

## THERAPEUTIC EFFECT OF GILOY ON CADMIUM INDUCED HAEMATO BIOCHEMICAL CHANGES IN BROILER CHICKEN

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### ABSTRACT

The present experimental study was conducted to assess the therapeutic effect of Giloy (*Tinospora cordifolia*) powder on cadmium (Cd) induced haemato-biochemical changes in broiler chicken for a period of 28 days. The study included 134 broiler chickens divided into six groups viz. B1 (Control), B2 (Giloy powder @ 1g/kg of feed), B3 (1/10<sup>th</sup> LD<sub>50</sub> of CdCl<sub>2</sub>), B4 (1/20<sup>th</sup> LD<sub>50</sub> of CdCl<sub>2</sub>), B5 (1/10<sup>th</sup> LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy powder @ 1g/kg of feed) and B6 (1/20<sup>th</sup> LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy @ 1g/kg of feed) with 21, 21, 23, 23 and 23 birds, respectively. Cadmium intoxication revealed significant (P<0.05) decrease in Haemoglobin (Hb), Packed Cell Volume (PCV) and Total Erythrocyte Count (TEC) depending on different doses given at 14 and 28 days. Mean Corpuscular Volume (MCV) was non-significantly increased and Mean Corpuscular Haemoglobin Concentration (MCHC) was non-significantly decreased in Cd treated groups. Biochemical studies revealed hypoproteinemia, hypoalbuminemia and significantly increased serum Aspartate Transaminase (AST), serum Alanine Transaminase (ALT), Blood Urea Nitrogen (BUN) and creatinine concentration in Cd treated groups as compared to respective control groups. Cd treated groups (B5 and B6) supplemented with Giloy powder showed non-significant improvement in haemato-biochemical parameters and proved negligible to mild therapeutic response of Giloy powder in cadmium induced hepatotoxic and nephrotoxic effect.

**Keywords:** Cadmium, Giloy, Haemato-biochemical changes.

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Poultry industry is one of the fastest growing segments in India which faces hurdles like infectious and non-infectious diseases. Among the non-infectious diseases, environmental pollution significantly affects the performance of birds (Abduljaleel and Shuhaimi-Othman, 2013). Cadmium (Cd), a non-essential heavy metal and well-known environmental pollutant, arising primarily from anthropogenic causes like fertilizer, plastic, battery, electro-plating and pigment industries etc. (Vig *et al.*, 2003). It bioaccumulates within the vital organs in poultry through contaminated water, feed ingredients of plant origin and also from fish meal, causing hepatotoxicity and nephrotoxicity by increasing the free radical production and promoting lipid peroxidation (Galadima *et al.*, 2011; Mallya *et al.*, 2017). A dietary supplementation of natural antioxidants is necessary to prevent effects of Cd toxicity. Giloy (*Tinospora cordifolia*) plant is a widely used herb in Indian medicinal system for several pharmacological properties and it was reported to have biosorptive potential in removing Cd ions (Panchabhai *et al.*, 2008; Sahu *et al.*, 2018). Studies have also showed the protective effect of supplementing Giloy stem extract in Cd induced oxidative stress, hepatotoxicity, nephrotoxicity and cardiotoxicity in rats (Padma *et al.*, 2016; Priya *et al.*, 2017; Baskaran *et al.*, 2018). However, there is paucity of data related to therapeutic effect of Giloy in Cd induced toxicity in

poultry birds. Hence, the present experimental study was planned to investigate the therapeutic effect of Giloy (*Tinospora cordifolia*) on Cd induced haemato-biochemical changes in broiler chicken for a period of 28 days.

### MATERIALS AND METHODS

The present study was conducted in broiler chicken procured from local private hatchery, Hisar. These birds were reared in cages under strict hygienic conditions with optimum temperature and proper ventilation at Animal House Facility in Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. All the birds were acclimatized for two weeks and provided with fresh, clean drinking water and fed *ad libitum* with commercially available standard balanced ration throughout the experimental study. The experimental design, general procedure and use of experimental birds were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the university (VCC/IAEC/560-82, dated: 18-03-2021).

**Chemical:** Cadmium chloride of Alfa Aser company (CdCl<sub>2</sub>, anhydrous, ACS, 99.0% min, FW: 183.32) was procured from authorized dealer. For chicken, oral 50 per cent lethal dose (LD<sub>50</sub>) for cadmium was already determined as 218.44 mg/kg body weight (Li *et al.*, 2013).

