The Pharma Innovation Journal 2021; SP-10(9): 359-363

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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(9): 359-363

www.thepharmajournal.com
Received: 19-07-2021
Accepted: 21-08-2021

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Histopathological changes in liver and kidney induced by lead and thiram alone and combined exposure in broilers

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Abstract
The experiment was designed to study the pathology of lead acetate, tetramethylthiuram disulfide and its combination induced toxic effects in broilers. Hundred day old broiler chicks divided into four groups, 25chicks in each group. The group 1 (control) fed with basal diet, group 2 (lead acetate) at the rate of 300 ppm per day, group 3 (tetramethylthiuram disulfide) at the rate of 60 ppm per day and group 4 birds were fed with combination of lead acetate and tetramethylthiuram disulfide with same dose. The duration of experiment for 5 weeks and the age of the birds were 6 weeks (first week for acclimatization). Histopathological changes in liver and kidney were recorded at end of the 1st, 3rd and 5th week of experiment. Histopathologically, alteration in liver and kidneys were severe on 5th week of experiment. Liver showed congestion, necrosis, and cirrhosis. Kidney sections revealed vacuolar degeneration, haemorrhages and necrosis. The lesions were pronounced in group 4 comparatively group 2 and 3.

Keywords: broilers, histopathology, lead acetate, liver, kidney, tetramethylthiuram disulfide

Introduction
Lead (Pb), a ubiquitous metal, is one of the most abundant elements present on earth. The common sources of lead is natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units, paint industries and so forth [9]. The TMTD (Tetramethylthiuram disulfide) is an organic sulphur compound and a systemic fungicide commonly used for treating corn and other grains intended for seed purposes and also for storing food grains [6]. The Pb inhibits the delta amino levulinic acid dehydrogenase enzyme (ALA) which is present in the erythrocytes [1]. The Pb is also known to reduce erythrocyte membrane stability [3]. Anemia accompanying with Pb poisoning was due to inhibitory effects of Pb on Heme biosynthesis [15]. Histologically liver and kidney sections of lead treated birds showednecrotic foci and congestion of liver, kidneys and distortion of cellular architecture in lead treated birds at 200 mg/Kg diet for 42 days [4].
Histologically liver and kidney sections of thiram treated birds showed severe dilation of central vein CV, mild dilatation of sinusoids, mild to moderate fatty change, hydropic degeneration of perivascular area and shrunken hepatocytes with thickened wall of the blood vessels and karyorrhexic and pyknotic nuclei observed. The kidney sections are showed moderate to severe lesions like intertubular dilatation, shrunken glomeruli. The tibial growth plate cartilage (TGPC) revealed severe lesions were characterized by grater thickening of proliferating zone, grater thinning of hyaline zone, absence of chromatin material and empty clefts like chondrocytes which were grouped in clusters [6].
In view of the above, the present study was designed to evaluate the toxic effects of individual (lead and thiram), combination of these compounds in Vencobb broiler chicks.

Materials and Methods
Chemicals
Lead acetate (PbAc) obtained from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, and thiram obtained from Seed Research and Technology Centre (SRTC) Professor Jayashankar Telangana State Agriculture University (PJTSAU).
Experimental animals: Hundred day old broiler chicks were procured from M/S Venkateshwara Hatcheries Pvt, Ltd, Hyderabad were randomly divided into 4 groups each group consisting of 25 chicks.

Group 1(control) was given basal diet, group 2 (lead group) was lead acetate (obtained from Thermo Fisher Scientific India Pvt. Ltd., Mumbai) @ 300 ppm daily in feed, group 3 was given TMTD (obtained from Seed Research and Technology Centre (SRTC) Professor Jayashankar Telangana State Agriculture University (PJTSAU), Rajendranagar) @ 60 ppm daily in feed and group 4 birds were fed with combination of PbAc + TMTD with same dose. The duration of experiment was for 5 weeks. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC-No. I / 2018 - 36).

Methods

To study the histopathology of Liver and kidney, Histopathological changes in liver and kidney were recorded at end of the 1st, 3rdand 5th week of experiment. Detailed necropsy was conducted, liver and kidney were collected in 10 percent Neutral Buffered Formalin. Samples were processed, sectioned (5µm) and stained with Hematoxylin and Eosin (H&E) as per the standard protocol given [8].

