Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.062

RESEARCH ARTICLE

Cadmium Toxicity in Female Japanese quail (*Coturnix japonica*) and Its Diminution with Silymarin

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ARTICLE HISTORY (16-360)

Received: January 01, 2017
Revised: June 20, 2018
Accepted: June 30, 2018
Published online: July 06, 2018

Key words:
Cadmium
Milk thistle
Oviduct
Quail
Toxicity

ABSTRACT

Present study was designed to investigate the toxico-pathological effects of cadmium in female Japanese quail (*Coturnix japonica*) and their attenuation by silymarin. A total of 120 quail chicks of four weeks of age were assigned into six groups and given basal feed, Cd_1 (150 mg.kg^{-1} feed), Cd_2 (300 mg.kg^{-1} feed) and SL (250 mg.kg^{-1} feed) alone and in combinations for 60 days. Hematological parameters, serum biochemical profile, feed intake, bodyweights, relative organ weights, egg production and histopathological alterations in kidney and oviduct were noted. Our results showed dose dependent decrease in mean relative weight of oviduct but increase in liver, intestine, kidney and spleen in groups treated with Cd only. Feed intake was decreased in Cd groups compared with control and bodyweight of Cd treated groups was lower as compared with control and SL groups. The Hb and PCV values were low but higher values of blood urea, creatinine, and serum activity of AST and ALT were observed in Cd treated groups only. Reduced height of mucosal folds collapsed tubular glands, nuclear degenerative changes and infiltration of plasma cells in parenchymatous tissue of oviduct were observed in Cd treated groups only. Moreover, Cd exposure resulted into hemorrhage, casts in tubules, infiltration of mononuclear cells, tubular necrosis and fibrosis in kidneys. Egg production was also lower in Cd treated groups as compared with control group. It may be concluded that Cd has strong nephrotoxic and gonadotoxic effects and SL may effectively ameliorate these toxic effects completely at lower doses but partially at higher doses of Cd.

To Cite This Article: Butt SL, Saleemi MK, Khan MZ, Khan A, Farooq M, Khatoon A, Bhatti SA, Tahir MW, Islam NU, Jamil H and Muhammad F, 2018. Cadmium toxicity in female Japanese quail (*Coturnix japonica*) and its diminution with silymarin. Pak Vet J, 38(3): 249-255. http://dx.doi.org/10.29261/pakvetj/2018.062

INTRODUCTION

Heavy metals toxicity is a major health problem of animal and human populations due to their bioaccumulation in body through water and food. Cadmium (Cd) is an important environmental pollutant that is continuously discharged through natural and industrial sources into environment (Adedapo and Adeoye, 2014). After absorption through gastrointestinal tract cadmium targets liver, brain, kidneys and gonads (George *et al*., 1996), however, higher residues of cadmium have been found in liver and kidney tissues. By producing reactive oxygen species, it leads to oxidative stress disturbing the antioxidant defense system (Thijissen *et al*., 2007) along with teratogenic effects like malformation of appendages, gut defects and ear abnormalities in a wide variety of animals including rats, mice and chicken. Silymarin acts as an antioxidant, regulates intracellular glutathione peroxidase. It modulates entry of hepatotoxic substances into hepatocytes by manipulating cell membrane permeability. Silymarin has protective effects against oxytetracycline toxicity (Vijayakumar *et al*., 2004) and

also attenuates nephrotoxic effects of arsenic (Prabu and Muthumani, 2012). Chtourou *et al*. (2013) reported that silymarin could protect hepatocytes against Mn-induced oxidative stress. Silymarin accelerates hepatocellular regeneration, lowering blood cholesterol level, by scavenging free radicals inhibits lipid peroxidation, hampered the tumor progression by stabilizing mast cells (Kim *et al.,* 2009). Silymarin reversed the cisplatin induced necroptosis in HK-2 cells showing nephroprotective effects against antibiotic toxicity. Silymarin supplementation also resulted into decreased end product of lipid peroxidation, MDA, and an increase in the RBC glutathione peroxidase, an antioxidant enzyme showing protective effects of silymarin on RBCs and hemoglobin synthesis (Kumaş *et al*., 2011). Srinivasan and Ramprasath (2012) reported that oral administration of silibinin @80 mg/kg normalized the peroxidation and also potentiated antioxidants in cadmium induced peroxidation of lipids. Another study indicated that Cd is bio transformed by plants (Sozubek *et al*., 2015). Although lot of work has been done to see the pathological effects of Cd on the mammalian renal and reproductive systems, but little is known about these effects in avian species. Therefore, this study was designed to investigate the Cd induced renal and reproductive pathology and their attenuation by silymarin in Japanese quail chicks.

