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Scientific Research and Essays

Full Length Research Paper

Biochemical studies on Ziram induced acute, subacute and subchronic toxicity in broiler chicken

Majid Shafi[1]*, Shayaib Ahmad Kamil[1], Masood Saleem Mir[1], Sheikh Adil[2], Mohammad Maqbool Darzi[1] and Abdul Shakoor Bhat[3]

[1]Division of Veterinary Pathology, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India.
[2]Division of Livestock Production and Management, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India.
[3]Division of Veterinary Pharmacology, Faculty of Veterinary Science and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India.

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The present experimental work was conducted to elucidate the biochemical changes in broiler chicken after ziram intoxication. For the study, 150 apparently healthy broiler chicks were reared together up to 2 weeks of age and thereafter allocated randomly to four groups, that is, control (n=30), acute (n=10), subacute (n=20) and subchronic (n=90). Toxicity was induced following single oral dose of Ziram at 100 mg/kg body weight for acute intoxication, 5 mg/kg body weight per day for 20 days in case of subacute group and 1 mg/kg body weight per day for the study period of 90 days in case subchronic intoxication. The mean values of liver function test viz. serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), serum alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH) and bilirubin in acute intoxicated birds at 24 h post intoxication, subacute and subchronic groups were significantly ($p<0.05$) higher when compared with the control group. Further in kidney function test, a significant ($p<0.05$) increase in the mean values of blood urea nitrogen (BUN) was recorded in acute ziram intoxicated birds at 24 h interval and subchronic groups but no significant ($p>0.05$) effect was observed in the subacute group when compared with the control. The creatinine levels increased significantly ($p<0.05$) in all the intoxicated birds compared to control. Moreover, a significantly ($p<0.05$) higher increase in the zinc levels was observed in all the intoxicated birds compared with the control. The mean serum copper and calcium levels were significantly ($p<0.05$) lower in all the intoxicated birds compared to the control. In conclusion, the results were suggestive of the fact that ziram caused hepatotoxicity and nephrotoxicity in broiler chicken.

Key words: Broiler chicken, kidney function test (KFT), liver function test (LFT), toxicity, minerals, Ziram.

INTRODUCTION

Dithiocarbamates (DTC) are organosulfur compounds which were first introduced as fungicides for commercial applications during World War II (Ware and Whitacare, 2004). Besides their wide use as fungicides for treatment

of crops, vegetables, seeds, and ornamental plants, they are also used as accelerators in the rubber industry, animal repellants, and biocides in many household products (Edwards et al., 1991; Kamrin 1997). Ziram is analogous dialkyl DTC with differences in their R groups used in preharvest agricultural applications. Ziram has been proven more toxic than ferbam and thiram in adult fowl (Rasul and Howell, 1974). These fungicides often find their way in poultry feed. Although there are benefits of using these pesticides but on the other hand these fungicides pose a potential threat to humans, birds and other animals. Since there is a very scanty literature available regarding ziram toxicity, so the present experiment was undertaken with the objectives of studying the effect of ziram intoxication in broiler chicken.

MATERIALS AND METHODS

A total number of 150 healthy broiler chicks was procured from the market and used for the study. The experimental protocol was approved by Institutional Animal Ethics Committee vide no. AU/FVSc/C-09/2012/0476-88. All the chicks were vaccinated against prevalent diseases. The chicks were given Furasol at ½ gram per litre of water for 5 days in the first week of experiment so as to prevent them from coccidiosis. The chicks were given broiler starter ration for two weeks followed by broiler finisher as per standards. The chicks were reared together up to 2 weeks of age. After that they were divided into four groups viz. acute, subacute, subchronic and control on random basis. The acute group consisted of 10 chicks, subacute 20 chicks, subchronic 90 chicks and control 30 chicks. The control group received fungicide free diet, where as acute, subacute and subchronic groups received fungicide (Ziram) at different dose rates in feed. The dose rate was selected on the basis of LD50 by dividing the number of days in each toxicity group. The LD50 of ziram in birds is 100 mg/kg body weight (Kamrin 1997). For inducing acute toxicity, single oral dose of ziram at 100 mg/kg body weight (LD50 dose) was given to the experimental birds. For inducing subacute toxicity, LD50 dose was given orally in divided doses daily for 20 days, that is, 5 mg/kg body weight per day. For inducing subchronic toxicity, LD 50 dose was given orally in divided doses daily for 90 days, that is, 1 mg/kg body weight per day.