**Preparation of Giloy (*Tinospora cordifolia*) powder:** Giloy stems were procured from Medicinal and Aromatic

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Plants Section of Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar. These stems were washed thoroughly with clean water and cut into small pieces and dried in shade for 7-10 days. After drying, the stems were grounded into powder form by using electric mixer grinder. Grounded powder was sieved, and finally stored in air tight containers. In the present experimental study, Giloy powder @ 1 gm/kg of feed was used.

### Experimental design

One hundred and thirty four, day old broiler chicks were acclimatized for 14 days and were divided into six groups viz. B1, B2, B3, B4, B5 and B6 with each group having 21, 21, 23, 23, 23 and 23 number of birds, respectively. Group B1 was kept as control and fed with basal diet. Group B2 was kept as Giloy control and supplemented with Giloy powder @ 1g/kg of feed. Groups B3 and B4 were orally administered with 1/10th and 1/20th LD<sub>50</sub> of CdCl<sub>2</sub>, respectively. Group B5 and B6 were orally administered with 1/10th LD<sub>50</sub> of CdCl<sub>2</sub> and Giloy powder @ 1g/kg of feed, and 1/20th LD<sub>50</sub> of CdCl<sub>2</sub> and Giloy powder @ 1g/kg of feed, respectively. Randomly selected six birds from each group were subjected for blood collection from the jugular vein on 14 and 28 DPCT (Days Post Cadmium Treatment)/28 and 42 DOA (day of age).

**Estimation of Haemato-biochemical parameters:** Blood samples were collected in sterile Ethylene Diamine Tetra Acetate (EDTA) coated vials for complete blood count i.e., Hb, PCV, TEC, MCV, MCH, MCHC and TLC by using Automatic haematological analyzer (MS4Se-Melet Schloesing Laboratories-France). The thin blood smears were prepared from fresh blood and stained with Wright-Giemsa staining method. The counting of different leucocytes was done by battlement method (Benjamin, 2013).

Serum samples were collected and analyzed for different biochemical parameters like Total protein, Albumin, AST, ALT and Creatinine by using Automatic Biochemistry Analyzer (Erba Mannheim-200, ERBA Diagnostics Mannheim GmbH, Germany) employing different kits procured from ERBA Diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.). Blood urea concentration was first analyzed using urease-GLDH method in Semi-automatic Biochemistry analyzer (Erba Mannheim Chem-5 Plus, Transasia) employing Erba urea kits and the BUN concentration is obtained by multiplying the urea concentration with the factor 0.467.

**Statistical analysis:** The data for haemato-biochemical parameters were subjected to statistical analysis by using one way analysis of variance (ANOVA). The pairwise comparison among different treatment groups was done using Duncan Multiple Range Test. Results were

expressed as Mean  $\pm$  SE. The statistical significance was considered if value of P is less than 0.05 (P<0.05). Statistical analysis of experimental data was determined by SPSS 20.0 version software.

### RESULTS AND DISCUSSION

The present study was conducted to evaluate the therapeutic effect of Giloy in cadmium induced haemato-biochemical changes in 134 broiler chicken.

Haematological studies conducted in the present experimental trial have been shown in Table 1 and 2. No significant alterations were observed in haematological parameters in both the control groups viz. Group B1 and Group B2, throughout the experimental study. It shows that Giloy powder @1g/Kg feed has no adverse effects when given as feed supplement to poultry birds. Bhardwaj *et al.*, (2012) also reported no adverse effects by feeding Giloy extract in broilers @ 1g/kg of feed.