MATERIALS AND METHODS

Experiment was designed by considering all the national legislation concerning protection of animal welfare and guidelines of the Graduate Studies and Research Board (GSRB) of the University were followed.

Birds management and experiment procedure: Female Japanese quail chicks were reared in wire cages under optimum housing and managemental conditions. A total of 120 female Japanese quail chicks of similar body weights and free from any apparent clinical ailment were procured from a local farm. After 7 days of acclimatization, birds were divided into six equal groups. Basal diet alone, different inclusion levels of Cd (Cd_1 @ 150 $mg.kg^{-1}$, Cd_2 @ 300 $mg.kg^{-1}$ feed), SL (250 $mg.kg^{-1}$ feed) and combination of Cd and SL was offered to these groups for a duration of 60 days (experiment period). Feed intake (daily basis), daily egg production, body weight gain (weekly basis), relative organ weights of all the groups were recorded.

Hematological and biochemical parameters: The six birds from each group were slaughtered on 30^{th} and 60^{th} day of experiment to collect blood for hematology and serum chemistry. Microhematocrit method (Sharaf *et al*., 2013) was used to estimate packed cell volume and hemoglobin concentration was measured by cyanmethemoglobin method. Total protein was measured by Biuret method and albumin by Bromocresol Green Dye Binding method. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were measured using commercially available colorimetric kits of Merck Company following instructional manual.

Pathomorphological studies: On each slaughtering, organs were collected, weighed and examined for gross pathology. liver, kidneys and oviduct tissues were fixed in 10% neutral buffered formalin for histopathology (Hussain *et al*., 2015).

Statistical analysis: The data obtained from the experimentation were subjected to analysis of variance test and different group means were compared by Duncan's Multiple Range test. $P<0.05$ was considered as significant. A statistical software package (MSTATC) was used for analysis.

RESULTS

Hematological parameters: At day 30 of experiment hematocrit of group 2 (Cd_1), 3(Cd_2) and 6 (Cd_2+SL) was significantly low from control group. At day 60 hematocrit of the group 5 (Cd_1+SL) and 6 (Cd_2+SL) was non-significantly different from control group showing curative effects of SL. While remaining groups were significantly lower when compared to control group indicating adverse effects of Cd. The hematocrit of group 4 (SL) was significantly higher at day 30 and 60 compared with control group. At day 30 and 60 of the experiment the hemoglobin concentration of groups 2 (Cd_1) and 3 (Cd_2) was significantly lower from control group (Table 1).

Serum biochemical parameters: At day 30 total serum protein of all groups was significantly lower compared with control group. At day 60 total protein of all groups except group 4 (SL) was significantly lower from control group. At day 30, albumin concentration of group 2, 3 and 6 was significantly lower, while all other groups were non-significantly different from control group. At day 60 albumen concentration of group Cd_1 and Cd_2 was significantly lower than that of control group while all other groups were non-significantly different from control group. The similar pattern with little variations was observed for globulin. At day 30 and 60, creatinine concentration of group Cd_2 and Cd_1 were significantly higher compared with control. Urea concentration of all groups remained significantly high compared with control group, however urea level of group 5 (SL) at day 30 and 60 was non-significantly different from control group (Table 2).

At day 30 and 60, serum concentration of ALT of groups 2 (Cd_1) and 3 (Cd_2) was significantly higher followed by groups 6 (Cd_2+SL), 5 (Cd_1+SL) and group 4(SL) was non-significantly different from control group. At day 30, serum AST concentration of groups 3, 2 and 6 was significantly high when compared with control group, however, other groups were non-significantly different. At day 60 significantly high concentration of AST was observed in groups 3 (Cd_2), 2 (Cd_1) and 6 (Cd_2+SL) (Table 2).