The parameters estimated were Liver function Test (SGOT, SGPT, Alkaline phosphatase ALP, Lactate dehydrogenase LDH and Bilirubin), kidney function test (Blood urea nitrogen and Creatinine) and mineral estimation (Calcium, Zinc and Copper). The liver and kidney function tests were estimated by using Olympus analyzer with compatible kits from aspen chemicals. For mineral estimation, 1 ml of serum and 5 ml of nitric acid were taken into 50 ml volumetric flask. The flask was then kept at room temperature for overnight and next day flask was put on a hot plate at simmering heat till the volume in the flask was reduced up to 0.5 ml. The final volume of flask was made up to 10 ml by diluting with distilled water. This 10ml solution was used for mineral estimation by using the Atomic absorption spectrophotometer.

Data obtained were analyzed by t-test, one-way ANOVA followed by Dunnet's test using SPSS software (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Liver function test (LFT)

The mean values of SGOT in acute intoxicated birds at 24 h post intoxication were significantly ($p<0.05$) higher when compared with the control group (Table 1). Further, in case of sub acute intoxicated birds the SGOT values were statistically non-significant ($p>0.05$) at day 10 but singnificant at day 20 post intoxication. In subchronic group highly significant ($p<0.05$) SGOT values were recorded from day 80 onwards. Similar findings have been reported earlier by Dalvi et al. (2002) and Kurata (1981) in rats with thiram and zineb toxicity. In the present study, the increase in SGOT levels observed in ziram intoxicated birds indicated the possible damage to the liver which could be substantiated by the presence of significant pathomorphological alterations in liver as observed by Robbins et al. (1984) in thiram intoxicated rats. Evaluation of SGPT mean values revealed significantly increased activity in acute intoxicated birds at 24 h interval when compared with mean values of control birds (Table 2). In case of sub acute group, there was no significant difference in the mean values of SGPT between intoxicated and control groups upto 10^{th} day, however, at day 20, the SGPT values in ziram intoxicated birds were significantly ($p<0.05$) higher when compared with the control group. Further, in the sub chronic group, the significantly ($p\leq0.05$) higher SGPT values were observed in intoxicated birds than control group from 20 day onwards. The present findings coincide with the reports of Li and Zhang (2007) who found higher SGPT values in thiram intoxicated broiler chicken. Higher SGPT levels have also been reported by Kurata (1981) in zineb carbamate treated rats. This increase might be due to the destructive changes in hepatic cells as observed by Sharma (1999) in fresh-water teleost after the adminstration of carbaryl. The mean values of alkaline phosphatase (ALP) in acute ziram intoxicated birds at 24 h post intoxication were significantly ($p\leq0.05$) higher than the control birds (Table 3). In sub acute group, the mean ALP values in ziram intoxicated birds were significantly ($p<0.05$) higher than control birds throughout the study and in subchronic group highly significant ($p<0.05$) increase in ALP values was found from 10^{th} day onwards. The present finding could be correlated to the similar results reported earlier by Mishra et al. (1998) in rats when thiram was fed at doses 5, 10 and 25 mg/kg/day for 180 days. Gera et al. (2009) also reported increase in level of ALP in carbofuran treated rats. The elevation in the level of serum ALP in the present study might be due to hepatotoxic effect of the fungicides as observed by Dikic et al. (2012) after exposure of Carbendazim in

*Corresponding author. E-mail: majidshafivet@gmail.com

Table 1. Effect of acute, sub-acute and sub-chronic ziram intoxication on SGOT (IU/L) in broiler chicken (mean±SE).

