Effect of milk thistle oil (Silymarin) on histopathological characteristics of local hens exposed to aflatoxins

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Abstract

The study aimed to assess Milk Thistle oi reducing the aflatoxin damage and improve the health status of local breed hens. The study was performed on 60 hens aged 35 weeks, weighing 2-2.5 kg of the body was obtained and housed on a private poultry farm in north of Baghdad (Altaji) for a period of 9 weeks from 22 October until 22 December 2020. Hens were divided to three treatments (20 hens). The first was fed a basal diet free of aflatoxin. The 2nd fed on a contaminated diet with aflatoxin (14.6 ppb) while the 3rd fed as in the 2nd with 0.5% of Milk Thistle oil /kg feed. A 5 hens from each group were slaughtered at the end of the trial for the histopathological inspection of liver, spleen and intestine tissues to evaluate the harmful effect of aflatoxin and the repairing role of Milk Thistle. In G2 Aflatoxin group there was leg weakness, lameness, abnormal pigmentation (shank, feet), lethargy, and the crop is free from food. While in the 3rd group, the macro and microscopic examination of the symptoms were less marked and almost invisible. We conclude that silymarin may have a highly effect in repairing the aflatoxin damage to the body tissues of laying hens.

Keywords: Milk thistle, Aflatoxins, Local hens.

Introduction

Mycotoxins found in all crops feed such as maize, peanuts, cottonseed, and tree nuts, which posed challenges for poultry farmers as well as caused huge economic losses in the world since the 1960ss in bird health, immune response, and production performance (3 and 9). Aflatoxin is one of the most important types of mycotoxins, held throughout the digestive system rapidly, extend during the body and mainly metabolized in the liver, then convert 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 (15). 18 types of aflatoxins have been discovered, including four main types B1, B2, G1, G2 , The most dangerous and prevalent of them is B1 (14).

In recent years, used of natural remedies in poultry diets to minimize toxic effects of mycotoxins. It has been shown that certain compounds in herbal medicines are able to prevent lipid peroxidation in biological membranes and increase the proliferation of new liver cells to replace the damaged ones. Milk thistle (MT) or *Silybum marianum*, is a well-known plant mainly interested in its hepatotoxic extract named Silymarin (4). For It is a natural additive to poultry diets 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 sallying, silychristine and silydianin, with sallying being the most biologically active (2). Radical scavenging and antioxidant properties of silymarin have given more attention in poultry research 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 (14).

By the way an excellent antioxidant effect of Milk thistle, it inhibits lipid peroxidation due to scavenge reactive oxygen ions (ROS) and thereby protects body cells (11). 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 (7). In addition to Milk thistle can protect against radiation-induced cell death and DNA damage after radiotherapy of cancer patients (13). Previously these toxins negatively affect the body, such as: Weight gain, feed intake, and performance status Chicken besides biochemical disorders and Immunity strength. However, herbal food supplements are now working to get rid of such cases (16). Milk thistle metabolically stimulates hepatic cells and activates the ribosomal RNA synthesis to stimulate protein formation. These findings suggest that Milk thistle might be used in chickens to prevent the toxic effects of AFB1 originating from contaminated feed. Effects of Milk thistle on higher body weight and increased hatchability were demonstrated in chickens and turkeys. It also prevented excessive adipose in birds. The Hepatoprotective effects of Milk thistle were also proved by biochemical and histopathological examinations (17 and 18). Therefore, this study aimed to evaluate milk thistle in

reducing the harmful effects of aflatoxin in body tissues (liver, spleen and intestines) of local breed hens.

Materials and Methods

The animals of the experiment:

The study was performed on 60 local breed hens aged 35 weeks, weighing 2-2.5 kg of the body that were obtained and housed on a private farm in Baghdad, for a period of 9 weeks from 22 October to 22 December 2020. All birds were offered feed adjusted at a rate of 4400 gm/day/group. The diets were formulated according to the recommendations of NRC (1994) with received water (*ad libitum*) throughout the experimental period. In this experiment the feed of groups two and three contaminated with aflatoxin were obtained from a private feed factory in Baghdad 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 treatments (20 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 as (in 2^{nd} group) with 0.5% of silymarin the active component of milk thistle to 1kg of feed. At age 44 weeks, 5 chickens from each group were selected randomly and slaughtered for the Histopathological examination.

