

PATHOMORPHOLOGICAL CHANGES IN OCHRATOXICOSIS AND ITS AMELIORATION USING DIATOMACEOUS EARTH AND VITAMIN E IN BROILER CHICKENS

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Abstract: *The study was conducted to evaluate the pathological and immunosuppressive effects of ochratoxin (OA) in broiler chicken and its amelioration using diatomaceous earth (DAE) and Vitamin E. The major gross lesions included congestion and hemorrhages of liver, kidney, heart etc. Lymphoid organs bursa of fabricious, thymus and spleen were edematous and enlarged and occasionally hemorrhagic lesions were also evident. Liver particularly was hard, fibrotic with degenerative changes. Liver and kidney also revealed many retrogressive changes, kidney revealed hydropic degeneration and necrosis. Microscopic lesions, characteristic to ochratoxicosis were prominent in kidneys, liver and lymphoid organs. Dietary inclusion of ochratoxin caused hepatorenal and immunotoxicity in birds. However, supplementation with DAE and Vitamin E was helpful in ameliorating the adverse effects of ochratoxicosis in broilers.*

Key words: Ochratoxin, broiler chicken, Diatomaceous earth, Vitamin E, Pathology

INTRODUCTION

Ochratoxins are toxic metabolites, commonly produced by toxic species of fungi, *Penicillium verrucosum* and *Aspergillus ochraceus* [1,2]. The primary toxin was identified as ochratoxin A and its less toxic dichloro analogue is ochratoxin B [3]. Ochratoxins, in recent years has received considerable attention not only because of its effect on animal performance and well being but also deleterious effects on humans where it is implicated in a fatal kidney disease known as Balken Endemic Nephropathy.

Ochratoxins are nephrotoxic, hepatotoxic, carcinogenic, immunotoxic and teratogenic. Exposure to low concentrations of ochratoxin is known to cause structural and functional changes in different organs

especially, the kidney and the liver of several domestic and experimental animals.

In poultry, contamination of feed and feed stuffs with ochratoxins results in the reduction of growth efficiency, lower feed conversion, and impaired resistance to diseases, reduced vaccination efficacy and damage to the liver, kidney and other organs. Further, it affects the lymphoid organs and particularly responsible for immuno suppression.

Considering the ill effects of the ochratoxins on health and performance of birds, it becomes essential to develop large scale, cost effective methods using non-toxic dietary additives to neutralize or eliminate the preformed toxin in the diet and that would boost birds resistance. Keeping these in mind, the present

study has been made to assess the efficacy of Diatomaceous earth a toxin binder to ameliorate the toxic effects of ochratoxin and vitamin E an antioxidant and an immune booster.

MATERIALS AND METHODS

Fungal culture: The toxigenic strain of *Aspergillus parasiticus* NRRL 2999 and *Aspergillus ochraceus* NRRL 3174 culture maintained at the Department of Veterinary Pathology, Veterinary College, KVAFSU Bengaluru were used in the study. Ochratoxin was produced on the broken wheat using *Aspergillus ochraceus* NRRL 3174 and quantified using the thin layer chromatography.

Experimental birds: A total of 320 day old commercial broiler chicks were procured from a reputed commercial hatchery. The chicks were weighed individually and reared in battery brooder fitted with electrical bulbs on raised wire mesh floor under optimum condition of brooding and management. They were fed with commercial broiler starter (0-3 weeks) and finisher feed (4-5). The feed samples were screened for mycotoxin content prior to use in feed. The chicks were divided into eight groups of 40 chicks each group. The control and experimental dietary treatment groups as detailed in table 1.

Ochratoxin feed: Powdered ochratoxin culture material was incorporated into the feed at the rate of 1 ppm. Vitamin E was mixed with the feed at the rate of 80mg/kg feed. Mycotoxin ameliorating agent diatomaceous earth (DAE) obtained from M/s Agripower, Australia was incorporated in feed at the

Table 1: Experimental design. 320 chicks were divided in to 8 groups, each containing 40 animals. They were treated as follow:

Treatments	Ochratoxin (ppm)	DAE (mg/kg feed)	Vitamin E (mg/kg feed)
Group I (Control)	0	0	0
Group II	0	2000	0
Group III	0	0	80
Group IV	0	2000	80
Group V	1	0	0
Group VI	1	2000	0
Group VII	1	0	80
Group VIII	1	2000	80

rate of 2000 mg / kg feed).

