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Research Article

EFFECT OF SUBLETHAL DOSE OF PENICILLIC ACID TOXICITY ON PATHOLOGICAL CHANGES IN BROILER CHICKENS

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ABSTRACT
Penicillic acid (PA) is a mycotoxin, produced by *Penicillium puberulum.* The present study was undertaken to find out the sublethal effects of penicillic acid mycotoxicoses on pathological changes in broiler chicken. Forty eight day-old broiler chicks were randomly allotted to four groups of 12 birds each and fed with 0, 7.5, 15 and 30 ppm of penicillic acid from 0 to 21 days of age respectively. On 21st day of trial, the birds were sacrificed to study the pathological changes. The clinical signs observed from first week onwards in the penicillic acid fed birds were inappetence, poor growth and depression without any mortality. Grossly, the liver was pale and enlarged or showed yellowish discolouration in the PA fed birds. Degenerative, necrotic and inflammatory changes were observed in the liver, kidneys, proventriculus, gizzard and duodenum in the PA toxin fed birds. Hyperplastic crop mucosa and crop muscle degeneration were also noticed in PA toxicity. This research indicated that pathological changes were observed even at sublethal dose of 7.5 ppm.

Keywords: *Broiler Chicken, Penicillic Acid Toxicity, Pathology*

INTRODUCTION
Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum* (Alsberg and Black, 1913). Natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products (Kurtzman and Ciegler, 1970). The penicillic acid, one of the extrolites produced from subgenus *Penicillium,* is shared with species in the genus *Aspergillus* and its teleomorphs (Frisvad *et al*., 2004). Penicillium mycotoxins exposure specifically Ochratoxin (OTA) and other mycotoxin combinations such as Citrinin (CIT), Patulin (PAT), Mycophenolic acid (MPA) or PA can alter the expression of Bovine Macrophages (BoMacs) enzymes that are involved in epigenetic regulation (Se-Young *et al*., 2013).

Sarmadha (2003) reported that a four weeks feeding of 50-480 ppm penicillic acid to broiler chicken resulted in gross changes of pale discolouration of liver and histopathological changes in different organs. Literature search supported the need for study on the lower levels of penicillic acid mycotoxicosis affecting the health of broiler chicken. Hence, the present study was undertaken to find out the sublethal effects of penicillic acid mycotoxicoses on pathological changes in broiler chicken.

MATERIALS AND METHODS
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, 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, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051, India.

Experimental Design

Forty eight day-old broiler chicks were randomly allotted to four groups of 12 birds each. They were fed with 0, 7.5, 15 and 30 ppm of penicillic acid mixed diets from 0 to 21 days of age respectively. During the study period, the clinical signs were recorded. On 21st day of trial, the birds were sacrificed.

Pathology

After collection of blood, the birds were sacrificed by cervical dislocation and a detailed post-mortem examination was conducted on sacrificed birds. Representative samples of tissues from liver, kidney, crop, proventriculus, gizzard, duodenum and pancreas were collected in 10 per cent formal saline. Paraffin embedded tissues were sectioned to 5 µm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination (Bancroft and Gamble, 2008).

RESULTS AND DISCUSSION

Fungal Culture

The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar and the culture material yielded 20–80 ppm penicillic acid. The toxigenic fungus *Penicillium cyclopium* NRRL 1888 growth characteristics in the subculture and solid substrates in the present study correlated with the earlier reports (Birkinshaw *et al*., 1936; Ciegler *et al*., 1972; LeBars, 1980; Sarmadha, 2003).

Clinical Signs

The clinical signs observed from first week onwards in the PA fed birds were inappetence, poor growth and depression. No mortality was observed during the trial period. The clinical signs of inappetence, poor growth and depression observed in the present study are in accordance with the previous reports of Sarmadha (2003) and Pazhanivel and Balachandran (2014b). However, poor and abnormal wing feathering reported by Sarmadha (2003) were not be observed in this study which might be due to the low level of toxin used in the present study.

