Dietary Silymarin (*silybum marianum*) to Improve The Health Status of Layer Chickens

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Abstract:*The study aimed to evaluate silymarin material (SM) in reducing the damage in the body caused by residual aflatoxin as well as to improve the health status and quality of the produced eggs in laying hens which were fed on a contaminated diet with aflatoxin. The experiment was conducted on 120 layer chickens (ISA Brown" strain) aged 240-day were randomly divided into three equal groups (40 hens). The first group was fed a basal diet free of aflatoxin. The 2nd group fed on contaminated diet with aflatoxin (14.6 ppb) while the 3rd group fed on the same diet (in 2nd group) with 0.5% of silymarin/kg feed. Ten hens from each group were slaughtered at the end of the experiment for the histopathological examination to evaluate the harmful effect of aflatoxin in tissues (liver, spleen and intestine) as well as, estimated the production and quality of the produced eggs during experiment study.*

Abnormal visible symptoms observed on 2nd group include decreased in feed intake and body weight, as well as, in some there was abnormal pigmentation (comb and wattles). The most frequent effects on eggs were poor egg quality as decreased egg production, reduced egg size, thin or rough egg shell. The most common pathological lesions associated with aflatoxin residue in chickens were found in liver, spleen and intestine organs. In advanced cases, these organs become enlarged, swollen and changed colour into yellowish. While in the 3rd group, the macroscopic examination of the same organs was less pronounced and almost invisible. We conclude that silymarin has a significant effect and is highly effective in repairing the damage caused by aflatoxin to the body tissues of laying hens. The production in terms of the quantity and quality of eggs was not affected as well as, improving the health status.

Keywords: Silymarin, Aflatoxins, layer

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1. INTRODUCTION

Mycotoxins are found worldwide in all feed resources, mainly in crops such as maize, peanuts, cottonseed, and tree nuts, and cause major economic losses through compact bird health, immune response, and performance [1,2]

Natural diet pollutants including mycotoxins are among the main challenges facing poultry men as well as producers [3]. One of the most important types of mycotoxins it is aflatoxin which is rapidly held throughout the digestive system and extend during the body and mainly metabolized in the liver where it converted into reactive and electrical entities by some hepatic cytochrome enzymes. Aflatoxins, are thermostable, resistant to some chemicals, soluble in polar solvents, and insoluble in fats and oils [4]. This toxin caused a lot of economic losses in the world since the beginning 1960s until the present day, 18 types of aflatoxins have been discovered, including four main types B1, B2, G1, G2 and the most dangerous and most prevalent of them is B1 [5].

In recent years, administration of natural remedies into poultry diets has received huge attention to minimize toxic effects and impaired liver function in mycotoxins affected birds. It has been shown that certain compounds in herbal medicines are able to prevent lipid peroxidation in biological membranes and increase proliferation of new liver cells to replace the damaged one [6,7] .

Silybum marianum, or milk thistle, is a well-known plant mainly interested in its hepatotoxic extract named Silymarin. It is administration of natural extract into poultry diets has received enormous attention to reduce toxic effects and impaired liver function in mycotoxins affected hens Milk thistle plant had been identified as a good source of various phytochemicals like flavanolignans silybin, silychristine and silydianin, with silybin being the most biologically active [8]. Free radical scavenging and antioxidant properties of silymarin have concerned more attention in poultry researchers where they have confirmed that silymarin restored the endogenous antioxidant enzymes (Superoxide dismutase, Glutathione peroxidase and Catalase) and non-enzymatic antioxidants (vitamins E and C) in the liver of the stressed laying hens [9,10,11] .

In addition Silymarin as an excellent antioxidant, inhibiting lipid peroxidation due to scavenges reactive oxygen ion (ROS) and thereby protect of body cells against ROS ([12]. Free radicals, including the superoxide radical, hydroxyl radical, hydrogen peroxide and lipid peroxide radicals have been implicated in liver diseases, cancer, inflammation and neurodegenerative diseases With all these properties, reveal hepatoprotective effects in human [13,14].

Silymarin can protect against radiation-induced cell-death and DNA damage [15]. Previously known that silymarin if given orally, it prevents the accumulation of external cholesterol in the liver and the development of diet-induced hypercholesterolemia [16]. Silymarin metabolically stimulates hepatic cells and activates the ribosomal RNA synthesis to stimulate protein formation This study aimed to evaluate the Silymarin in reducing harmful effects of aflatoxin in body tissues and improve performance the produced eggs of laying hens [17,18].

