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Cadmium and Thiram Induced Nephrotoxicity in Experimental Broiler Chicken

A. Uma Choudary[1], M. Lakshman[1], D. Madhuri[2], Banothu Anilkumar[3*] and Y. Ravikumar[1]

[1]Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, INDIA
[2]Department of Veterinary Pathology, College of Veterinary, Science, Korutla, Jagtial District, Telangana, INDIA
[3]Department of Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, INDIA

**Corresponding author: A Banothu; E-mail: anilvetpharma@gmail.com*

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ABSTRACT

The experiment was designed to study TMTD and $CdCl_2$-induced toxicity in broiler birds. 100-day-old chicks were distributed into 4 groups of 25 each. Group 1 birds were fed with a basal diet, group 2 fed with 60 ppm of TMTD, group 3 fed with 100 ppm of $CdCl_2$ and group 4 fed with TMTD + $CdCl_2$ for 35 days. Six birds from each group were sacrificed on the 14th, 28th and 42nd day of the experiment and the serum samples were collected. Significantly, increased levels in serum creatinine, uric acid and TBARS and decreased levels of GSH in groups 2, 3, and 4 were recorded on the 14th, 28th and 42nd day. Kidney sections showed degeneration followed by desquamation of cells and presence of casts on the 14th day, severe congestion of renal artery, and intertubular haemorrhages were observed on the 28th day. Additional lesions like degeneration of tubular epithelial cells with pyknotic nuclei, swollen to shrunken glomeruli with hypercellularity and increased Bowman's space were also observed on the 42nd day of the experiment. In conclusion, the present study suggested that TMTD, $CdCl_2$ and its combination are responsible for significant changes which could be due to free radicals which led to impairment of antioxidant defences and resulted in macroscopic and microscopic pathological changes in kidneys.

HIGHLIGHTS

- TMTD and $CdCl_2$ causes changes in the kidney due to release of free radicals.
- The occurrence of mixed toxicity could be possible due to increased environmental pollutants (Industrial and Agricultural) in developing countries more particularly in India.
- The combined toxicity is more severe than individual toxicity and envisages the synergistic action of the toxicants.

Keywords: Cadmium (Cd), Thiram (TMTD), Nephrotoxicity, Broiler chicken

Thiram (TMTD) is a systemic fungicide commonly used for treating corn and other grains intended for seed purposes and also for storing food grains. Treated grains occasionally find their way into the market (Osman *et al*., 2012). The TMTD is a proven fungicide which produces pathological alterations in birds both naturally and experimentally (Pavani *et al*., 2022). The TMTD mainly affects bone and also induces changes in vital organs such as the liver, bile duct, kidneys, heart, crop, gizzard, intestine, and lymphoid organs including the bursa of Fabricius, spleen, caecal tonsils, brain and bone which influence the health and performance of broiler chicken (Zhang *et al*., 2018). Heavy metals are known toxic substances and cause various pathological changes in the vital organs of humans and animals. Among existing toxic metals, the most important are lead, cadmium and mercury, which are harmful to animal health (Suttle, 2010). Environmental pollution with heavy metals was considered a very serious concern because these metals cannot be degraded and stay permanently in the environment (Sankhla *et al*., 2016). The Cd is a naturally occurring element present in soil and water. The Cd is broadly distributed in the

