



Protective Effects of Rhino-Hepato Forte in Broiler Chickens During Aflatoxicosis

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A RESEARCH was conducted to investigate the counteracting effect of Rhino-Hepato (RHF), against aflatoxin B1 effect on the hematology, serum biochemical parameters and its immunomodulatory effect on Newcastle disease antibody titer in broilers. One hundred twenty , of one day old commercial Ross 308 broiler chicks were divided into four groups: G1 [Control group, without addition of Aflatoxin (AF) or (RHF) ; G2 [feed contaminated with 500ppb AF]; G3 [feed contaminated with 500ppb AF + 1 ml / L of RHF in drinking water]; G4 [feed contaminated with 500ppb AF + 2 ml/L of RHF in drinking water]. Birds were vaccinated with Newcastle and lasota vaccines at 8 and 28 days of age. At the end of the experiment (42 days), 72 birds were slaughtered and blood samples from them were collected for evaluation of (RHF) counter effect against aflatoxin B1. Results showed that AFB1 was negatively affecting hematological parameters by reducing RBCs, PCV and Hemoglobin, WBCs and increasing H/L stress factor ratio. AFB1 also had a deleterious effect on serum biochemistry by increasing triglycerides , cholesterol, AST, ALP and creatinine, while reducing glucose, total protein and calcium. Antibody titers against NDV vaccine were significantly reduced by contaminating feed with AFB1. Inclusion of (RHF) at two doses (1 and 2 ml/L) was responsible for restoring all the previous parameters to those of the normal ones. It is concluded that(RHF), containing silymarin and other active ingredients protect birds against adverse effects of (AFB1) one hematological, biochemical and NDV antibody titers.

Keywords: Aflatoxicosis, Broiler, Milk Thistle, Serum Biochemistry.

Introduction

Aflatoxins are a group of closely related biologically active toxic small molecular weight secondary fungal metabolites, Twenty metabolites have been identified as aflatoxins. They mainly include Aflatoxins B1, B2, G1, and G2 which categorized as a Group I carcinogen for humans [1]. Among them AFB1, exhibits the highest toxigenic effects and the most commonly found metabolite in cereals [2]. They are produced by some strains of *Aspergillus flavus*, *A. parasiticus* and *A. nomius* [3], which are normally encountered in a wide range of tropical and subtropical feeds. Food and Agricultural

Organization (FAO), estimated that up to 25% of the world's food crops and a higher percentage of the world's animal feedstuffs are contaminated significantly by mycotoxins [4]. Exposure to mycotoxins takes place mostly by the ingestion of contaminated cereals such as corn, wheat, peanuts and sorghum, as well as other raw materials, which are used in preparing animal feed [5]. Here in Iraq, outbreaks of Aflatoxicosis had been reported in broiler farms [6];, and a variable amount of aflatoxins were actually reported in commercial broiler feeds [7]. Much work has been done on Aflatoxin B1 (AFB1), the most potent of these mycotoxins, exploring hepatotoxic, carcinogenic,