2. MATERIAL AND METHODS

a- The animals of the experiment:

The study was performed on 120 laying hens "ISA Brown" strain aged 240 day, weighing 4-4.5 kg of body that were housed in a private poultry farm in Diyala Governorate, Iraq for a period of sixty days from 22 October 2019 until 20 December 2019. All birds were offered feed adjusted at a rate of 4400 gm/day/group. The diets were formulated according to the recommendations of NRC in1994 [19] with received water (*ad libitum*) throughout the experimental period. The feed contaminated with aflatoxin used in this experiment were obtained from a private feed factory in Diyala province and examined in the central laboratories of Veterinary Directorate. The feed exam showed that feed contains (14.6 ppb) of aflatoxin. Birds were randomly divided into three equal groups (40 hens). The first group was fed a basal diet free of aflatoxin. The 2nd group fed on contaminated diet with aflatoxin (14.6 ppb) while the 3rd group fed on the same diet (in 2nd group) with 0.5% of silymarin/kg feed. At age 242 the birds were vaccinated with attenuated IB (4/91) + ND Clone 30 via drinking water. At aged 300 day, ten chickens from each group were selected randomly and slaughtered for the Histopathological examination.

b- Chemicals

1. Milk thistle oil (Silymarin) cold-pressed unrefined, pure-oils and-crude-dietary supplement-product by brand Ol'Vita. Co.UK. 0.9%.
2. Physiological saline solution was purchased from Eczacibasi Chemical Company (Istanbul, Turkey).
3. Formalin (10%) BDH-England.
4. Paraffin wax from local market –Iraq while the stain was Hematoxylin and eosin from BDH-England.

Grossly examination

All macroscopic symptoms observed on birds were fixed and all the readings obtained every ten days were entered, such as the rate of morbidity and the quality of the eggs in terms of size, where the size was classified into 5 groups based on the weight of the egg: Gumbo up 70g, Extra large 64-70 g, Large 56-63 g, Medium 46-55 g and Small down to 46. The shell of the egg was classified into 3 groups based on the quality of the shell as good, thin and rough. Data were analyzed using available software (IBM SPSS Statistics for windows, version 22, the chi-square test was used for comparisons between variables with a level of significance in lower than 0.05.

Histological examination

Tissue specimens were collected from the liver, intestine, and spleen of the poultry groups at 300 d of age and fixed in 10% neutral buffered formalin solution, the specimens were washed in tap water and then passed through the routine paraffin embedding procedures (dehydration in ascending grades of the ethyl alcohol, clearing in a series of xylene baths and then infiltrated with melted paraffin wax, embedded and put in paraffin blocks). Sections 3-5 microns thick were stained with hematoxylin and eosin. [20].

3. RESULTS

Grossly examination

Abnormal macroscopic symptoms observed on G_2 hens (aflatoxicosis), include decreased feed intake and body weight. In many cases there was an increase in leg problems, abnormal pigmentation (shank, feet) and leg weakness. In some cases, there were anemia and abnormal blood clotting, increased incidence of abnormal behavior bruising and down grading, and nervous syndrome ,any one of these signs submit as morbidity rate . The most frequent effects on eggs were poor egg quality as decreased egg production, reduced egg size, thin or rough egg shell.

The most common pathological lesions associated with aflatoxicosis in chickens were found in liver, spleen and intestine organs. In advanced cases this organ was enlarged, swelling, and has changed colour to yellowish red or pale. Whereas in the silymarin group G_3, the macroscopic changes in these organs were less pronounced and almost invisible.

Observation signs (egg weights, sheet of eggs and morbidity rate) of the three groups Pearson Chi-Square, Value, Asymp. Sig. (2-sided), Chi-Square Tests

