edema (thick	
	arrow) and endotheliosis (arrowhead) (H&E x 300).	93
47	Bursa of Fabricius (gp.4) showing depletion of	
	lymphocytes from lymphoid follicle (thin arrow) with	
	interfollicular edema (arrowhead) and focal hemorrhage	
	(thick arrow) (H& E x 300).	94

48	Bursa of Fabricius (gp.4) showing congestion of bursal	
	blood vessels (arrows) with necrosis of some lymphoid	
	follicles (arrowhead) (H&E x 300)	94
49	Intestine (gp.4) showing fusion of some intestinal villi	
	tips (arrows) (H&E x 300)	95
50	Kidney (gp.5) showing mild perivascular tubular necrosis	
	(arrows) with congestion of blood vessels (arrowhead)	
	(H&E x 300).	95
51	Kidney (gp. 5) showing tubular vacuolation (arrows)	
	(H&E x 1200)	96
52	Liver (gp. 5) showing hypertrophied endothelium of	
	hepatic arterioles (arrow) (H&E x 1200)	96
53	Liver (gp. 5) showing proliferation of bile ductules	
	(arrows) (H&E x 300)	97
54	Bursa of Fabricius (gp. 5) showing mild interfollicular	
	edema (arrows) (H&E x 300)	97
55	Spleen (gp. 5) showing mild subcapsular edema (arrow)	
	(H&E x 300)	98
56	Intestine (gp. 5) showing mild thickening of intestinal	
	muscular coat (arrow) (H&E x 300)	98
57	Kidney (gp.6) showing degenerative changes of renal	
	tubules (arrow) with focal area of leukocytic cell	
	infiltration in the renal medulla (arrowhead) (H&E x 300)	99
58	Kidney (gp. 6) showing dilation of some renal tubules	
	(arrows) with cast formation (arrowhead) (H&E x 1200)	99
59	Kidney (gp. 6) showing regenerative attempts from	
	tubular epithelia (arrow) (H&E x 1200)	100
60	Liver (gp. 6) showing focal and diffuse infiltration of	
	mononuclear cell (arrow) with atrophied hepatocyte	
	(arrowhead) (H&E x 300).	100
61	Bursa of Fabricius (gp.6) showing mild atrophy of some	
	lympholid follicles (arrows) with slight depletion of	
	lymphocyte from its center (arrowhead) (H&E x 300)	101

62	Intestine (gp.6) showing partial hyalinization of muscle	
	fibers of muscular layer (arrows) (H&E x 300)	101
63	Liver (gp. 7a) showing mild perivascular lymphocytic	
	cell infiltration (H&E x 300)	102
64	Liver (gp.7b) showing mild congestion of hepatic blood	
	vessels (H&E x 300)	102
65	Kidney (gp.7b) showing normal renal parenchyma (H&E	
	x300).	103
66	Bursa of Fabricius (gp.7a) showing mild depletion of	
	lymphocyte from some lymphoid follicle (H&E x 300).	103
67	Intestine (gp. 7b): showing normal mucosa and	
	submucosa (H&Ex300).	104
68	Liver (gp.8) showing normal tissue architecture and	
	cellular details (H&E x 300).	104
69	Spleen (gp.8) showing normal tissue (H&E x 300).	105
70	Bursa of Fabricius (gp. 8) showing normal lymphoid	
	follicles (H&E x 300).	105
71	Liver (gp.1) showing strong peroxidase positive	
	expression for caspase-3 (x 1200)	106
72	Kidney (gp.1) showing weak peroxidase positive	
	expression for BCL-2 (x 1200)	106
73	Kidney (gp.1) showing moderate peroxidase positive	
	expression for P53 ( x 300)	107
74	Liver (gp.2) showing moderate peroxidase positive	
	expression for caspase-3 ( x 300).	107
75	Kidney (gp.3) showing weak peroxidase positive	
	expression for BCL-2 (x1200).	108
76	Spleen (gp.3) showing weak peroxidase positive	
	expression for p53 (x 300)	108
77	Kidney (gp.4) showing strong peroxidase positive	
	expression for caspase -3 ( x 1200).	109

78	Kidney (gp.4) showing strong peroxidase positive	
	expression for p53 (x 1200).	109
79	Liver (gp.4) showing moderate peroxidase positive	
	expression for caspase- 3 ( x 1200)	110
80	Liver (gp.5) showing positive moderate peroxidase	
	expression for p53 (x 1200).	110
81	Liver (gp.5) showing positive moderate peroxidase	
	expression for caspase-3 ( x 1200).	111