mutagenic, teratogenic and immunosuppressive effects in many animal species including poultry [8]. The Toxic effects of Aflatoxin on poultry hematological and blood biochemistry [9], Immunological [10], gross and histopathology. were well documented [11]. Furthermore, small amounts of AFB1 and its metabolites can be found in several edible tissues [12]. Determination of Aflatoxin biochemical toxic effects is important for toxicosis diagnosis in broilers [13]. This toxicity of Aflatoxin in broilers was demonstrated by decreased serum concentrations of total protein, albumin and total cholesterol [14], uric acid [15] and increased hepatic enzyme activities such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) [16]. In Japanese quail, a decrease was observed in the levels of serum total protein, albumin, globulin, glucose, cholesterol, ALT and an increase of AST and Gamma-glutamyltransferase (GGT) and variable Alkaline phosphatase (ALP) levels were also observed in the groups treated with the toxin [17]. Also, the activity of serum enzymes such as AST has been extensively employed as a measure of aflatoxin toxicity in chickens [18]. Among medicinal plants, milk thistle (*Silybum marianum*) has been used for centuries as a natural remedy for digestive troubles of the upper gastrointestinal tract, especially liver and biliary tract diseases. The Silymarin, which is an extract from the seeds of milk thistle (*Silybum marianum*), is a complex made up of four flavonolignans, that have strong antioxidant and free radical scavenging activity [19]. Its active constituents, the flavonolignans silybin, isosilybin, silydianin and silycristin, are well-known for their hepatoprotectivity [20]. Antiinflammatory, cytoprotective, and anticarcinogenic effects [21]. These extracts are also used to successfully treat hepatitis patients [21] It has been shown that milk thistle seed (MTS) protects birds from the adverse impacts of Aflatoxin B1 (AFB1) [22] and it also serves as immunostimulant [23]. The pharmacological profile of silymarin demonstrated that it has antioxidant and free radical scavenging properties, improvement of the antioxidative defense by prevention of glutathione depletion, and antifibrotic activity. This suggests that silymarin may contribute to preventing aflatoxicosis-induced damage.

Taking into consideration the medicinal value of milk thistle, this study was conducted to investigate the efficiency of RHINO-HEPATO (RHF) containing milk thistle (MTS) on serum

biochemistry, lipid profile, liver enzymes and immune performance of the broilers against Newcastle disease (ND) in broiler chickens contaminated with AFB1 through exposure up to 42 days. As far as our knowledge is concerned, this is the first work concerning the effect of Aflatoxin on 42 day old broilers and the assessment of RHINO-HEPATO (RHF) containing milk thistle (MTS) MTS on AFB1 adverse effects.

Materials and Methods

The research was conducted at the animal house, department of veterinary public health, college of veterinary medicine, University of Mosul, during the period from 1 / 8 / 2018 to 12 / 9 / 2018 in order to investigate the counter-acting effect of Rhino-Hepato ® against aflatoxin B1 effect on the hematology, serum biochemical parameters and its immunomodulatory effect on Newcastle disease antibody titer in broiler chicks.

Experimental design

The experiment was carried out in a completely randomized block design (CRBD), with two factors: (i), which represents the level of milk thistle and (ii), which is the level of aflatoxin. One hundred twenty (120) Ross 308, commercial one day old broiler of approximately the same weight were purchased from the local hatchery. Chicks were divided into four groups: G1 [Control group, feed without Aflatoxin (AF) or Reno-hepato forte (RHF)*; G2 [feed contaminated with 500ppb AF only without addition of RHF]; G3 [feed contaminated with 500ppb AF + 1 ml/L of RHF in drinking water]; G4 [feed contaminated with 500ppb AF + 2 ml/L of RHF in the drinking water]. Each group involved three replicates with 6 chicks per replicate. The birds were raised in 4×4 square foot wooden pens on conventional deep litter. All the pens were located in the same house in order to have identical environment, where each pen was provided with separate feeder and drinker, supplemented with artificial cold during water. Feed and water were offered *ad libitum*. Birds were vaccinated by Newcastle and Lasota vaccines at 8 and 28 days of age.

Production of aflatoxin B1 contaminated poultry feed:

Aflatoxigenic strain of *Aspergillus flavus* already isolated on Sabouraud agar from contaminated poultry feed sample was used for the production of aflatoxins, using a rice culture as described by [24]. Aflatoxin B1 was estimated to be 50ppm using Neogen Enzyme linked immunosorbent assay (ELISA) kit (Neogen company, USA).

