ASSESSMENT OF APOPTOSIS AND NECROSIS IN SPLEEN AND THYMUS UNDER SUBLETHAL DOSE OF AFLATOXIN AND OCHRATOXIN

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Abstract

A study was undertaken to assess the induction of apoptosis and necrosis in splenocytes and thymocytes of broiler chicks on feeding sublethal dose of aflatoxin (AF) or ocharatoxin (OA). Three week old broiler birds were fed 100ppb of AF or OA and were sacrificed at 24, 48, 72 and 96h post-treatment (PT). Spleen and thymus of the birds were taken and the induction of apoptosis and necrosis were studied by flow cytometry and histopathology. AF and OA groups showed significant (P<0.05) induction of apoptosis and necrosis in splenocytes at 72h PT and maximum induction of apoptosis in thymocytes at 96h and necrosis at 72h PT as determined by flowcytometry. In the spleen and thymus of AF fed birds, clusters of apoptotic cells were observed. In OA fed birds, isolated apoptotic cells in spleen and cluster of apoptotic cells in thymus were observed. Isolated lymphoid cells in spleen and thymus revealed necrosis characterized by cell swelling, eosinophilia of cytoplasm, pyknosis and karyorrhexis.

Key words: *Apoptosis, Aflatoxin, Ochratoxin, Thymus, Spleen*

Mycotoxins are secondary metabolites of fungi mostly of Aspergillus, Penicillium and Fusarium species causing pathological and physiological alterations and impairing the health and productivity (D'Mello and MacDonald, 1998). Aflatoxin (AF) and ochratoxin (OA) are the most frequently encountered mycotoxins. Poultry feed samples found positive for mycotoxin contamination had AF with levels ranging from 1 ppb to 12 ppm (Gupta *et al*., 1985; Shetty *et al*., 1987; Purwoko *et al*., 1991; Jindal *et al*., 1993) and OA from 0.1 to 16 ppm (Hamilton *et al*., 1982; Bauer and Gareis, 1987; Sakthivelan and George, 1998). Mycotoxicosis caused by ingestion of low

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levels AF and OA have been well documented in broiler chicken (Coulombe, 1993), but reports on its effect on immune system by sublethal doses of these toxicities are scanty. Van Engeland *et al*. (1998) reported that loss of plasma membrane asymmetry is an early event in apoptosis, independent of the cell type, resulting in the exposure of phosphatidylserine (PS) residues at the outer plasma membrane leaflet. Annexin V was shown to interact strongly and specifically with PS and can be used to detect apoptosis by targeting for the loss of plasma membrane asymmetry. Hence, the present work was formulated to assess apoptosis in spleen and thymus using Annexin V in AF and OA fed broiler chicken.

Materials and Methods

Thirty six newly hatched broiler chicks were fed on control diet up to three weeks of age. Subsequently, the birds were randomly distributed to three groups of 12 chicks each and fed with control, AF (100 ppb) and OA (100 ppb) diets. Three birds from each group were sacrificed at 24, 48, 72 and 96h after treatment. Spleen and thymus of birds of each group were collected and made into a single cell suspension and the concentration was adjusted to $1x10^6$ cell/ml. Flow cytometric (Becton and Dickinson, USA) analysis was done using annexin kit to assess apoptosis and necrosis in splenocytes and thymocytes in control and toxin fed birds. Spleen and thymus samples were collected in 10 per cent neutral buffered formalin for histopathological studies

Results and Discussion

Mean ± SE percent apoptosis and necrosis assessed by flowcytometry at different hours PT in spleen and thymus of broiler chicks fed with AF and OA mixed feed are presented in Tables 1 to 4. Cells that are in the early stages of apoptosis bind only with annexin V, while those in late stages of apoptosis or necrosis bind with both annexin V and propidium iodide. There was a significant ($P<0.05$) difference between the control and toxin fed groups for apoptosis and necrosis. AF (100 ppb) and OA (100 ppb) groups showed significant ($P<0.05$) induction of apoptosis and necrosis in splenocytes at 72h PT. There was a significant ($P<0.05$) difference between the control and mycotoxin fed groups with maximum resultant induction of apoptosis in thymocytes at 96h and necrosis at 72h PT for both AF (100 ppb) and OA (100 ppb) treated groups as determined by flowcytometry.