Results

Liver

Normal histological features of liver were observed in group 1 (Fig 1) and the liver sections of group 2 birds showed cellular swelling, vacuolar degeneration of cytoplasm followed by dilation of sinusoids on 1st week of experiment. Majority sections exhibited hyperchromatic and pyknotic nuclei which resembles to inclusion bodies. Few hepatocytes showed focal areas of infiltration of inflammatory cells with loss of architecture, dilation of CV and cirrhosis of hepatic parenchyma. In the same group, the changes after 3rd week of experiment were similar to lesions observed on 1st week but the lesions were more prominent. Fibrous tissue proliferation and necrosis along with haemosiderin like pigment in degenerated hepatocytes were observed. Bile duct hyperplasia, perivascular and sinusoidal fibrosis were also noticed. In the same group, the changes after 5th week of experiment were similar to the lesions observed on 1st and 3rd week lesions with additional features like complete loss of architecture, congestion, periportal fibrosis, haemosiderin like pigment with karyorrhectic nuclei. (Fig 2).

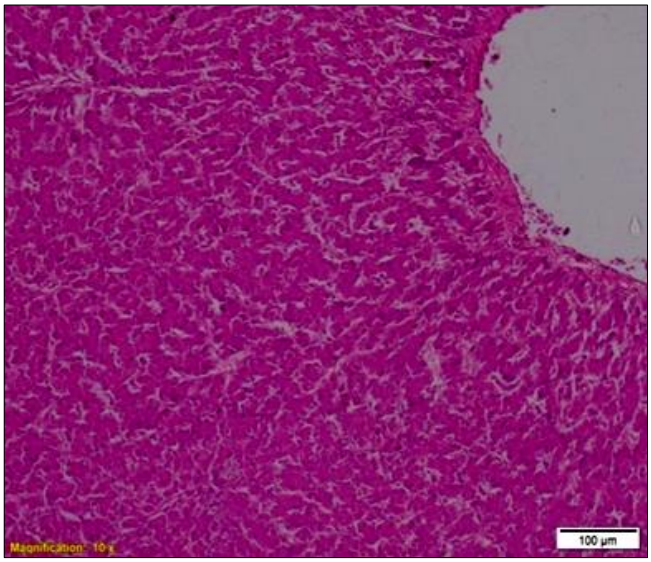

Fig 1: Photomicrograph of liver showing normal architecture (Group 1, 5th week): H&E 100 µm

Fig 2: Photomicrograph of liver showinghyperplasia of bile duct and fibrous tissue proliferation around portal vein (Group 2, 5thweek): H&E 50 µm

On 1st week of experiment the lesions in the liver of TMTD group (3) were characterized by dilatation and haemorrhages of sinusoids; mild thickening of wall of CV, necrosis, vacuolar degeneration of hepatocytes and diffuse fibrous tissue proliferation. On 3rd week of experiment similar lesions were noticed but they were moderate to severe and progressive. Additionally, degeneration, necrosis and mild proliferation of Kupffer cells, perivascular fibrosis, peripotal infiltration of inflammatory cells and hyperplasia of bile duct were exhibited.

Similar lesions were observed in group 3 sections on 5th week of experiment, but the lesions were diffuse, severe and progressive. Few sections revealed focal areas of lymphoid aggregates. (Fig 3).

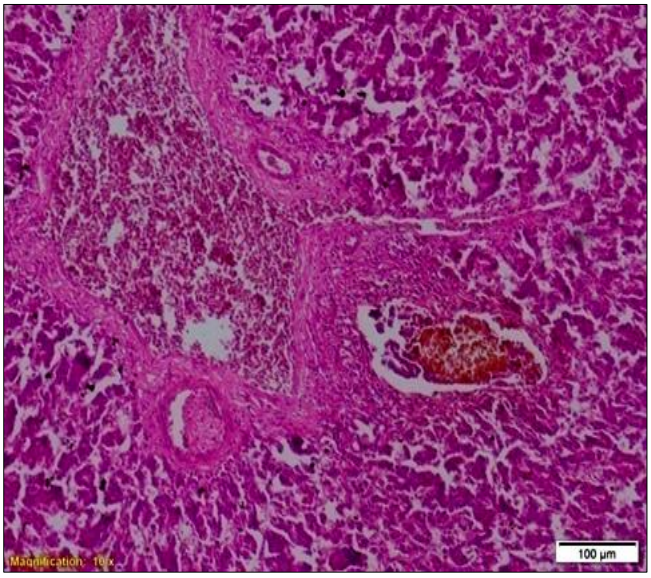

Fig 3: Photomicrograph of liver showing severe congestion, hyperplasia of bile duct, necrosis and dilated sinusoids (Group 3, 5th week): H&E 100 µm