Blood biochemical parameters

At the end of the experiment (42 days), seventy two (72) birds slaughtered (6 birds from each replicate) and blood samples were collected from jugular vein for determination of serum biochemical parameters. Blood samples (1.5 ml) were drawn to 3 ml syringes with a 23 gauge needle from jugular vein causing minimum stress to the bird for determination of serum biochemical parameters. Blood samples were later collected into labeled serum tubes. Serum was separated by centrifugation at 6000 rpm for 1 and a half minute. Clear serum was divided into two Eppendorf tubes for measuring biochemical profiles and NDV antibodies. Samples were then stored at (-20°C) till analysis. Serum biochemical profile, lipid profile and liver enzymes were carried out, using spectrophotometer. Tests were include Alkaline Phosphatase (ALP) (U/L), Aspartate Aminotransferase (AST) (U/L), glucose mg/dl, Calcium mg/dl, Triglycerides mg/dl, Cholesterol mg/dl and Creatinine mg/dl were measured by commercial kits (bioMérieux S.A. France). Total protein in serum was measured by Biuret test and absorbance was measured at 550 nm. [25].

Newcastle antibody titer

A total of 72 serum samples were collected and tested to evaluate serum antibody titer level against Newcastle disease by Enzyme linked immunosorbent assay (ELISA) (Bio Check Immunoassays, Product code ck119, UK)[26].

Rheno-Hepato Forte (Germany)

Liquid complementary feed for poultry to prevent and treat liver and kidney disorders
Analytical constituents and levels: 1% potassium, 2% methionine
Additives per L: Vitamin B12, 15.000 µg; L-carnitine, 20.000 mg; Betaine, 40.000 mg; Choline chloride, 80.000 mg; Sorbitol, 200.000 mg; Flavour, 2.000 mg; 1,2-propanediol, 50.000 mg; Magnesium sulphate, 10.000 mg; Total seasoning flavorings, 40.000 mg; Milk thistle extract, 32.000 mg; Artichoke extract, 8.000 mg.

Statistical analysis

The results (group means) were analyzed by means of the analysis of variance (ANOVA) using SPSS package, version 20. Means were subjected to Duncan's test and the statistical significance was accepted at $P \leq 0.05$.

Results

As shown in Table 1, addition of 500 ppb of AFB1 to the diet of broiler chicks (group B) clearly resulted in a significant reduction

($P < 0.05$) of RBC number to $\{2.41 \pm 0.06 \times 10^6 / \mu\text{l}\}$. The addition of both RHF doses (1 and 2 ml/L) to the drinking water (groups C and D), were effectively ameliorating AFB1 negative effect on RBCs, and were $\{2.59 \pm 0.05 \times 10^6 / \mu\text{l}\}$ and $\{2.60 \pm 0.24 \times 10^6 / \mu\text{l}\}$ respectively.

White blood cells were significantly affected ($P < 0.05$) by the addition of AFB1 in their increasing number to $\{26.47 \pm 1.20 \times 10^3\}$, compared to control group (group A) $\{23.66 \pm 0.28 \times 10^3\}$. Addition of RHF at its two levels (1 and 2 ml/L) (group C and D), were able to restore WBC values to values of $\{24.27 \pm 0.78 \times 10^3\}$ and $\{24.22 \pm 0.85 \times 10^3\}$ of the control one.

Packed cell volume values show pronounced changes with the addition of AFB1 at a rate of 500ppb to broilers feed, and were significantly ($P < 0.05$) reduced to $27.10 \pm 1.39\%$ compared to the control value of $32.26 \pm 1.41\%$. Amending groups (C and D) with RHF at two levels (1 and 2 ml/L) in broilers drinking water, had positively counteracted the negative AFB1 effect on PCV values, by their restoring their values to those of control one and were being $\{29.15 \pm 1.46\%$ and $30.18 \pm 0.50\%$ }.

The same picture of PCV was noticed with Hemoglobin values, through the addition of AFB1, since they were significantly ($P < 0.05$) reduced to 8.13 ± 0.98 g/dl, when compared to the control value of 12.46 ± 0.46 g/dl. Amending groups (C and D) with RHF at two levels (1 and 2 ml/L) in drinking water, had positively counteracting the negative effect of AFB1 on Hb values, in their restoring them to vales of control one $\{10.13 \pm 0.50$ g/dl and 10.33 ± 0.41 g/dl }.