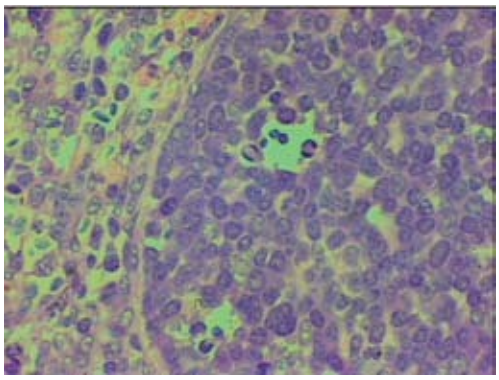

Fig. 1. Spleen – Germinal centre. 96h-AF-100 ppb– Cluster of apoptotic cells with chromatin margination. H&E. x OM 1000

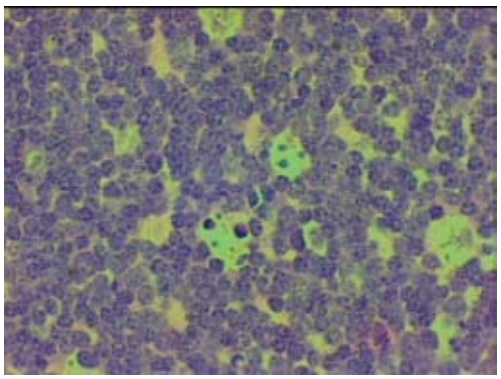

Fig. 2. Thymus. 96h-OA-100 ppb – Cluster of apoptotic cells with chromatin margination. H&E. x OM 1000

Examination of paraffin embedded spleen and thymus revealed presence of apoptotic cells characterized by chromatin margination along the nuclear envelope, shrinkage of cells with a clear halo formation and without any inflammatory reactions in all mycotoxin fed birds. In the spleen (Fig. 1) and thymus of AF (100 ppb) fed birds, clusters of apoptotic cells were observed. In OA (100 ppb) fed birds, isolated apoptotic cells in spleen and cluster of apoptotic cells in thymus (Fig. 2) were seen. Isolated lymphoid cells in spleen and thymus revealed necrosis characterized by cell swelling, eosinophilia of cytoplasm, pyknosis and karyorrhexis.

Table 1. Mean ± SE of percentage apoptosis in spleen at different hours in AF and OA treated broiler chicks (n = 3)

Groups	24h	48h	72h	96h
Control	3.10 ± 0.68	8.62 ± 0.23	3.79^{b} ± 0.23	8.90 ± 0.23
AF (100 ppb)	3.48 ± 0.83	10.14 ± 3.51	17.45^{a} ± 2.90	18.91 ± 0.38
OA (100 ppb)	4.61 ± 0.85	15.02 ± 0.20	12.13^{a} ± 4.96	15.58 ± 0.85

Means with same superscripts within a column do not differ from each other (P>0.05)

Table 2. Mean ± SE of percentage necrosis in spleen at different hours in AF and OA treated broiler chicks (n = 3)

Groups	24h	48h	72h	96h
Control	5.43 ± 1.14	1.66 ± 0.06	4.76^{b} ± 0.17	3.21 ± 0.23
AF (100 ppb)	6.79 ± 2.80	3.03 ± 0.91	8.25^{a} ± 2.16	6.69 ± 1.37
OA (100 ppb)	4.05 ± 0.85	1.90 ± 0.20	7.94^{a} ±2.79	4.48 ± 1.70

Means with same superscripts within a column do not differ from each other (P>0.05)

Table 3. Mean ± SE of percentage apoptosis in thymus at different hours in AF and OA treated broiler chicks (n = 3)

Groups	24h	48h	72h	96h
Control	2.27 ± 0.54	3.20 ± 0.23	3.23^{b} ± 0.23	3.32^{b} ± 0.23
AF (100 ppb)	2.12 ± 0.82	3.01 ± 0.26	4.05^{a} ± 1.77	7.32^{a} ± 1.11
OA (100 ppb)	2.01 ± 0.75	2.75 ± 0.39	3.81^{a} ± 1.39	6.05^{a} ± 0.83

Means with same superscripts within a column do not differ from each other (P>0.05)

Sublethal levels of AF and OA (100 ppb) induced peak apoptosis and necrosis in spleen and thymus of broiler chicks by 72 to 96h of feeding indicating their short term effect. Gounalan (2005) reported induction of apoptosis in splenocytes and thymocytes of layer chicks fed 500 ppb of AF or OA with peak induction at 24 h PT and necrosis with peak induction at 36 h PT. The apoptotic cells were characterized by chromatin margination along the nuclear envelope, shrinkage of cells with a clear halo formation and without any inflammatory reactions in the spleen and thymus of layer birds fed 500 ppb of either AF or OA.

Table 4. Mean ± SE of percentage necrosis in thymus at different hours in AF and OA treated broiler chicks (n = 3)

Groups	24h	48h	72h	96h
Control	1.79 ± 0.80	0.85 ± 0.02	0.80^{b} ± 0.17	3.10^{b} ± 0.23
AF (100 ppb)	0.81 ± 0.17	0.36 ± 0.13	1.41^{a} ± 0.67	1.03^{a} ± 0.16
OA (100 ppb)	2.08 ± 0.62	0.38 ± 0.02	1.23^{a} ±0.55	1.17^{a} ± 0.06

Means with same superscripts within a column do not differ from each other (P>0.05)

The present study revealed that the feeding of broiler chicks will sublethal dose of AF or OA will cause apoptosis and necrosis of splenocytes and thymocytes by 72 to 96h indicating their short term effect. Hence it could be concluded that even low levels of AF or OA in feed of broiler birds could affect the immune system and indirectly affect the production.

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