Biochemical study: 5 ml of blood was collected from each birds at weekly interval (I to V week) in a dry test tube and serum was separated and analyzed for serum urea using Lab Met clinical analyzer as per the procedure described in the manual by using reagent kits supplied by Swemed Diagnostics Ltd., Bengaluru.

Histopathological study: All birds were sacrificed on day 7, 14, 21, 28 and 35. The liver, kidney, heart, lymphoid organs, thymus and spleen were dissected out and fixed in Bouin's fluid fixative for 24 hours. The tissues were washed, dehydrated and paraffin blocks were prepared. 10 μm sections were cut and processed for staining. Sections were stained with Haematoxylin and Eosin and desired portion was photographed.

Statistical Analysis: The data generated from different parameters of the experimental study was subjected to one way analysis of variance (ANOVA) test using graph pad prism software.

Explanation of figures

Fig. 1: Gross photograph from a bird fed with ochratoxin alone (Group V) at 7th day of age showing severe paleness with haemorrhages on liver and slight hepatomegaly.

Fig. 2: Section of liver from ochratoxin treated bird showing congestion of portal vein, periportal fibrosis, bile duct epithelial hyperplasia and mild infiltration of inflammatory cells. H&Ex200

Fig. 3: Section of the liver from ochratoxin treated bird supplemented in Vitamin E showing an appreciable improvement in the architecture of liver lobule at 35th day, however with persistence of mild peri portal fibrosis and infiltration of inflammatory cells. H&Ex200

Fig. 4: Section of kidney from ochratoxin treated bird showing cystic dilatation of the some of the tubules at 35th day of the experiment. H&Ex200

Fig. 5: Section of kidney from ochratoxin treated bird showing degeneration of tubular epithelial cells and severe infiltration of inflammatory cells in the interstitium at 28th day of the experiment. H&Ex200

Fig. 6: Section of kidney from ochratoxin treated bird and supplemented with Vitamin E showing a moderate improvement in the architecture of the tubules at 21st day of the experiment. H&Ex200

Fig. 7: Section of bursa from ochratoxin treated bird showing atrophy of bursal follicle and multiple microcysts in the epithelial plica at 35th day of the experiment. H&Ex100