Relative organ weights: At day 30 and 60, relative weight of intestine was significantly higher in groups 2 (Cd_1), 3 (Cd_2) and 6 (Cd_2+SL) whereas all other groups were non-significantly different from control group. At day 30 and 60, relative weight of kidneys of groups 2, 3 and 6 was significantly higher than control group. At day 30 and 60, relative weight of oviduct of all groups was significantly low compared to control group however birds of groups 2 (Cd_1) and 3 (Cd_2) were having the lowest

relative weight of oviduct. At day 30 and 60 of experiment relative weight of spleen of group 3 (Cd_2) was significantly higher than that of control group (Table 3).

Body weights: During 1st week of experiment, body weights of all the groups were non-significantly different from control group. During 2nd through 6th week body weight of Cd1, Cd2, and Cd2+SL groups remained significantly low as compared with control group however all other groups were non-significantly different from control group. Birds in SL group showed significantly high body weights compared to control group. During 7th week all the groups were significantly lower than control group however body weight of birds in SL groups were non-significantly different to control group. During 8th week of age body weight of all the groups was significantly lower than that of control group however body weight of Cd1 and Cd2 groups was significantly lower than that of all other groups (Table 4).

Feed intake: Feed intake (g/bird) of all the groups has been shown in Table 5. During 1st week, feed intake of the birds in group Cd1 and Cd2+SL was significantly lower than control group. At week 2 the feed intake of the groups Cd1, Cd2, and Cd1+SL were significantly lower than control group while all other groups were non-significantly different from control group. During week 3 and 4 feed intake of birds in group SL was significantly higher while that of group Cd2 was significantly lower than the control group. Feed intake of all other group was non-significantly different from control group. At week 5 feed intakes of the birds of group Cd1and Cd2+SL was significantly lower while it was nonsignificant in all other groups compared with control. During week 6 feed intakes of birds of group SL was significantly higher than control group however all other groups were non-significantly different from control group. During week 7 feed intakes of groups, SL, and Cd2+SL was significantly higher than control group whereas Cd1and Cd2 remained significantly lower than control group. During week 8 feed intake of Cd2 remained significantly low than control group however feed intake was significantly high in all other groups.

Egg production: The average daily egg production (%) of each week for all the groups has been shown in Table 6. During week 1 of the experiment, there was no egg production in all the groups. In week 2, egg production started in control and SL groups and was 5.36 and 3.57 percent, respectively. In week 3, the egg production in control, Cd1, Cd2, Cd1+SL, and Cd2+SL was 20.54, 2.04, 0, 5.71 and 4.40 percent, respectively. In weeks 4-9 the egg production remained high in control and SL group, moderate in Cd1+SL and lowest in Cd1, Cd2 and Cd2+SL. At the end of the experiment the highest production was observed in control group, followed by SL and was least egg production in Cd2.

Table 1: Hematological parameters of birds treated with cadmium alone and in combination with silymarin

Parameter/ experimental days	Groups					
	1 (Control)	2 (Cd_1)	3 (Cd_2)	4 (SL)	5 (Cd_1+SL)	6 (Cd_2+SL)
Hematocrit						
30	34.50±1.87b	26.67±3.01d	23.50±2.43e	42.00±1.41a	32.50±1.05bc	32.00±1.79c
60	35.83±3.31b	30.83±2.14c	27.67±1.75d	42.33±1.75a	34.30±3.39b	33.67±2.16b
Hemoglobin						
30	9.930±0.06bcd	08.60±0.26e	8.41±0.60e	10.81±0.73ab	09.64±0.21cd	09.30±0.17d
60	12.97±0.67abc	10.07±1.14d	8.90±1.29d	14.17±0.85a	11.81±1.54c	12.19±0.98bc

Values (Mean±SD) bearing different alphabets in a row differ significantly (P≤0.05). Control=Basal feed; Cd_1 & Cd_2=150 & 300mg $CdCl_2$ kg^{-1} feed, respectively; SL=Silymarin 250mg.kg^{-1} feed; Cd_1+SL=150 mg $CdCl_2$+Silymarin 250 mg.kg^{-1} feed; Cd_2+SL=300 mg $CdCl_2$+Silymarin 250 mg.kg^{-1} feed.