Age	%		Control g.	Aflatoxin g.	Sylimarin g.	Chi –square test X2		
						Value	df	Asymp. Sig
250days	Egg weight	Gumbo up 70g	18	-	-	62.757[a]	8	0.001
		Extra larg 64-70 g	15	-	-			
		Large 56-63 g	44	25	-			
		Medium 46-55 g	23	46	-			
		Small down of 46		28	-			
	Sheet of eggs	Good	100	58	80	21.832[a]	4	0.001
		Thin	-	6	5			
		Rough	-	10	15			
	Morbidity			2		4.068[a]	2	0.131
260 days	Egg weight	Gumbo up 70g	17.5	-	15	36.219[a]	8	0.001
		Extra larg 64-70 g	15	-	17.5			
		Large 56-63 g	45	26	40			
		Medium 46-55 g	22.5	46	22.5			
		Small down of 46	-	28	5			
	Sheet of eggs	Good	92.5	64	90	15.253[a]	4	0.004
		Thin	-	15	3			
		Rough	7.5	21	7			
	Morbidity		-	3	-	2.181[a]	2	.336
270 days	Egg weight	Gumbo up 70g	17.5	-	15	36.219[a]	8	0.001
		Extra larg 64-70 g	15	-	17.5			
		Large 56-63 g	45	26	40			
		Medium 46-55 g	22.5	46	22.5			
		Small down of 46	-	28	5			
	Sheet of eggs	Good	92	64	90	15.253[a]	4	0.004
		Thin		15	3			
		Rough	8	21	7			
	Morbidity		-	3	-	2.181[a]	2	0.336
280 days	Egg weight	Gumbo up 70g	17	-	8	79.582[a]	8	0.001
		Extra larg 64-70 g	17	-	15			
		Large 56-63 g	63	11	45			
		Medium 46-55 g	3	40	16			
		Small down of 46	-	49	16.5			
	Sheet of eggs	Good	94	35	85	30.422[a]	4	0.001
		Thin	-	26	5			
		Rough	6	39	10			
	Morbidity		-	15	2	8.728[a]	2	0.013
290 days	Egg weight	Gumbo up 70g	17.5	-	18	89.628[a]	8	0.001
		Extra larg 64-70 g	12.5	-	26			
		Large 56-63 g	67.5	7	48			
		Medium 46-55 g	2.5	30	8			
		Small down of 46	-	63	-			
	Sheet of eggs	Good	92	27	75	52.929[a]	4	0.001
		Thin	3	50	9			
		Rough	5	23	16			
	Morbidity		-	25	3	18.215[a]	2	0.001
300 days	Egg weight	Gumbo up 70g	18	-	9	60.109[a]	8	0.001
		Extra larg 64-70 g	25	-	31			
		Large 56-63 g	40	8	56			
		Medium 46-55 g	10	48	3			
		Small down of 46	7	44	3			
	Sheet of eggs	Good	90	40	84	22.648[a]	4	0.001
		Thin	5	28	8			
		Rough	5	32	8			
	Morbidity		-	37	10	22.637[a]	2	0.001

Histopathology

a- Liver

Although there was no macro or micro lesion observed in the liver of the control after 60 days of treatment, Microscopic examination of liver sections in G_2 group of aflatoxin revealed vascular congestion and dilatation (A), mononuclear cellular infiltration and granulomatous inflammation (B), coagulative necrotic and apoptotic hepatocytes (C) sometimes observed fatty degeneration of hepatocytes areas of necrosis surrounded by multinucleated giant cells (D), large basophilic intra-nuclear inclusion bodies in hepatocytes compatible prominent evidence of pathological lesions(E), such as necrosis. Necrosis was not observed in silymarin group ($G_{3)}$ but mild mononuclear cellular infiltration and apoptotic cells were seen when compared with other groups (G_1,G_2) (H&E X 400).

b- Spleen

In spleen, there was no visible or micro lesion observed in the spleen of G_1or G_3 groups but in G_2 there was coagulative necrosis (F), lymphocyte depletion, and hemorrhages in follicles, interstitial edema (G).

c- Intestine

There was no histopathological changes observed in the intestine of the birds in the control group,. On the otherwise in G_2 were observed sever mononuclear cellular infiltration(H) and apoptotic cells disruption of villi within epithelial cells of atrophic villi(I), fusion of villi and goblet cell hyperplasia (J) .