Mean Hb and PCV were significantly (P<0.05) decreased at 14 DPCT and mean TEC was significantly (P<0.05) decreased at 28 DPCT in group (B3) treated with high dose of CdCl<sub>2</sub>, as compared to control group (B1). However, in group B4, mean Hb concentration was comparable to both group B3 and control group (B1) up to 28 DPCT, but no significant difference was observed in PCV throughout the experimental study. Thevenod and Lee, (2013) reported that the decreased Hb and PCV might be due Cd induced disruption of the Fe absorption in the intestine leading to Fe deficiency and haemolysis of RBC leading to anaemia. Decrease in TEC might be due to the Cd induced haemopoietic tissue degeneration and decreased level of erythropoietin hormone (Gabol *et al.*, 2014). Similar decrease in mean Hb, PCV and TEC values were reported by earlier researchers in cadmium intoxicated chicken (Choudhary *et al.*, 2018). Cadmium treated groups supplemented with Giloy powder (B5 and B6) showed non-significant increase in Hb, PCV and TEC values as compared to Cd treated groups without Giloy powder supplementation (B3 and B4) throughout the experimental study. Bhardwaj *et al.* (2012) reported similar increase in Hb and TEC values in broilers fed with Giloy stem aqueous extract and stem juice concentrate @ 1g/kg of feed. Sharma and Pandey (2010) reported a moderate therapeutic effect of supplementing aqueous extract of Giloy stem and leaves to mice exposed to lead.

Mean value of MCV was non-significantly increased and MCHC was non-significantly decreased in group B3 and B4 as compared to group B1 (control) up to 28 DPCT. Similar results were reported by earlier authors in chicken intoxicated with cadmium (Abdo and Abdulla, 2013). Group B5 and B6 showed almost similar changes as observed in group B3 and B4. However, Sharma and

**Table 1. Hematological parameters (Mean ± S.E.) of broiler chicks in different experimental groups (B1, B2, B3, B4, B5 and B6) at different intervals**

DOA/DPCT	Group	Hb (g/dl)	PCV (%)	TEC (x10 <sup>6</sup> /cu mm)	MCV (fl)	MCH (pg)	MCHC (%)
28/14	B1	8.70 <sup>b</sup> ± 0.68	28.58 <sup>b</sup> ± 0.80	3.98 <sup>ab</sup> ± 0.31	73.76 <sup>a</sup> ± 5.62	22.64 <sup>a</sup> ± 2.78	30.47 <sup>a</sup> ± 2.23
	B2	8.73 <sup>b</sup> ± 0.55	28.34 <sup>b</sup> ± 0.97	4.09 <sup>b</sup> ± 0.34	71.17 <sup>a</sup> ± 5.16	21.76 <sup>a</sup> ± 1.49	30.68 <sup>a</sup> ± 1.07
	B3	6.24 <sup>a</sup> ± 0.76	23.81 <sup>a</sup> ± 1.57	2.93 <sup>a</sup> ± 0.30	86.22 <sup>a</sup> ± 10.33	22.28 <sup>a</sup> ± 2.94	27.09 <sup>a</sup> ± 4.42
	B4	6.53 <sup>ab</sup> ± 0.79	24.54 <sup>ab</sup> ± 1.60	3.06 <sup>ab</sup> ± 0.43	86.38 <sup>a</sup> ± 9.40	25.12 <sup>a</sup> ± 6.33	27.53 <sup>a</sup> ± 4.31
	B5	6.47 <sup>ab</sup> ± 0.70	23.93 <sup>a</sup> ± 1.55	2.96 <sup>a</sup> ± 0.33	85.30 <sup>a</sup> ± 9.49	23.08 <sup>a</sup> ± 2.83	27.55 <sup>a</sup> ± 3.26
	B6	6.74 <sup>ab</sup> ± 0.85	24.71 <sup>ab</sup> ± 1.52	3.10 <sup>ab</sup> ± 0.33	85.94 <sup>a</sup> ± 14.45	23.85 <sup>a</sup> ± 4.59	27.90 <sup>a</sup> ± 4.09
42/28	B1	8.81 <sup>b</sup> ± 0.81	29.16 <sup>b</sup> ± 0.60	4.27 <sup>b</sup> ± 0.30	70.43 <sup>a</sup> ± 6.30	21.09 <sup>a</sup> ± 2.60	30.39 <sup>a</sup> ± 3.07
	B2	8.80 <sup>b</sup> ± 0.55	28.75 <sup>b</sup> ± 0.86	4.23 <sup>b</sup> ± 0.30	70.19 <sup>a</sup> ± 6.50	21.70 <sup>a</sup> ± 2.78	30.94 <sup>a</sup> ± 2.59
	B3	5.77 <sup>a</sup> ± 0.70	23.49 <sup>a</sup> ± 2.07	2.69 <sup>a</sup> ± 0.39	98.02 <sup>a</sup> ± 17.86	24.77 <sup>a</sup> ± 5.68	26.13 <sup>a</sup> ± 4.71
	B4	6.72 <sup>ab</sup> ± 0.57	23.63 <sup>a</sup> ± 1.78	2.98 <sup>a</sup> ± 0.40	88.29 <sup>a</sup> ± 16.52	23.91 <sup>a</sup> ± 2.65	28.87 <sup>a</sup> ± 2.53
	B5	5.92 <sup>a</sup> ± 0.81	23.35 <sup>a</sup> ± 1.59	2.75 <sup>a</sup> ± 0.38	95.51 <sup>a</sup> ± 16.90	24.25 <sup>a</sup> ± 5.97	25.41 <sup>a</sup> ± 2.92
	B6	7.01 <sup>ab</sup> ± 0.74	23.79 <sup>a</sup> ± 1.70	3.03 <sup>a</sup> ± 0.34	81.87 <sup>a</sup> ± 7.56	23.66 <sup>a</sup> ± 1.96	29.86 <sup>a</sup> ± 2.99

