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STUDY OF APOPTOSIS IN THE SUBLETHAL DOSE OF PENICILLIC ACID TOXICITY IN BROILER CHICKENS

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ABSTRACT

Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum*. The present study was undertaken to study the apoptosis in the sublethal dose of penicillic acid mycotoxicosis in broiler chicken. Eighteen day-old broiler chicks were fed with control diet for the period of three weeks. Subsequently, the birds were randomly distributed to two groups of nine birds each and fed with control and 15 ppm of penicillic toxin diets. Three birds from control and treated groups were sacrificed at 24, 48 and 72 h after treatment in the acute toxicity trial. Similarly, six birds (3 control and 3 penicillic acid–15 ppm) were used for subacute toxicity trial (21 days) and sacrificed to study the apoptosis in the spleen and thymus by using flow cytometric analysis with the Annexin V kit to assess apoptosis and necrosis in splenocytes and thymocytes. This research indicated that peak induction of apoptosis was observed at 24 h treatment of penicillic acid (15 ppm).

Key words: Broiler chicken, Penicillic acid toxicity, apoptosis

INTRODUCTION

Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum* (Alsberg CL and Black OF, 1913). Later it was found that *P. cyclopium* Westling produced relatively larger amounts of penicillic acid (Bentley R and Keil JG, 1962). Penicillic acid occurred at high concentrations in corn (Kurtzman CP and Ciegler A, 1970) and was also produced concomitantly with other mycotoxins in poultry feed (Bacon CW *et al*., 1973). Natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products. Penicillic acid, a mycotoxin produced by *Penicillium* and *Aspergillus* species, was isolated from blue-eye diseased corn, poultry feed, commercial corn, dried beans, cheese and tobacco products (LeBars J. 1980). The present work was undertaken to study of apoptosis in the sublethal dose of penicillic acid toxicity in broiler chickens.

MATERIALS AND METHODS

1. PENICILLIC ACID PRODUCTION

The *Penicillium cyclopium* NRRL 1888 culture was obtained from National Center for Agricultural

Utilization Research, Microbial Genomics and Bioprocessing Research Unit, 1815 N University Street, Peoria, Illinois 61604, USA. The penicillic acid toxin was produced on maize (LeBars J. 1980). The maize samples were pre-tested for the presence of mycotoxins. The penicillic acid from ground maize culture samples were quantified by using thin layer chromatography at the Central Animal Feed and Food Residue Laboratory, Directorate of Centre for Animal Health Studies, TANUVAS, Chennai. The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar and the culture material yielded 20–80 ppm penicillic acid.

2. EXPERIMENTAL DESIGN

Eighteen day-old broiler chicks were fed on control diet up to three weeks of age. Subsequently, the birds were randomly distributed to two groups of nine birds each and fed with control and 15 ppm of penicillic toxin diets. Three birds from control and treated groups were sacrificed at 24, 48 and 72 h after treatment in the acute toxicity trial. Similarly, six birds (3 controls and 3 penicillic acid–15 ppm) were used for subacute toxicity trial (21 days) and sacrificed to study the apoptosis in the spleen and thymus. Flow cytometric (Becton and Dickinson, USA) analysis was done using Annexin V kit to assess apoptosis and necrosis in splenocytes and thymocytes in the control and toxin fed birds. The spleen and thymus were collected in 10 per cent formalin for histopathological examination. The paraffin embedded tissue sections were cut into 4 to 6 μm thickness and stained with haematoxylin and eosin as per the standard procedures described by Bancroft JD and Gamble G, (2008).

3. STATISTICAL ANALYSIS

The data generated from different experimental trials were subjected to one-way analysis of variance (ANOVA) using SPSS version 10 software for windows.

RESULTS

ACUTE TOXICITY STUDY

Cells in the early apoptotic stage binds only with Annexin V and cells in the late apoptotic and necrotic stages bind with both Annexin V and propidium iodide.

SPLEEN

Mean ± SE apoptosis and necrosis values of spleen in the penicillic acid (15 ppm) treated broiler chicken are presented in Table 1. Significant ($P<0.05$) differences were observed between the control and penicillic acid treated groups for splenic apoptosis at 24 h treatment. No significant differences were observed for necrosis between control and penicillic acid treated broiler chicken. There was significant ($P<0.05$) increase in the splenic lymphocyte apoptosis values at 24 h in the penicillic acid treated group when compared to the control group.

Table 1

Mean (±SE) per cent apoptosis and necrosis in the spleen at different hours in penicillic acid fed broiler chicks (n=3)

Groups	24 h	48 h	72 h
Apoptosis			
Control	$1.46^{b} \pm 0.13$	3.15 ± 0.05	4.04 ± 0.16
PA (15 ppm)	$14.78^{a} \pm 0.09$	2.04 ± 1.83	1.67 ± 0.78
Necrosis			
Control	1.11 ± 0.85	3.37 ± 0.18	2.39 ± 0.23
PA (15 ppm)	1.02 ± 0.89	3.11 ± 0.22	1.83 ± 0.39

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

Thymus

Mean (SE thymic apoptosis and necrosis values of penicillic acid (15 ppm) treated broiler chicken are presented in Table 2. There was significant ($P<0.05$) difference between the control and penicillic acid treated birds at 24 h treatment for apoptosis. There was significant ($P<0.05$) increase in the apoptosis of thymic lymphocytes in the penicillic acid treated group at 24 h when compared to the control group. There was no significant difference between the control and penicillic acid treated birds for thymic necrosis.