Histological examination

Tissue specimens were collected from the liver, intestine, and spleen of the poultry groups and treatment as mentioned by authors Bancroft and Marilyn in 2002 to obtain tissue sections for histopathological assessment (5).

Results and Discussion

Gross examination

The poultry

Abnormal macroscopic symptoms observed in G_2 hens (aflatoxicosis), include leg weakness, lameness, lethargy, and the crop is free from food … figure 1- a. While in the 3rd group, the macroscopic examination of the symptoms was less marked and almost invisible.

The organs (liver, spleen and intestine)

The most common pathological lesions associated with aflatoxicosis was enlarged in the liver, spleen and intestine organs. In advanced cases gross changes including fatty degeneration with variable areas of petechial haemorrhages, Congestion, fibrosis and large whitish focus of necrosis …. Figure 1-b. Whereas in the silymarin group G_3, the macroscopic changes in these organs were less pronounced and almost invisible..

Histopathology

Liver

There was no micro lesion observed in the liver of the control G_1 after 9 weeks of trial, but microscopic examination of liver sections in the G_2 group of aflatoxin revealed vascular congestion and dilatation (A), mononuclear cellular infiltration and granulomatous inflammation (B), coagulative necrotic and apoptotic hepatocytes (C) … Figure 2 A,B,C.). Also 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 necrosis lesions (E). Necrosis was not observed in silymarin group ($G_{3)}$ but mild mononuclear cell infiltration and apoptotic cells were seen when compared with other groups (G_1,G_2) … Figure 2 D,E,F (H&E X 400).

a- 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, hemorrhages in the follicles and interstitial edema (G) … Figure.2 F, G.

b- Intestine

There were no histopathological changes observed in the intestine of the birds in the control group. On the otherwise in G_2 were observed severe mononuclear cell infiltration(H) and apoptotic cells disruption of villi within epithelial cells of atrophic villi (I), a fusion of villi and goblet cell hyperplasia (J) … Figure 2 G, H, J and Figure 3

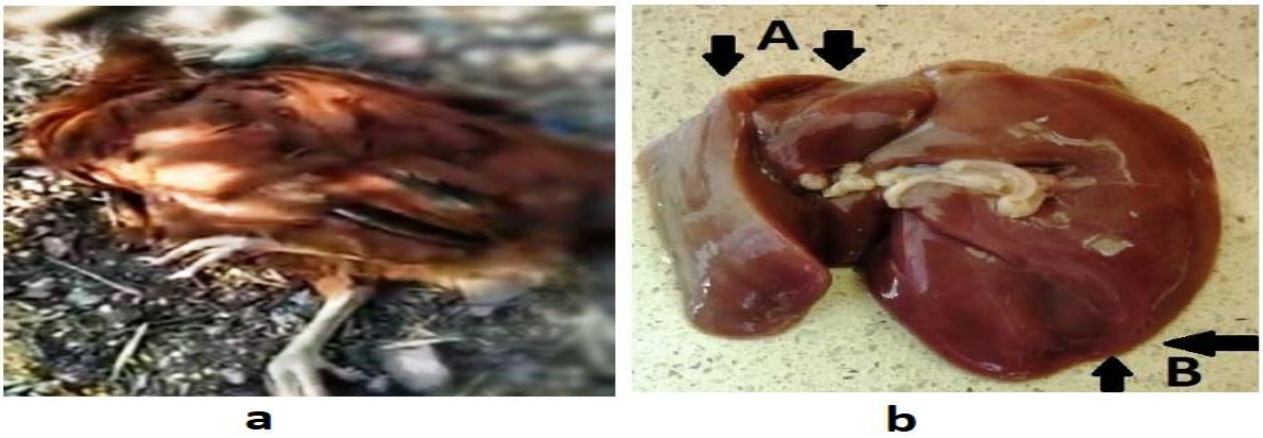

Figure 1:Abnormal macroscopic symptoms observed in G2 hens (aflatoxicosis):

a- Case of leg weakness, lameness lethargy , and the crop is free from food.
b- Gross patho changes in liver of local hens. (A) paleness and yellow discoloration (B) haemorrhages on liver (arrows).

