Original Research

Experimental Study on Pathology of Aflatoxicosis in Broiler Chicks and Its Amelioration by *Emblica officinalis* (Amla) Supplementation

R. S. Khetmalis[1*], B. K. More[2], C. S. Mote[2], S. N. Jadhav[3], B. P. Kamdi[2] and V. S. Dhaygude[4]

[1]Division of Pathology, ICAR- IVRI, Izatngar, Bareilly- 243122, Uttar Pradesh, INDIA
[2]Department of Veterinary Pathology, K.N.P. College of Veterinary Science, Shirwal -412801, INDIA
[3]Department of Veterinary Biochemistry, K.N.P. College of Veterinary Science, Shirwal -412801, INDIA
[4]Department of Veterinary Pathology, B. V. C. Parel, Mumbai - 400012, Maharashtra, INDIA

***Corresponding author:** dr.rhushiksh.k@gmail.com

Rec. Date:	Mar 27, 2018 17:26
Accept Date:	May 24, 2018 16:51
DOI	10.5455/ijlr.20180327052604

Abstract

An experiment was conducted to study the protective role of herb Emblica officinalis in induced aflatoxicosis in broilers. Thirty day old broiler chicks divided into three groups. Birds of group T0 were fed with standard basal rations. Group T1 birds were fed with standard feed mixed with aflatoxin (AF) B1@150 ppb and group T2 birds were fed with standard feed mix with AFB1@150 ppb and E. officinalis @5 g/kg of feed. Skin thicknesses were measured in six birds from each group on 21st day for cell mediated immune response. Group T1 and T2 showed significant differences in weight gain and significant reduction in cell mediated immune response was noted as compared to T0 group. Gross examination of group T1 birds showed yellowish discoloration of liver and hepatomegaly, mild lesions in kidneys. Microscopically, liver parenchyma showed moderate hepatic derangement, focal formation of microgranuloma, multiple foci of hepato-cellular swelling, necrosis in some hepatocytes, congestion of blood vessels, mononuclear cells infiltrations around blood vessels, focal bile duct hyperplasia and sinusoidal aggregation of erythrocytes. In T2 group birds, microscopically liver, kidney, spleen and bursa of Fabricius showed mild changes as compared to T1. Present study suggest that supplementation of herb E. officinalis could reduce aflatoxin toxicity in broilers.

Key words: Aflatoxin (AF) B1, Broiler, Cell Mediated Immune Response, *Emblica officinalis*, Hepatomegaly

How to cite: Khetmalis, R., More, B., Mote, C., Jadhav, S., Kamdi, B., & Dhaygude, V. (2018). Experimental Study on Pathology of Aflatoxicosis in Broiler Chicks and Its Amelioration by *Emblica officinalis* (Amla) Supplementation. International Journal of Livestock Research, 8(10), 287-297. doi: 10.5455/ijlr.20180327052604

Introduction

DOI 10.5455/ijlr.20180327052604

Indian poultry sector has made remarkable progress during last few decades growing from backyard farming to large commercial industry. Consequently the modern farming practices to fulfil the increasing demand of poultry birds are making birds prone to various infections and toxicities due to contamination of feed and water that produces great economic losses. Contamination of feed with mycotoxins is major problem (Mohanamba *et al.,* 2007) encountered in poultry industry. Aflatoxin (AF) is most common mycotoxin produced by fungi *Aspergillus flavus* and *Aspergillus parasiticus* which contaminating the grains and cereals at any time before and or after or during harvesting, storage, transportation and processing of feed ingredients. Among aflatoxin B1, B2, G1, and G2, AF B1 is most toxic. Metabolites of AF are stable and resistant to degradation (Park, 2002; Desphande, 2002). Consumption of AF contaminated feed causes aflatoxicosis in poultry characterized by reduced feed intake, weight gain, feed utilization (Bailey *et al*., 2006; Shi *et al.,* 2006, 2009), increased susceptibility to environmental, microbial stresses and mortality (Leeson *et al.,* 1995 ; Jand *et al*., 2005). Aflatoxin is responsible for damage of liver, kidney and impaired immunity (Ibrahim *et al.,* 2000; Oguz *et al.,* 2003). AFB1 has immunosuppressive properties by affecting cell-mediated immunity (Meissonnier *et al.,* 2008). Chronic exposures of chicken to aflatoxins depress the phagocytic efficiency and the delay hypersensitivity reactions in birds (Kadian *et al.*, 1988). AFB1 at low dose decrease both mRNA and protein levels of lymphocytic IL-2, IFNγ and affected macrophage functions along with IL-1α, IL-6 and TNF production (Giambrone, 1978 and Dugyala and Sharma, 1996). Also AF causes deleterious effect by producing free radicals that is injurious to DNA, protein and lipid membrane (Yang *et al.,* 2000). This deleterious effect of free radical could be effectively ameliorated by the antioxidant defence of the body and thus controlling aflatoxicosis (Yarru *et al.,* 2009).