Gross Pathology

Control group revealed no gross changes. The liver was congested to pale in colour in the 7.5 ppm, pale and enlarged in the 15 ppm and pale to yellowish and enlarged in the 30 ppm groups (Figure 1). Congestion and paleness was noticed in the liver in the 7.5 ppm, while enlargement in the 15 ppm and with yellowish discoloration were found in the 30 ppm group which concurred with the earlier findings (Sarmadha, 2003; Pazhanivel and Balachandran, 2014a, b).

Histopathology

Microscopically, control group revealed no histopathological changes. The 7.5 ppm penicillic acid toxin fed birds showed congestion of vessels and sinusoids, mild diffuse vacuolar degeneration of hepatocytes (Figure 2) and hyperplastic bile duct epithelial cells extending into the parenchyma of liver. Two cases showed Kupffer cell hyperplasia.

Two cases showed degeneration of cholangiocytes with the bile duct lumen containing desquamated and necrotic cells. Kidneys revealed congestion of interstitial blood vessels. Proximal convoluted tubular epithelial cells showed mild vacuolar degeneration (Figure 3) and single to multiple cell necroses. Crop revealed focal hyperplastic changes in the mucosal layer. Proventriculus revealed mononuclear cell infiltration in the mucosa. Gizzard revealed empty and dilated glands with focal necrosis and mononuclear cell infiltration. Duodenum showed shortening of villi and increased goblet cell activity.

The 15 ppm penicillic acid fed birds showed venous congestion, moderate vacuolar degeneration of hepatocytes and focal microgranulomas in the liver. Focal hepatic necrosis and mononuclear cell infiltration were also observed. Crop showed extensive mucosal hyperplasia and parakeratosis with bacterial colonies.

Crop muscle showed vacuolar degeneration. Proventricular mucosa revealed diffuse mononuclear cell infiltration. Focal areas revealed full thickness mucosal necrosis in the proventriculus. Glandular epithelium of the proventriculus showed vacuolar degeneration, necrosis (Figure 4) and apoptotic cells. Gizzard revealed dilatation of glands with eosinophilic secretion. Pancreas showed mild degeneration of acinar cells and focal necrosis.

The 30 ppm penicillic acid fed birds showed diffuse vaculolar degeneration of hepatocytes and bile duct hyperplasia in the liver. Hyperplastic biliary epithelium showed papillary-like projections into the lumen. Kidneys revealed moderate to diffuse vacuolar degeneration of tubular epithelial cells and necrosis (Figure 5). Occasionally, mononuclear cell infiltration was observed in the interstitium predominantly consisting of lymphocytes. Crop showed extensive mucosal hyperplasia and muscular degeneration. Proventriculus showed partial to full thickness necrosis of mucosal epithelium with mononuclear cell infiltration. Two cases showed submucosal fibrosis. Gizzard gland showed vacuolar degeneration of epithelial cells and focal cystic dilatation (Figure 6). Duodenum showed shortening and thinning of villi with catarrhal changes.