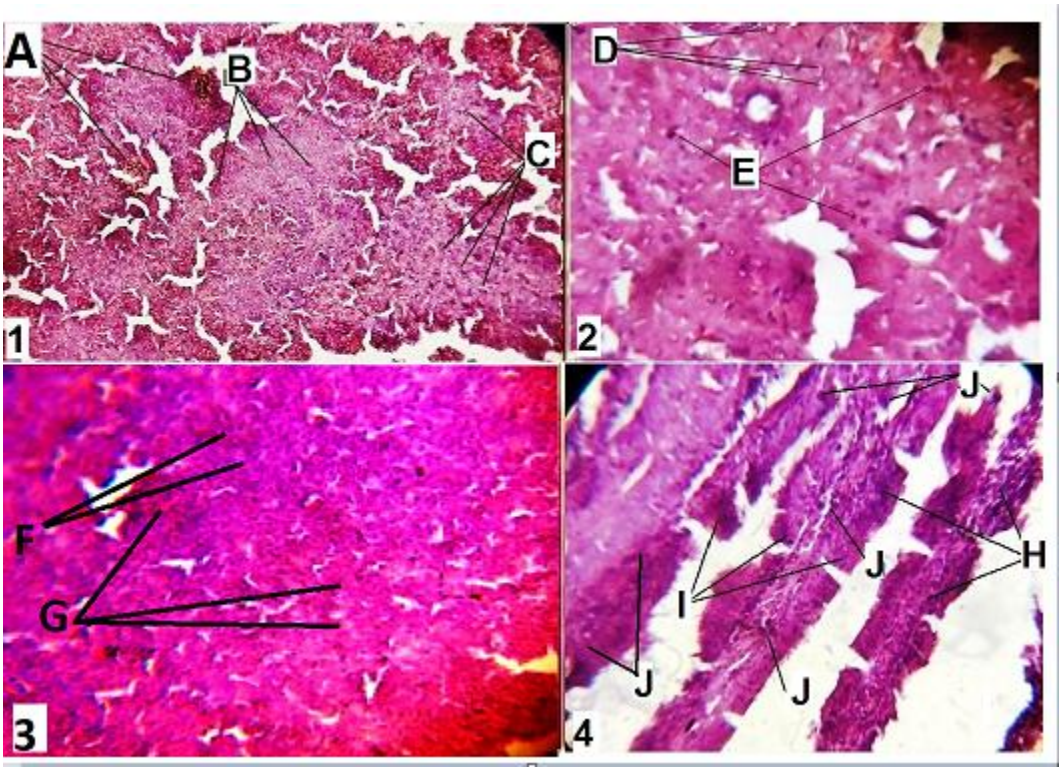

Figure 2: Histopathology sections of Liver, Spleen and Intestine in G_2 group:

1 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) X100 & 2 X400.

3: Spleen: in G_2 group there was coagulative necrosis (F), lymphocyte depletion, and hemorrhages in the follicles, interstitial edema (G). Photomicrograph (H&E X 400)

4: Intestine: in the G_2 group were observed severe mononuclear cell infiltration (H) and apoptotic cells disruption of villi within epithelial cells of atrophic villi andreduce the surface (I), a fusion of villi and goblet cell hyperplasia (J) Photomicrograph (H&E X 400).