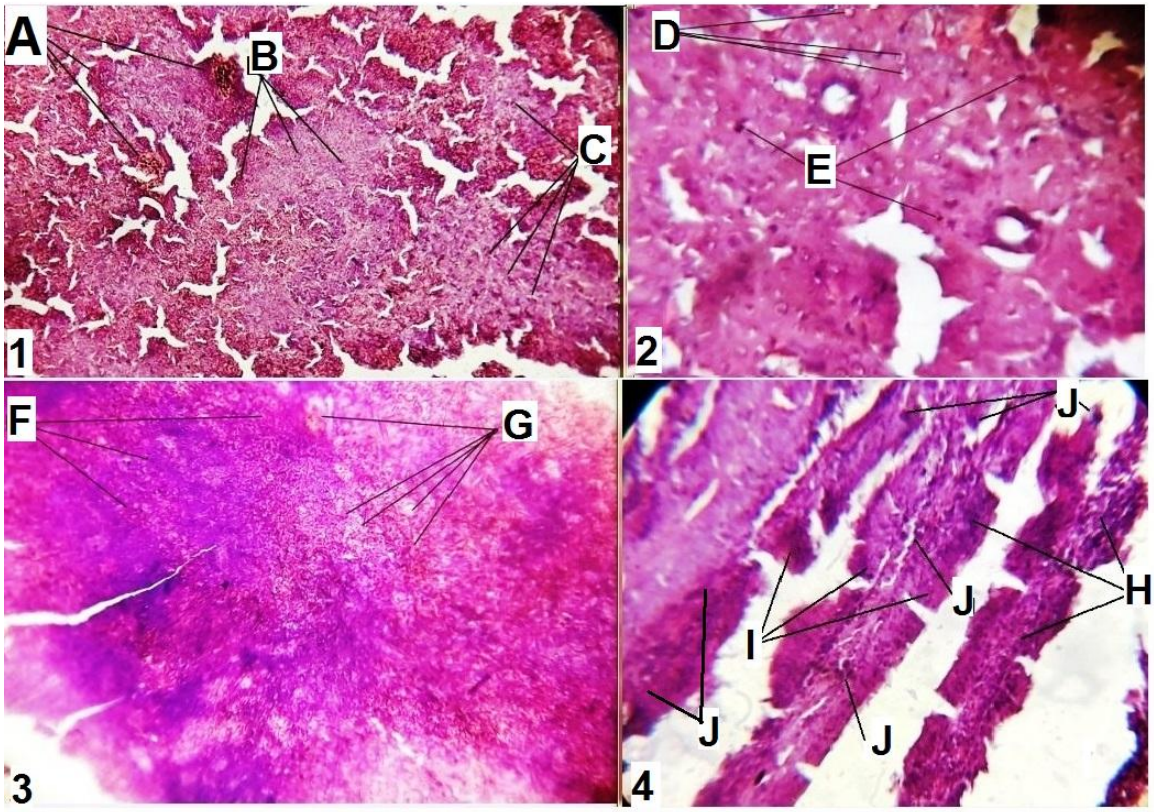
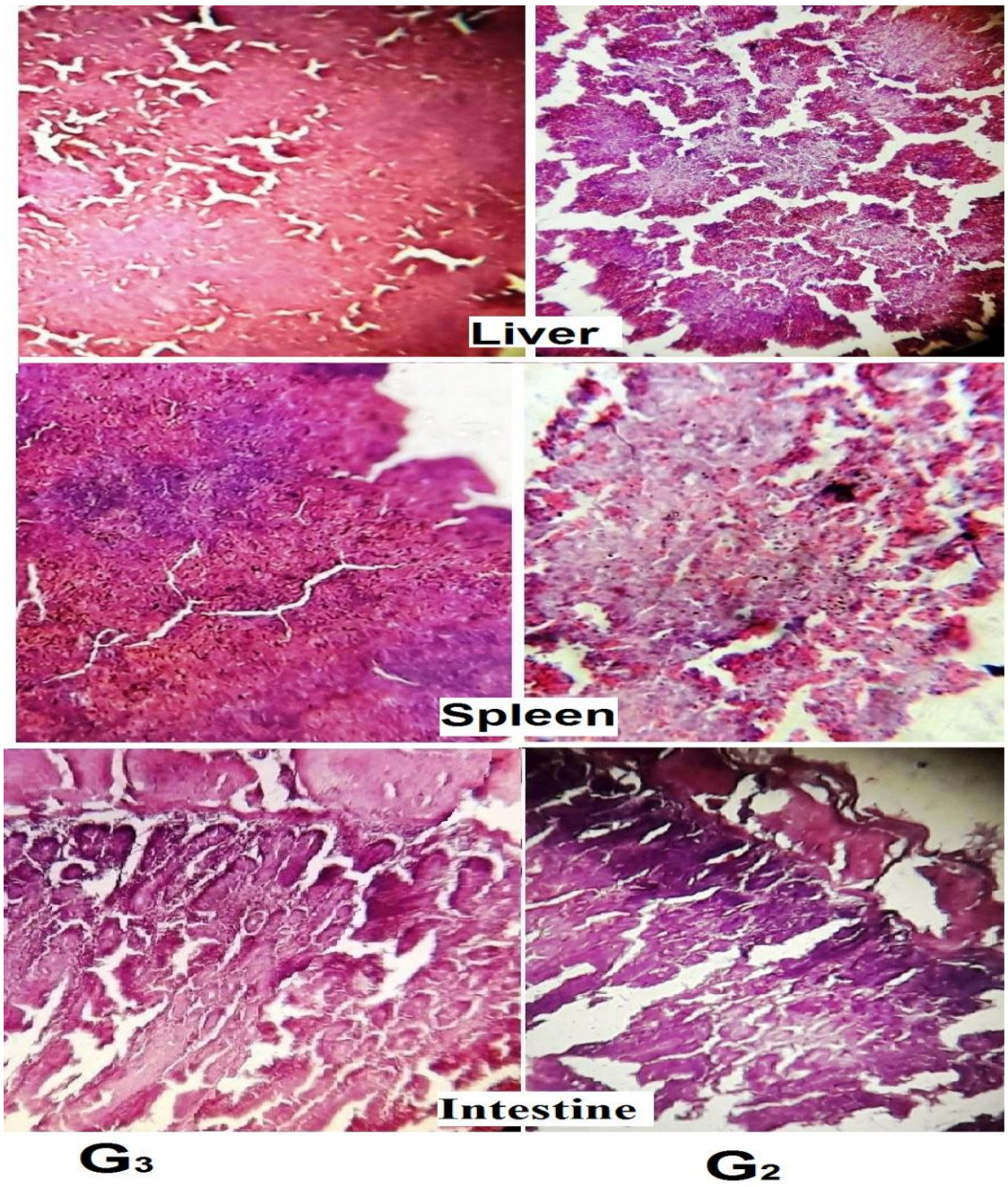