Use of chemical growth promoters and immunomodulator has been criticized due to adverse residual effects on consumers. There is an increase in demand for organic meat and eggs. Also practical and cost effective methods to detoxify mycotoxin present in feed stuffs on a larger scale basis are not available. In view of this, herbal and plant derivatives might be a valuable alternative to promote growth, immunomodulation and health in poultry as there is no residual toxicity. Wide use of *E. officinalis* in the Ayurveda is believed to increase defence against diseases. It has beneficial role in treatment of cancer, diabetes, hepatic and cardiac ailments, intestinal ulcers, anaemia and various other diseases. Also, it used as an antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective agent (Bhagat, 2014). Hence, considering the above facts, the present study was planned to study the preventive role of *E. officinalis* supplementation on Aflatoxicosis.

Material and Method

Chicken Management and Diet

DOI 10.5455/ijlr.20180327052604

Study was conducted in thirty day-old Ven-Cobb-400 broiler chicks. The chicks were divided into three equal groups with ten birds in each group having similar body weight within a group as well as between groups. Standard managemental procedures were followed throughout the course of experiment. The given diets were free of any growth promoter, toxin binders, antibiotic or known contaminants that would interfere with the study objectives. The ration was formulated as per BIS (2007) specification for broiler chicken.

Aflatoxin B1

The aflatoxin used in present study was procured from HiMedia lab, India. Pure crystalline AFB1 was incorporated into the diets by dissolving AFB1 in chloroform (1mg/10mL) followed by mixing the solution with appropriate quantities of ground feed in experimental groups (Denli *et al.,* 2009; Kaoud, 2013). The premix feed was kept overnight at room temperature for the solvent to evaporate and was then mixed into the basal diet to provide the desired level of 150 ppb AF B1/ kg of feed.

***Emblica officinalis* (Amla) Powder**

Commercially available *E. officinalis* powder was purchased from local ayurvedic market. The desire level of 5g *E. officinalis* /kg of feed was obtain by well mixing and homogenizing feed before offering to birds.

Experimental Groups

The birds of group T0 were kept as control group and was fed on basal diet without any supplementation throughout experimental period, birds of group T1 were fed basal diet supplemented with AF B1 @150 ppb per kg of feed from day eight onwards till the end of experiment and birds of group T2 were fed basal diet supplemented with *E. officinalis* @ 5 g/kg of feed +AF B1 @150 ppb per kg of feed from day eight onwards till the end of experiment. During experiment all the clinical signs were noted.

Organ Weights and % Organ Weight to Body Weight Ratios (Weight Index)

The live body weights (g) of six birds from each treatment were recorded just before slaughter. Six birds from each treatment were sacrificed by humane method at the end of experiment. Visceral organs viz. Liver, heart, bursa of Fabricius and spleen were dissected out from carcass and weighed and weight index was calculated as percentage of the weight of visceral organ to the total body weight of bird.

Cell Mediated Immune (CMI) Response by 2, 4-Dinitrochlorobenzene (DNCB)

Skin contact sensitivity by DNCB was used to asses CMI in different group. Test in each group was performed on 21st day described by More (1996) and Tiwari and Goel (1985). The skin thickness was measured before and 24, 48 and 72 hours after sensitization. One per cent (1%) solution of 1-Chloro-2, 4-dinitrobenzene (HiMedia) in acetone was prepared by dissolving one gram in 100 ml acetone and used to

DOI 10.5455/ijlr.20180327052604

assay cell mediated immune response. DNCB (0.1 ml of 1 percent) was injected to six birds of each group intra-dermally at inter-digital space between third and fourth digit of right leg using one ml tuberculin syringe. This was allowed to dry immediately by blowing so as to avoid the solution running down the sides. The thickness of the skin at the site was measured using digital slide Vernier calliper before challenge (0 hour) and 24, 48 and 72 hours after challenge and expressed in millimetres.