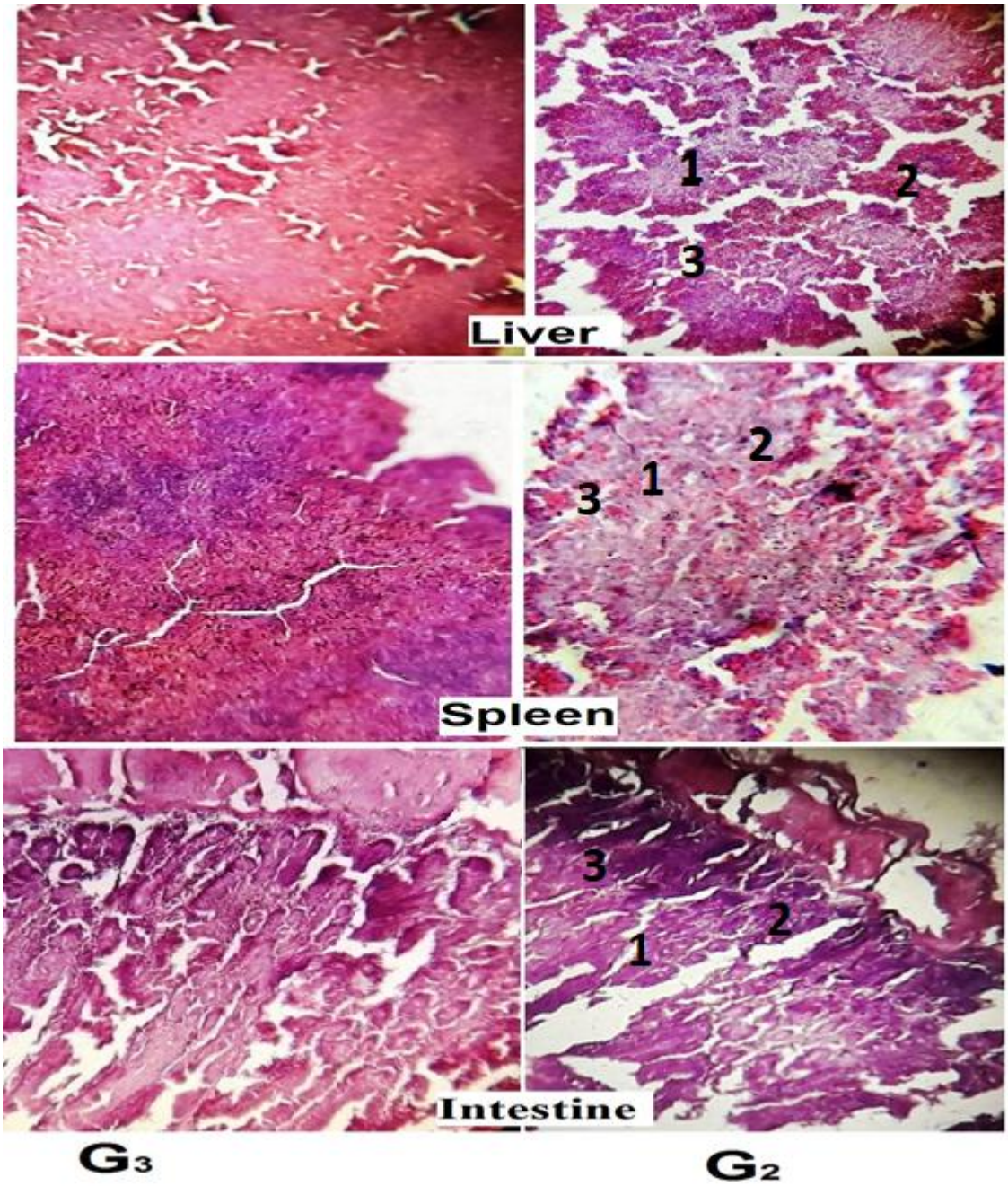

Figure 3: The histopathological lesions in (liver, spleen and intestine):
1: lymphocytic or mononuclear cell infiltrations, 2: necrosis and congestion or yellowing due to lipid accumulation haemorrhage and 3: hyperplasia or altered in some cells in G2 (aflatoxinosis) besides G3: the repairing to all these histopathological changes by a diet that includes silymarin.