Table 2: Serum biochemical parameters of birds treated with cadmium alone or in combination with silymarin

Parameter/experimental days	Groups					
	1 (Control)	2 (Cd_1)	3 (Cd_2)	4 (SL)	5 (Cd_1+SL)	6 (Cd_2+SL)
Total plasma protein (g/dL)						
30	5.77±0.12a	4.88±0.06c	4.41±0.18d	5.23±0.11ab	5.29±0.28bc	4.97±0.12bc
60	6.28±0.18a	5.45±0.15bc	4.62±0.14e	6.40±0.17a	5.61±0.17b	5.15±0.19cd
Albumen (g/dL)						
30	1.28±0.01a	1.23±0.01b	1.21±0.01b	1.29±0.03a	1.31±0.04a	1.23±0.03b
60	1.27±0.01ab	1.19±0.02d	1.18±0.03d	1.31±0.02a	1.25±0.03bc	1.27±0.02bc
Globulin (g/dL)						
30	4.50±0.62a	3.65±0.06cd	3.20±0.18d	3.94±0.11bc	3.65±0.52cd	4.04±0.26abc
60	5.00±0.69a	4.17±0.16bc	3.41±0.16e	5.09±0.49a	4.36±0.16b	3.88±0.19cd
Creatinine (mg/dL)						
30	0.19±0.01d	0.33±0.02b	0.41±0.06a	0.20±0.01cd	0.22±0.01cd	0.20±0.03cd
60	0.19±0.01d	0.32±0.02ab	0.33±0.01a	0.17±0.04d	0.29±0.07bc	0.32±0.03ab
Urea (mg/dL)						
30	3.930±0.34d	9.01±0.39b	11.93±1.03a	3.98±0.18d	7.05±0.77c	7.75±0.71bc
60	4.750±0.34e	7.90±0.76bc	10.40±0.71a	5.28±0.49 e	7.45±1.27bc	7.15±0.92cd
Alanine aminotransferase ALT (IU)						
30	8.2±0.35e	12.8±0.30a	13.5±0.45a	9.1±0.64de	10.0±0.49cd	11.3±2.12b
60	9.8±0.47e	17.4±0.84b	19.8±0.54a	9.9±0.34e	12.2±0.83d	13.7±0.43c
Aspartate aminotransferase AST (IU)						
30	244.3±21.53cd	319.5±14.40ab	347.3±62.03a	219.5±40.60d	246.7±24.79cd	328.2±22.8ab
60	268.7±14.88fg	355.7±26.23b	402.2±24.51a	257.7±14.58g	272.3±23.19fg	317.8±21.66cd

Footnote of this table remains the same as mentioned in Table 1.

Table 3: Relative organ weights of organs (g) of the birds treated with cadmium alone or in combination with silymarin

Organ/experimental days	Groups					
	1 (Control)	2 (Cd_1)	3 (Cd_2)	4 (SL)	5 (Cd_1+SL)	6 (Cd_2+SL)
Intestines						
30	7.55±0.81cd	9.84±0.78ab	10.90±1.50a	5.84±0.36e	8.08±1.04c	10.09±0.07ab
60	7.60±0.72cd	10.4±0.49ab	11.09±1.29a	5.99±0.45e	8.21±0.98c	10.38±0.89ab
Kidneys						
30	0.77±0.03cd	0.99±0.05b	1.17±0.10a	0.87±0.07c	0.83±0.11c	0.96±0.09b
60	0.80±0.06d	1.09±0.03b	1.23±0.09a	0.88±0.08d	0.88±0.06d	0.98±0.09c
Oviduct						
30	3.79±0.31a	2.60±0.19c	2.15±0.35d	3.39±0.35b	3.35±0.25b	3.34±0.11b
60	4.00±0.40a	2.73±0.12e	2.10±0.8f	3.70±0.09bc	3.49±0.12cd	3.85±0.26d
Spleen						
30	0.04±0.02b	0.05±0.01b	0.13±0.02a	0.07±0.03b	0.06±0.01b	0.08±0.02b
60	0.08±0.02b	0.09±0.01b	0.15±0.01a	0.11±0.01b	0.10±0.01b	0.12±0.01b

Footnote of this table remains the same as mentioned in Table 1.