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environment and is present in minor levels in seawater and in a wide range of animal and plant species (Pandey and Madhuri, 2014). The common sources of environmental contamination of Cd are industrial & mining activities, plastic stabilizers, batteries and also in the use of pigments which may result in widespread into the environment and agricultural fields (Akan *et al*., 2010 and Ravikumar *et al*., 2020). Increased concentration of Cd in agricultural soil comes from the application of phosphate fertilizers, sewage sludge and pesticides (Yadala *et al*., 2020). The Cd affects many target tissues viz. heart and blood vessels, kidneys and lungs and also affects appetite and pain centres in the brains of cattle, sheep, chickens and camels (Bendeddouche *et al*., 2014; Anilkumar *et al*, 2013). The animals exposed to Cd compounds result in an accumulation of the ions abundantly in the kidneys, heart, and liver and to a lesser degree, in the pancreas and brain (Kumar and Reddy, 2012). Intake of Cd in male chickens results in accumulation in different organs and the amount accumulated depends on the interval of exposure, a quantity of ingestion, production and reproduction phase, age and breed of the animals (Sankhla *et al*., 2016). After distribution to different tissues, Cd modulates the immune function and produces toxins in animal tissues (Magrone and Jirillo, 2013). The various toxic effects of Cd included morphological and functional damage in hepatic and renal tissues of rats (Sun *et al*., 2021). The toxicity of Cd mainly affects the kidneys, liver and lungs (Yadala *et al*., 2020). Adequate literature was published about TMTD and $CdCl_2$-induced toxicity in animals and experimental broilers. On perusal of literature on mixed toxicity ($CdCl_2$ + TMTD) in animals and broilers is obscure, but there is a possibility for mixed contamination due to an increase in environmental pollutants (Industrial and Agricultural) in developing countries, more particularly in India. Hence, the present study was designed with the objective of the study of nephrotoxicity by mixed toxicants ($CdCl_2$ + TMTD) in broilers.

MATERIALS AND METHODS

Location

The present study was carried out in the Department of Veterinary Pathology, Ruska Labs and Poultry Experimental Station (PES), Instructional livestock farm complex (ILFC), College of Veterinary Science, Rajendranagar, Hyderabad-30.

Experimental Animals

A total of 100-day-old broiler chicken (Cobb strain) weighing 45-50g were procured from M/S Venkateshwara Hatcheries Ltd, Hyderabad. They were divided into four groups, with twenty-five chicks in each group. The chicks were raised under uniform managemental conditions; fresh feed and water were provided regularly ad libitum throughout the experimental period (42 days). During the first week, all chicks were fed with basal diet and allowed for acclimatization. From the second week onwards, the experimental diet was fed to the respective groups and observed for clinical signs and mortality if any, during the entire period of the experiment. The experiment was carried out according to the guidelines and prior approval of the Institutional Animal Ethics Committee (IAEC) bearing no: I/2018-37/IAEC/ C.V.Sc, Hyderabad, dated: 16-07-2018.

Chemicals

The $CdCl_2$ was obtained from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, and TMTD was procured from SRTC (Seed Research and Technology Centre), Prof. Jaya Shankar Telangana State Agriculture University (PJTSAU), Rajendranagar was used for inducing toxicity in broiler chicken through the feed for a period of 35 days (2^{nd} – 6^{th} week). Other chemicals used in the study were obtained from authorized dealers.

Experimental Design

A total of 100 broiler chicks were randomly divided into 4 groups consisting of 25 chicks in each.

1^{st} Group: Normal controls (fed with regular poultry feed-Basal diet).

2nd Group: Birds fed with Basal diet +TMTD @ 60 ppm.

3^{rd} Group: Birds fed with Basal diet + $CdCl_2$ @ 100 ppm.

4^{th} Group: Birds fed with Basal diet +TMTD @ 60 ppm + $CdCl_2$ @ 100 ppm.

Collection of blood for biochemical parameters

Prior to blood collection, the selected experimental birds from each group were allowed to fast for 12 h. On the day of sacrifice, approximately 2 mL of blood was collected from 6 chicks in each group on the 14th, 28th and 42nd day of the experiment. All the biochemical parameters were analyzed with help of an automatic ELISA reader (μ-Quant Biotech Instruments) by using Erba Mannheim Diagnostic Kits, at the Directorate of Poultry Research (DPR), Rajendranagar, Hyderabad.

Tissue antioxidant profile

Small pieces of kidneys were collected and stored at -20ºC for estimation of tissue oxidative stress markers (reduced GSH and TBARS/MDA). Thiobarbituric acid reacting substances (TBARS) analysis from kidneys was performed as per the method described by Ohkawa *et al*. (1979) and GSH as per the method described by Cohen *et al*. (1970).