A flatoxicosis is a hepatic disease caused liver damage and cirrhosis, but the gastrointestinal tract is the first site where contact and absorption the contaminated feed with aflatoxin and affected. The fusion

of villi and goblet cell hyperplasiahad reported after 9 weeks of dietary exposure to Aflatoxin, its noted catarrhal enteritis with lymphocytic or mononuclear cell infiltrations in the intestine. However, this part habitually was neglected by mycotoxin researchers, but the histopathological changes of villi and crypts can indicate the potential toxic effect on the product which consumed by humans. (6).

The hen's spleen is the principal organ of systemic immunity. It is considered the second largest lymphoid organ after the primary lymphoid organs (thymus and bursa). This study with previous studies confirmed that spleen has a very complex lymphatic and vascular system also has multiple functions in diseases resistance and it's the major source of antibody production. Spleen is the main organ for the proliferation of plasma cells which takes place in the red pulp, this cells carries antigen presenting cells (APCs) and it plays a significant role in maintaining the immunologic homeostasis and tissue regeneration processes (10).

The histopathological assessment of group G_2 appeared hyperplasia in all liver, spleen and intestine perform as alteration in some cells, hyperchromatic irregular direction cells, excessive vesicular tissue and sometime few mitotic figure due to aflatoxin, these features may be consider as pre-cancerous lesions. Teratogenic and other genetic effects occurred in chronic exposure to aflatoxin. Several studies have shown that according to the FDA group B2 of aflatoxin a possibly carcinogenic to humans, also demonstrated several studies proved that carcinogenicity occurs naturally in mixtures of aflatoxins B1, G1 and M1 (1, 8).

The harmful effects of aflatoxicosis in poultry involve immunologic, digestive, and hematopoietic defects were repaired by milk thistle. Previous comparative medicine studies have shown, that milk thistle may be used in liver disorders including hepatitis, alcoholic liver diseases and cirrhosis (12).

Conclusion

Milk thistle is effective in reducing the damage caused by aflatoxin in poultry. The presence of mycotoxins in poultry feed cause large losses in production. To control this problem the efforts must be duplicate and improve the policies in agricultural management, production and feed storage systems. Milk thistle oil product (silymarin) can be safely used as an alternative antidote in aflatoxin overdose in local hens breed.

Conflict of interest

The authors have no conflict of interest.

References

1- **Ahmed, M.A.E.; K. Ravikanth; D. S. Rekhe and Maini, S. 2009**. Histopathological alterations in Aflatoxicity and its amelioration with herbomineral toxin binder in broilers. Veterinary World, 2(10): 390-392.

2- **Albadrany Y.M.; A. S. Naser and Hasan M.M. 2021.**Study the analgesic effect of diclofenac and silymarin Coad ministration in chicks. Iraqi Journal of Veterinary Sciences, 35: 25-31, Supplement I. DOI: 10.33899/ijvs.2021.127065.1453

3- **Al-Taee, Z. and M. Gh. Saeed. 2023**. Correlation incidence between infectious bursal disease and aflatoxicosis in broilers chicken farms in Nineveh province, Iraq. Iraqi Journal of Veterinary Sciences, 37(1): 183-190. DOI: 10.33899/ijvs.2022.133881.2315.

4- **Bagherzadeh K.F. and M. Mehri. 2015**. Effects of a multi-strain probiotics against aflatoxicosis in growing Japanese quails. Livestock Science, 177:110–116. https://doi.org/10.1016/j.livsci.2015.04.018

5- **Bancroft, J. D. and G. Marilyn.2002.** Theory and Practice of Histological Techniques, 5th ed. Churchill-Livingstone Publication. London. UK.

6- **Bennett, J. W. and M. Klich. 2003**. Mycotoxins. Clinical Microbiology Reviews, 16 (3): 497-516. DOI: 10.1128/CMR.16.3.497-516.