The significant ($P < 0.05$) increase in Stress factor (H/L ratio) (0.59 ± 0.07) after the addition of AFB1 to the diet was a reflection of the significant ($P < 0.05$) increase in the total number of Heterophils ($9.32 \pm 1.9 \times 10^3 / \mu\text{l}$). Ameliorating effect by the addition of 1 and 2 ml/L of RHF in drinking water was conducted through the significant reduction of heterophils number to $(6.83 \pm 1.5 \times 10^3 / \mu\text{l})$ and $(6.33 \pm 1.4 \times 10^3 / \mu\text{l})$ respectively.

The negative effect of AFB1 was not restricted to the blood picture but also includes biochemistry profile as shown in table (2) through the significant reduction of glucose (55.49 ± 31.60 mg/dl), creatinine (5.33 ± 2.06 mg/dl), calcium (97.99 ± 5.47 mg/dl) and total protein (4.49 ± 0.14 g/dl) in comparison to the control group. Counteracting the negative effect of AFB1

on the mentioned parameters was obtained by the addition of both RHF doses (1 and 2 ml/L) to the drinking water (groups C and D), and for glucose were (114.48± 15.35 mg/dl and 116.41± 12.62 mg/dl respectively); for calcium (117.40± 8.85mg/dl and 124.21± 9.95 mg/dl) respectively; for total protein (5.61± 0.13 g/dl and 5.69± 1.24 g/dl), and for creatinine level (4.55±1.70mg/dl and 3.66± 0.36 mg/dl) respectively.

As shown in the same Table 2, the addition of 500 ppb of AFB1 to the diet of broiler chicks (group B) has clearly resulted in a significant increase (P<0.05) of lipid profiles (triglycerides and cholesterol) (132.62± 7.65 mg/dl , 154.35±21.87 g/dl), when compared to that of the control group (group A)(98.07± 5.37 mg/dl, 107.40±15.29 g/dl) respectively. The addition of both RHF doses (1 and 2 ml/L) to the drinking water (groups C and D), were effective in restoring cholesterol level to that of (118.80± 7.35g/dl, and 110.64± 11.09 g/dl) and cholesterol to (112.90±14.33 mg/dl , 108.35±9.06mg/dl).

The effect of feeding AFB1 has also resulted in a significant (P<0.05). increase in liver enzymes, namely ALP (151.16 ± 14.03 U/L) and AST (262.83±14.07U/L), in comparison with the control group. A significant reduction of AFB1 negative effect on ALP and AST was evident after the addition of RHF doses (1 and 2 ml/L) to the drinking water (groups C and D) MT and returning values significantly (P<0.05) of ALP and AST to (120.50 ± 43.40 U/L) and (203.33±44.00 u/l) respectively.

Diagrams presented in Fig. 1 demonstrate the mean titer of the antibodies after NDV vaccination by the addition of AFB1 to the diet and RHF at two doses i.e., 1 ml and 2ml/L to the drinking water using ELISA technique. It reveals that contamination of feeds with 500ppb AFB1 was significantly decreased (P<0.05) the mean titer of the antibodies and were ranged between 0-3000 (not more than log₁₀ 3.47) versus 4000-5500 (log₁₀ 3.47- log₁₀ 3.74) of the control uncontaminated diet. The negative effect of AFB1 on the NDV antibody titer was significantly (P<0.05) counteracted by the addition of RHF at its two doses (1and 2 ml/L) with the drinking water, since the range of antibodies titer were ranged between 3000-5500 (log₁₀ 3.47- log₁₀ 3.74) after amending water with 1 ml/L of RHF and between 3500-5500 (log₁₀ 3.54- log₁₀ 3.74) with a higher dose of 2 ml/L of drinking water.

TABLE 1. Effects of AFB1 and Rheno-Hepato Forte (RHF) on hematological parameters of broiler chickens at the age 42 days.