Post Mortem Examination and Histopathology

Representative birds from each group were opened and all the visceral organs were examined for alteration if any. Representative samples of liver, kidneys, heart, spleen and bursa of Fabricius were collected from each group and fixed in 10 percent neutral buffered formalin (NBF). Tissue sections were cut at 3-5µ thickness and stained with routine Haematoxylin and Eosin method (Culling, 1974). Histo-pathological examination was done at the end of experiment.

Statistical Analysis

The means and SE of different parameters in different treatment groups were calculated and the effects of the treatments were analyzed as per Snedecor and Cochran, (1994). The differences between the means were tested at $P \leq 0.05$ by ANOVAs.

Result

The present experiment was conducted to study the effect of *E. officinalis* (Amla) on growth, immunomodulatory response and pathological changes in organs of birds fed with AF B1 in feed.

Growth Performance

Birds from T1 showed slight reduced feed intake, poor growth and depression than control groupT0. T2 group showed improvement on feed intake than T1. No mortality was noted throughout the experimental period in any of the group studied. The birds in group T0 and T2 did not show any clinical signs and were healthy throughout the experiment.

Table 1: Effect of AF (150ppb/kg feed) and *E. officinalis* (5g/kg feed) on Mean live body weight (g) and % relative organ weights at 42 DPT

S. No.	Parameter	T0	T1	T2
I	Live wt of birds*	2029±1.0	1918±1.41	1965±2.17
II	Spleen (%)*	0.163^{a}	0.135^{c}	0.151^{bc}
III	Liver (%)*	2.562^{b}	3.044^{a}	2.78^{ab}
IV	Bursa (%)*	0.139^{a}	0.109^{c}	0.124^{b}
V	Heart (%) **	0.53	0.51	0.522

***Significant; **Non- significant*

 DOI 10.5455/ijlr.20180327052604

Growth, Live Body Weights (g) and Relative Organ Weights (%)

The average live body weights of broiler chicken at six weeks of age recorded for the treatment T0, T1 and T2 were 2029±1.0, 1918±1.41 and 1965±2.17 gm respectively (Table 1). The average live body weight of broiler chicken fed diet supplemented with AF B1 (T1) was significantly (P<0.05) lower than the broiler chicken fed on basal diet (T0) and diet supplemented with AF B1 and *E. officinalis* (T2). The relative weights (%) of liver, spleen, bursa of Fabricius and heart of broiler fed with basal diet (T0) were 2.562, 0.163, 0.139, and 0.530 while in birds fed with aflatoxin (T1) were 3.044, 0.135, 0.109, and 0.510 respectively. The corresponding values in birds supplemented with AF B1 and *E. officinalis* (T2) were 2.78, 0.151, 0.124, and 0.522 (Table 1). The results of present study indicated that the relative organ weights of liver increased significantly (P<0.05) in AFB1 treated group (T1) than other groups, while lymphoid organ weights viz. spleen and bursa of Fabricius were decreased significantly (P<0.05). The relative weight of heart was unaffected in all groups.

Cell Mediated Immune Response –Skin Contact Sensitivity

The skin thicknesses (mm) on 21st day of AF B1 (T1) and AF B1 + *E. officinalis* (T2) group birds were significantly lower (P<0.05) as compared to T0. There was no statistical significant difference (P<0.05) in the mean skin thickness across groups at time point zero. The difference in mean skin thickness between AF B1 (T1) and AF B1+ *E. officinalis* (T2) fed chicks was statistically non-significant at all other time points. The mean skin thickness between AF B1 (T1) or AF B1+ *E. officinalis* (T2) and or control (T0) group was significant (P<0.05) at 24, 48 and 72 hrs. Skin thickness increased slowly and reached maximum at 24 hrs after DNCB challenge and reduced subsequently. Histopathology of skin section at 24 hrs after DNCB challenge revealed congestion and oedema with mononuclear cell infiltration. The study clearly indicated that the AF B1 caused immunosuppression in birds.

Table 2: Mean± S.E of DNCB delayed skin hypersensitivity test result (mm) at 21st day

Time (Hrs)	T0	T1	T2
0	1.426±0.007	1.413±0.009	1.413±0.001
24	2.113±0.015[a]	1.936±0.010[b]	1.946±0.011[b]
48	1.983± 0.045[a]	1.806±0.010[b]	1.816±0.006[b]
72	1.480±0.015[a]	1.426±0.004[b]	1.430±0.007[b]

Means bearing different superscript within the same row differ significantly (P<0.05).