Period post intoxication	Control	Intoxicated
Acute group		
Day 0	16.80±1.90	19.19±0.51
24 h	17.67±0.66[a]	38.45±1.75[b]
Subacute group		
Day 0	20.18±2.50	18.95±0.60
Day 10	17.82±1.30	18.74±0.61
Day 20	19.78±1.48[a]	28.98±1.07[b]
Subchronic group		
Day 0	17.32±0.64	18.63±0.58
10 days	19.18±0.52[a]	25.83±0.70[b]
20 days	19.58±1.24[a]	27.62±0.94[b]
30 days	24.12±2.26[a]	31.07±1.09[b]
40 days	34.52±1.45	31.68±0.98
50 days	35.10±1.18	31.66±1.20
60 days	35.05±1.51	33.77±1.11
70 days	35.21±2.18	39.59±1.60
80 days	34.65±1.78[a]	48.15±1.56[b]
90 days	34.92±0.74[a]	51.03±1.32[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 2. Effect of acute, sub-acute and sub-chronic ziram intoxication on SGPT (IU/L) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	37.07±0.97	31.12±0.85
24 h	39.47±1.73[a]	42.91±1.74[b]
Subacute group		
Day 0	35.84±0.65	30.61±0.62
Day 10	36.14±1.16	31.13±0.56
Day 20	32.23±2.10[a]	40.68±1.20[b]
Subchronic group		
Day 0	35.84±0.65	33.35±0.80
10 days	46.57±0.88	44.09±1.28
20 days	47.65±0.20[a]	54.03±1.47[b]
30 days	49.14±0.51[a]	58.94±1.11[b]
40 days	48.95±0.71[a]	62.36±1.06[b]
50 days	49.42±1.04[a]	63.45±0.98[b]
60 days	50.65±0.33[a]	64.01±0.88[b]
70 days	50.01±0.86[a]	64.61±0.99[b]
80 days	48.57±1.20[a]	64.88±0.96[b]
90 days	48.68±2.14[a]	67.30±1.03[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 3. Effect of acute, sub-acute and sub-chronic ziram intoxication on ALP (IU/L) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	103.65±1.37	119.15±0.84
24 h	105.62±2.01[a]	169.25±1.08[b]
Subacute group		
Day 0	104.87±2.04	120.07±0.86
Day 10	108.31±0.88	120.59±0.96
Day 20	104.68±1.83	138.06±1.52
Subchronic group		
Day 0	103.60±1.45	105.76±0.73
10 days	110.81±0.91[a]	146.96±1.09[b]
20 days	118.11±1.61[a]	145.46±3.48[b]
30 days	114.72±1.40[a]	160.34±1.18[b]
40 days	117.80±1.67[a]	162.67±0.96[b]
50 days	119.56±1.29[a]	167.98±0.91[b]
60 days	118.40±1.95[a]	172.04±0.77[b]
70 days	118.10±1.30[a]	176.51±0.83[b]
80 days	121.29±0.97[a]	176.61±1.06[b]
90 days	118.59±2.09[a]	176.32±0.99[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

Swiss mice for a study period of 28 days. At 24 h post intoxication in acute group, the mean values of serum lactate dehydrogenase (LDH) in ziram intoxicated birds were significantly ($p<0.05$) higher when compared with the control (Table 4). In sub acute group, the significantly ($p<0.05$) higher LDH values were observed thrroughout the study period, however, a significant ($p<0.05$) increase in LDH in subchronic group was oserved from 10th day onwards. Sastry et al. (2009) have also reported an increased level of LDH due to carbamate toxicity in freshwater snakehead fish. Similarly, carbamate toxicity has been reported to cause an increase in LDH in ruminants which they have attributed to the fact that these toxins cause plasma membrane damage and decrease glutathione content in hepatocytes (Radostitis et al. 2009). The mean Bilirubin levels in acute intoxicated birds were significantly higher at 24 h intervals when compared with the control birds (Table 5). In sub acute group, significant ($p\leq0.05$) increase in bilirubin levels in ziram intoxicated birds was oberved at day 20 of intoxication and in subchronic group from 10 day onwards when compared to the control. These finding could be correlated to reports of Adjrah et al. (2013) who reported increase in the bilirubin levels of wister rats after exposure to mancozeb carbamate. The increase in the bilirubin level after the exposure to zineb carbamate in rats has also been reported by Mountie et al. (1983). The reason might be the red blood cell destruction, hepatitis and cirrhosis of the liver as noticed by Mach (1986) in rats after the administration of disulfiram.