Parameters	Groups							
	A		B		C		D	
	AF	RHF	AF	RHF	AF	RHF	AF	RHF
RBC(x106/ μ l)	0	0	2.74± 0.06 ^a	0	2.41± 0.06 ^b	2.59± 0.05 ^{ab}	0	2.60± 0.24 ^{ab}
WBC(x103/ μ l)	0	0	23.66± 0.28 ^b	0	26.47± 1.20 ^a	24.27± 0.78 ^b	1 ml	24.22± 0.85 ^b
PCV(%)	0	0	32.26± 1.41 ^a	0	27.10± 1.39 ^c	29.15± 1.46 ^{bc}	500 ppb	30.18± 0.50 ^{ab}
HB(g/dl)	0	0	12.46± 0.46 ^a	0	8.13± 0.98 ^c	10.13± 0.50 ^b	1 ml	10.33± 0.41 ^b
Heterophils(x103/ μ l)	0	0	5.70± 1.2 ^b	0	9.32± 1.9 ^a	6.83± 1.5 ^b	500 ppb	6.33± 1.4 ^b
Lymphocytes(x103/ μ l)	0	0	15.88± 1.3 ^a	0	15.65± 1.5 ^a	15.32± 1.4 ^a	2ml	15.77± 1.4 ^a
Ratio of (H/L)	0	0	0.35± 0.02 ^b	0	0.59± 0.07 ^a	0.44± 0.06 ^b	2ml	0.40± 0.04 ^b

TABLE 2. Effects of AFB1 and Rheno-Hepato Forte (RHF) on lipid profiles, serum biochemistry and some liver enzymes of broiler chicken at 42 days of age.

Groups	Treatments		Lipid profiles			Biochemical profiles				Liver enzymes	
	AFB1 (ppb)	RHF ml	Triglycerides (mg/dl)	cholesterol (g/dl)	Glucose (mg/dl)	creatinine (mg/dl)	calcium (mg/dl)	total protein (g/dl)	alkaline phosphatase (U/L)	AST (U/L)	
A	0	0	98.07±5.37 ^{d*}	107.40±15.29 ^b	121.04±10.87 ^a	2.50±0.24 ^c	129.74±6.23 ^a	6.22±0.80 ^a	87.89±14.03 ^b	202.50±43.19 ^b	
B	500 ppb	0	132.62±7.65 ^a	154.35±21.87 ^a	55.49±31.60 ^b	5.33±2.06 ^a	97.99±5.47 ^b	4.49±0.14 ^b	151.16±43.40 ^a	262.83±14.07 ^a	
C	500 ppb	1 ml	118.80±7.35 ^b	112.90±14.33 ^b	114.48±15.35 ^a	4.55±1.70 ^{ab}	117.40±8.85 ^a	5.61±0.13 ^a	130.09±30.53 ^{ab}	235.66±10.89 ^{ab}	
D	500 ppb	2ml	110.64±11.09 ^c	108.35±9.06 ^b	116.41±12.62 ^a	3.66±0.36 ^{bc}	124.21±9.95 ^a	5.69±1.24 ^a	120.50±26.82 ^a	203.33±44.00 ^b	

* (a,b) Main effect means within a column lacking a common superscript differ significantly (P<0.05). [Main effect values are mean ± SD from 6 observations each].

