



Determination of Optimal Toxic Concentration and Accumulation of Cadmium in Broiler Chicks

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Cadmium is considered one of the most toxic, non biodegradable heavy metal for the human and animals. The purpose of the present study was to investigate the changes in biochemical parameters of blood and accumulation of cadmium in various tissue caused by various levels of dietary cadmium chloride (CdCl₂) in broiler chicks. CdCl₂ was administered through drinking water to broiler chicks. In spectral analysis, CdCl₂ treatment caused a significant increase in Glutamate pyruvate transaminase (GPT), creatinine and uric acid levels in all treated groups. Intriguingly, the GPT, creatinine, and uric acid levels were significantly higher at 75 mg/kg as compared to the groups treated with high doses (100, 125 and 150 mg/kg) of CdCl₂. Atomic Absorption Spectrophotometer (AAS) was used for the determination of Cd accumulation in kidney, liver and Breast muscles. AAS analysis revealed that Cd accumulation is increased in breast muscles as compared to liver and kidney at higher doses of Cd than 75 mg/kg.

Key words: Cadmium, Broiler chicks, Toxicity, Biochemical parameters

INTRODUCTION

Cadmium is a heavy metal that is broadly distributed in the environment and is present in minor levels in sea water and in a wide range of animal and plant species (Klaassen *et al.*, 2001). After absorption, cadmium is circulated in blood, bound mainly to blood cells and albumin. It primarily distributed to the liver and then redistributes progressively to the kidney as cadmium-metallothionein (Cd-MT). After distribution, approximately 50% of the total-body burden is found in the liver and kidney (Akyolcu *et al.*, 2003). Cadmium is an environmental pollutant that is able to modulate the immune function (Sant'Ana *et al.*, 2005). In animals, cadmium was shown to be toxic to all tissues. The various toxic effects of cadmium include those of morphological and functional damage in hepatic (Dehn *et al.*, 2004) and renal tissues (El-Sharak *et al.*, 2007), testicular necrosis (Lorico *et al.*, 2002), morphological and biochemical changes in lung, and gastrointestinal tract (Weisman *et al.*, 1998). The toxicity of cadmium mainly affects the kidney, liver and

lung (Thevenod *et al.*, 2003; Satarug and Moore, 2004). It may disrupt the bone homeostasis (Wang *et al.*, 2003).

The serum enzyme glutamic pyruvate transaminase (GPT) is an important indicator of chemically induced hepatotoxicity in experimental animal studies (Ernest and Robret, 2002). Uric acid is the last step in the purines breakdown pathway (Kumar and Clark, 2005). Uric acid is a catabolite of purine metabolism as derived from nucleic acid or nucleotide cofactor (Remington, 2000).

Creatinine is the waste product of creatine metabolism and is the common excreted compound. Normally, serum creatinine is 1 to 5 mg/dl and higher values indicate glomerular damage or cardiac insufficiency (Remington, 2000).

In spite of being a known toxic compound, there is no or very little data reported for the toxic concentration and accumulation of cadmium in broiler chicks' tissues especially at muscle at higher doses. In present study, effects of cadmium chloride on biochemical parameters were studied in broiler chicks and optimal toxic concentration (at which the body gives maximum response in the form of GPT, creatinine and uric acid) was determined. The accumulations of cadmium were investigated in kidney, liver and in breast muscles. It was found that the broiler biochemical parameters are most severely affected at a dose rate of 75 mg/kg as compared to other dose rates. It was also found that cad-

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mium accumulation was increased in muscles at more than 75 mg/kg dose rate while in liver and in kidney remains the same at 75 mg/kg or more dose level of cadmium as 25 to 50 mg/kg.