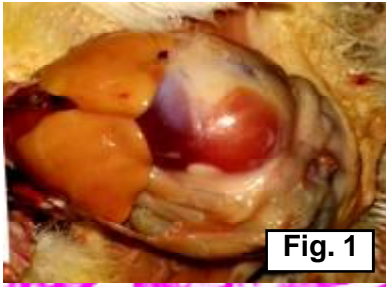


Fig. 1

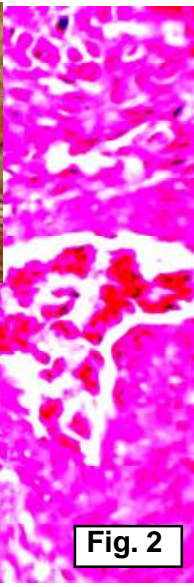


Fig. 2

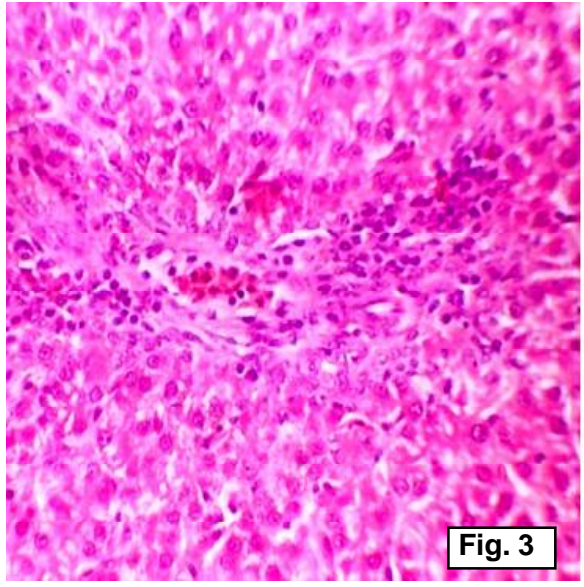


Fig. 3

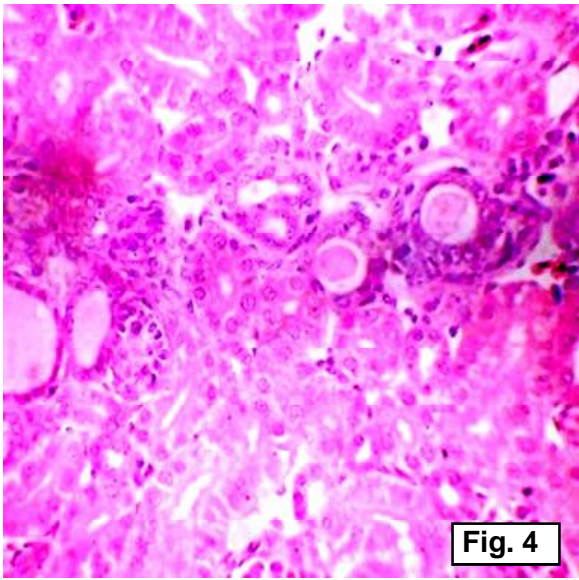


Fig. 4

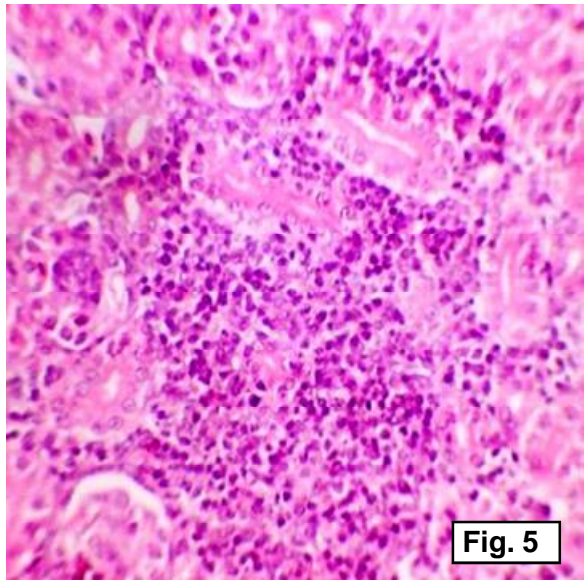


Fig. 5

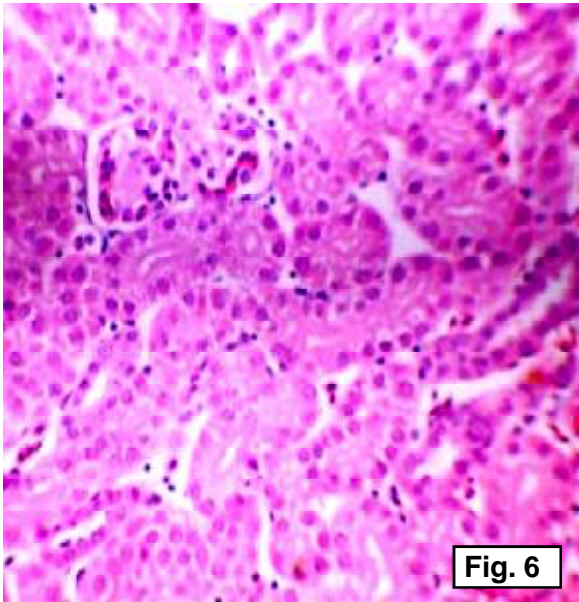


Fig. 6

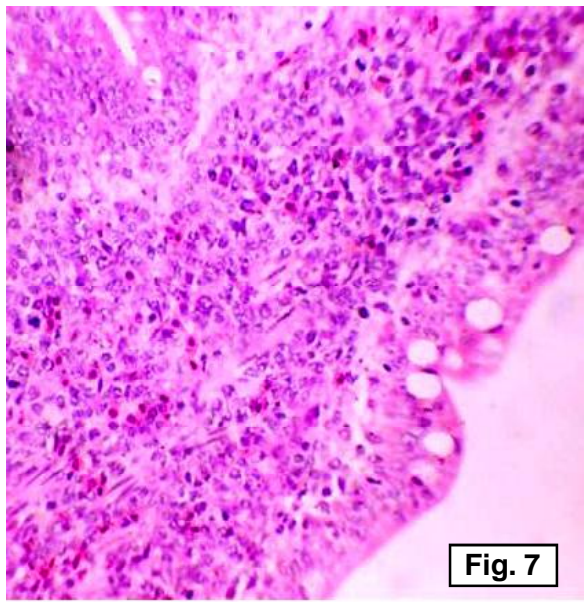


Fig. 7

RESULTS

Gross pathological changed in body organs: The birds of Group I (Control), Group II (diatomaceous earth supplemented), Group III (vitamin E supplemented) and Group IV (both diatomaceous earth and vitamin E supplemented) revealed normal morphological appearance. The pathomorphological changes observed in birds of Group V to VIII are detailed out as hereunder.

Animals of group V, which were treated with 1 ppm ochratoxin in the diet revealed enlarged, pale, friable and occasionally congested livers (Fig 1). Hematomas along the borders of livers were first noticed from second week onwards, which became gradually prominent in the subsequent weeks. The gall bladders in these birds were distended with greenish viscous bile. These changes were consistent throughout the period of study in all toxin fed groups. However, the magnitude and severity of lesions were less pronounced in birds fed with ochratoxin and supplemented with diatomaceous earth, vitamin E (Group VII), and both diatomaceous earth and vitamin E treated groups on 21st, 28th and 35th day of the experiment.

The kidneys were pale, tumified and hamorrhagic in a few birds and swollen and congested in others fed with ochratoxin in the diet. Urate deposits were occasionally seen along with distended ureters in ochratoxin fed birds (Group V). Patchy haemorrhages were seen in birds during 3rd, 4th and 5th week of experiment.

The intensity of the above said lesions were less pronounced in birds which received toxin along with DAE (Group VI), vitamin E (Group VII) and combined treatment with both diatomaceous earth and vitamin E (Group VIII). Heart appeared pale in a few birds and congested, swollen in others fed with toxin in the diet. Atrophy of bursa of Fabricius and thymus were observed in the toxin treated birds (Group V). In few cases edema and petechiae