B1 - Control (Basal diet), B2 - Giloy control (Giloy powder @ 1g/kg of feed), B3 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B4 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B5 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy powder @ 1g/kg of feed, B6 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy powder @ 1g/kg of feed. DOA - Days of age, DPCT- Days Post Cadmium Treatment.

a, b, c, d: Means with unlike superscripts in a column differ significantly, P<0.05

**Table 2. Total leucocyte count (x10<sup>3</sup>/cu mm) and Differential leucocyte count (DLC, %),(Mean ± S.E.)of broiler chicks in different experimental groups (B1, B2, B3, B4, B5 and B6) at different intervals**

DOA/DPCT	Group	TLC (x10 <sup>3</sup> /cu mm)	Heterophil (%)	Eosinophils (%)	Basophil (%)	Lymphocyte (%)	Monocytes (%)
28/14	B1	18.29 <sup>a</sup> ± 0.96	37.67 <sup>a</sup> ± 0.99	0.67 <sup>a</sup> ± 0.33	0.50 <sup>a</sup> ± 0.22	55.00 <sup>c</sup> ± 1.51	6.17 <sup>a</sup> ± 0.87
	B2	18.26 <sup>a</sup> ± 1.19	40.50 <sup>ab</sup> ± 1.71	1.50 <sup>ab</sup> ± 0.43	0.50 <sup>a</sup> ± 0.22	52.00 <sup>bc</sup> ± 1.29	5.50 <sup>a</sup> ± 0.56
	B3	24.08 <sup>b</sup> ± 2.51	45.00 <sup>b</sup> ± 1.41	3.83 <sup>cd</sup> ± 0.40	0.50 <sup>a</sup> ± 0.34	44.33 <sup>a</sup> ± 1.12	6.33 <sup>a</sup> ± 1.12
	B4	22.85 <sup>ab</sup> ± 2.04	41.83 <sup>ab</sup> ± 0.75	2.17 <sup>b</sup> ± 0.48	0.67 <sup>a</sup> ± 0.33	49.00 <sup>ab</sup> ± 1.13	6.33 <sup>a</sup> ± 0.88
	B5	23.67 <sup>ab</sup> ± 2.22	40.83 <sup>ab</sup> ± 2.68	4.17 <sup>d</sup> ± 0.65	0.33 <sup>a</sup> ± 0.21	48.67 <sup>ab</sup> ± 2.44	6.00 <sup>a</sup> ± 1.55
	B6	21.12 <sup>ab</sup> ± 1.12	39.67 <sup>ab</sup> ± 1.76	2.67 <sup>bc</sup> ± 0.49	0.50 <sup>a</sup> ± 0.22	49.67 <sup>b</sup> ± 1.54	7.50 <sup>a</sup> ± 0.99
42/28	B1	18.94 <sup>a</sup> ± 0.38	37.67 <sup>a</sup> ± 1.89	0.83 <sup>a</sup> ± 0.31	0.50 <sup>a</sup> ± 0.34	55.33 <sup>d</sup> ± 1.50	5.67 <sup>a</sup> ± 1.09
	B2	18.55 <sup>a</sup> ± 0.94	39.00 <sup>ab</sup> ± 1.29	1.17 <sup>ab</sup> ± 0.48	0.33 <sup>a</sup> ± 0.21	53.50 <sup>cd</sup> ± 1.18	6.00 <sup>a</sup> ± 1.32
	B3	25.92 <sup>b</sup> ± 2.38	44.50 <sup>c</sup> ± 0.96	3.50 <sup>cd</sup> ± 0.50	0.17 <sup>a</sup> ± 0.17	44.33 <sup>a</sup> ± 1.86	7.50 <sup>a</sup> ± 1.18
	B4	25.11 <sup>b</sup> ± 2.25	42.50 <sup>bc</sup> ± 1.65	2.50 <sup>bc</sup> ± 0.50	0.50 <sup>a</sup> ± 0.34	48.67 <sup>ab</sup> ± 1.41	5.83 <sup>a</sup> ± 1.22
	B5	25.76 <sup>b</sup> ± 1.96	43.67 <sup>bc</sup> ± 1.43	4.33 <sup>d</sup> ± 0.71	0.33 <sup>a</sup> ± 0.21	45.83 <sup>ab</sup> ± 1.64	5.83 <sup>a</sup> ± 0.70
	B6	25.02 <sup>b</sup> ± 2.40	40.67 <sup>abc</sup> ± 1.58	2.50 <sup>bc</sup> ± 0.43	0.50 <sup>a</sup> ± 0.22	49.83 <sup>bc</sup> ± 1.22	6.50 <sup>a</sup> ± 1.34