Discussion

Aflatoxin B1 is known to be the most toxic metabolite, particularly in the sensitive species such as poultry particularly on their performance [27], hematological [28], biochemical and immunological parameters [29]. In this study, the inclusion of 500ppb of AFB1 to the diet of broilers had detrimental effect on the hematological, biochemical and immunological parameters, confirmed by the other studies through the inclusion of AF at a rate of 250 and 500ppb on BW gain, feed intake, FCR and relative organ weights [30]. The significant improvement of hematological, biochemical and immunological profile in this experiment after amending the birds with Rhino-hepato® at two levels 1 and 2 ml/l of drinking water, is due to its composition, which play excellent role in potassium, methionine, Vitamin B12, L-carnitine, Betaine, Choline chloride, Sorbitol, Flavour, 1,2-propanediol, Magnesium sulphate, seasoning flavorings, Milk thistle extract, Artichoke extract it contained. These components are reported to improve overall birds metabolism, through providing antioxidant and hepato-protective action, preventing the infiltration of AFB1 into hepatic cells, and also stimulates the regeneration of hepatocytes and normalize their functioning. Our results were in agreement with [31], in that silymarin (one of the Rhino-hepato® components) contributed to preventing the aflatoxicosis-induced damage, through keeping the levels of hematological and serum biochemical parameters in normal ranges and the reduction of AFB1 immunosuppressive effects. Our study also went in the same line with [32], who found that 500ppb AFB1 when fed alone to broilers was responsible for elevation of AST,GGT, enzymes and in reduction of total protein, but when given with silymarin, the later was able to counteract the reduction of serum urea, GGT and LDH and improving AST, albumin and total protein.

The multifacted positives of Silymarin, is due to its role as hepatoprotective against different liver (a vital organ in blood, liver enzymes and immunoglobulin formulation) ailments like AFB1 toxicity, [21]. The mode of action, by which silymarin might protect liver cells, includes the stabilization of membranes and free radical scavenging [33]. In addition, silymarin can be used to restore the levels of various hepatic enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [34].