haemorrhagic spots were also observed on the thymus. The atrophy of the lymphoid organs was consistently seen throughout the experimental period. The spleen showed slight enlargement from second week onwards and the same was prominent during fourth and fifth week of age. The size of the lymphoid organs of birds supplemented with DAE (Group VI), vitamin E (Group VII) and both DAE and vitamin E

(Group VIII) revealed slight improvement in comparison to toxin fed birds.

Microscopic pathology: The histopathological changes consequent to different dietary treatment (Group II to VIII) in comparison with the untreated control (Group I) as noticed at different time intervals have been detailed out hereunder.

Liver: Examination of liver section from Group I (untreated control birds) and birds of Group II (DAE), Group III (vitamin E) and Group VI (combined DAE and vitamin E supplemented) appeared normal. Microscopic changes observed in the liver of ochratoxin fed birds (Group V) included changes such as mild to moderate congestion of vessels and sinusoidal space, mild degree of hydropic degeneration and areas of haemorrhages, necrosis along with infiltration of heterophils and lymphoid cells in the portal areas. These changes were observed at the end of second week. In addition to the above changes, moderate degree of bile duct proliferation, portal and periportal infiltration of heterophils and lymphoid cells along with hepatocytes with pyknotic nuclei, cholangitis, perivascular, periductular fibrosis (Fig 2) in some birds were conspicuous during 3rd, 4th and 5th week of experiment in ochratoxin fed birds. The histopathological lesions observed in the livers of birds fed with toxin and supplemented with DAE (Group VI) and vitamin E (Group VII) included mild degree of congestion of vessels and sinusoidal spaces, occasional areas of haemorrhages and infiltration of inflammatory cells along with mild fatty changes. The changes noticed were of less severe intensity and frequency in DAE and vitamin E supplemented birds throughout the period of experiment (Fig 3). Liver of birds from Group VIII almost appeared normal with occasional lymphoid aggregates throughout the period of experiment.

