Ameliorative Effect of Spirulina Platensis against Cadmium Toxicity in Broiler Chickens

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1. INTRODUCTION

Cadmium chloride is one of the top 126 pollutants, and its toxicity is an alarming problem due to its vast range of health risks it poses to all organisms on this earth (Khafaga et al., 2019). The World Health Organization has classified Cd as a significant food contaminant, while the International Agency for Research on Cancer has listed it as a class I carcinogen (Ataei et al., 2019). Cd has two sources (natural and anthropogenic). Natural sources of Cd include volcanic eruptions, migration of soil elements by windblown and woods fires. Anthropogenic sources include copper and nickel smelters, fossil fuel burning, phosphate fertilizers produced from contaminated rocks, incorporation of sewage sludge into the soil nickel-Cd batteries, hue plants, cigarette smoke, soldering producers, and petroleum distillation (Waisberg et al., 2003; Obianime et al., 2011). Humans and animals are exposed to Cd through air, water, and food. The oxidative stress and production of reactive oxygen species mediated by Cd-induced free radicals may have the substantial effect in Cd toxicity (Dua et al., 2015). Cd is fatal in even small doses and has major toxic consequences in both people and animals, including nephrotoxicity, carcinogenesis, teratogenicity, and endocrine and reproductive toxicities (Torabifard et al., 2017).

Spirulina platensis has been shown to have hepatoprotective, nephroprotective and neuroprotective properties against oxidative damage caused by chemicals and environmental contaminants (Jeyaprakash and Chinnaswamy, 2005; Sharma et al., 2007; Banji et al., 2013). Spirulina not only boosts antioxidant enzymes i.e. SOD, CAT, GSH, GSH-PX, and reduces lipid peroxidation but also acts as a free radical scavenger (Kurd and Samavati, 2015). The antioxidant properties of Spirulina could be attributed to the presence of two major phycobiliproteins component, phycocyanin and allophycocyanin, that act mostly against superoxide radicals (Chaiklahan et al., 2010). The polysaccharides derived from Spirulina platensis were shown to have potent scavenging activities on DPPH and hydroxyl radicals (Kurd and Samavati, 2015). This study aimed to evaluate the efficacy of Spirulina platensis as natural antioxidant against oxidative damage, tissue residues, biochemical and histopathological alterations induced by Cd in broiler chickens.

2. MATERIAL AND METHODS

2.1. Chemicals, Spirulina and Diagnostic Kits
Lead acetate was purchased from Algomhoria Company. Spirulina platensis (SP) powder was obtained from Green...
Gold Farms, Ismailia, Egypt. Diagnostic kits of malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC) and for liver & kidney function tests were supplied from BioMed company, Cairo, Egypt.

2.2. Animals and Experimental Design
Sixty broiler chicks (one- day -old, Cobb 500) were supplied from El-Watania company with an average body weight of 40-45g on arrival. Experiments were conducted based on the guidelines for care of laboratory animals approved by the ethical animal committee of Benha University (Approval no. BUVFM 03-10-21).

Prior to the start of the experiment, the chicks were acclimated to the room environment for a week. On day seven post arrival, chicks of all groups were weighed (average body weight; 100-120 g) and vaccinated against NDV orally and randomly subdivided into four equal groups (15 chicks each) as following:

Group I: (control group) administered distilled water.
Group II: received Spirulina platensis at dose of 3g/kg (Selim et al., 2018).
Group III: received Cd chloride at dose of 50 mg/kg B.wt orally (Subhan et al., 2011).
Group IV: received both Spirulina platensis (3g/kg of diet) and Cd chloride (50 mg/kg B. wt.) orally. At day 14, chicks were vaccinated against IBDV orally, and received booster vaccination of NDV at day 21. After 4 weeks of the experiment, chickens were individually weighed and sacrificed.

2.3. Body Weight and Feed Conversion Ratio
Body weight and feed intake of chickens in all the experimental groups were checked weekly and FCR of each group was calculated by dividing the feed given by the gain in animal body weight.

2.4. Sampling
Blood samples were collected from randomly chosen chickens from each group at the end of the experiment from wing vein into plain centrifuge tubes for serum biochemical analysis and antioxidant evaluation as previously described (Akbarzadeh et al., 2008). Specimens from liver, kidney, breast, and thigh muscles were collected from sacrificed chickens for Cd residue analysis and histopathological examination.

2.5. Serum Biochemical Analysis
The serum level of liver function enzymes including aspartate amino transferase (AST), alanine amino transferase (ALT) and gamma glutamyl transferase (GGT) were evaluated in all the groups as previously described (Reitman and Frankel, 1957; Szasz, 1969). Total protein and albumin were also assessed (Henry, 1964; Doumas et al., 1971). Kidney function tests including the assessment of the levels of uric acid level and serum creatinine were also conducted (Caraway, 1963; Di Giorgio, 1974).

2.6. Cd Residues
Cd residues were estimated in liver, kidney, breast and thigh muscles using Atomic Absorption Spectrophotometer model SensAA Australia (AOAC, 1990) at Animal Health Research Institute, Dokki, Egypt.