MATERIALS AND METHODS

Animals and experimental design. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee, which entirely fulfill the international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985). A day old broiler chicks, purchased from a commercial supplier at Lahore (Pakistan), and 20 days old chicks were used in this study. All The chicks were freely allowed to the commercial grower broiler diet, and tap water. The 20th days old chicks were considered as 0th day at the start of experiment. Thirty five chicks were divided in to seven respective groups (A, B, C, D, E, F and G) with an average body weight of about 600 gm at the start of experiment. The group G was kept as control and the other were treated at a dose rate of 25, 50, 75, 100, 125 and 150 mg/kg respectively. Our unique pattern of selected dose level based on previous experiments, to determine the most toxic concentration level of cadmium in broiler chicks. The medication was done through oral route by using drinking water. Fresh solution of cadmium chloride was prepared in tap water as ppm (part/million) during the medication period.

Chemicals and solutions. Cadmium chloride ($\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from BDH Laboratory supplies Poole, Bh 15 ITD, England. Known concentration of cadmium chloride was used according to the experimental design. Anticoagulants agent, EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$) was purchased from Sigma-Aldrich Laborchemikalien GmbH and was used at the rate of 1 mg/ml of blood. Various diagnostic kits, GPT CRESCENT (Saudi Arabia), creatinine CRESCENT diagnostic kit (Germany) and uric acid AXIOM diagnostics kits (Saudi Arabia) were used for the analysis of GPT, creatinine and uric acid respectively.

Assays.

GPT, Creatinine and Uric acid determination: The level of the serum GPT, creatinine and uric acid were determined in groups (A, B, C, D, E, F, and G with dose level of 25, 50, 75, 100, 125, 150 mg/kg and control respectively) before and after treatment at 0th, 5th, 15th, 25th and 30th day of experiment. The fresh experimental solution was prepared from the cadmium chloride (CdCl_2) for the treatment. Totally, 2 ml blood were taken from the wing vein of chicks and placed in the micro tube. The blood samples serum were separated by centrifugation at 4000 rpm/5 minutes. Shimadzu UV-Visible double beam Spectrophotometer 1700 Pharma (Japan) was used to determine GPT (505 nm), crea-

tinine (492 nm) and uric acid (520 nm) level using isolated plasma.

Cadmium concentration in tissue: The Atomic Absorption Spectrophotometer (AAS, PERKIN EMER, Model Spectro-AA90-Varian Tectron, Australia) was used to determine the cadmium concentration in kidney, liver and breast muscle using the method described by Uyanik *et al.*, 2001. Dry ashing procedure was used for analysis of cadmium in various organs. In brief, a 9 gm sample of wet tissue from each group was dried at 100°C for 2 hours. Dried samples were transferred to cool muffle furnace and the temperature was slowly raised to 450°C and ashed overnight, then dissolved in 2 ml of HNO_3 plus de-ionized water. After filtering, the digest was made up to 100 ml with de-ionized water. The concentration of cadmium was determined by using AAS.

Statistical analysis: All data presented as the mean values \pm SD (standard deviation) of triplicate independent experiments. The data were evaluated by SPSS software; the data was analyzed by two-way analysis of variance (ANOVA). The significance of the differences between the means was determined. Statistical significance was at $p < 0.05$.

RESULTS

Level of GPT, Creatinine and Uric acid in blood serum.

In the present study; the serum GPT, creatinine and uric acid level was changed by the treatment of cadmium chloride which was analyzed by the Spectrophotometer in blood at different doses and time interval. The level of GPT was determined for various groups (A, B, C, D, E, F, and G with dose level of 25, 50, 75, 100, 125, 150 mg/kg and control respectively) before and after treatment at the following