Alteration in Visceral Organs

The six birds were sacrificed randomly from each group on 42 day post treatment (DPT) of the study and the gross changes were noted by conducting systematic post mortem examination. The birds from group T0 and T2 did not reveal any appreciable gross changes in any of the organs studied. The birds from group T1 showed gross pathological changes in visceral organs viz. liver showed slight paleness and was icteric.

 DOI 10.5455/ijlr.20180327052604

However, heart muscles, spleen, kidneys and bursa of Fabricius were without any appreciable gross changes.

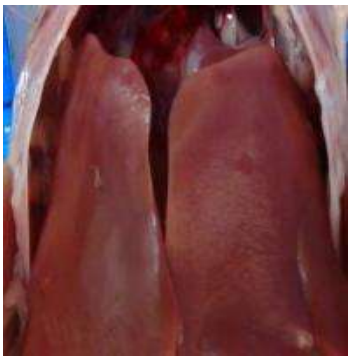

Fig. 1: Normal liver of Control group (T0)

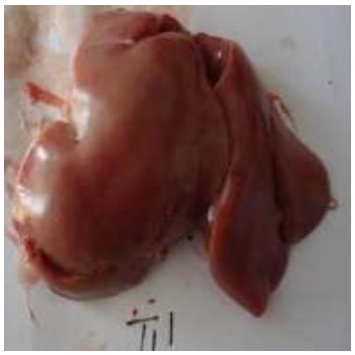

Fig. 2: Icteric liver of AF fed group (T1)

Histopathology

The visceral organs viz., liver, heart, kidneys, spleen and bursa of Fabricius of the birds from all groups sacrificed on 42 day of age of experiment were subjected to histo-pathological examination. The microscopic examination of liver, kidney, spleen, heart and bursa from the birds of control group (T0) showed normal histological features. Mild to moderate histopathological changes were observed in the birds fed diet with aflatoxin (T1). The histopathology of liver showed moderate changes of derangement of hepatic parenchyma, focal formation of microgranuloma, multiple foci of hepato-cellular swelling, vacuolar cytoplasmic changes, loss of nuclei in some hepatocytes, congestion of blood vessels, mononuclear cells infiltrations around blood vessels, focal bile duct hyperplasia and sinusoidal congestion (Fig. 4.). Kidney of these birds showed cellular swelling with degenerative changes (Fig. 6.).

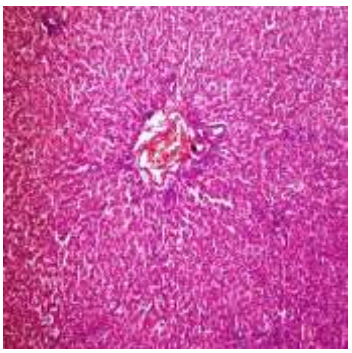

Fig. 3: Group T0 (Control), Liver with normal parenchyma, H&E x 100

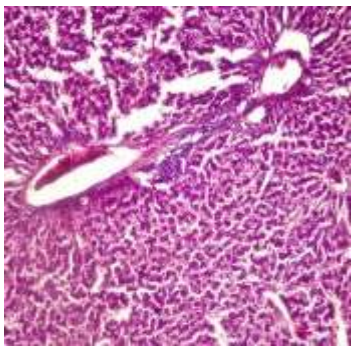

Fig. 4: Group T1 (AFB1 150ppb) Liver showing degenerative, necrotic changes, cellular swelling and bile duct hyperplasia, H&E x 200

Spleen and bursa of Fabricius showed lymphocytic depletion. Whereas, heart did not reveal any observable changes on histo-pathological examination. The histo-pathological examination of liver tissues from birds

DOI 10.5455/ijlr.20180327052604

treated with AF B1 and *E. officinalis* (T2) showed mild histo-pathological changes as compared to T1. The hepatic parenchyma showed focal cellular swelling and congestion with mild degenerative changes in hepatocytes and occasional foci of necrosis (Fig. 5).