Pathological changes of liver sections in mixed toxic group (4) after 1st week of experiment were similar to that of individual toxic groups (2 and 3). Few sections of liver

showed focal areas of lymphoid aggregates with dilatation of CV, sinusoids and portal vessels. Severe cirrhosis with pyknotic nuclei giving a inclusion body like appearance were also observed, few other sections revealed vacuolar degeneration, focal areas of haemosiderin pigment. On 3rd week of experiment the liver sections showed severe infiltration of MNC and necrosis. Sections of liver on 5th week of experiment showed severe lesions than that of the lesions observed on 1st and 3rd week of experiment (Fig 4).

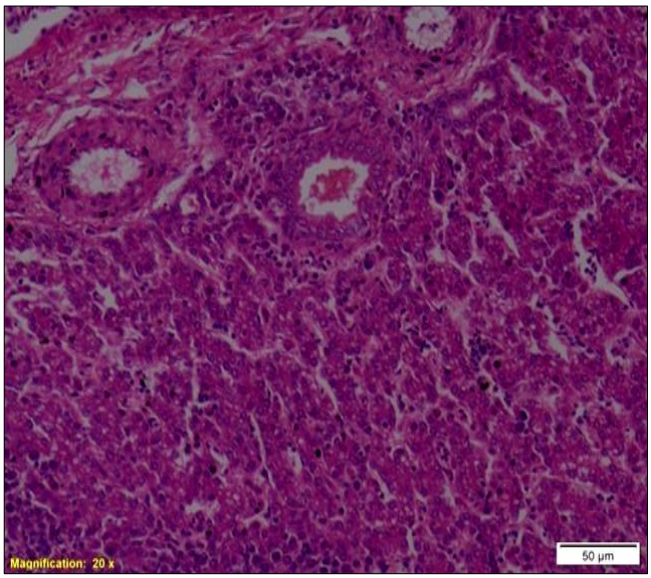

Fig 4: Photomicrograph of liver showing periportal and perivascular infiltration of MNC and sinusoidal dilatation (Group 4, 5th week): H&E 50 µm

Kidneys

The kidney sections of group 1 birds showed normal histological features (Fig 5). The group 2 (PbAc) birds kidney sections revealed loss of histological architecture of renal parenchyma, degeneration of tubules and focal areas of necrosis on 1st week of experiment. Intertubular hemorrhages, sloughing of epithelial cells, cystic dilation of tubules were also observed in few sections. Additional features like atrophy of glomeruli and vacuolar degeneration was noticed. On 3rd week kidney sections were characterized by focal areas of tubular necrosis, intertubular congestion and hemorrhages, focal areas of massive lymphoid aggregates and cystic dilation of tubules. Few other sections showed haemorrhages, cloudy swelling, pyknotic nuclei, MNC infiltration and deposition of haemosiderin like pigment. The lesions were similar but the degree of severity varied in group 3 birds on 5th week of experiment. The lesions were diffuse, severe and progressive. Additionally, few sections showed focal areas of hypercellularity in interstitium and glomeruli where in Bowman's space was increased. The nuclear changes noticed were pyknotic and karyorrhectic. (Fig 6).

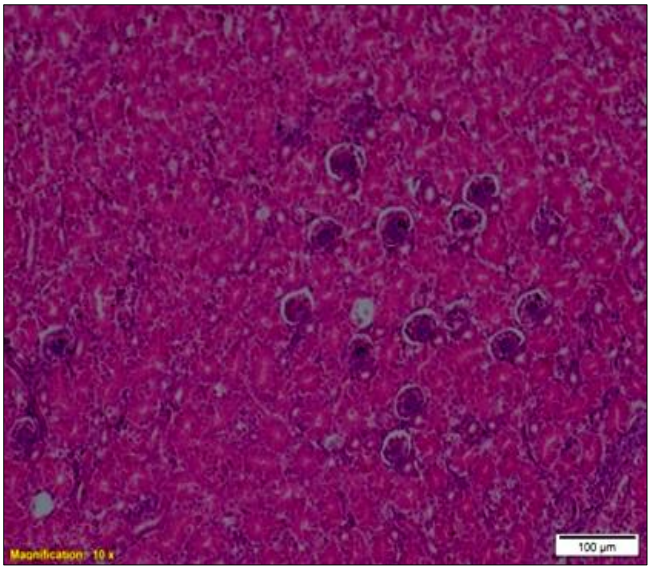

Fig 5: Photomicrograph of kidney showing normal architecture, (Group 1, 3rd week): H&E 100µm