Table 4: Body weights (g) of birds offered various levels of cadmium and silymarin for 60 days

Group	Experimental weeks							
	1	2	3	4	5	6	7	8
Control	91.3±3.14ab	120±3.52a	130.5±4.37b	134.3±3.67b	152.8±6.5b	161.0±4.86ab	171.6±6.62a	174.3±5.9a
Cd1	89.5±1.64b	97.2±3.66c	106.7±4.68e	109.5±3.4d	114.8±5.27d	109.8±4.15d	102.8±5.4d	99.3±4.7d
Cd2	88.8±3.31b	91.0±3.52d	98.5±6.22f	100.0±4.29e	109.8±4.17d	107.7±4.93d	100.3±4.27d	99.7±11.91d
SL	94.7±4.72ab	121.0±2.83a	139.3±2.50a	147.5±5.09a	172.0±5.97a	167.6±5.01a	163.0±18.2a	162.6±7.12b
Cd1+SL	90.5±4.85b	119.8±4.45a	125.6±4.89bcd	132.3±8.62bc	148.3±3.78b	142.6±5.20bc	138.5±9.65c	143.0±10.8c
Cd2+SL	97.8±4.96a	112.7±5.54b	124.2±3.13d	127.3±4.08c	128.8±5.15c	135.5±3.51c	131.3±5.24c	143.0±10.43c

Values (Mean±SD) in each column followed by different small letters are statistically different (P≤0.05). Other Footnote about experimental groups remains the same as mentioned in Table 1.

Table 5: Feed intake (g/bird/day) of birds treated with cadmium alone and in combination with silymarin

Experimental weeks	Groups					
	1 (Control)	2 (Cd_1)	3 (Cd_2)	4 (SL)	5 (Cd_1+SL)	6 (Cd_2+SL)
1	24.2±1.07c	21.5±1.09de	23.5±1.19cd	25.6±1.00abc	24.7±2.178bc	20.1±1.30e
2	28.0±1.18ab	24.0±2.30c	23.0±0.93c	29.8±1.68a	22.6±4.23c	30.0±1.38a
3	28.5±2.65bc	28.4±1.22bc	25.5±2.12d	35.9±2.55a	26.4±2.62cd	26.4±1.94cd
4	30.3±4.14bc	29.7±3.97bcd	25.7±2.84d	33.4±3.66ab	28.8±3.75cd	28.9±3.91cd
5	31.0±1.18ab	26.2±2.21de	28.3±2.30bcd	32.4±1.71a	29.5±4.14abc	27.5±3.55cde
6	29.8±2.30bc	28.5±2.59c	29.0±2.61c	33.4±3.55b	27.5±2.31c	31.5±5.66bc
7	25.9±2.19c	23.1±0.72d	20.7±1.06e	34.0±1.48a	26.0±1.73c	32.0±1.60b
8	27.2±0.64d	29.8±2.45c	24.3±0.95e	35.9±1.88b	30.7±1.24c	35.3±2.11b

Footnote of this table remains the same as mentioned in Table 1.

Gross and histopathology: Pale, swollen kidneys with distended ureters were observed in groups kept upon cadmium contaminated feed only. Oviducts were atrophied in birds of group 2 (Cd_1) and 3 (Cd_2). Size of oviducts in groups fed silymarin was similar to that of control group. Atrophied tubular glands, patent lumen with reduced height of mucosal folds in magnum and isthmus and surface epithelium only in magnum were observed in oviduct from birds treated with Cd (Fig. 2a & 2b). At some places fragmented nuclei were indicative of degenerative/necrotic changes. Increased number of plasma cells and macrophages were present at the basal border of surface epithelium. There were also aggregates of mononuclear cells in stroma of folds of magnum indicating inflammatory conditions over there. Tubular glands of all groups were having normal pattern but collapsed tubules resting stage nuclei with prominent nucleoli, atrophied glands, with increased connective tissue proliferation in the middle of folds were observed in group 2 (Cd_1) and 3 (Cd_2) as shown in Fig. 2a & 2b. Tubular necrosis, aggregates of mononuclear cells in parenchyma along with increased cellular infiltration around proximal convoluted tubules was noted in kidney parenchyma of Cd treated birds (Fig. 1a). Homogenous pinkish proteinaceous casts were present in lumen of tubules (Fig. 1b & 1c). Collapsed tubules with pinkish proteinaceous and sloughed tubular cells from basement membrane were suggestive of chronic degenerative changes (Fig. 1a & 1c).

DISCUSSION

Cadmium is an environmental pollutant present in soil, water and air (Shagirtha and Pari, 2011). Drinking water contaminated by industrial effluent and the plants grown on Cd contaminated soil are potential sources of Cd exposure to animal and human population. After getting in circulatory system, major target organs of Cd include liver, brain, kidneys and digestive tract (George *et al.*, 1996). Silymarin is now frequently be used for the treatment of liver and kidney disorders. It was assumed that it may also protect the birds from Cd induced toxicopathological effects in Japanese quail. In order to confirm this hypothesis, four combinations of Cd and SL were used in the feed. Different parameters were noted to evaluate the pathological effects of Cd and ameliorative effect of SL against these alterations in kidney and oviduct.