B1 - Control (Basal diet), B2 - Giloy control (Giloy powder @ 1g/kg of feed), B3 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B4 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B5 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy powder @ 1g/kg of feed, B6 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub> + Giloy powder @ 1g/kg of feed.

a, b, c, d: Means with unlike superscripts in a column differ significantly, P<0.05

Pandey (2010) reported that supplementing aqueous extract of Giloy stem and leaves normalized the affected MCV and MCHC levels in mice exposed to lead. MCH showed no significant difference between the Cd treated and control groups.

Mean TLC was significantly (P<0.05) increased in group B3 after 14 DPCT as compared to group B1, but the mean TLC in group B4 was significantly (P<0.05) increased on 28 DPCT. This might be due to Cd induced inflammatory reaction. Gabol *et al.* (2014) and Guttyj *et al.* (2019) also reported increased TLC in cadmium intoxicated chicken. On the contrary, decreased TLC was reported in

chicken administered with Cd @ 1000 ppm/kg feed (Choudhary *et al.*, 2018). Similarly, increased TLC was observed in cadmium intoxicated groups supplemented with Giloy powder (B5 and B6) indicative of a lack of ameliorative effect of Giloy powder. Differential leucocyte count revealed that mean relative heterophil count and eosinophil count in Cd treated groups (B3 and B4), was significantly (P<0.05) increased and mean relative lymphocyte count was significantly (P<0.05) decreased as compared to control group (B1) at different time intervals. However, no significant difference was noticed in the mean relative monocyte and basophil counts as compared to control group (B1). Similar DLC results were reported

**Table 3. Biochemical parameters (Mean ± S.E.) of broiler chicks in different experimental groups (B1, B2, B3, B4, B5 and B6) at different intervals**