Kidney function test (KFT)

A significant ($p<0.05$) increase in the mean values of BUN were recorded in acute ziram intoxicated birds at 24 h interval when compared with the control birds (Table 6). In case of sub acute group, no effect on BUN levels was observed throughout the experimental period. Similarly, in subchronic group, significant ($p<0.05$) effect on BUN levels was observed only on day 10 and 20 of the study but no significant effect was observed afterwards, though the values were higher in intoxicated birds than control group. A significant increase in BUN levels have also been reported by Sakr et al. (2013) and Francesconi et al. (1986) in rats after the administration of Metiram carbamate and pyridostigmine carbamate respectively. The hyperurecemia in the ziram intoxicated birds clearly reflected that repeated use of ziram for prolonged period may have induced marked renal dysfunction. This fact has been fully substantiated by pathomorphological observations of the kidney that is congestion and degeneration of bowmans capsule, hydropic degeneration with accumulation of hyaline mass in tubular lumens as reported by Wael et al. (2013) in kidney of albino rats. Further, the serum creatinine values in acute intoxicated birds were significantly higher when compared with the control birds (Table 7). The mean

Table 4. Effect of acute, sub-acute and sub-chronic ziram intoxication on LDH (IU/L) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	844.66±36.67	1298.00±59.98
24 h	908.00±84.30[a]	3371.50±128.68[b]
Subacute group		
Day 0	844.66±41.69[a]	1315.00±62.71[b]
Day 10	897.00±56.78[a]	1325.00±39.91[b]
Day 20	952.00±69.47[a]	2511.20±92.53[b]
Subchronic group		
Day 0	838.66±31.92	1013.40±42.46
10 days	932.00±60.63[a]	1619.90±56.20[b]
20 days	908.33±100.00[a]	1851.00±57.16[b]
30 days	891.66±74.56[a]	2114.20±83.91[b]
40 days	908.33±66.83[a]	2277.70±70.16[b]
50 days	871.33±85.19[a]	2529.00±84.33[b]
60 days	915.66±92.83[a]	2639.90±105.06[b]
70 days	1022.66±76.14[a]	3515.00±77.62[b]
80 days	878.33±92.71[a]	3467.90±57.28[b]
90 days	868.33±52.84[a]	3528.60±66.65[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 5. Effect of acute, sub-acute and sub-chronic ziram intoxication on Bilirubin (mg/dl) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	2.93±0.19	3.90±0.13
24 h	2.873±0.3[a]	4.91±0.19[b]
Subacute group		
Day 0	2.65±0.23[a]	3.58±0.15[b]
Day 10	2.93±0.11	3.50±0.16
Day 20	2.86±0.20[a]	4.83±0.17[b]
Subchronic group		
Day 0	2.79±0.16	3.53±0.12
10 days	3.00±0.09[a]	5.08±0.21[b]
20 days	2.92±0.25[a]	4.71±0.17[b]
30 days	2.89±0.13[a]	4.91±0.13[b]
40 days	3.29±0.29[a]	4.85±0.09[b]
50 days	2.78±0.28[a]	4.75±0.16[b]
60 days	2.88±0.19[a]	4.77±0.16[b]
70 days	3.32±0.38[a]	4.85±0.21[b]
80 days	3.43±0.33[a]	4.99±0.24[b]
90 days	3.11±0.06[a]	4.82±0.12[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

values of creatinine in sub acute group were significant ($p<0.05$) higher in intoxicated birds at day 20 and no such effect was oberved at day 10. Moreover, a highly significant ($p<0.05$) effect on creatinine levels was