Our findings showed atrophied oviduct both in Cd1 and Cd2 depicting the apoptosis inducing ability of Cd in germinal cells. The Cd not only induced apoptosis in ovarian tissue leading to rudimentary fallopian tube but also disrupted the normal cascade of reproductive hormone like FSH and LH in mice (Toman *et al.*, 2005; Obianime *et al*., 2011).

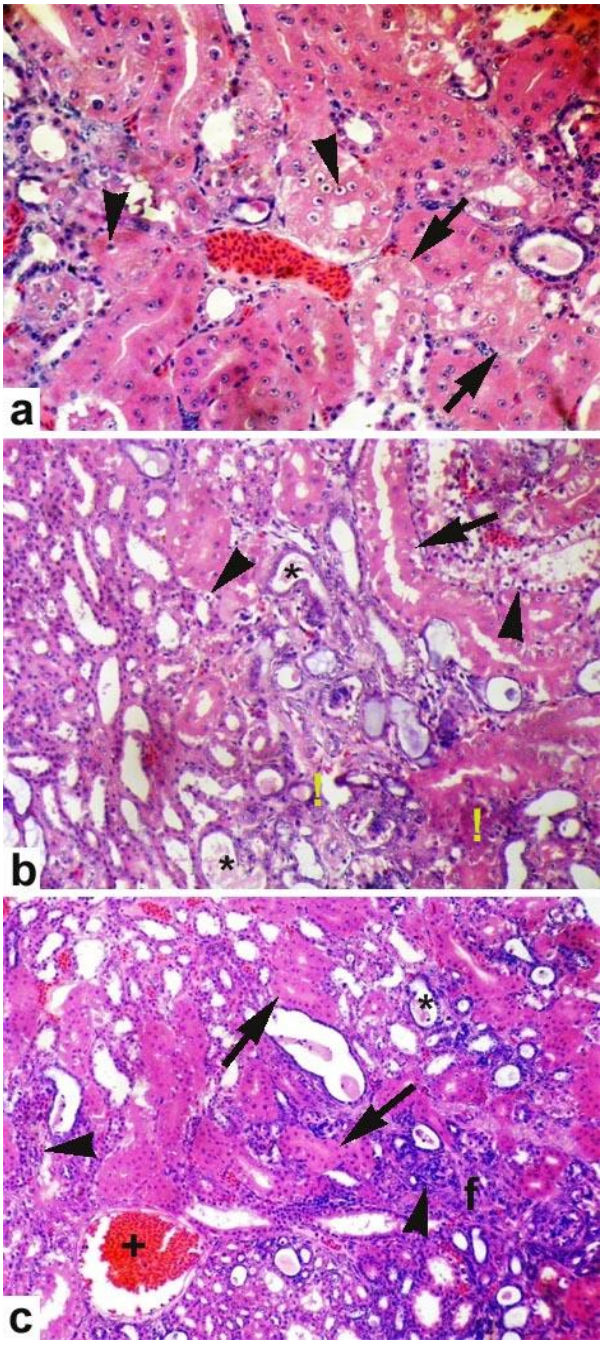

Fig. 1: Photomicrograph of kidneys of Japanese quail chicks treated with cadmium showing 1a) tubular necrosis (arrows) characterized by pyknotic (arrow heads), karyorrhexis and karyolysis in the cells of the tubules; 1b) degenerative changes characterized by collapsed tubules (!), presence of casts in tubules (*), tubular necrosis (arrows), pyknotic nuclei (arrow heads), and sloughed tubular cells from basement membrane and 1c) congestion (+), hemorrhage, infiltration of mononuclear cells (arrow heads), (*), tubular necrosis (arrows), and fibrosis in parenchymatous tissue (f). H and E Stain. a) 400X; b and c: 200X.