DOA/DPCT	Group	Total serum protein concentration (g/dl)	Serum albumin concentration (g/dl)	Serum globulin concentration (g/dl)	Serum albumin globulin ratio	AST (IU/L)	ALT (IU/L)	BUN (mg/dl)	Creatinine concentration (mg/dl)
28/14	B1	4.09 <sup>a</sup> ± 0.41	1.47 <sup>b</sup> ± 0.21	2.62 <sup>a</sup> ± 0.48	0.70 <sup>b</sup> ± 0.16	199.45 <sup>a</sup> ± 7.07	10.77 <sup>a</sup> ± 0.85	2.11 <sup>a</sup> ± 0.27	0.33 <sup>a</sup> ± 0.01
	B2	4.10 <sup>a</sup> ± 0.17	1.45 <sup>b</sup> ± 0.10	2.65 <sup>a</sup> ± 0.20	0.58 <sup>ab</sup> ± 0.08	201.97 <sup>a</sup> ± 18.17	10.73 <sup>a</sup> ± 1.18	2.05 <sup>a</sup> ± 0.26	0.33 <sup>a</sup> ± 0.04
	B3	3.41 <sup>a</sup> ± 0.24	0.88 <sup>a</sup> ± 0.15	2.53 <sup>a</sup> ± 0.23	0.37 <sup>a</sup> ± 0.07	279.25 <sup>b</sup> ± 13.91	17.20 <sup>b</sup> ± 2.79	2.99 <sup>a</sup> ± 0.37	0.69 <sup>b</sup> ± 0.12
	B4	3.90 <sup>a</sup> ± 0.17	1.11 <sup>ab</sup> ± 0.18	2.79 <sup>a</sup> ± 0.25	0.44 <sup>ab</sup> ± 0.11	258.68 <sup>b</sup> ± 14.37	16.13 <sup>ab</sup> ± 2.50	2.61 <sup>a</sup> ± 0.37	0.61 <sup>ab</sup> ± 0.10
	B5	3.66 <sup>a</sup> ± 0.25	1.07 <sup>ab</sup> ± 0.16	2.59 <sup>a</sup> ± 0.34	0.48 <sup>ab</sup> ± 0.11	266.42 <sup>b</sup> ± 13.69	17.08 <sup>b</sup> ± 1.89	2.80 <sup>a</sup> ± 0.36	0.64 <sup>b</sup> ± 0.15
	B6	3.98 <sup>a</sup> ± 0.15	1.16 <sup>ab</sup> ± 0.14	2.82 <sup>a</sup> ± 0.09	0.42 <sup>ab</sup> ± 0.05	239.97 <sup>ab</sup> ± 29.05	16.01 <sup>ab</sup> ± 1.83	2.55 <sup>a</sup> ± 0.39	0.54 <sup>ab</sup> ± 0.09
42/28	B1	4.18 <sup>b</sup> ± 0.17	1.50 <sup>b</sup> ± 0.16	2.68 <sup>a</sup> ± 0.18	0.58 <sup>a</sup> ± 0.09	218.83 <sup>a</sup> ± 11.38	10.83 <sup>a</sup> ± 1.07	2.25 <sup>a</sup> ± 0.43	0.35 <sup>a</sup> ± 0.02
	B2	4.16 <sup>b</sup> ± 0.40	1.49 <sup>b</sup> ± 0.12	2.67 <sup>a</sup> ± 0.32	0.59 <sup>a</sup> ± 0.06	208.93 <sup>a</sup> ± 5.50	10.93 <sup>a</sup> ± 1.19	2.31 <sup>a</sup> ± 0.43	0.34 <sup>a</sup> ± 0.01
	B3	3.10 <sup>a</sup> ± 0.10	0.82 <sup>a</sup> ± 0.12	2.28 <sup>a</sup> ± 0.16	0.39 <sup>a</sup> ± 0.08	290.45 <sup>b</sup> ± 27.47	17.68 <sup>b</sup> ± 2.47	4.16 <sup>b</sup> ± 0.40	0.85 <sup>b</sup> ± 0.12
	B4	3.73 <sup>ab</sup> ± 0.26	1.03 <sup>a</sup> ± 0.12	2.70 <sup>a</sup> ± 0.27	0.41 <sup>a</sup> ± 0.08	289.18 <sup>b</sup> ± 21.73	16.31 <sup>ab</sup> ± 2.48	3.88 <sup>b</sup> ± 0.67	0.71 <sup>b</sup> ± 0.13
	B5	3.35 <sup>ab</sup> ± 0.25	0.95 <sup>a</sup> ± 0.16	2.41 <sup>a</sup> ± 0.18	0.40 <sup>a</sup> ± 0.08	284.97 <sup>b</sup> ± 18.25	17.41 <sup>b</sup> ± 2.41	3.97 <sup>b</sup> ± 0.40	0.74 <sup>b</sup> ± 0.15
	B6	3.80 <sup>ab</sup> ± 0.28	1.15 <sup>ab</sup> ± 0.16	2.65 <sup>a</sup> ± 0.33	0.51 <sup>a</sup> ± 0.14	283.50 <sup>b</sup> ± 15.62	16.09 <sup>ab</sup> ± 1.96	3.75 <sup>ab</sup> ± 0.63	0.63 <sup>ab</sup> ± 0.11

B1 - Control (Basal diet), B2 - Giloy control (Giloy powder @ 1g/kg of feed), B3 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B4 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>, B5 - 1/10<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>+ Giloy powder @ 1g/kg of feed, B6 - 1/20<sup>th</sup> of LD<sub>50</sub> of CdCl<sub>2</sub>+ Giloy powder @ 1g/kg of feed.

a, b, c, d: Means with unlike superscripts in a column differ significantly, P<0.05

in Cd intoxicated chicken at different time intervals (Choudhary *et al.*, 2018). In Cd treated groups supplemented with Giloy powder (B5 and B6), the DLC changes were non significantly reduced as compared to group B3 and B4.