Gross and Histopathology

Six birds from each group were sacrificed on the 14th, 28th and 42nd day of the experiment by dislocation of the Atlanta occipital joint and a detailed necropsy examination were carried out as per standard procedure followed at the Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar. The kidneys were observed for gross lesions if any. Slices of kidneys from each group were collected and fixed in 10 per cent neutral buffered formalin (NBF) for histopathology. The fixed tissues were processed and stained with Haematoxylin and Eosin (H & E) stain as described by Luna (1968).

STATISTICAL ANALYSIS

Data obtained were subjected to statistical analysis by applying one-way ANOVA using a statistical package for social sciences (SPSS) version 16.0. Differences between means were tested by using Duncan's multiple comparison tests and the significance level was set at $P < 0.05$ (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Kidney sero-biochemical profile

Significantly ($P<0.05$) increased mean values of serum creatinine and serum uric acid were recorded in groups 2, 3 and 4 on the the 14th, 28th and 42nd days than in group 1 (Table 1 and 2). A significant ($P<0.05$) increase in serum creatinine and uric acid levels in groups 2, 3 and 4 were observed than group 1. The group 2 birds showed increased serum creatinine and urea levels which agreed with the observations of Lakshman (2011), Anilkumar *et al.* (2010) and of Majid *et al.* (2017). They explained that this might be due to nephrotoxicity leading to hindrance in glomerular filtration rate (GFR) resulting in increased excretion of uric acid. A significant increase in serum

Table 1: Serum creatinine (mg/dL) in different groups

Sl. No.	Group	Day 14	Day 28	Day 42
1	Control	0.61 ± 0.045^{b}	1.03 ± 0.238^{b}	1.06 ± 0.210^{c}
2	TMTD	1.73 ± 0.222^{a}	2.3 ± 0.252^{a}	2.52 ± 0.210^{b}
3	Cd	1.60 ± 0.075^{a}	2.77 ± 0.088^{a}	3.16 ± 0.099^{a}
4	Cd + TMTD	1.82 ± 0.044^{a}	2.84 ± 0.064^{a}	3.59 ± 0.094^{a}

Table 2: Serum uric acid (mg/dL) in different groups

Sl. No.	Group	Day 14	Day 28	Day 42
1	Control	7.95 ± 0.400^{a}	8.21 ± 0.410^{b}	8.85 ± 0.412^{c}
2	TMTD	8.04 ± 0.595^{a}	9.55 ± 0.357^{a}	10.33 ± 0.326^{ab}
3	Cd	8.22 ± 0.336^{a}	9.88 ± 0.299^{a}	9.46 ± 0.472^{ac}
4	Cd + TMTD	8.41 ± 0.534^{a}	10.53 ± 0.503^{a}	11.32 ± 0.361^{b}

creatinine and uric acid were observed in group 3, which was in accordance with the findings of Cinar *et al.* (2011), Subhan *et al*. (2011) and Singh *et al*. (2016). This might be due to oxidative damage to the renal tissue due to the nephrotoxic effect of $CdCl_2$ that affects GFR. In the mixed toxic group, serum creatinine and urea levels were elevated; it might be due to the synergistic action of TMTD and $CdCl_2$. These observations were supported by histopathological changes in kidney functional units such as degeneration, cellular swelling of the tubules, protein casts in tubules and necrosis (Zubair *et al*., 2013).