2.7. Oxidative Stress Markers in Serum
The levels of antioxidants as SOD, TAC and MDA were measured in the sera collected from sacrificed chickens (Uchiyama and Mihara, 1978; Sun et al., 1988; Koracevic et al., 2001).

2.8. Histopathological Examination
Liver and kidney specimens collected from all groups were fixed in buffered neutral formalin 10% for 72 hrs. Fixed tissue specimens were trimmed, dehydrated in descending grades of ethyl alcohol, cleared in xylene and embedded in melted paraffin wax at 56°C in hot air oven. Paraffin tissue blocks were sectioned at 5 μm thickness by slide microtome. The sliced tissue sections were collected on glass slides in a water bath, dried, deparaffinized, stained by hematoxylin & eosin stain (H&E), cleared and cover-slipped as previously described (Suvarna et al., 2018).

2.9. Statistical analysis
Data were expressed as the mean ± standard error of the mean (SEM) for each group. Differences and variations between groups were analyzed using one-way analysis of variance (ANOVA). P value of < 0.05 was considered to be statistically significant.

3. RESULTS

3.1. Body weight and feed conversion ratio
There was no difference in B.wt. and FCR between groups I and II. Cd intoxicated chickens showed significant decline in their B.wt. Spirulina in group IV mitigated the loss induced by Cd evidenced by an increase in the B.wt. of chickens compared to Cd intoxicated chickens (group III) but still less than control and Spirulina groups. In contrast, a marked increase in the FCR was noticed in group III (Cd) compared to group IV (Cd+ Spirulina) which was slightly higher than control and Spirulina groups (Table 1).

<table>
<thead>
<tr>
<th>Period</th>
<th>Item</th>
<th>Control</th>
<th>Spirulina</th>
<th>Cd + Spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>B.WT</td>
<td>0.27±0.00ª</td>
<td>0.25±0.00ª</td>
<td>0.14±0.00ª</td>
</tr>
<tr>
<td>week</td>
<td>FCR</td>
<td>1.77±0.03ª</td>
<td>1.69±0.02ª</td>
<td>1.79±0.24ª</td>
</tr>
<tr>
<td>2nd</td>
<td>B.WT</td>
<td>0.86±0.01ª</td>
<td>0.75±0.00ª</td>
<td>0.27±0.02ª</td>
</tr>
<tr>
<td>week</td>
<td>FCR</td>
<td>1.40±0.01ª</td>
<td>1.70±0.01ª</td>
<td>4.14±0.11ª</td>
</tr>
<tr>
<td>3rd</td>
<td>B.WT</td>
<td>1.00±0.02ª</td>
<td>1.13±0.01ª</td>
<td>0.50±0.02ª</td>
</tr>
<tr>
<td>week</td>
<td>FCR</td>
<td>2.51±0.01ª</td>
<td>2.20±0.02ª</td>
<td>3.86±0.07ª</td>
</tr>
<tr>
<td>4th</td>
<td>B.WT</td>
<td>1.96±0.03ª</td>
<td>1.41±0.04ª</td>
<td>0.70±0.03ª</td>
</tr>
</tbody>
</table>

B.WT: Body weight, FCR: Feed conversion ratio. The data are presented as mean ± S.E. (n = 15). Different superscript letters within a row denotes significantly different mean values (p ≤ 0.05).

3.2. Biochemical parameters
The levels of liver (ALT, AST and GGT) and kidney (uric acid and creatinine) function markers in the sera of group II were nearly similar to control group. In Cd intoxicated chickens, there was significant increase in the serum levels of these markers with marked decrease in the levels of total protein and albumin. The serum level of ALT, AST, GGT enzymes, uric acid and creatinine were restored in group IV to nearly normal level with a marked decline in their levels compared to Cd intoxicated chickens. An increase in the levels of total protein and albumin was also reported in group IV compared to group III (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Spirulina</th>
<th>Cd + Spirulina</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/l)</td>
<td>23.00±1.0³</td>
<td>18.00±1.7³</td>
<td>62.60±2.2³</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>40.00±7.1³</td>
<td>37.00±1.2³</td>
<td>88.60±2.7³</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>14.60±1.4³</td>
<td>11.20±0.3³</td>
<td>61.60±1.3³</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>4.00±0.3³</td>
<td>3.24±0.1³</td>
<td>7.56±0.2³</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.00±0.3³</td>
<td>0.96±0.3³</td>
<td>2.56±0.1³</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.76±0.12³</td>
<td>6.08±0.06³</td>
<td>4.80±0.10³</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.56±0.07³</td>
<td>3.62±0.06³</td>
<td>2.76±0.08³</td>
</tr>
</tbody>
</table>

AST: Aspartate transaminase, ALT: Alanine transaminase, GGT: Gamma glutamyl transferase. The data are presented as mean ± S.E. (n = 15). Different superscript letters within a row denotes significantly different mean values (p ≤ 0.05).