Kidney: Histologically, sections of kidneys did not show any lesions of pathological significance in untreated control birds (Group I), DAE (Group II), vitamin E (Group III), and their combined DAE and vitamin E supplemented (Group IV) birds.

The kidneys of the birds fed with ochratoxin (Group V) showed congestion, haemorrhages, mild to moderate degree of vacuolar degeneration with loss of brush borders along with necrosis of epithelial cells of the proximal convoluted tubules. Several PCT showed distension, desquamation of tubular

epithelium, along with accumulation of eosinophilic homogenous material in the birds. The glomeruli were enlarged in some cases and vacuolated. The interstitium was infiltrated with lymphocytes in a few birds examined at 21 days of age (Fig 4). In addition to the above mentioned lesions, massive infiltration of heterophils in the interstitium, dilated, misshapened tubules with reduced lumen and degenerative changes of glomeruli were observed in the subsequent days. The above lesions were noticed till the end of the experiment (Fig 5).

The microscopic lesions of kidneys in birds of Groups VI and VII included mild congestion of vessels, occasional haemorrhages, distended and misshapened PCT with loss of brush borders. But, these lesions were of less severe intensity as compared to only toxin fed birds during the entire period of study. In the group VIII birds, in addition to congestion and haemorrhages there was appreciable improvement in the architecture of the tubules during 21st to 35 days of experiment (Fig 6).

Heart: The histopathological examination of heart in birds of Group I to IV revealed normal appearance during the entire study period. Whereas, examination of heart in toxin fed birds revealed congestion, focal areas of infiltration with mononuclear cells and degeneration of cardiac myocyte. The heart of birds in Groups VI, VII and VIII also revealed mild degree of above lesions as compared to Group V birds.

Bursa of Fabricius: Microscopically, the bursa of Fabricius of control birds (Group I) showed normal bursal follicles. The birds of Group II to IV showed mild to moderate hyperplasia and with presence of secondary follicles filled with large number of proliferating lymphoblasts. The birds fed with toxin showed moderate to severe degree of lymphocytolytic activity characterized by cellular sparsity along with interstitial connective tissue proliferation. The plical epithelium revealed hyperplasia and corrugation with numerous cystic spaces with occasional area of haemorrhages and varying degree of heterophil infiltration in the interstitium. Additionally, lymphophagocytosis was also evident in these birds (Fig 7). The histopathological changes observed in birds fed with toxin and supplemented with DAE (Group VI), vitamin E (Group VII) and both (Group VIII)

revealed mild to moderate degree of lymphocytolysis and depletion, regeneration and repopulation of cells and few microcyst in plical epithelium. However, these changes were of mild degree in comparison to the toxin fed birds (Group V).

Thymus: The birds of Group I to IV revealed normal appearance of thymus throughout the experimental period. Histopathological changes observed in thymus of ochratoxin fed birds revealed moderate degree of congestion, multifocal areas of haemorrhage, moderate degree of lymphocytolytic activity accompanied by depletion and reduction in the thickness of cortex, along with multifocal areas of heterophil infiltration in the medulla in the first two weeks of experiment. In addition, degeneration of Hassal's corpuscles, severe lymphocytolytic activity and presence of eosinophilic necrotic material in the medullary region with increased heterophil infiltration were the additional features observed in the birds from 28 to 35 days of age. The thymus of birds fed with toxin and supplemented with DAE and vitamin E (Group VI) to VIII) revealed mild degree of lymphocytolytic activity and increased lymphophagocytosis with stray infiltration of heterophils in the medulla. However, some birds in the above group revealed normal appearance of thymic architecture.