Table 6: Mean daily egg production (%) of different groups fed various levels of cadmium, and silymarin for 60 days

Weeks	Control	Cd1	Cd2	SL	Cd1+SL	Cd2+SL
1	0	0	0	0	0	0
2	05.36	0	0	03.57	0	0
3	20.54	02.04	0	14.29	4.40	0
4	25.89	09.52	05.19	15.18	10.71	2.86
5	58.92	21.42	14.28	41.00	25.98	9.52
6	33.92	15.58	09.52	21.42	18.18	9.52
7	26.78	12.78	07.14	20.40	18.18	8.80
8	26.79	10.72	07.14	18.36	12.98	6.66

All other designations are as outlined in footer of Table 1.

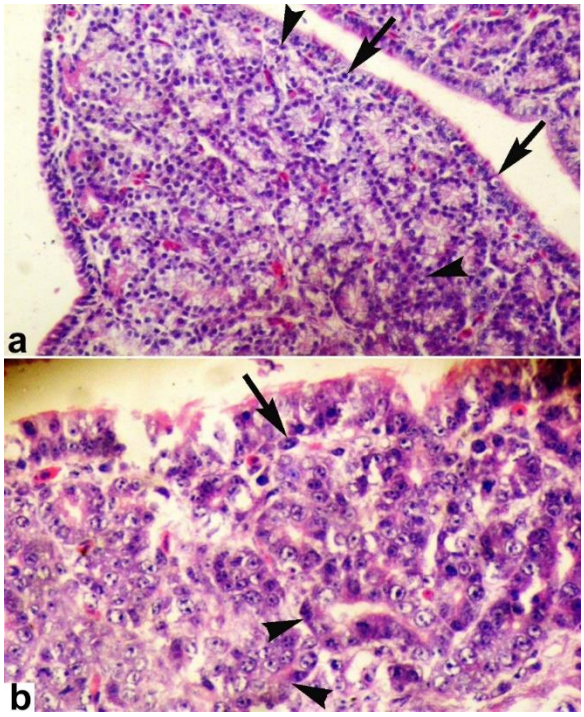

Fig. 2: Photomicrograph of oviduct (magnum) of Japanese quail chicks treated with cadmium showing 2a) low height of epithelium, round prominent resting nuclei (arrows) and collapsed tubules (arrow heads) and 2b) degenerative changes characterized by collapsed tubular glands (arrow heads), condensed nuclei, proliferation of plasma cells beneath the surface epithelium (arrow head). H and E stain. Magnification a) 200X; b) 400X.

Lowest PCV and Hb were noted in group 3 (Cd_2) followed by group 2(Cd_1) showing adverse effects of Cd on hematopoiesis. This decrease was dose dependent as hypochromic microcytic anemia was more pronounced in birds receiving Cd @ 300 mg Kg^{-1} of feed compared to control and Cd @ 150 mg $Kg^{-1}$of feed. Similar types of results have been reported by Altef and Al-Attar (2005) in fish exposed to Cd in which low level of Hb and PCV was a consistent feature. Our study is strengthened by Siakpere and Ikomi (2011) in fish and by Zhang *et al.* (2005) in chicken. However, groups fed upon SL supplemented feed only did show higher values of Hb and PCV compared to control showing its ameliorative effects on hematopoiesis. The effect of SL upon hematological parameters in Japanese quail is might be first time being reported by our experiment. In the present experiment, total protein and albumin concentration Cd-treated groups were significantly lower compared to control. Similar adverse effects were reported in quails by Tahir *et al*. (2017) and Uyanik *et al.* (2001) in chicken.

In present study serum concentration of urea and creatinine was significantly high in birds kept upon Cd contaminated feeds compared to control group showing renal impairment by Cd. Our findings had also been supported by Abdel-Moneim *et al.* (2007) who reported that Cd administration to male rats elevated the urea and creatinine concentrations in the blood.

In the present study, there was a significant increase in the serum level of ALT and AST in Cd-treated groups compared to control group. This shows impairment in the integrity of hepatocytes by Cd as reported by Olsvik *et al*. (2016) in carps. Tahir *et al.* (2017) observed significantly

increased serum activity of ALT and AST in serum of quails exposed to Cd. However, in feed SL-supplementation @ 250 mg.kg^{-1} lowered the serum activity of ALT and AST when compared to control. Similar hepatoprotective effects of SL have been reported in poultry in ochratoxins toxicity (Khatoon *et al*., 2013).