Table 6. Effect of acute, sub-acute and sub-chronic ziram intoxication on BUN (mg/dl) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	26.88± 1.76	26.74±0.62
24 h	27.73± 1.32[a]	32.90±1.53[b]
Subacute group		
Day 0	28.50±0.69	26.65±0.63
Day 10	30.26±1.33	26.63±0.46
Day 20	28.49±1.12	32.02±1.27
Subchronic group		
Day 0	29.65±0.37	27.39±0.48
10 days	28.65±0.49[a]	39.39±1.20[b]
20 days	28.90±0.90[a]	37.28±1.17[b]
30 days	28.90±2.39	34.34±1.68
40 days	31.77±2.26	31.09±1.58
50 days	30.96±1.19	34.62±2.01
60 days	28.70±1.55	30.46±1.36
70 days	28.33±2.20	31.67±1.53
80 days	28.61±2.04	32.56±1.77
90 days	30.44±1.08	34.04±1.44

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 7. Effect of acute, sub-acute and sub-chronic ziram intoxication on creatinine (mg/dl) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	3.03± 0.24	3.66±0.21
24 h	2.70 ± 0.36[a]	8.24±0.34[b]
Subacute group		
Day 0	3.83±0.14	3.77±0.20
Day 10	3.80±0.20	3.69±0.22
Day 20	3.80±0.05[a]	6.28±0.40[b]
Subchronic group		
Day 0	3.10±0.2 3	3.71±0.20
10 days	2.90±0.34	4.57±0.28
20 days	3.73±0.08	4.60±0.20
30 days	3.26±0.23[a]	5.32±0.28[b]
40 days	3.16±0.31[a]	5.65±0.22[b]
50 days	3.60±0.15[a]	5.55±0.25[b]
60 days	3.03±0.21[a]	6.92±0.16[b]
70 days	3.30±0.30[a]	6.86±0.32[b]
80 days	4.26±0.23[a]	7.68±0.32[b]
90 days	4.20±0.20[a]	8.36±0.25[b]

Means within the same row with different superscripts are significantly different ($p<0.05$)

observed from day 30 onwards in intoxicated birds of subchronic group when compared to the control.group. Similar result have been mentioned by Radostitis et al. (2009) in ruminants and Veerappan et al. (2012) in albino

Table 8. Effect of acute, sub-acute and sub-chronic ziram intoxication on Zinc (ppm) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	0.60±0.05	0.79±1.69
24 h	0.90±0.35[a]	2.64±0.17[b]
Subacute group		
Day 0	0.79±0.38	1.36±0.12
Day 10	0.96±0.29	1.32±0.12
Day 20	1.36±0.43[a]	2.32±0.16[b]
Subchronic group		
Day 0	1.40±0.17	1.31±0.12
10 days	1.33±0.08	1.53±0.20
20 days	1.20±0.15	1.4±0.22
30 days	1.13±0.49[a]	2.14±0.17[b]
40 days	1.90±0.15	2.37±0.16
50 days	1.00±0.32[a]	2.81±0.15[b]
60 days	1.20±0.40[a]	2.74±0.12[b]
70 days	1.73±0.12[a]	2.75±0.09[b]
80 days	1.93±0.20[a]	2.79±0.07[b]
90 days	1.76±0.43[a]	2.90±0.11[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

rats as a result of zineb carbamate and carbamate toxicity respectively. A significant increase in serum creatinine concentration in ziram intoxicated groups may be due to the ill effect of these toxins on kidneys and as could be correlated with gross and histopathological changes observed in kidneys Khanam et al. (2011).