Spleen: The spleen of untreated control (Group I) birds revealed normal appearance. The microscopic changes observed in the spleen of birds supplemented with DAE (Group II), vitamin E supplemented (Group III), both DAE and toxin vitamin E fed birds (Group IV) revealed moderate degree of lymphoid hyperplasia with increased cellularity. The microscopic examination in the spleen of ochratoxin fed birds during the first week of treatment revealed mild degree of congestion, occasional areas of haemorrhage and sparse cellularity of splenic corpuscles. Mild degree of lymphocytolytic activity with presence of histiocytes were evident during 28 days of observation. In addition to the above changes, increased lymphophagocytosis with degenerating lymphocytes and presence of hyperplastic secondary follicles were observed at 35 days of age. The birds fed with toxin and supplemented with DAE, vitamin E and together (Groups VI, VII and VIII) revealed the above mentioned changes as mild lesions along with formation of prominent secondary follicles.

DISCUSSION

The gross lesions observed in liver of OA fed birds (Group V) were enlarged, congested and friable liver and distended gall bladder with thick bile. The increase in serum concentration of enzymes in the toxin fed birds was indication of hepatic damage occurred during ochratoxicosis [5-8]. These lesions were of less intensity in birds belonging to Group VI, VII and VIII.

The toxin fed birds in the present study showed that the kidneys were tumefied, haemorrhagic and swollen. Increased size of kidney could be due to dilatation of PCT and accumulation of proteinaceous material along with infiltration of inflammatory cells. Similar findings during ochratoxicosis of broilers thus lending support to the present investigation [9-12].

The birds fed with toxin, grossly showed atrophic changes in bursa of Fabricius, thymus and spleen. These findings draws support from the fact that the relative weights of these organs were decreased consequent to lymphocytolytic activity observed in these organs. Similar observations were also reported by several workers [12-15]. However, the above said lesions were less pronounced in birds fed with toxin and supplemented with DAE, vitamin E and their combination (Groups VI, VII and VIII, respectively).

In the present study, the liver of toxin fed birds showed congestion of vessels sinusoids, occasional area of haemorrhages and necrosis of hepatocytes, biliary hyperplasia with heterophilic and lymphocytic infiltration in focal areas and some birds also revealed cholangitis with perivascular and periductular fibrosis. These changes were in accordance with earlier findings of [5,8]. Increase in serum concentration of liver enzymes confirmed the high degree of liver damage in ochratoxicosis.

However, the liver from birds fed with toxin and supplemented with DAE, and vitamin E (Group VI and VII) showed histologically mild fatty changes and biliary hyperplasia. The severity of these lesions were of mild intensity as compared to only OA fed birds. The same could be attributed to partial binding of toxin by DAE/toxin binder and protective role of vitamin E against ochratoxin as also reported by earlier workers (10,11,13}. Further, the birds

supplemented with DAE and vitamin E together in toxin treated groups (Group VI, VII and VIII) showed reduced severity of lesions and thus indicating the beneficial effect when supplemented together in alleviating the toxic effect of ochratoxin. This could also draw support from the fact that weight gain and FCR were better in the toxin fed and combined supplementation of DAE, vitamin E and their combined group in that order.

In the present study, kidney of OA treated birds revealed distended misshapen PCT with loss of brush border and presence of eosinophilic proteinaceous material in their lumen. These findings were in accordance with others investigators [5,16]. Increased size of kidney in toxin fed birds observed grossly could be due to enormous dilatation of the tubules and infiltration of inflammatory cells seen histologically. Tubular damage along with thickened glomerular basement membrane indicated the impaired renal function, which in turn reflected the cause of increased serum creatinine levels observed in this study as also indicated by earlier workers [9,17].

In the present study, lymphocytolysis coupled with cellular sparcity with infiltration of histiocyte, presence of micro cyst in mucosa along with proliferation of connective tissue were the lesions observed in thymus and bursa of Fabricius in bird fed with OA.

The histopathological lesions in spleen of birds fed with OA revealed lymphocytolytic activity with increase in number of histiocytes in splenic corpuscles, along with haemorrhage, congestion and lymphoid hyperplasia, a feature also reported earlier workers [5,18]

Supplementation of DAE, vitamin E and both together (Group VI, VII and VIII) showed lesions of less severity when compared to only toxin treated birds. However, spleen showed formation of secondary follicle with stray infiltration of heterophils. In addition, thymus and bursa of Fabricius also revealed moderate lymphoid hyperplasia with mild haemorrhage and focal areas of mild lymphocytolysis and depletion with increased lymphophagocytosis in birds fed with toxin and supplemented with DAE, vitamin E and both combined together (Group VI, VII and VIII).