## SUMMARY

This work was done to study the clinical signs and pathological changes in some organs (liver, kidney, spleen, Bursa of Fabricius and intestine) of broiler chickens (Cubb breed) fed on contaminated ration with aflatoxin B1 (AFB1) or ochratoxin A (OTA) for 36 day. Study the protective effects of either cholestyramine or oxihumate in ameliorating the toxicity via evaluation the pathological lesions, some immunohistochemistry markers and tissue residues of both AFB1 and OTA). So in this aspect, the work was conducted on using one hundred and forty-four healthy, one-day-old commercial broiler chicks (cubb breed) which were purchased from Alkahira Poultry Company and kept under standard hygienic conditions. The broiler chicks equally divided into nine equal groups, each of sixteen chicks. Group (1): Chicks were given ration contaminated with 2 ppm AFB1/kg ration for the whole of the experimental period. Group (2): Chicks were given 2 ppm AFB1 /kg ration together with chemical detoxifying agent cholestyramine (170 µg/ mg aflatoxin in the ration) (340  $\mu$ g/ kg ration) for the whole of the experimental period. Group (3): Chicks were given 2 ppm AFB1/kg ration together with physical detoxifying agent oxihumate (3.5 g/kg ration) for the whole the experimental period. Group (4): Chicks were given ration contaminated with 100 ppb OTA/kg ration for the whole the experimental period. Group (5): Chicks were given 100 ppb OTA/kg ration together with chemical detoxifying agent cholestyramine (170 µg/mg ochratoxin) (17 µg/kg ration) for the whole of the experimental period. Group (6): Chicks were given100 ppb OTA/kg ration with physical detoxifying agent oxihumate (3.5 g/kg ration) for the whole of the experimental period. Group (7): Sixteen chicks were divided equally into two sub-groups with 8 chicks each and were given chemical detoxifying agent cholestyramine. **Group** (8): Chicks were given physical detoxifying agent oxihumate (3.5 g/kg ration) for the whole of the experimental period **Group** (9): Control group which chicks were given the recommended ration, free from mycotoxins for all the experimental period.

Our investigation revealed that the chickens received AFB1 (group 1) showed severe illness in the form of reduction of body weight, feed intake, ruffling broken feather, dropping of head and wings, depression, lameness and yellowish diarrhea. The mortality rate was 25% during the whole experiment. Macroscopically, marked enlarged friable and yellow liver beside multifocal hemorrhage on its capsule mean while other cases showed dark color liver with presence of nodules of variable size on its surface. Congestion of kidneys was noticed in most cases and other cases showed enlargement and paleness with petechial hemorrhage. The intestinal mucosal surface showed severe hemorrhagic spots. Severe reduction in the relative size of bursa of Fabricius was recorded. Enlarged spleen was also noticed in some cases. Microscopically, the hepatic parenchyma showed degenerative changes and coagulative necrosis of the hepatocytes. The portal area in many cases was infiltrated with numerous leukocytic cells particularly around proliferated bile ductules. The portal blood vessels were congested with perivascular fibrosis in some cases. Kidneys showed focal area of intertubular leukocytic cells infiltrations with mild inter tubular extravasated erythrocytes and congestion. The spleen and Bursa of Fabricius showed depletion of lymphocytes, congestion and hemorrhage. Intestine showed vacuolation of glandular epithelium with severe interglandular leukocytic cells infiltration. Group (2) showed mild signs and lesions in comparison to group (1). Meanwhile group (3) showed moderate signs comparable to group (1). The previous results in groups 2 and 3 were due addition of chemical detoxifying agent cholestyramine and physical detoxifying agent oxihumate which minimized the adverse effects of aflatoxins in the exposed chicks of these groups.

The chickens received OTA in group (4) showed weakness, restlessness, reduce weight gain and increased water consumption. Diarrhea, poorly feathers and paleness of comb and wattles were detected in some cases. Many degree of anorexia was also noticed. The mortality rate was 31.4%. Macroscopically, severe congested and enlarged kidneys were observed in majority of cases. Enlargement of liver size with yellowish coloration were detected. Hyperemic mucosal surface of intestine was noticed in some cases. Some chicken showed thickening of intestinal wall with excessive amount of mucous.Reduction in both bursa of Fabricius and Spleen size were detected. Microscopically, kidneys showed severe congestion of peritubular capillaries with mild degeneration of some renal tubules. Focal intertubular lymphocytic cells infiltration with intertubular extravasated erythrocytes together with degeneration of some renal tubules was also noticed. Some renal tubules in some cases showed urates deposition. Liver showed severe perivascular fibrosis with congestion of hepatic blood vessels in most cases. Other cases revealed dissociation of hepatocytes with severe congestion of hepatic blood vessels and diffuse von kuppfer cells proliferation in addition to severe perivascular leukocytic cells infiltration. Spleen and bursa of Fabricius showed depletion of lymphocyte. Intestine of most chickens showed congestion of intestinal blood vessels with vacuolation of the intestinal gland. Other cases showed necrotic area beneath submucosa. Fusion of intestinal villi was seen in some cases.

Chickens of **group** (5) showed mild clinical signs, macroscopic lesions and microscopic lesions comparable to group (4) also moderate clinical signs, macroscopic lesions and microscopic lesions were seen in **group** (6) in comparison to group (4).The addition of chemical detoxifing agent cholestyramine and physical detoxifing agent oxihumate minimized the adverse effects of ochratoxin in the exposed chicks of these groups.

Mild side effects were associated with oxihumate in chicks of **group** (8) while milder effects of cholestyramine were encountered in chicks of **group** (7).

**Immunohistochemical** results showed strong expression in both intoxicated groups with AFB1 and OTA while mild to moderate expression were noticed in treated group by chemical and physical detoxifier.

Higher residual level were recorded in liver then kidney and finally muscle in aflatoxicated chicken while in ochratoxicated chickens higher level were in muscle than liver and finally kidney.

Finally, it could be concluded that ochratoxicosis occur less frequently in poultry than aflatoxicosis but is more lethal because of its acute toxicity. The use of both chemical detoxifying agent cholestyramine and physical detoxifying agent oxihumate alleviated the adverse effects of AFB1and OTA. The use of chemical detoxifying agent cholestyramine was more effective with a minimal side effect.