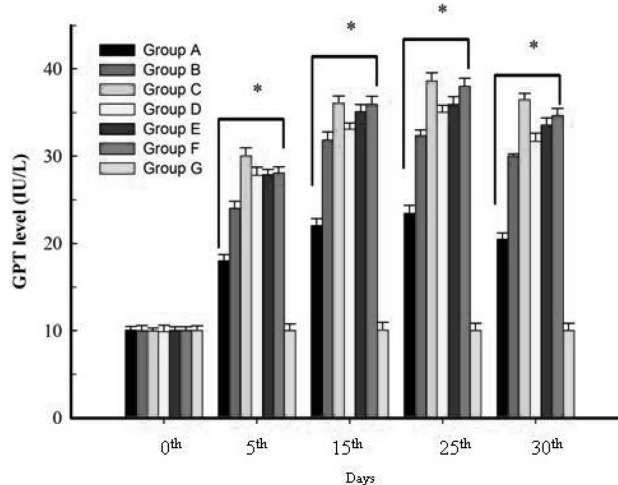


Fig. 1. Determination of GPT level at various doses of cadmium in blood serum. * $p < 0.05$ compared with control.

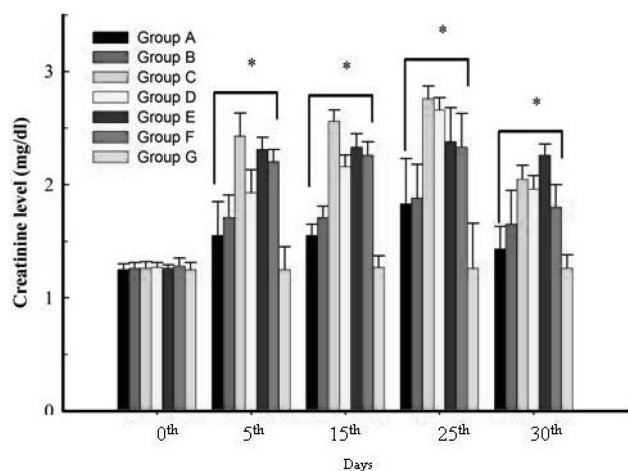


Fig. 2. Determination of creatinine level at various doses of cadmium chloride in blood serum. * $p < 0.05$ compared with control.

time intervals; 0th (before), 5th, 15th, 25th (during) and at 30th day (after) of experiment. There was significant increase ($P < 0.05$) in GPT in all the intoxicated groups as compared to control. The group C intoxicated with cadmium at dose rate of 75 mg/kg showed highest level of GPT as compared to groups (D, E and F) intoxicated with dose rate of 100, 125 and 150 mg/kg respectively in Fig. 1.

The spectrophotometer analysis of blood shows that cadmium also affects the creatinine level. As cadmium dose was increased to a certain limit (75 mg/kg), creatinine level also increased in blood and, onward as the cadmium dose was increased but creatinine level tended to decrease. The highest effect on creatinine was observed in group C intoxicated with cadmium at dose rate of 75 mg/kg when compared with groups (D, E and F) intoxicated with dose rate of 100, 125 and 150 mg/kg respectively as in Fig. 2.

The uric acid level of all groups was determined in the blood samples taken from the chicks during experiment. There was significant increase ($P < 0.05$) in uric acid level when the dose of cadmium was increased. The maximum effect was observed when a dose of 75 mg/kg (Group C) was administered, and, no increase in uric acid level was observed at higher than 75 mg/kg of CdCl_2 as shown in Fig. 3. It has been revealed that the kidney function was more significantly affected at the dose rate of 75 mg/kg as compared to other doses levels in the form of maximum uric acid secretion.

Analysis of Cadmium levels in various tissues. The AAS was used for the determination of cadmium organs concentration in kidney, liver and breast muscles of treated and control groups. The level of the cadmium in the group G (control) was $0.03 \pm 0.003 \mu\text{g/gm}$, $0.07 \pm 0.012 \mu\text{g/gm}$ and $0.15 \pm 0.021 \mu\text{g/gm}$ in the breast muscle, kidney and