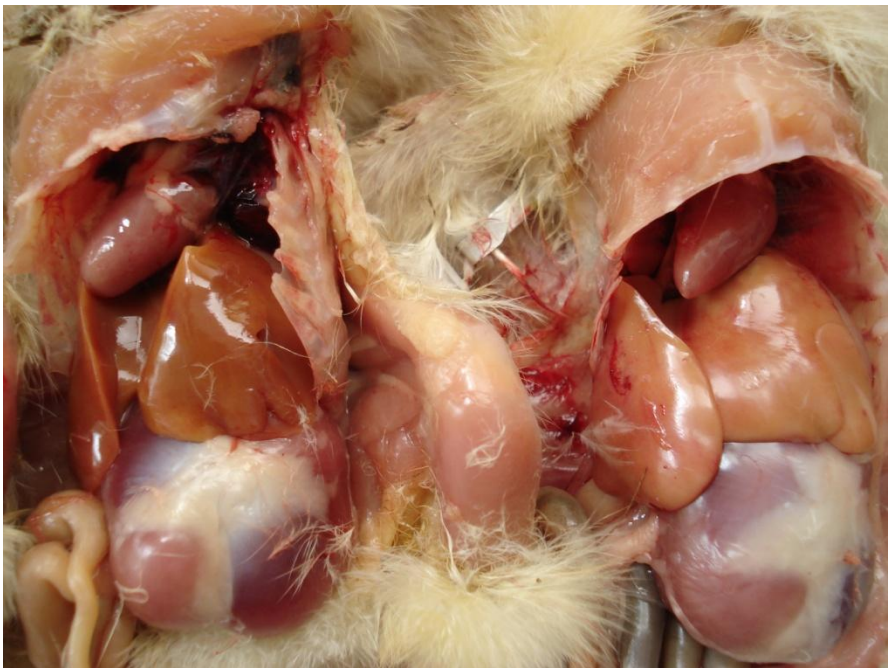

Figure 1: PA 30 ppm – Liver - pale and enlarged

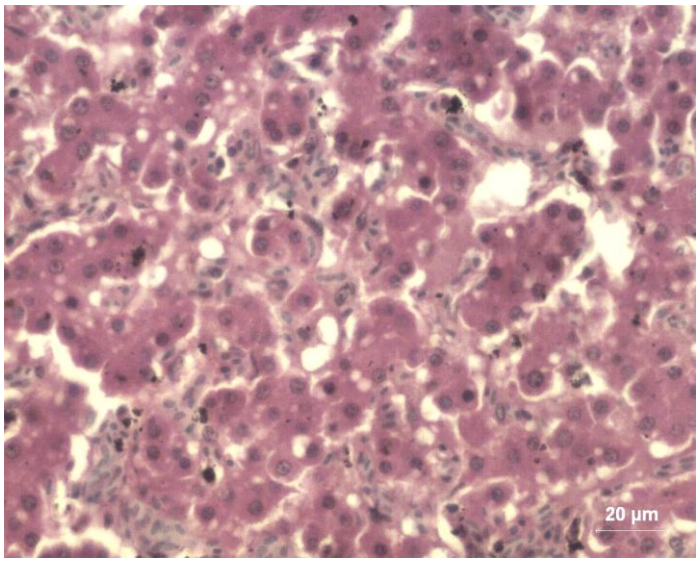

Figure 2: PA 7.5 ppm – Liver – Mild diffuse vacuolar degeneration of hepatocytes Scale bar H&E 20 µm

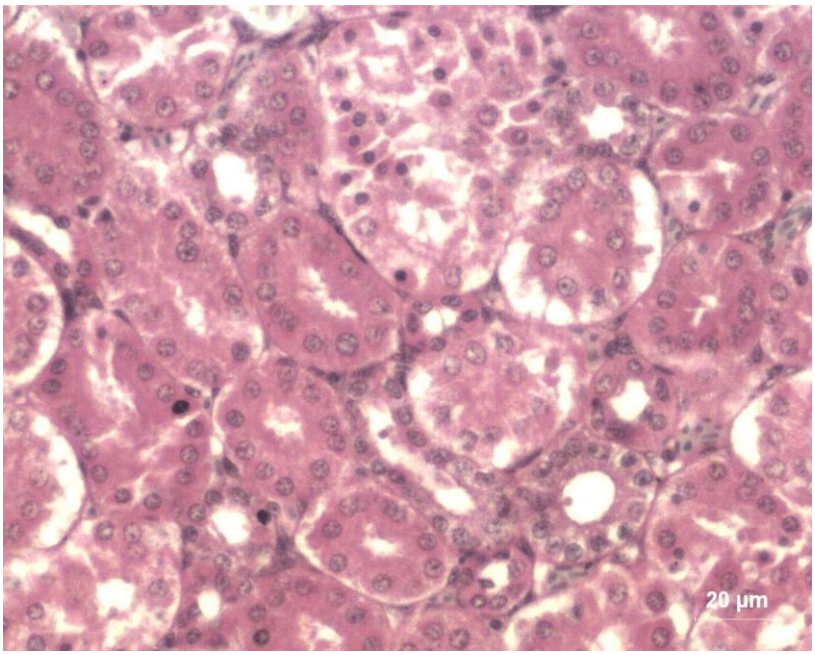

Figure 3: PA 7.5 ppm – Kidney – Proximal convoluted tubular epithelial cells showed mild vacuolar degeneration Scale bar H&E 20 µm