Antioxidant profile

The mean values of kidney TBARS / MDA levels were significantly increased in groups 2, 3 and 4 compared to group 1 on the 14th, 28th and 42nd day of the experiment. Significantly (P<0.05) elevated levels of MDA mean values were recorded in groups 2, 3, and 4 than that of group1 on the 14th, 28th and 42nd day of the experiment (Table 3). Group 2 bird tissue samples showed a significant increase in MDA levels in accordance with Perry *et al*. (2010), and Khalid Mehmood *et al*. (2017). The authors documented increased levels of MDA in the tissue of TMTD-treated birds, and they opined that this could be due to TMTD-induced damage to liver and kidney tissue which prevents the antioxidative enzymatic activity of GSH and SOD resulting in elevated levels of lipid peroxides of the cell membrane. The group 3 birds showed a significant increase in tissue MDA levels which agreed with the findings of several authors viz. Bharavi *et al*. (2011) and Omid Karimi *et al*. (2015). They opined that the damage to the protective antioxidant barrier might be due to the generation of ROS produced by direct/ indirect lipid peroxidation mechanisms. In group 4, increased oxidative stress to the hepatic parenchyma and renal functional unit resulted from the action of combined toxicants causing increased production of tissue MDA levels as was discussed in individual toxicants.

There was a significant (P<0.05) decrease in kidney-reduced GSH mean values in groups 2, 3 and 4 as compared to group 1 on the 14th, 28th and 42nd day of the experiment (Table 4). The decrease in GSH in Group 2 birds was in accordance with the findings of Rana *et al*. (2002). They explained that the TMTD interferes with glutathione concentration in the growth plate leading to disturbance in cellular homeostasis and depletion of GSH which may induce pathological changes and cell death. Decreased levels in tissue GSH of group 3 birds were similar to the findings of Bharavi *et al*. (2011), Yadala Ravikumar *et al.* (2020) and Imer *et al*. (2017). They opined that this might be due to an imbalance between oxidative and antioxidative mechanisms viz; change in SOD levels, CAT, GST, GSH and MDA. Continuous exposure of cells to CdCl2 resulted in depletion of GSH levels and increased production of MDA which stimulated the formation of β2-microglobulin in urine that induced renal impairment. In the present study, significantly reduced GSH levels were observed

Table 3: Thiobarbituric acid reacting substances: (TBARS nM of MDA released/g tissue) in different groups of kidneys

Sl. No.	Group	Day 14	Day 28	Day 42
1	Control	22.58±0.868^{c}	22.73±0.229^{c}	25.58±0.964^{c}
2	TMTD	43.32±0.585^{b}	44.97±0.368^{b}	47.47±0.502^{b}
3	Cd	53.3±0.895^{a}	55.77±0.731^{c}	58.22±0.938^{a}
4	Cd + TMTD	52.37±0.768^{a}	56.5±0.606^{c}	58.82±0.334^{a}

Table 4: Reduced Glutathione levels (Reduced GSH µM /g tissue) of kidney in different groups

Sl. No.	Group	Day 14	Day 28	Day 42
1	Control	55.1±0.601a	55.07±0.267a	54.87±0.081a
2	TMTD	46.52±0.435b	41.02±0.401b	38.55±0.486b
3	Cd	43.3±0.367c	39.73±0.817ab	38.32±0.715b
4	Cd + TMTD	42.65±0.171c	38.57±0.473c	36.87±0.402c

All Values are Mean ± SE (n=6); One way ANOVA; All Means with different superscripts in a column differ significantly at P<0.05.