Figure 1: Histopathology sections of Liver, Spleen and Intestine:
1 X100 & 2 X400 Liver: in G_2 group of aflatoxin revealed vascular congestion and dilatation (A), mononuclear cellular infiltration and granulomatous inflammation (B), coagulative necrotic and apoptotic hepatocytes (C) fatty degeneration of hepatocytes areas of necrosis surrounded by multinucleated giant cells (D), large basophilic intranuclear inclusion bodies in hepatocytes compatible prominent evidence of pathological lesions(E). Photomicrograph (H&E)
3:Spleen: in G_2 group there was coagulative necrosis (F), lymphocyte depletion, and hemorrhages in follicles, interstitial edema (G). Photomicrograph (H&E X 400)
4:Intestine: in G_2 group were observed sever mononuclear cellular infiltration(H) and apoptotic cells disruption of villi within epithelial cells of atrophic villi and reduce the surface (I), , fusion of villi and goblet cell hyperplasia (J) Photomicrograph (H&E X 400).



The common histopathological lesions in three organs (liver, intestine and spleen) such as necrosis and congestion or yellow due to lipid accumulation haemorrhages and hyperplasia or alteration in some cell in G2 (aflatoxinosis) and the repairing to all these histopathological changes by a diet that includes silymarin in G3

4. DISCUSSION

In the current study the negative impacts of aflatoxin on laying hens, was observed various unhealthy signs and low performance on the layers in G2 including bad quality of eggs, decrease egg production and high morbidity rate. The research have been considered since the early development of the poultry industry and huge number of reports have been appeared in literature that aflatoxin had adversely affects on weight gain, feed intake, feed conversion efficiency, pigmentation, processing yield, egg production and may be cause recurrent infection as a result of immunity suppression, gastrointestinal dysfunction, reduced reproductivity, anemia, and jaundice [20].

Aflatoxin type B1 adversely influences egg quality by decreasing shell thickness, egg weight and egg energy deposition. In addition, aflatoxin in laying hen feed can result an aflatoxin residue in the eggs (feed to egg aflatoxin B1 transmission ratio was approximately 5000:1); therefore it is very important to control aflatoxin concentrations in feeds for laying hens. Increasing feed Aflatoxin B1 concentration lead to residue in laying hens tissues and organs like liver, kidney, legs, and gizzard [1].