Table 2

Mean (±SE) per cent apoptosis and necrosis in the thymus at different hours in penicillic acid fed broiler chicks (n=3)

Groups	24 h	48 h	72 h
Apoptosis			
Control	$0.29^{b} \pm 0.01$	4.44 ± 0.58	3.87 ± 0.17
PA (15 ppm)	$1.53^{a} \pm 0.10$	2.07 ± 0.65	5.08 ± 3.23
Necrosis			
Control	4.12 ± 0.41	7.85 ± 2.27	2.39 ± 0.66
PA (15 ppm)	3.26 ± 1.76	4.38 ± 1.40	2.95 ± 1.80

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

HISTOPATHOLOGY

Numerous apoptotic bodies were seen in the lymphocytes of spleen and thymus in the 24 h treated birds characterized by chromtin margination as shown in Figure 1 and cell shrinkage as shown in Figure 2.

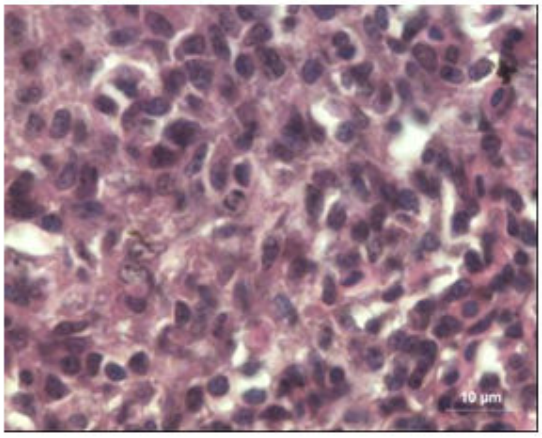

Fig. 1.Spleen-Apoptotic cell-Chromatin margination H&E Scale Bar=10µm

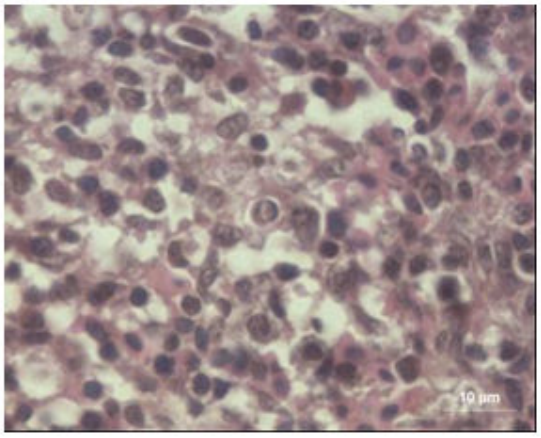

Fig.2 Spleen- Apoptotic cell- Cell shrinkage and Chromatin margination H&E Scale Bar=10µm

SUBACUTE TOXICITY STUDY

Mean ± SE splenocyte and thymocyte apoptosis and necrosis values of penicillic acid (15 ppm) treated broiler chicken for 21 days are presented in Tables 3. There was no significant difference between the control and treated groups for splenocyte and thymocyte apoptosis and necrosis values.

Table 3

Mean (±SE) per cent apoptosis and necrosis in the spleen and thymus in subacute toxicosis in penicillic acid fed broiler chicks

Groups	Spleen		Thymus	
	Apoptosis	Necrosis	Apoptosis	Necrosis
Control	1.99 ± 0.52	0.62 ± 0.60	1.14 ± 0.43	0.27 ± 0.14
PA (15ppm)	1.29 ± 0.06	1.26 ± 0.19	4.99 ± 3.29	0.24 ± 0.11

HISTOPATHOLOGY

Moderate lymphoid depletion in the spleen and no appreciable changes with thymus were observed in penicillic acid (15 ppm) treated birds for 21 days.

DISCUSSION

ACUTE TOXICITY STUDY

During 72 h penicillic acid treatment, peak induction of apoptosis was observed at 24 h in the splenocytes and thymocytes. The results of apoptosis study indicated that the lymphoid cell depletion might be due to apoptosis up to 24 h following exposure to 15 ppm toxin level. The results of acute toxicity study also suggest that the histological observation of lymphoid cell depletion in the penicillic acid treated birds over 21 to 28 days might be mostly due to necrotic changes in the lymphocytes rather than apoptotic changes.

SUBACUTE TOXICITY STUDY

No significant differences were observed in the splenocyte and thymocyte apoptosis and necrosis values of broiler chicken fed penicillic acid toxin (15 ppm) for 21 days. The results indicated that at the end of the 21st day, the definite evidence of lymphoid cell depletion found might be due to the direct injury to the lymphocytes in the spleen and thymus. There is no literature for comparison of apoptosis study associated with pencillic acid mycotoxin while Penicillic acid (PCA) inhibits FasL-induced apoptosis and concomitant loss of cell viability in Burkitt's lymphoma Raji cells (Bando M et al., 2003). Other mycotoxin like Ochratoxin A OTA induces apoptosis by disrupting mitochondrial function in human T lymphocytes (Assaf H et al., 2004).

CONCLUSION

This research indicated that the peak induction of apoptosis was observed at 24 h treatment of penicillic acid (15 ppm).

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