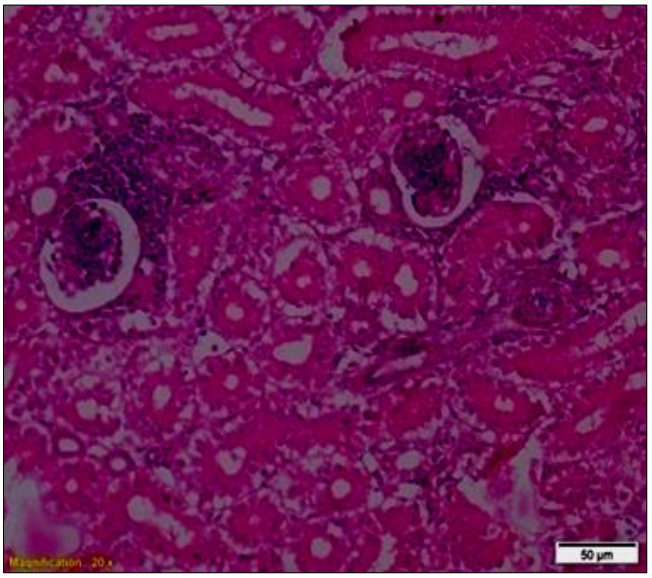

Fig 6: Photomicrograph of kidney showing necrosis of tubules, atrophy of glomeruli, periglomerular infiltration of MNC and increased Bowman's space (Group 2, 5th week): H&E 100µm

Lesions in the kidneys of group 3 birds (TMTD) on 1st week of experiment showed mild to moderate congestion, intra and intertubular haemorrhages, cystic dilation of tubules, moderate to severe vacuolar degeneration, sloughing of epithelial cells and presence of tubular hyaline casts. Glomeruli atrophy with vacuolation and shrunken to swollen

glomuruli in different areas were observed. Similar lesions were also noticed on 3rd week of experiment; however the lesions are progressive and moderate to severe. Additionally, mild infiltrations of MNC, necrosis of tubular epithelial cells were observed on 5th week of experiment; however the lesions are progressive and severe with increased Bowman's space and infiltration of inflammatory cells into interstitium (Fig 7).

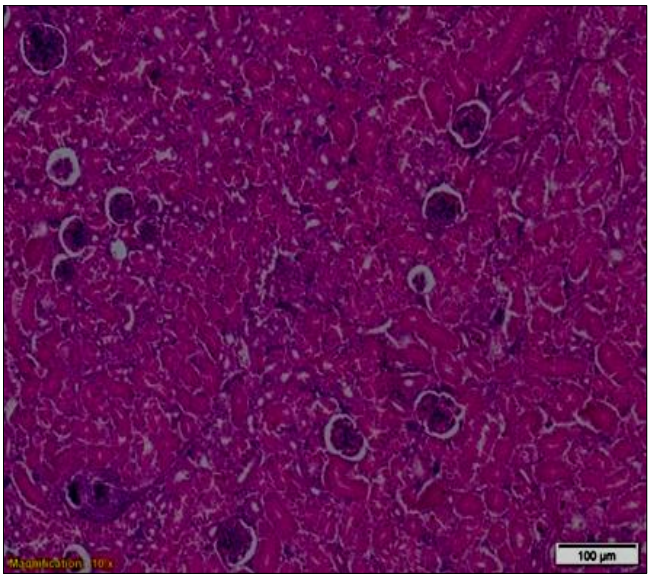

Fig 7: Photomicrograph of kidney showing increased Bowman's space, necrosis of tubular epithelial cells (Group 3, 5th week): H&E 100µm

On 1st week of experiment group 4 birds (mixed toxicity) kidney sections revealed severe vacuolar degeneration of tubules, mild intertubular haemorrhages, necrosis of tubular epithelial cells, cystic dilation of tubules. In few sections few areas showed glomerular atrophy and in few other areas detachment of glomeruli from basement membrane was also observed. Glomeruli were congested, swollen with mild hypercellularity and depositions of haemosiderin like pigment in few kidney sections were also noticed. On 3rd week of experiment kidney section revealed similar lesions but they were moderate to severe and progressive than the lesions observed on 1st week of experiment. Additionally, changes like proliferation of connective tissue, necrosis and infiltration MNC into interstitium and glomerular tufts. On 5th week of experiment the lesions were similar but severe and progressive with additional features like focal areas of lymphoid aggregation, intertubular infiltration of inflammatory cells and increased Bowman's' space than that of 1st and 3rd week lesions (Fig 8).