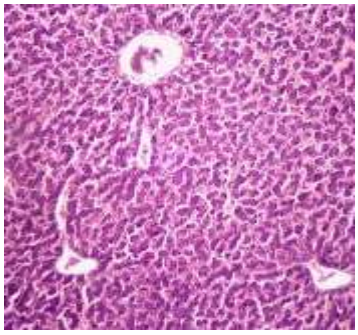

Fig. 5: Group T2 (AFB1 + *E. officinalis*), Liver showing mild cellular swelling, H&E x 200

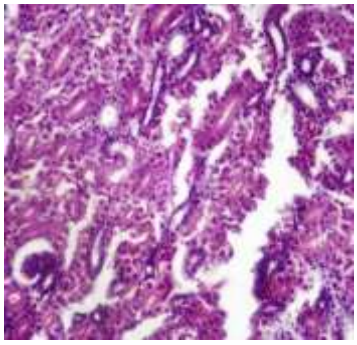

Fig. 6: Group T1 (AFB1), Kidney showing cellular swelling, 100 x H&E

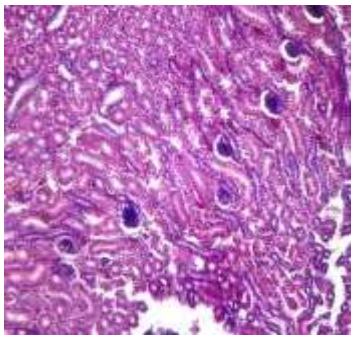

Fig. 7: Group T2 (AFB1+ *E. officinalis*), Kidney with mild cellular swelling, 100 X H&E

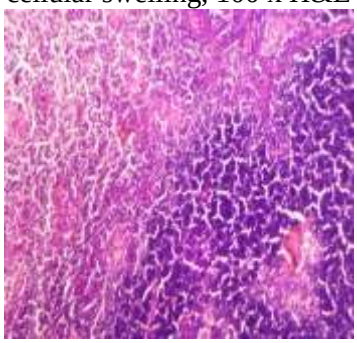

Fig. 8: Group T0 (Control), Spleen showing normal cellular population in red and white pulp, 100 x H&E

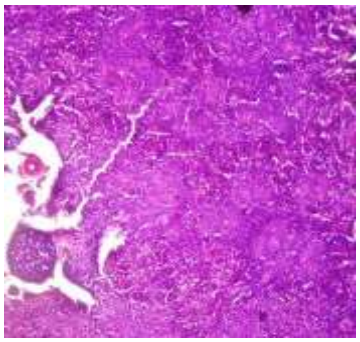

Fig. 9: Group T1 (AFB1), Spleen showing mild depletion of cell population in red and white pulp and intermixing of pulps, 100 x H&E

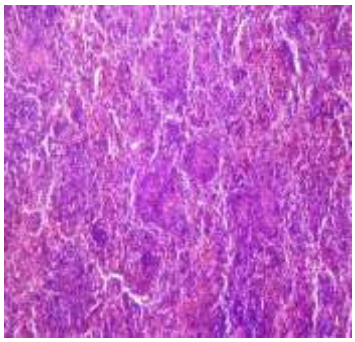

Fig. 10: Group T2 (AFB1 + *E. officinalis*), Spleen showing focal minimal depletion of cell population in red and white pulp, 100 x H&E

DOI 10.5455/ijlr.20180327052604

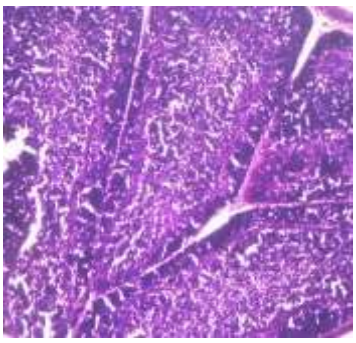

Fig. 11: Group T1 (AFB1) Bursa of Fabricius showing cortico-medullary lymphoid depletion in bursal follicles, 100 x H&E

The kidneys of T2 birds showed mild degenerative changes with cellular swelling (Fig. 7). Spleen and bursa of Fabricius showed mild depletion of cells from parenchyma. Heart sections did not show any observable changes.

Discussion

In the present study body weight, CMI response and various alterations in different visceral organs were taken into consideration to investigate the effect of AF B1 (150ppb/kg of feed) and *E. officinalis* (5 g/kg of feed) supplementation.