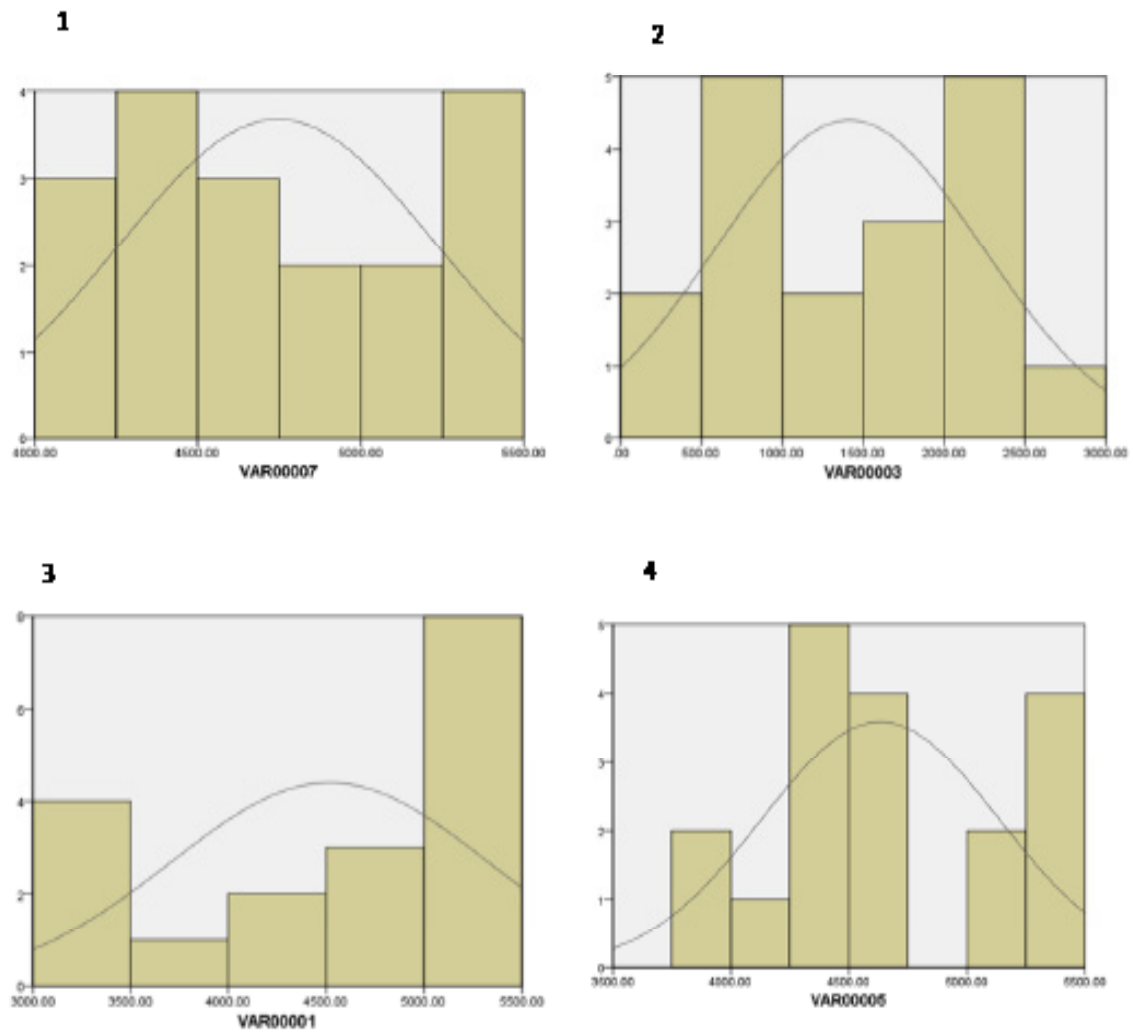


Fig. 1. Diagrammatic representation of the effect of (AF) toxin and (RHF) on NDV antibody titers of 42 days old broilers.

1= Control

2 = 500 ppb AF

3= 500ppb AFB1+ 1 ml/L RHF

4= 500ppb AFB1 + 2 ml/L RHF

Aflatoxin was reported to affect the primary lymphoid organs, spleen [35] and bursa [36], by increasing the weight of the first and decrease the weight of the second in broilers when given over 300ppb. And between 80-250 ppb [37]. In the present study, supplementation of Rhino-hepato® to the aflatoxin contaminated broilers diet was able to reverse the negative effect of aflatoxin on the number of lymphocytes [37].

Aflatoxin is an oxidative agent that could lead to immune suppression and increase the susceptibility of animals to different diseases

[38]. In the experiment done by [37] aflatoxin was reported to be significantly reduced the titers of antibodies against vaccines of Newcastle disease virus (ND), infectious bronchitis virus (IBV) and infectious bursal disease (IBDV), which were ameliorated after the addition of 10 g silymarin / kg feed. These results went in the same line with our findings, through the significant counteracting negative AFB1 effect by amending broilers drinking water with Rhino-hepato (containing silymarin) on ND antibody titers.

This is attributed to the immunomodulatory beneficial effects of silymarin by stimulation hepatocyte protein synthesis [33]. Moreover, silymarin is capable of moderating the immune system by boosting I L-4, IFN- γ , and IL-10, [23,39,40] and preventing glutathione depletion. [21].

Moreover the ameliorating action of Rhino-hepato® could also traced to carnitine as one of the antioxidants, and antistress factors and could ameliorate the oxidative negative AFB1 effect on the immunity by strengthening the immune system [41,42], which was reflected here on Newcastle antibody titer. The positive results of using Rhino-hepato® in drinking water may be related to methionine, which contained, as a hepatoprotective agent, through the activation of vitamins (cyanocobalamin, ascorbic and folic acid), enzymes, proteins, and the decreasing of cholesterol concentration and increasing of blood phospholipids concentration and elimination of some toxic substances by methylation [33,34,35,43].

The two other Rhino-hepato® important components are betaine and sorbitol, which play by their sides a vital role in counteracting aflatoxicosis in broilers, since betaine as trimethyl glycine derivative, as hepatoprotective which activates methylation, metabolic reactions, metabolism of fat, and stimulates digestion, in addition, sorbitol plays an important role in detoxification and energy metabolism [44].

Artichoke, one of the Rhino-hepato® component is a potent antioxidant thereby protecting liver from oxidative damage and inflammation, and could lower blood cholesterol, and balances the intestinal microbiota by increasing beneficial microorganisms in the gut [45,46].