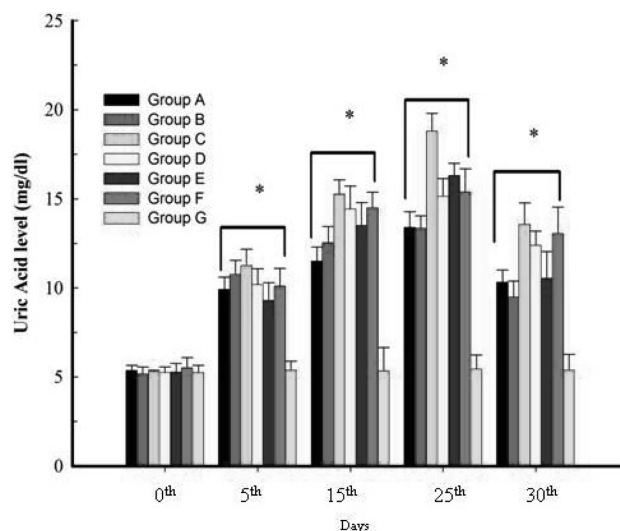


Fig. 3. Determination of uric acid level at various doses of cadmium chloride in blood serum. * $p < 0.05$ compared with control.

liver respectively. The overall accumulation of cadmium was high in kidney in all the groups as compared to the liver and muscles. The maximum level of cadmium ($32 \pm 0.069 \mu\text{g/gm}$) was determined in group B intoxicated with a dose rate of 50 mg/kg. There was a maximum value for cadmium in liver ($35 \pm 0.056 \mu\text{g/gm}$) in only one group (B) intoxicated with cadmium at dose rate of 50 mg/kg. The rest of the groups had low level of cadmium in liver. The maximum level of cadmium in muscle ($14 \pm 0.011 \mu\text{g/gm}$) was found in group D which was intoxicated with cadmium at dose rate of 100 mg/kg. It has been concluded that as the dose rate increased (at 75 or more than 75 mg/kg), its elimination and cumulative capability in muscles increased. The results have been shown in Fig. 4.

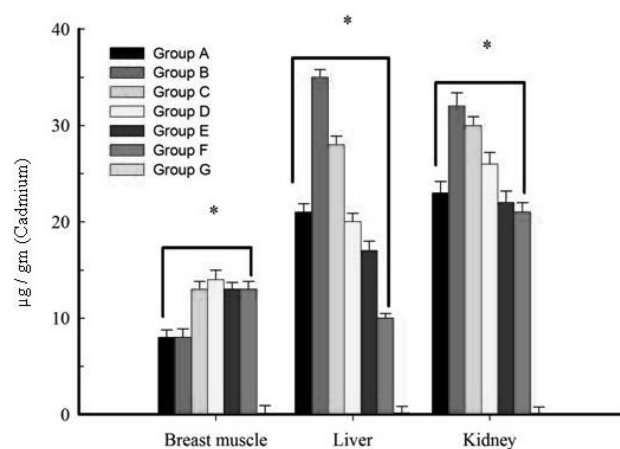


Fig. 4. Determination of cadmium level in kidney, liver and breast muscles. * $p < 0.05$ compared with control.

DISCUSSION

The level of biochemical parameters of groups A, B, C, D, E, F, and G (with dose level of 25, 50, 75, 100, 125, 150 mg/kg and control respectively) was analyzed by the spectrophotometer in the blood samples taken from the chicks before and after treatment at the time intervals of; 0th, 5th, 15th, 25th and 30th day of experiment. The maximum effect was observed in the form of secretion and cadmium cumulative capability when a dose of 75 mg/kg was administered.

The association of the CdCl₂ dose level and their accumulation in breast muscles is of unique characteristic that at certain dose of CdCl₂ (75 mg/kg to 100 mg/kg) shows an increased level of accumulated cadmium in breast muscles of the treated chicks as compared to high doses.

As the cadmium dose increased, there was paradoxical decrease in the kidney and liver level of cadmium while muscle levels increased, suggesting that elimination and accumulation in muscle of cadmium was increased. This view also matches well with the creatinine, uric acid or GPT data that showed a plateau from 75 mg/kg. More importantly, this is in accord with the previous finding in rats (Savage *et al.*, 1991).