The reduction in growth of broiler in AF B1 fed group were in agreement with Campbell *et al*. (1983) and Bakshi *et al*. (2000) who reported declined growth in birds fed with diet containing AF B1. Also Teleb *et al.* (2004) showed a significant decrease in live body weight of chicken fed on diet containing 30 ppb AF B1 for 45 days. The reduced weight gain is due to impaired liver function and decreased utilization of nutrients from feed affected the chicken weight gain and general health (Oguz and Kurtoglu, 2000). Addition of *E. officinalis* (5 g/kg) in feed found to improve the performance of broiler in present experiment was similar to the findings of Sapcota *et al.* (2006) by feeding of *E. officinalis* @ 2.5 g/kg in experimentally aflatoxin (300 ppb). The supplementation of *E. officinalis* in diet of broiler chicken might have improved the relative weights of liver, spleen and bursa of Fabricius due to improved feed efficiency (Sapcota *et al.*, 2006), absolute body weight gain (Maini *et al.*, 2007 and Mode *et al.*, 2009); and free radicals scavenging effect (Kanchana *et al.*, 2013).

The DNCB hypersensitivity test at 21st day showed the lower in skin thicknesses in both of AF B1 fed and AF B1+ *E. officinalis* fed groups indicating lowered CMI responses. Findings of the present study in agreement with the Kalorey *et al.* (2005) who found significant depression of delayed hypersensitivity in chicken fed AF B1@ 200ppb/kg of feed. Aflatoxin inhibits the chemotactic and phagocytic abilities of leucocytes and heterophils, respectively (Giambrone *et al*., 1978a; Ghosh *et al.*, 1991) these immune cells are important in CMI. Aflatoxin also reacts with the T cells and affects the CMI (Thaxton *et al.*, 1974).

 DOI 10.5455/ijlr.20180327052604

AF mainly targets the liver, Kidney, Lymphoid organs viz. spleen and bursa of Fabricius. In this study the relative organ weight of liver was increased significantly (P<0.05) in AFB1 treated group than other groups, while lymphoid organ weights viz. spleen, bursa of Fabricius were decreased significantly (P<0.05) was similar to the finding of Kalorey *et al.* (2005) and Sakhare *et al.* (2007) feeding of AFB1 @ 0.2 ppm. The relative weight of heart was unaffected in all the groups. The increase in liver weight might be due its swelling caused by fatty infiltration and impaired lipid transport caused by aflatoxicosis. In aflatoxin fed group, the relative organ weights of lymphoid organs were decreased due to the lymphoid cells depletion. In this study histopathology of liver, kidneys, spleen and bursa of Fabricius found significant moderate changes in the birds fed diet with AF B1 (150 ppb) similar to the finding of AF B1 feeding at lower level (50-100 ppb) Giambrone *et al.* (1985) and Ortatatli *et al.* (2005). Also the similar changes were noticed by Sakhare *et al*. (2007) and Rathod *et al.* (2013) of AF B1 feeding @200 ppb and @100-150ppb respectively. The histo-pathological examination of liver, kidney, spleen and bursa of Fabricius from birds treated with AF B1 and *E. officinalis* showed mild histo-pathological changes. Feeding of *E. officinalis* (5g/kg feed) for 42 day reduced the severity of AF lesions. Bhattacharya *et al.* (1999) reported that antioxidant property of tannoid principal of *E. officinalis* which had vitamin C like property. Kaleem *et al.* (2014) reported the effect of *E. officinalis* derived tannins on humoral immune responses and their protective efficacy against *Eimeria* infection in chickens.

Conclusion

Findings of the present study proved that Aflatoxin B1 at lower doses produces its detrimental effect on health of birds which reduces the production of poultry. Whereas feeding of *E. officinalis* through feed reduces the severity of toxin and losses in poultry. Hence *E. Officinalis* could be used in feed mix during unavoidable circumstances of aflatoxicosis in chickens.

Acknowledgement

We thank the Associate Dean and Department of Poultry science, KNP College of Veterinary Science, Shirwal, Satara for providing necessary facilities and support to carry out this work.

Conflict of Interest

Authors has declared no conflict of interest.

References

1. Bailey, C. A., Latimer, G. W., Barr, A. C., Wigle, W. L., Haq, A. U., Balthrop, J. E. and Kubena, L. F. (2006). Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *Journal of applied poultry research*, 15(2):198-206.