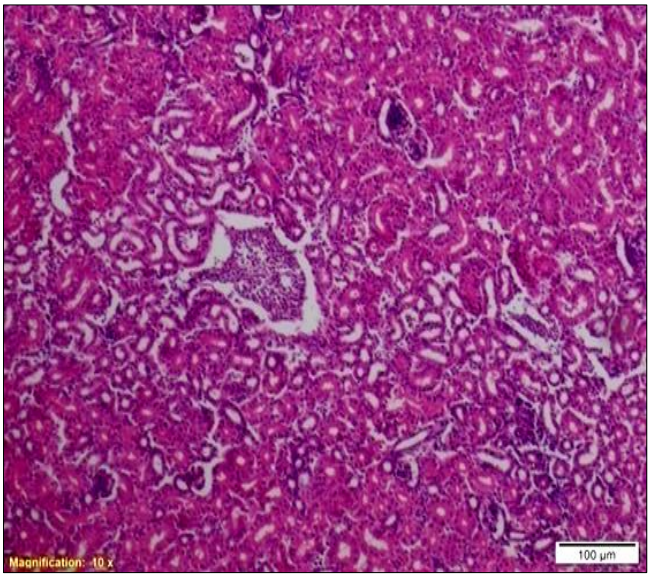

Fig 8: Photomicrograph of kidney showing severe congestion, cystic dilatation of tubules and shrunken glomeruli with basophilic appearance (Group 4, 5th week): H&E 100µm

Discussion

Liver

Alteration in hepatocytes of group 2 birds showed cellular swelling, vacuolar degeneration of cytoplasm followed by dilation of sinusoids, hyperchromatic, pyknotic, karyorrhectic nuclei (resembles the inclusion bodies) with increased peri nuclear vesiculation. Focal areas of inflammation, dilation of CV, presence of haemosiderin like pigment, hyperplasia of bile duct, sinusoidal fibrosis, diffuse and periportal cirrhosis and necrosis observed in present study were in accordance

with histopathological changes and hypothesis published [11, 2, 10, 4]. Hypothetically, these manifestations might be due to the interaction of proteins and enzymes in the hepatic tissue which could have interfered with the antioxidant defence mechanisms and resulted in generation of ROS, which in turn might have initiated inflammatory response [10].

Additionally, mild thickened wall of CV, vacuolar degeneration, sinusoidal haemorrhages, diffuse fibrous tissue proliferation, proliferation of Kupffer cells, perivascular fibrosis, peripotal infiltration of inflammatory cells and hyperplasia of bile duct were noticed. Focal areas of lymphoid aggregates and necrosis were also observed in group 3 birds, which were in accordance with previous observations [5, 12].

The liver cells would have lost its effective detoxifying mechanism, thereby toxic compounds and its metabolites were alarmingly increased and acted on degenerating hepatocytes [5, 12]. This could have further aggravated the cellular damage, mitochondrial dysfunction and resulted in and leakage of enzymes. The lesions in mixed toxic group were similar to that of groups 2 and 3 with increase in severity of lesions.

Kidneys

Kidney sections of toxic groups (2, 3 and 4) revealed loss of histological architecture of renal parenchyma *viz*. cloudy swelling, vacuolar degeneration, sloughing of epithelial cells, cystic dilation of tubules, intertubular congestion and haemorrhages focal areas of tubular necrosis, and massive lymphoid aggregates was also observed. In addition, presence of haemosiderin like pigment, hyaline casts, infiltration of MNC into interstitium along with swollen to pyknotic and karyorrhectic nuclei of tubular epithelial cells were observed. Hypercellularity and atrophy of glomeruli, increased Bowman's space was also observed. The lesions observed in kidney sections of the present study were in agreement with previous studies of PbAc induced neprotoxicity in broilers [2, 10, 4]. In TMTD induced kidney changes in experimental broilers [7, 13, 6, 14]. The authors were opined that these changes could be due to accumulation of PbAc protein complex which triggers discernible changes in PCT lining epithelial cells due to deposition PbAc predominantly in PCT, and the TMTD might have interfered in the aggravation of renal lesions along with PbAc, as these compounds have to be excreted through kidneys. Hence, severe lesions were observed in mixed toxicity.

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