CONCLUSION

Study shows that supplementation of DAE and vitamin E individually had shown some improvement in all the above biochemical parameters. Supplementation of DAE and vitamin E together reduced the severity of toxicity, thus indicating that they were helpful in alleviating the adverse effects of ochratoxin in broilers.

REFERENCES

- [1] Scott, P.M., Lawrence, J.W. and Van Walbeek, W.: *Appl. Microbiol.*, 20: 839-842 (1970).
- [2] Frisvad, J.C. and Samson, R.A.: Mycotoxins produced in species of *Penicillium* and *Aspergillus* occurring in cereals. In: *Cereal grain Mycotoxins, Fungi and quality drying and Storage Chelkowski* (Ed). Elsevier, Amstardam, pp 441-476 (1991).
- [3] Vander Merwe, K.J., Steyn, P.S., and Fourie, L.: *J. Chem. Soc.* Vol. 7083 (1965).
- [4] Luna, L.G.: *Manual of Histologic Staining Methods of the Armed Forces Institute Pathology*. 3rd Edn. McGraw-Hill Book Company, Lonodn (1968).
- [5] Peckham, J.C., Douppnik Jr. B. and Jones, J.R.O.H.: *Appl. Microbiol.*, 21: 492-494 (1971).
- [6] Huff, W.E., Wyatt, R.D. and Hamilton, P.B.: *Appl. Microbiol.*, 30: 48-51 (1975).
- [7] Thyagarajan, D., Sundarasu, V. and Narahari, D.: *Effect of graded dietary ochratoxin A on broiler performance*. Proc. 20th World Pould. Cong., New Delhi, 4: 433-439 (1996).
- [8] Kumar A., Jinda, N., Shukla, C.L., Yash Pal, Ledoux, D.R. and Rottinghaus, G.E.: *Avian Dis.*, 47: 415-424 (2003).
- [9] Anil Kumar, P.: *Pathology of alfatoxicosis, ochratoxicosis and ameliorating effect of vitamin E and selenium in broiler chicken*. M.V.Sc. Thesis, University Agricultural Science, Bangalore (2002).
- [10] Bhanuprakash., *Pathology of experimental alatoxicosis, ochratoxicosis and assessment of the efficacy of toxin binder in broiler chickens*. M.V.Sc. Thesis, University Agricultural Science, Bangalore (2002).
- [11] Shivappa, —?—: *Assessment of comparative efficacy of toxin binder in ameliorating the combined toxic effect of aflatoxin and ochratoxin in broiler chickens*. M.V.Sc. Thesis University Agricultural Science, Bangalore (2005).
- [12] Anand V.P.: *Studies on Pathology and immunosuppressive effect of ochratoxin in broiler and its Amelioration with diatomaceous earth*. Ph.D. thesis, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar (2015).
- [13] Amita, P.: *Pathology of experimental ochratoxicosis in broilers and assessment of efficacy of a toxin binder*. M.V.Sc. Thesis, University Agricultural Science, Bangalore (2001).
- [14] Rajeev, K.: *Pathology of ochratoxin and T-2 toxin and their combined toxicity in broiler chickens*. M.V.Sc. Thesis, University Agricultural Science, Bangalore (2001).
- [15] Anilkumar P., Satyanarayana, M.L., Vijayasarathi, S.K., Sreenivas Gowda, R.N. and Suguna Rao: *Indian J. Vet. Pathol.*, 27: 102-106 (2003).
- [16] Dwivedi, P. and Burns, R.B.: *Res. Vet. Sci.*, 36:117-121 (1985).
- [17] Prakash, G.C.: *Pathology of combined effects of alfatoxin and ochratoxin in broiler chickens*. M.V.Sc. Thesis, University Agricultural Science, Bangalore (2001).
- [18] Sakhare, P.S., Harné S.D., Kalorey D.R. Warke, S.R., Bhandarkar, A.G., and Kurkure, N.V.: *Vet. Arhiv.*, 77(2): 129-146 (2007).