DOI 10.5455/ijlr.20180327052604

2. Bakshi, C. S., Sikdar, A., Johari, T. S., Meenakshi, M. and Singh, R. K. (2000). Effect of graded dietary levels of aflatoxin on humoral immune response in commercial broilers. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*, 21(2): 163-164.
3. Bhattacharya, A., Chatterjee, A., Ghosal, S. and Bhattacharya, S. K. (1999). Antioxidant activity of active tannoid principles of Emblica officinalis (amla). *Indian journal of experimental biology,* 37:676-680.
4. Bhagat, M. (2014). Indian Gooseberry (Emblica officinalis): Pharmacognosy Review. *Utilisation and Management of Medicinal Plants,* 2: 471-487.
5. Campbell Jr, M. L., May, J. D., Huff, W. E. and Doerr, J. A. (1983). Evaluation of immunity of young broiler chickens during simultaneous aflatoxicosis and ochratoxicosis. *Poultry Science*, *62*(11): 2138-2144.
6. Culling, C. F. A. (1974) Handbook of histopathological and histochemical techniques, 3rd Edn., Butterworth and Co. Ltd., 29-221.
7. Denli, M., Blandon, J. C., Guynot, M. E., Salado, S. and Perez, J. F. (2009). Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poultry Science, 88(7): 1444-1451.
8. Desphande, S.S. (2002) fungal toxins, In S. S. Desphande (Ed.), *Handbook of food toxicology*, New York: Marcel Decker, 387-456.
9. Dugyala, R.R. and Sharma, R.P. (1996). The effect of aflatoxin B1 on cytokine mRNA and corresponding protein levels in peritoneal macrophages and splenic lymphocytes. *International journal of immunopharmacology*, *18*(10):599-608.
10. Ghosh, R. C., Chauhan, H. V. S. and Jha, G. J. (1991). Suppression of cell-mediated immunity by purified aflatoxin B1 in broiler chicks. *Veterinary immunology and immunopathology*, *28*(2): 165-172.
11. Giambrone, J. J., Ewert, D. L., Wyatt, R. D. and Eidson, C. S. (1978). Effect of aflatoxin on the humoral and cell-mediated immune systems of the chicken. *American Journal of Veterinary Research*, 39(2): 305-308.
12. Kaoud, H. A. (2013). Innovative methods for the amelioration of aflatoxin (AFB1) effect in broiler chicks. *Sci J Appl Res*, 1: 15-19.
13. Ibrahim, I. K., Shareef, A. M. and Al-Joubory, K. M. T. (2000). Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. *Research in Veterinary Science*, *69*(2): 119-122.
14. Jand S. K., Kaur, P. and Sharma, N. S. (2005). Mycoses and mycotoxicosis in poultry: A review, *Indian J. Anim. Sci.*, 75(4): 465-476.
15. Kadian, S. K., Monga, D. P. and Goel, M. C. (1988). Effect of aflatoxin B1 on the delayed type hypersensitivity and phagocytic activity of reticuloendothelial system in chickens. *Mycopathologia*, 104(1): 33-36.
16. Kaleem, Q. M., Akhtar, M., Awais, M. M., Saleem, M., Zafar, M., Iqbal, Z. and Anwar, M. I. (2014). Studies on Emblica officinalis derived tannins for their immunostimulatory and protective activities against coccidiosis in industrial broiler chickens. *The scientific world journal*, *2014*.
17. Kalorey, D. R., Kurkure, N. V., Ramgaonkar, J. S., Sakhare, P. S., Warke, S. and Nigot, N. K. (2005). Effect of polyherbal feed supplement "Growell "during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. *Asian-Australasian J. Anim. Sci*, 18: 375-383.
18. Kanchana, K., Kumar, S. S., Shanthi, P. and Sachdanandam, P. *(*2013). Ameliorating effect of *kalpaamruthaa* on antioxidant defense system in thymus and spleen of aflatoxin B1 induced hepatocarcinogenic rat and its possible role as a potent immunostimulant, *International Journal of Biological & Pharmaceutical Research*, 4(8): 568-576.
19. Leeson, S., Diaz, G. and Summers, J. D. (1995). Poultry Metabolic Disorders and Mycotoxins, University Books, Canada, pp: 248–279.
20. Maini, S., Rastogi, S.K., Korde, J.P., Madan, A.K. and Shukla, S.K. (2007). Summer Stress and its Amelioration in Broilers, *The Journal of Poultry Science*, 44:339-347.