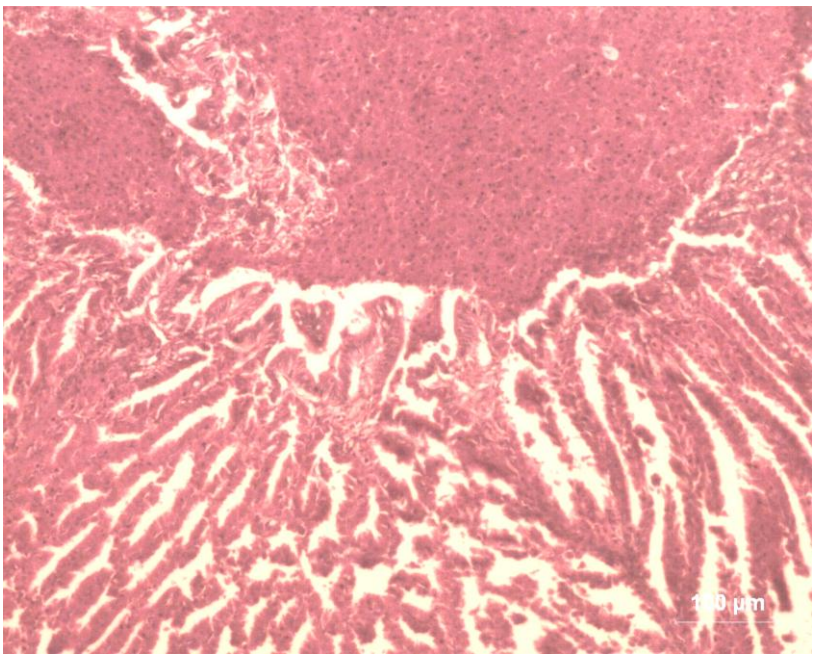

Figure 4: PA 15 ppm- Proventriculus - Glandular necrosis Scale bar H&E 20 µm

The 7.5 ppm PA fed birds revealed mild diffuse vacuolar degeneration of hepatocytes, bile duct hyperplasia and Kupffer cell hyperplasia while 15 ppm group showed focal hepatocellular necrosis and microgranulomas in the liver and 30 ppm PA fed birds showed diffuse vacuolar degeneration of hepatocytes, and papillary hyperplasia of biliary epithelium. In 7.5 ppm and 15 ppm PA fed birds, tubular epithelial cell degeneration and necrosis were observed in the kidneys while 30 ppm group also showed interstitial nephritis characterized by mononuclear cell infiltration.

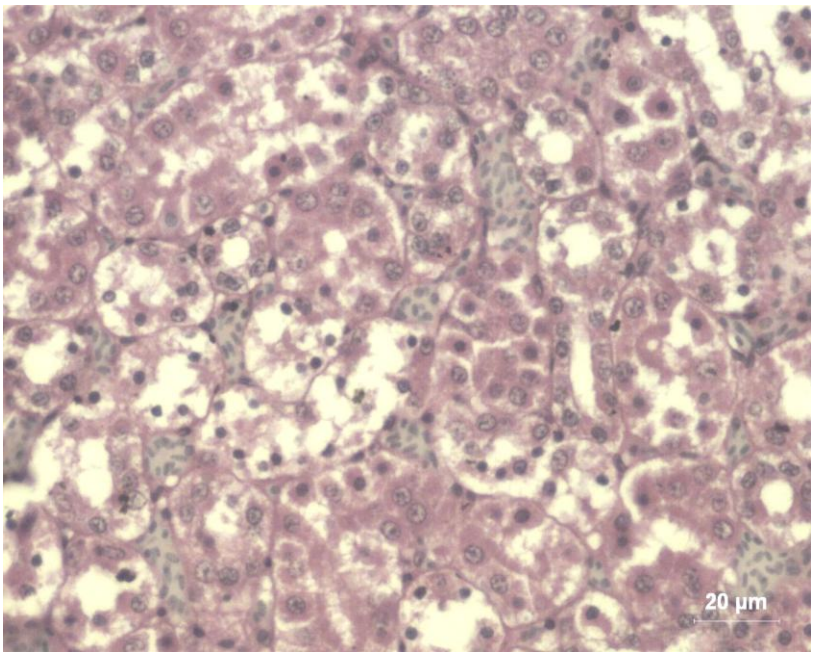

Figure 5: PA 30 ppm-Kidney- Tubular epithelial cell degeneration and necrosis Scale bar H&E 20 µm