It is concluded that silymerin and other components of Rhino-hepato®, could protect birds against adverse effects of Aflatoxin B1 (AFB1) as hepatoprotective, anti-inflammatory and cytoprotective effects suggesting that especially silymarin could contribute to the prevention of the aflatoxicosis-induced damage, an antioxidant, reducing free radical mediated damage in the tissues and eventually inhibiting lipid peroxidation [21, 22, 47]. it plays also an important role in hepatic protein synthesis by DNA-dependent RNA polymerase I activation and thus improves liver cell regeneration [48].

Conclusion

It is concluded that Rhino-Hepato® (RHF), containing silymarin is protects birds against negative effects of aflatoxin B1 (AFB1) and contribute to prevent the aflatoxicosis through increasing the WBC and decreasing of the enzymes (ALT and AST) and immunity strengthening.

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Conflict of interest

The authors declares no conflict of interest

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التأثيرات الوقائية للسائل العلاجي الكبدي في فروج اللحم خلال التسمم بسموم الافلا

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اجري البحث لغرض اظهار التأثير المعاكس للسائل العلاجي ضد تأثير التسمم التجريبي بسم الافلا نوع (B1) على قياسات الدم والقياسات الكيموحيوية لمصل الدم والتأثيرات المناعية للمعيار الحجمي لاضداد مرض نيوكاسل في فروج اللحم . استخدم في هذا البحث (١٢٠ صوصي) من فروج اللحم التجاري نوع (روز ٣٠٨) بعمر يوم واحد ، اذ قسم العدد الى اربع مجموعات : المجموعة الاولى (مجموعة السيطرة وهو العلف بدون سم الافلا وبدون السائل العلاجي) ، المجموعة الثانية (المجموعة الحاوية على العلف الملوث بسم الافلا بواقع ٥٠٠ ميكروغرام / كغم علف) ، المجموعة الثالثة (المجموعة الحاوية على العلف الملوث بسم الافلا بواقع ٥٠٠ ميكروغرام / كغم علف مع اضافة ١ مل سائل علاجي / لتر ماء شرب) المجموعة الرابعة (المجموعة الحاوية على العلف الملوث بسم الافلا بواقع ٥٠٠ ميكروغرام / كغم علف مع اضافة ٢ مل سائل علاجي / لتر ماء شرب) . الطيور لقت بلقاح نيوكاسل ولاسوتا عند الاعمار ٨ و ٢٨ يوم . في نهاية فترة التجربة (بعمر ٤٢ يوم) ، تم ذبح ٧٢ طير وجمعت عينات الدم ، وشملت الدراسة تقييم السائل العلاجي ضد سم الافلا (B1). اظهرت النتائج ان سم الافلا يؤثر سلبا على صفات الدم عن طريق تقليل كريات الدم الحمراء وحجم الخلايا المرصوصة والهيموكلوبين ، بينما الخلايا البيضاء وعامل (H/L) ازدادت . ان سم الافلا (B1) كذلك يؤثر على صفات مصل الدم الكيموحيوية عن طريق زيادة الكليسيريدات الثلاثية والكولسترول وانزيمات AST و ALP والكرياتينين ، بينما تقلل الكلوكوز والبروتين الكلي والكالسيوم . ان المعيار الحجمي لاضداد مرض نيوكاسل كان قد قل معنويا عن طريق تناول سم الافلا . ان تقديم السائل العلاجي بجرعتين لـ (١ مل) و (٢ مل) لكل لتر ماء شرب تعمل على ارجاع القياسات المتأثرة السابقة الذكر بمعدلات طبيعية . يستنتج من ذلك ان السائل العلاجي المحتوي على مادة السليمارين وبقية المكونات الفعالة تحمي الطيور ضد التأثيرات المعاكسة لسم الافلا (B1) على قياسات الدم وصفات الدم الكيموحيوية والمعيار الحجمي لاضداد مرض نيوكاسل.

الكلمات المفتاحية : التسمم بالافلا ، فروج اللحم ، نبتة شوكة الحليب ، صفات الدم الكيموحيوية.