DOI 10.5455/ijlr.20180327052604

21. Meissonnier, G. M., Pinton, P., Laffitte, J., Cossalter, A. M., Gong, Y. Y., Wild, C. P. and Oswald, I. P. (2008). Immunotoxicity of aflatoxin B1: impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. *Toxicology and applied pharmacology*, *231*(2): 142-149.
22. Mode, S.G., Funde, S. T., Waghmarc, S. P. and Kolta, A. Y. (2009). Effect of herbal immunodulator on body weight gain in immunosuppressed broiler birds. *Vet World*, *2*(7): 269-270.
23. Mohanamba, T., Rao, M. R., and Habibi, S. M. M. (2007). Aflatoxin Contamination In Animal Feeds, *Indian Vet J*., 84: 416.
24. More, B. K. (1996). Pathology of lymphoid organs of chicken with special emphasis on immunosuppression by infectious bursal disease, M. V. Sc. Thesis submitted to University of Agricultural Sciences, Bangalore.
25. Oguz, H. and Kurtoglu, V. (2000). Effect of clinoptilolite on performance of broiler chickens during experimental aflatoxicosis, *British Poultry Science*, 41: 512–517.
26. Oguz, H., Hadimli, H. H., Kurtoglu, V. and Erganis, O. (2003). Evaluation of humoral immunity of broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure, *Revue Méd. Vét.*, 154-7:483-486.
27. Ortatatli, M., Oguz, H., Hatipoglu, F. and Karaman, M. (2005). Evaluation of pathological changes in broilers during chronic aflatoxin (50 and 100 ppb) and clinoptilolite exposure, *Research in Veterinary Science*, 78:61–68
28. Park, D. L. (2002). Mycotoxins and food safety, In: *Advances in experimental medicine and biology* Eds: DeVries JW; Trucksess MW; Jackson LS 504: 173-179.
29. Rathod, P. R., Kulkarni, G. B. and Gangane, G. (2013). Pathological effect of low grade aflatoxicity in Broilers, *The Bioscan*, *8* (3): 1115-1118.
30. Sakhare, P. S., Harne, S. D., Kalorey, D. R., Warke, S. R., Bhandarkar, A. G. and Kurkure, N. V. (2007). Effect of Toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers, *Veterinarski Arhiv*, *77* (2): 129-146.
31. Sapcota, D., Islam, R. and Upadhyaya, T. N. (2006). Dietary supplementation of *Emblica officinalis* for amelioration of experimental aflatoxicosis in commercial broilers, *Animal Nutrition and Feed Technology*, 6-1: 65- 71.
32. Shi, Y. H., Xu, Z. R., Feng, J. L. and Wang, C. Z. (2006). Efficacy of modified montmorillonite nanocomposite to reduce the toxicity of aflatoxin in broiler chicks, *Animal Feed Science Technology*, 129: 138–148.
33. Shi, Y., Xu, Z., Sun, Y., Wang, C. and Feng, J. (2009). Effects of two different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. *Turkish Journal of Veterinary and Animal Sciences*, *33*(1), 15-20.
34. Snedecor, G. W. and Cochran, W. G. (1994) *Statistical methods (eighth edition)*, Calcutta, India: Oxford & IBH Publishing Co.
35. Teleb, H. M., Hegazy, A. A. and Hussein, Y. A. (2004). Efficiency of Kaolin and Activated Charcoal to Reduce the Toxicity of Low Level of Aflatoxin in Broilers, *Scientific Journal of King Faisal University (Basic and Applied Sciences)*, 5-1 1425: 145-159.
36. Thaxton, J.P., Tung, H.T. and Hamilton, P. B. (1974). Immunosuppression in chicks by aflatoxin, *Poultry Science*, 53: 721-725.
37. Tiwary, B. K., & Goel, M. C. (1985). Contact sensitivity to DNCB in normal and cell-mediated-immunity deficient chickens: in vivo detection and correlation with lymphocyte transformation and graft-versus-host reaction. *Veterinary immunology and immunopathology*, 8(4): 329-339.
38. Yang, C. F., Liu, J., Wasser, S., Shen, H. M., Tan, C. E. L. and Ong, C. N. (2000). Inhibition of ebselen on aflatoxin B1-induced hepatocarcinogenesis in Fischer 344 rats. *Carcinogenesis*, 21(12):2237-2243.
39. Yarru, L. P., Settivari, R. S., Gowda, N. K. S., Antoniou, E., Ledoux, D. R. and Rottinghaus, G. E. (2009). Effects of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. *Poultry Science*, *88*(12): 2620-2627.

DOI 10.5455/ijlr.20180327052604