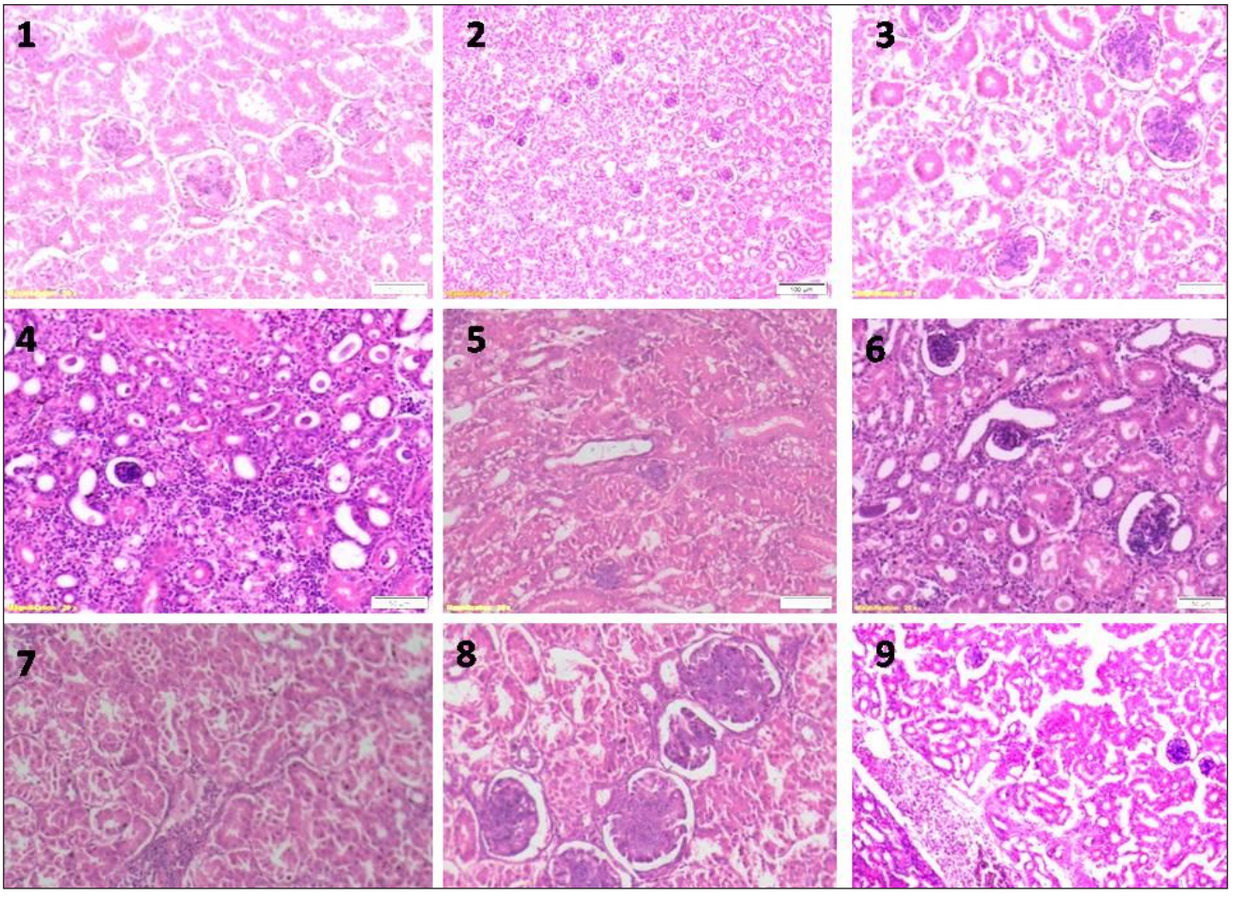

Fig. 1: Photomicrograph of kidney showing cystic dilatation of tubules, infiltration of MNC's, focal areas of congestion and haemorrhages and hyaline cast formation (Group 2, 14 days): H&E x20. **Fig. 2:** Photomicrograph of kidney showing shrunken glomeruli, mild increased Bowman's space, necrosis and cystic dilatation of tubules (Group 2, 28 days): H&E x10. **Fig. 3:** Photomicrograph of kidney showing shrunken glomeruli, degeneration and necrosis of tubular epithelial cells, interstitial inflammatory cells infiltration (Group 2, 42 days): H&E x20. **Fig. 4:** Photomicrograph of kidney showing shrunken glomeruli, degeneration and necrosis of tubular epithelial cells, hyaline casts in tubules and MNC infiltration (Group 3, 14 days): H&E x10. **Fig. 5:** Photomicrograph of kidney showing cystic spaces, atrophied glomeruli (Group 3, 28 days): H&E x20. **Fig. 6:** Photomicrograph of kidney showing swollen to atrophied glomeruli, increased Bowman's space, degenerated tubules (Group 3, 42 days): H&E x20. **Fig. 7:** Photomicrograph of kidney showing shrunken to atrophied glomeruli, necrosis of tubular epithelium and mild intertubular infiltration of round cells (Group 4, 14 days): H&E x20. **Fig. 8:** Photomicrograph of kidney showing swollen basophilic glomeruli arranged in rows with different stages of degeneration (Group 4, 28 days): H&E x20. **Fig. 9:** Photomicrograph of kidney showing severe congestion of renal arteries and shrunken glomeruli with increased Bowman's space (Group 4, 42 days): H&E x20.