Feed intake of the birds fed upon Cd supplemented feed remained significantly lower from control group showing that Cd had an inhibitory effect upon feed consumption. Similar type of observation has been reported in broiler (Uyanik *et al*., 2001; Swapna *et al*., 2010). In contrast to our study, Schiavone *et al*. (2007) reported that addition of only silymarin in feed put a negative effect on feed intake of broilers. However, feed intake of birds fed upon SL supplemented feed remained significantly higher from control group throughout experiment showing that SL has an additive effect on feed consumption. Similarly feed intake of birds fed with Cd and SL remained either significantly higher or non-significantly different from control group. This observation suggested that SL might have an ameliorative effect upon Cd induced anorexia in birds.

Body weights of the birds of Cd fed groups was significantly lower from control group and these findings were also reported in Japanese quail (Sant' Ana *et al*., 2005) and in chicken (Uyanik *et al*., 2001; Swapna *et al*., 2010; Bharavi *et al*., 2010). Body weight of the birds fed upon Cd contaminated feed decreased only at the end of the exposure, which indicates a cumulative effect of the metal that might be associated with the action of the metallothioneins (MTs). Long-term exposure to Cd causes depletion of liver and muscular glycogen, due to its action on enzymes involved with glycogenesis (Tourry *et al*., 1985), resulting in alterations in metabolic processes. The body weights of the birds of group SL were significantly higher than that of control group which indicated that there was additive effect of silymarin on growth rate.

In present study poor FCR in terms of egg production was observed in birds fed upon Cd contaminated feed. This may be due to disruption of hormonal mechanisms leading to late maturity and irregular egg production as reduced egg production was observed by cadmium in Japanese quail (Rahman *et al*., 2007). Some investigators explained this phenomenon due to suppression of gene transcription for yolk protein leading to reduced egg production. However, birds fed upon silymarin supplemented feed alone or in combination with Cd at low dose (150mg/kg of feed) showed better egg production compared with groups fed only Cd supplemented feed.

Histopathological alterations in the kidney of the quail of Cd_1 group were congestion, tubular dilatation and degeneration, proteinaceous casts in the lumen of the tubules, degenerative changes in glomeruli and proliferation of epithelial and mesengeal cells in bowman capsule, proliferation of cells (mononuclear cells and fibroblasts) in inter tubular spaces, condensed chromatin in epithelial cells of tubules, disappearance of nuclei in tubular epithelium. Glomerulonephritis, proliferation of cells in intertubular spaces and proteinaceous casts in dilated tubules were also reported in chicken (Uyanik *et al*., 2001). Abdel-Moneim *et al.* (2007) reported cell swelling, in the lining of proximal and distal convoluted tubules along with cytoplasmic vacuolation in male Wistar rats authenticating our findings. Similarly dilated peritubular capillaries, vacuolar degeneration of endothelial cells and disruption of microvilli in tubules had been reported in Cd induced renal toxicity by Olsvik *et al*. (2016). Sant'Ana *et al*. (2005) supported our results by reporting focal glomerulonephritis and degeneration of proximal convoluted tubules in kidney. Groups received SL supplemented feed showed normal cellular details like that of control suggesting that SL did not have adverse effects on the parenchyma of kidney. As renal corpuscles were having clear urinary spaces and proteinaceous casts were scarcely present in distal convoluted tubules indicating that SL had protected birds against toxic effects of Cd. Shahbazi *et al*. (2012) reported nephroprotective effects of silibnin in different species.

The magnum of the Cd-treated groups only (2 & 3) showed overall atrophy of glands with reduced thickening and height of mucosal fold, low height of surface epithelial columnar cells, reduction in number of goblet cells, fragmentation of nuclei of tubular glands, presence of plasma cells under the base of surface epithelial cells and proliferation of mononuclear cells in the stroma of folds. However, these lesions were rarely observed in SL treated groups (5 & 6). These finding had not been reported before.

Results of present experimental study are highly suggestive that Cd has strong nephrotoxic and gonadotoxic effects and SL has the ability to attenuate these toxic effects. The inclusion level of SL (250 mg.kg^{-1} feed) in this study ameliorated the alterations induced by Cd up to 150 mg.kg^{-1} feed. However, to assure alleviation of Cd induced pathological alteration in kidney and oviduct of quail, the inclusion level of SL has yet to be determined.

Authors contribution: MZK, MKS, AK, SR, SLB, MF designed the research experiment, and supervised the research and involved in manuscript preparation. SLB, NU, SAB, MWT and MKS performed the experiment and actively remained involved in experiment execution, laboratory analysis.

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