Total amount of CdCl₂ present in the body are not in the active form to cause damage to specific tissue structure or inhibit various enzyme activities (Rajannat *et al.*, 1984). So at a specific amount of CdCl₂ (75 mg/kg) the levels of biochemical parameters were at the maximum state.

It has been revealed that CdCl₂ at high dose have lesser effect on kidney function than 75 mg/kg also is due to accumulation in muscles and increased secretion, which shows low level of availability in systemic circulation to impair kidney function. It was concluded from the present result that the level of GPT, creatinine and uric acid was higher at 75 mg/kg of CdCl₂ because the CdCl₂ has the capability to accumulate in muscles at high concentration as compared to other organs (liver and kidney) when the cadmium dose increased and the excess cadmium is not available in systemic circulation to show its toxic effect on kidney. The uric acid level was also higher at 75 mg/kg than 100~150 mg/kg in the obtained data. It is concluded from the result that 75 mg/kg is the most toxic dose for the broiler chicks and all the biochemical's parameter are at the high level at this dose than higher dose. The previous result also investigated that increase plasma concentration of cadmium shows increase toxicity levels in living body (Reynders *et al.*, 2006).

Our data shows that 75 mg/kg of cadmium is the toxic concentration on a weight basis for the poultry and or at the this amount the broiler chicks has the maximum body function in the form of GPT, creatinine and uric acid and maximum accumulation of cadmium occur in muscles at more than 75 mg/kg of CdCl₂.

The AAS analysis was performed to test out the level of

accumulated cadmium in the kidney, liver and breast muscles of intoxicated group as well as control group. The result showed that maximum level of cadmium accumulated in the kidney and liver up to at 75 mg/kg of CdCl₂ in the medicated groups. This result is also in accord with previous result, suggesting that kidney and liver is the main organ for the accumulation of heavy metals (Haouen *et al.*, 2007). At high doses the level of the accumulated cadmium increased in the breast muscles and tended to decreased in the kidney and lever by increasing the secretion.

In the present study, live weight of the treated broiler chicks was significantly lower than the control. The live weight of all the groups (A, B, C, D, E and F) reduced with the increasing level of cadmium exposure (Uyanik *et al.*, 2001; Sant'Ana, *et al.*, 2005; Berzina *et al.*, 2007) the data are not shown. The feed consumption was not recorded during the experiment.

In the present study the post mortem examination was carried out to study the effect of cadmium chloride on internal organs (liver, kidney, lungs, heart and gizzard). No mortality was recorded in control group (G). There were gross pathological lesions on Liver (Sant'Ana *et al.*, 2005), Kidney (Jin *et al.*, 1986), Lung, Gizzard and Heart (Krish, 2008). There was discoloration of liver as well as enlargement (El-Sharak *et al.*, 2007). Kidneys were also swollen. Hemorrhages were present in intestine, gizzard and the lung. It was also investigated that cadmium causes morphological and functional damage in hepatic (Dehn *et al.*, 2004) and renal tissues (El-Sharak *et al.*, 2007), testicular necrosis (Lorico *et al.*, 2002). No any gross lesion was found in the chicks slaughtered from the control group.

CONCLUSION

From the present study it has been concluded that cadmium alter the GPT, creatinine and uric acid levels in broiler chicks. It shows that cadmium mainly affect kidney and liver. The toxic effect was more prominent when 75 mg/kg of cadmium chloride was administered which is justified by the spectrophotometer analysis of blood. After post-mortem examination, it was found that internal organs such as liver, kidney, lung, and gizzard were also severely affected at 75 mg/kg dose rate. The accumulation of cadmium was high in kidney as compared to liver and breast muscles. We observed that the cadmium accumulation was continually increased in muscle at dose dependent manner while highest accumulation in liver and kidney were at 75 mg/kg and further tended to decrease with increasing dose level.

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