in groups 2, 3 and 4 throughout the experimental period is indicative of increased oxidative stress. These findings and hypothesis is in accordance with earlier publications as stated above.

Gross and histopatholgy

After sacrifice, all birds were thoroughly examined for gross changes, if any. Abnormalities were observed in the kidneys of toxic groups. Swollen and congested kidneys were observed in groups 2, 3 and 4. Additionally, petechial haemorrhages on kidneys were also noticed in group 4 birds on the 42nd day of the experiment. Photomicrograph of the kidney (Group 2) showed variation in shape and size of glomeruli, cystic dilatation of tubules, infiltration of MNCs, focal areas of congestion and haemorrhages and

hyaline cast formation on the 14th day of the experiment (Fig. 1). Kidney sections showed swollen to shrunken glomeruli, focal areas of atrophied glomeruli, thickened renal arteries, mild increase in Bowman's space of the capsule and infiltration of mononuclear cells in the interstitium on the 28th day of the experiment (Fig. 2). The group 2 sections of the kidney on the 42nd day of the experiment showed swollen to shrunken tufts of glomeruli, severe increase in Bowman's space of some glomeruli, necrosis of tubular epithelial cells with infiltration of round cells in interstitium of the tubules. Some sections revealed detachment of epithelial cells, degeneration of glomerular tufts and tubular epithelial cells (Fig. 3). Photomicrograph of kidney of Group 3 showed shrunken to swollen glomeruli with mild hypercellularity, cystic dilatation of tubules, and increased inter-tubular space on the 14th day of the experiment (Fig. 4). Group 3 slices of kidneys showed similar lesions 28th day along with inter-tubular haemorrhages, mild degeneration, necrosis and vacuolization of tubular epithelial cells, and atrophied glomeruli with casts (Fig. 5). Slices of kidneys on the 42nd day of the experiment showed swollen to atrophied glomeruli, increased Bowman's space, cystic dilatation of tubules and formation of hyaline casts in tubules. Some sections showed severe infiltration of MNCs in the interstitium and denudation of epithelial cells of tubular structures resulting in cast formation (Fig. 6). These changes observed in groups 2 and 3 were in accordance with the findings of Emiola *et al*. (2013) and Singh *et al*. (2016) in group 3 and Lakshman (2011) in group 2. Kidney sections of group 4 revealed similar lesions to TMTD and CdCl2-induced groups but the lesions are more severe and progressive. Group 4 showed on the 14th day of the experiment, swollen PCT and DCT, shrunken to atrophied glomeruli, necrosis of tubular epithelium and mild intertubular infiltration of round cells (Fig. 7). On the 28th day of the experiment, section of the kidney showed distorted tubular cell architecture, degeneration and detachment of tubular epithelial cells, and inter-tubular haemorrhages with disintegrating erythrocytes that were basophilic to eosinophilic in nature (Fig. 8). Similar histopathological lesions were observed on the 42nd day in mixed toxicity-induced birds but the lesions were more severe with hyaline cast formation (Fig. 9). These changes might be due to excess production of ROS imbalance between oxidative and antioxidative mechanisms.

CONCLUSION

In conclusion, the present study suggested that TMTD, $CdCl_2$ and its combination are responsible for significant changes in biochemical parameters, oxidative stress enzymes alterations in kidneys which could be due to abnormal generation of free radicals which led to impairment of antioxidant defences. Finally, these changes resulted in macroscopic and microscopic pathological changes in kidneys.

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