Biochemical studies (Table 3) revealed that values of serum total protein and albumin concentration was lowest in group (B3) as compared to control group (B1) at different time intervals. Significant (P<0.05) decrease in serum total protein was observed at 28 DPCT and albumin concentration at 14 DPCT in group B3 as compared to control group B1. This decreased in serum total protein and albumin concentration might be due to Cd induced hepatotoxicity and nephrotoxicity which leads to decreased synthesis of albumin in the liver and loss of proteins through damaged nephrons leading to hypoproteinemia (Tennant and Center, 2008; Gutyj *et al.*, 2019). Almost similar changes were reported earlier in Cd induced toxicity in chicken (Hashem *et al.*, 2019). However, mild therapeutic effect of Giloy powder supplementation in Cd treated groups (B5 and B6) was observed as non-significantly increased mean total serum protein and albumin concentration as compared to Cd treated groups without Giloy powder supplementation (B3 and B4) throughout the experimental study. The therapeutic effect of Giloy powder supplementation may be due to hepatoprotective and nephroprotective effect of Giloy as reported by other workers (Padma *et al.*, 2016; Baskaran *et al.*, 2018). Bhardwaj *et al.* (2012) also reported similar increase in serum total protein and albumin concentration in broilers fed with Giloy stem aqueous extract and stem juice concentrate @ 1g/kg of feed.

Mean serum globulin concentration did not show

any significant difference in all the Cd treated groups (B3, B4, B5 and B6) as compared to control group (B1) up to 28 DPCT. Similarly, Abou-Kassem *et al.* (2016) reported a lack of significant change in globulin values in quails treated with Cd @ 40, 80 and 120 mg/kg feed as compared to the control group. The mean serum albumin globulin ratio (A/G) was non-significantly decreased in Cd treated groups (B3 and B4) throughout the experimental study. Similar changes of globulin and A/G ratio were observed in cadmium intoxicated groups supplemented with Giloy powder (B5 and B6) and all the groups (B4, B5 and B6) were comparable to both control groups (B1 and B2).

Mean serum AST, ALT, BUN and creatinine values were higher in group (B3) treated with high dose of Cd as compared to control group (B1), throughout the experimental study. Significant (P<0.05) increase in AST, ALT and creatinine values were observed at 14 DPCT and BUN at 28 DPCT. In group B4 treated with low dose of Cd, significant (P<0.05) increase in AST levels were observed at 14 DPCT, BUN and creatinine values at 28 DPCT. AST and ALT are enzymes present in the cytoplasm and mitochondria of hepatocytes and an increased activity of these enzymes might indicate mitochondrial and hepatocellular injury of liver in groups treated with Cd. Similar results of increased AST and ALT values were noticed by other workers in cadmium intoxicated chicken (Gutyj *et al.*, 2019; Hashem *et al.*, 2019). Cadmium induced increase in BUN and creatinine levels were also reported in chicken (Singh *et al.*, 2009; Reddy and Swapna, 2011). Increased BUN and creatinine concentration might be due to the cadmium induced nephrotoxicity and tubular epithelium degeneration. In Cd treated groups



supplemented with Giloy powder (B5 and B6), mean serum AST, ALT, BUN and creatinine levels were non-significantly decreased as compared to Cd treated groups without Giloy powder supplementation (B3 and B4) throughout the experimental study. Similar, therapeutic effect was reported by Sharma and Pandey (2010) in mice exposed to lead. They observed that supplementing aqueous extract of Giloy stem and leaves normalized the increased the AST, ALT levels. Hamsa and Kuttan (2012) reported a similar therapeutic effect of Giloy by reduction of BUN and creatinine levels in urotoxicity induced rats.

It is evident from the above results that Giloy powder supplementation mildly restored the cadmium induced haemato-biochemical alterations. Thus, it can be concluded that Giloy powder supplementation @ 1 gm/kg feed showed negligible to mild therapeutic effect on cadmium toxicity in broiler chicken.

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