Mineral estimation

A significantly ($p<0.05$) higher increase in the zinc levels was observed at 24 h in intoxicated birds of acute group when compared with the control (Table 8). In the sub acute group, the values significantly increased in the intoxicated group at 20th day. Moreover, from 30 days onwards significant ($p<0.05$) increase in zinc levels was oberved in subchronic intoxicated birds compared to the control. Increase in zinc levels have also been reported earlier by Kim et al. (1999) after administration of Pyrrolidine dithiocarbamate in bovines and Jones et al. (1992) in rats after thiram carbamate toxicity. The reason for increase in the zinc levels in present study might be due to the fact that the fungicide ziram belongs to zinc carbamate family and contains maximum amount of zinc (80%). The mean serum copper levels in acute ziram intoxicated birds at 24 h were significantly ($p<0.05$) lower when compared with the control (Table 9). In subacute group no effect was observed until day 20 where significantly ($p<0.05$) lower copper levels were observed in intoxicated birds. Further, significant reduction in copper levels were also observed in subchronic group from day 70 onwards compared to the control. Jyotsna et al. (2009) also reported decrease in the serum copper levels after the exposure to atrazine fungicide. The reason might be that the zinc and copper are antagonistic to each other and as ziram contains maximum amount of zinc. Fischer and Giroux (1981) have reported that zinc exerts its antagonistic effect by inducing the synthesis of a copper-binding ligand thionein. This may be a possible mechanism by which zinc decreases copper absorption and leads to a decrease in the level of copper. The mean values of calcium in the acute group showed a significant ($p<0.05$) decrease at 24 h intervals when compared with the control group (Table 10). In the subacute group, the significantly ($p\leq0.05$) decreased ca levels in intoxicated birds were noticed at day 20. Likewise, a non-significant ($p>0.05$) reduction in ca levels in subchronic intoxicated birds was observed from day 60 onwards when compared to the control group. The decrease in the calcium level in rats was also reported by Rangoonwala and Pnaday (2007) after the administration of Mipcin carbamate which might be due to the effect on the calcitonin. The carbamate toxicity has been reported to disturb intracellular calcium homeostasis in the albino rats (Veerappan et al., 2012).

Conclusion

In conclusion, the blood biochemical alterations found in

Table 9. Effect of acute, sub-acute and sub-chronic ziram intoxication on Copper (ppm) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	0.20±0.03	0.20±0.019
24 h	0.10±0.02[a]	0.05±0.005[b]
Subacute group		
Day 0	0.22±0.04	0.20±0.020
Day 10	0.18±0.04	0.20±0.015
Day 20	0.18±0.04[a]	0.05±0.006[b]
Subchronic group		
Day 0	0.21±0.02	0.19±0.01
10 days	0.22±0.03	0.20±0.02
20 days	0.15±0.03	0.21±0.02
30 days	0.15±0.04	0.21±0.02
40 days	0.19±0.05	0.22±0.01
50 days	0.22±0.03	0.20±0.02
60 days	0.17±0.01	0.21±0.02
70 days	0.19±0.05[a]	0.05±0.00[b]
80 days	0.18±0.05[a]	0.09±0.00[b]
90 days	0.19±0.05[a]	0.05±0.00[b]

Means within the same row with different superscripts are significantly different ($p<0.05$).

Table 10. Effect of acute, sub-acute and sub-chronic ziram intoxication on calcium (mg/dl) in broiler chicken (mean±SE).

Period post-intoxication	Control	Intoxicated
Acute group		
Day 0	8.88± 0.17	8.79±0.21
24 h	8.69± 0.30[a]	6.52±0.39[b]
Subacute group		
Day 0	8.78±0.43	8.78±0.31
Day 10	9.56±0.89	8.81±0.21
Day 20	8.86±0.69[a]	6.89±0.65[b]
Subchronic group		
Day 0	9.29±0.49	8.81±0.22
10 days	9.66±0.45[a]	14.95±0.71[b]
20 days	9.66±0.45[a]	14.15±0.67[b]
30 days	10.03±0.20[a]	12.00±0.48[b]
40 days	9.48±0.66	10.90±0.30
50 days	9.73±0.26	10.10±0.4
60 days	10.10±0.43	9.55±0.37
70 days	8.69±0.34	8.50±0.27
80 days	8.69±0.34	7.87±0.20
90 days	9.21±0.55	7.68±0.16

Means within the same row with different superscripts are significantly different ($p<0.05$).

The ziram intoxicated birds were suggestive of ziram induced hepatotoxicity and nephrotoxicity in broiler

chicken.

Conflict of interest

The authors have not declared any conflict of interest

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