Aflatoxicosis is primarily a hepatic disease, in this study was acute like liver damage, liver cirrhosis [21, 2]. The gastrointestinal tract is the first site where contact and absorption of feed that contaminated with aflatoxin and should be expected to be affected by aflatoxin with greater potency as compared to other organs. However, this aspect of aflatoxicosis is the often neglected area of mycotoxin research and available literature, But the histological changes that occur in the villi and crypts can be an indication of the potency of a toxic effect on the products which consumed by humans. In this regard, the fusion of villi and goblet cell hyperplasia has been reported after 60 days of dietary exposure to Aflatoxin however noted catarrhal enteritis with lymphocytic or mononuclear cell infiltrations in the intestine. Supported by the recent observations regarding aflatoxicosis in layers [22,23]

The Chicken spleen is the principal organ of systemic immunity. It considered as a second the largest lymphoid organ after the primary lymphoid organs like thymus and bursa. Spleen has a very complex lymphatic and vascular organization and has multiple functions.This organ important in disease resistance and is the major source of antibody production. It is the main organ for the proliferation of plasma cells which takes place in the red pulp into the chicken spleen. It carries APCs (antigen presenting cells). It also plays a significant role in maintaining the immunologic homeostasis and tissue regeneration processes. The pathological effect in this study of aflatoxin on the spleen such as cell necrosis, lymphocyte depletion, and haemorrhages in follicles, interstitial edema, is sufficient to disrupt all the above-mentioned functions of the spleen, This is confirmed by many studies in this field [2].

Aflatoxins are carcinogenic, but tumor formation is rare with natural disease, probably because the little period of this study in layers and the animals do not live long enough for this to occur in broilers. so,in this study not induction of tumours , teratogenic and other genetic effects which occurred in chronic exposure to aflatoxin Although there were hyperplasia and alteration in some cells due to Aflatoxin, especially in group G2 as in all three organs intestine when appeared hypertrophy, hyperchromatic irregular direction cells, and excessive vesicular tissue with few mitotic figure. Several studies

have shown that according to FDA Group 2B a possibly carcinogenic to humans, also is demonstrated several study proved the carcinogenicity naturally occur in mixtures of aflatoxins B1, G1 and M1. It is worth noting that there are many cases of animal tumors, around which many questions about their role in the occurrence of tumors in humans are raised, and they are still the subject of controversy and research [24,25].

Silymarin in this study has shown significant effects of efficiency in repairing of aflatoxin spoiled in the tissues of laying hens, as well as, on the production status of chickens in terms of the quantity and quality of eggs and reducing the rate of morbidity. So, harmful factors and elements of aflatoxicosis in poultry involve immunologic, digestive, and hematopoietic and repair all these effects of aflatoxin by silymarin. Previous studies have shown, silymarin has been primarily used in liver disorders including hepatitis, alcoholic liver diseases (ALD) and cirrhosis [26] and is also useful for toxin-induced liver toxicity including poisoning from a fungus called death cap mushroom (*Amanita Phalloides*) [16,27] .

The anti-proliferative, anti-inflammatory, and immunomodulatory of silymarin in this study very tangible. This suggested that silymarin could act, directly at the membrane of hepatocyte. It is known that silymarin exerts antioxidant and membrane stabilizing activities, attributes important for liver secretion and uptake of plasma lipoproteins as previous studies have shown [28,29].

5. CONCLOSION

The presence of mycotoxins in poultry feed can limit losses in the poultry production system. It can be seen the effects of fungal pollution and mycotoxins, which are reflected in the production of poultry. Control depends on the application of policies in agricultural management, production and storage systems, which are the root of the problem. Research in these areas should be developed to reflect better results in economic and productive poultry farming, as well as to improve healthy food for human consumption.
Silymarin was effective in reducing the damage caused by the presence of aflatoxin in experimental animal models. Further work is required to evaluate the potential synergistic effect of silymarin dosing. Thus silymarin can be safely used as an alternative antidote in aflatoxin overdose poisoning. However, further trials are needed to decipher the mechanism underlying the protective effects of silymarin and the dose-dependent effects of silymarin against aflatoxin-induced toxicity in experimental hens.

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