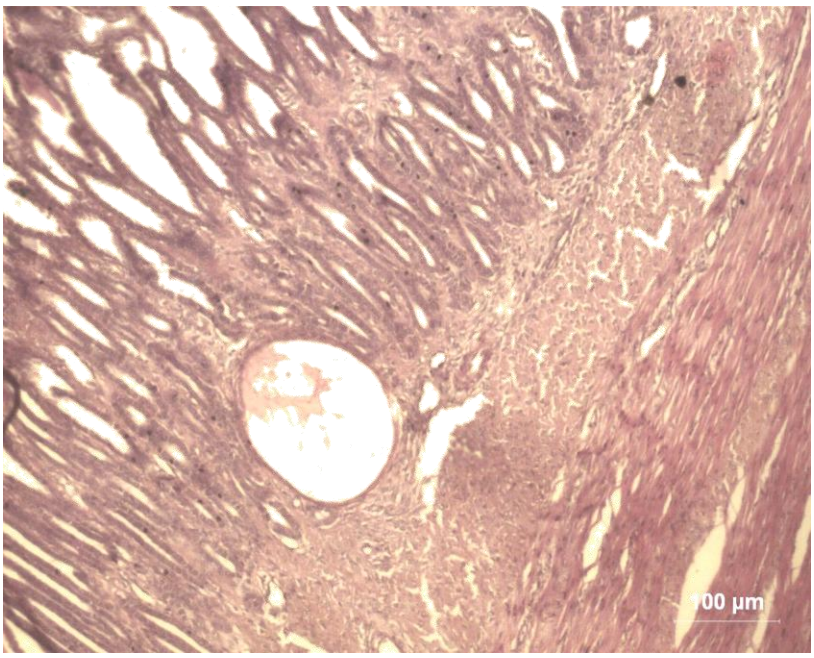

Figure 6: PA 30 ppm-Gizzard - Gizzard gland showed focal cystic dilatation Scale bar H&E 100 µm

The crop revealed focal mucosal hyperplasia at 7.5 ppm and diffuse hyperplasia accompanied by parakeratosis at 15 ppm level. Crop muscle degeneration was also noted at 15 ppm level compared to 7.5 ppm level. Similar but extensive changes were observed at 30 ppm level.

The proventricular changes were mucosal epithelial degeneration and necrosis at 15 ppm level and in addition submucosal necrosis was also found at 30 ppm level while 7.5 ppm PA fed birds showed proventriculitis.

The 7.5 ppm PA fed birds showed ventriculitis with focal necrosis. Dilated glands with eosinophilic secretion was observed at 15 ppm PA fed birds whereas 30 ppm PA fed birds showed degeneration of glandular epithelial cells and focal cystic dilatation of glands. Catarrhal duodenitis and shortened villi were observed at 15 ppm level. The pancreatic acinar cell degeneration and necrosis were observed at 15 ppm level. Pazhanivel and Balachandran (2014a) also observed mild vacuolar degeneration of hepatocytes and focal necrosis with mononuclear cell infiltration in the liver, bile duct hyperplasia with periductular mononuclear cell infiltration and necrosis of cholangiolar epithelium, eosinophilic amorphous substances within the bile duct lumen and mild periductular fibrosis and crop mucosal hyperplasia in the 15 ppm PA fed birds.

The above changes are in accordance with the findings of Sarmadha (2003) but were reported at higher levels of penicillic acid toxicity (50-480 ppm) in broiler chicken. However, no hepatic pyogranuloma, basement membrane thickening in the glomeruli, ingluvitis and proventricular crypt elongation were observed in this study as reported by Sarmadha (2003). Probably, it requires higher level of penicillic acid to induce such pathological changes as employed by Sarmadha (2003).

Conclusion

It could be concluded that the pathological changes were dose dependent and the changes were milder at 7.5 ppm, moderate at 15 ppm and severe at 30 ppm levels. The above study indicated that penicillic acid even at 7.5 ppm level could induce pathological changes in broiler chicken.

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