7- **Federico A.; M. Dallio and Loguercio C. 2017**. Silymarin/silybin and chronic liver disease: a marriage of many years. Molecules.22:191. **https://doi.org/10.3390/molecules22020191**

8- **Gowda, N. K. S.; D. R. Ledoux; G. E. Rottinghaus; A. J. Bermudez and Chen, Y.C. 2008**. Efficacy of turmeric (*Curcuma longa*), containing a known level of curcumin, and a hydrated sodium calcium aluminosilicate to ameliorate the adverse effects of aflatoxin in broiler chicks. Poultry Science, 87:1125–1130. https://doi.org/10.3382/ps.2007-00313

9- **He, J.; K. Y. Zhang; W. Chen; M. Ding; D. Feng and Ao, X. 2013**. Effects of maize naturally contaminated with aflatoxin B1 on growth performance, blood profiles and hepatic histopathology in ducks. Livestock Science, 152:192–199. https://doi.org/10.1016/j.livsci.2012.12.019

10- **Kannan, T.A.; S. Ramesh Geetha; S. Ushakumari; G. Dhinakarraj and Vairamuthu S. 2017.** Age related changes in T cell subsets in thymus and spleen of layer chicken (*Gallus domesticus*). International Journal of Current Microbiology and Applied Sciences, 6(1): 15-19. doi: http://dx.doi.org/10.20546/ijcmas.2017.601.002

11- **Kshirsagar, M.; V. Mahash; P. Srinivas and Mangala, L. 2013**. Evaluation of the protective effect of silymarin on doxorubicin induced chronic testicular toxicity in rats. International Journal of Pharmacy and Biological Sciences, 4: 473 – 484.

12- **Makki, F.; N. Omidi, Afzali; H. Sarir; M. Frouzanmehr and Shibak, A. 2014.** Efficacy of *Silybum marianum* seeds in ameliorating the toxic effects of Aflatoxin B1 in Broilers. Iranian Journal of Toxicology, 8(24): 977-982. http://ijt.arakmu.ac.ir/article-1-293-en.html

13- **Manish, A.; D. Atlar; A. Jawahar; I. Veselin; S. Vijay; Ch. Raman; K. Raj; S. Rakesh; K. Yana; G. Veselina and Rajesh, A. 2013**. *In vitro* studies on radio protective efficacy of silymarin against g - irradiation. International Journal of Radiation Biology, 89(3): 200–211.

https://doi.org/10.3109/09553002.2013.741285.

14- **Mohammed, S. F.; P. Mustafa and Mustafa, S. 2019.** Antioxidant, Antibacterial and Antifungal Activities of Different Extracts of *Silybum marianum* Collected from Duhok (Iraq). International Journal of Secondary Metabolite, 6(4): 317–322. https://doi.org/10.21448/ijsm.581500

15- **Pappas, C.; E. Tsiplakou; D. I. Tsitsigiannis; M. Georgiadou; K. Iliadi; K. Sotirakoglo and Zervas G. 2016**. The role of bentonite, binders in single or concomitant mycotoxin contamination, of chicken diets. Br. Poultry Science, 57(4): 551–558. https://doi.org/10.1080/00071668.2016.1187712

16- **Sawale, K.; C. Gosh; K. Ravikanth; S. Maini and Rekhe, S. 2009**. Experimental mycotoxicosis in layer induced by ochratoxin A and its amelioration with herbomineral toxin binder 'Toxiroak'. International Journal of Poultry Science, 8(6): 798-803. DOI: 10.3923/ijps.2009.798.803

17- **Sumit, R.; Ji. E. Kim and Jr, R. C. 2010.** Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. Research in Veterinary Science, 89(3): 325-331. https://doi.org/10.1016/j.rvsc.2010.04.011

18- **Taleb, A.; A. Ahmad; U. Ihsan; J. Qu; N. Lin; K. Hezam; N. Koju and Hui L. 2018.** Antioxidant effects and mechanism of silymarin in oxidative stress induced cardiovascular diseases. Biomedicine and Pharmacotherapy, 102(6): 689-698. https://doi.org/10.1016/j.biopha.2018.03.140

19- **Tedesco, D.; S. Steidler; S. Galletti; M. Tameni; O. Sonzogni and Ravarotto L. 2004.** Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B[1] in broiler chicks. Poultry Science, 83(11): 1839-1843. https://doi.org/10.1093/ps/83.11.1839