3.3. Oxidative markers
There was a significant decrease in level of MDA and an increase in TAC and SOD in Spirulina treated chickens.
compared to control chickens while there was a significant decline in TAC and SOD with an increase in level of MDA in Cd intoxicated chickens compared to control and Spirulina treated chickens. Spirulina elevated the levels of TAC and SOD and reduced the level of MDA in group IV compared to group III (Table 3).

### 3.4. Cd residues in different tissues

There was an increase in Cd concentration in Cd intoxicated chickens compared to control group. A significant reduction in Cd residues was recorded in all examined tissues (livers, kidneys, breast, and thigh muscles) of group IV compared to group III but was still higher than control and Spirulina groups. Breast and thigh muscles had less than 0.01 μg/gm Cd in group II.

### 3.5. Histopathology:

There were no significant microscopic lesions in the livers of most broiler chickens in both control and Spirulina treated chickens (Figure 1A). In Cd intoxicated chickens, the predominant microscopic lesion was in form of diffuse severe hepatocellular degeneration (hydropic degeneration) (Figure 1B). Multifocal areas of hepatic necrosis represented by fine granular eosinophilic and basophilic substances infiltrated with many mononuclear inflammatory cells and few hepatocytes were also evident in some sections in this group (Figure 1C). There was prominent bile ductal hyperplasia with marked peribiliary fibrosis and leukocytic cellular infiltrations in many Cd intoxicated chickens (Figure 1D). Spirulina attenuated Cd induced hepatic toxicity in group IV evidenced by null to mild degenerative changes and mild Kupffer cell activation in most livers in this group (Figure 1E). Foci of extramedullary hematoepoiesis were seen in some livers in this group (Figure 1F). There were no significant alterations in the renal histoarchitecture in both control and Spirulina treated chickens (Figure 2A). The majority of examined glomeruli in Cd intoxicated chickens were suffered from mesangio proliferative glomerulopathy and interstitial hemorrhage and mononuclear inflammatory cellular infiltration (Figure 2B-C). Multifocal tubular degeneration and necrosis with intraluminal basophilic casts were also evident in some sections (Figure 2D-E). In group IV, most renal tubules showed nearly normal histological appearance while few had mild tubular degeneration in form of cloudy swelling (Figure 2F).

### 4. DISCUSSION

Cd is a heavy metal that has been widely used and discharged in significant quantities from factories, leading to an increase in its level in water, soil, and food, thus contaminating the human environment (Abdelrazek et al., 2016). Chronic Cd exposure causes severe hepatic and renal damage as well as bone loss in animals and human (Torabifard et al., 2017; Buha et al., 2019). Previous literature indicated that Spirulina possesses antioxidant properties that could attenuate heavy-metal toxicity. In this study, we assessed Spirulina as hepatorenal protective agent against experimental induced cadmium toxicity in broiler chickens.

Our results showed that Cd severely decreased the body weight of intoxicated chickens which might be explained by the reduction in feed intake, and the increase in catabolism of proteins and lipids associated with Cd toxicity. These findings are consistent with other prior studies (Erdogan et al., 2005; Liao et al., 2017). On the other hand, feed conversion ratio was significantly increased in Cd intoxicated chickens, and this may be attributed to the drop in weight gain of chickens of this group. Cd intoxicated chickens co-treated with Spirulina platensis were heavier than Cd intoxicated chickens which could be due to the optimization in the feed utilization efficiency (Kaoud, 2012). The improvement in FCR in chickens cotreated with Spirulina may be due to the balanced bacterial population in gastro-intestinal tract that play a role in boosting minerals and vitamins absorption and enhance performance and health of broiler chickens (Shanmugapriya and Saravana Babu, 2014).

Nephrotoxicity is one of the well-known adverse effects of Cd exposure (Ibrahim et al., 2018). In accordance, nephrotoxicity was also reported in Cd intoxicated chickens in this study demonstrated by the rise in the levels of both uric acid and creatinine. This could be due to direct toxic effect of Cd on renal tubules and glomerular filtration which eventually results in failure of kidneys to excrete Cd causing further damage and intoxication. The glomerulonephrophy and renal tubular degeneration seen in Cd intoxicated chickens further confirm the nephrotoxicity and this is also consistent with another previous study (El-Sheshtawy et al., 2019). Spirulina had a nephroprotective effect against Cd induced renal injury in our experiment evidenced by restoration of renal functions and histological structure. It has been shown that phycocyanin derived from Spirulina protected against oxidative stress and renal dysfunction in diabetic mice and Cd induced renal toxicity in rats (Zheng et al., 2013; Ibrahim et al., 2018). Based on FAO...
recommendations, the maximum permissible Cd level in tissues and meat is 0.5 mg/kg (Amfo-Otua et al., 2014). Our results further confirmed the concomitant significant decrease (P ≤ 0.05) in Cd concentration in liver (0.28 ± 0.02 μg/gm) and renal (0.65 ± 0.07 μg/gm) tissues in Spirulina platensis co-treated group compared to corresponding Cd levels in these organs in Cd intoxicated group (2.90 ± 0.09 and 2.41 ± 0.30 μg/gm consequently).

The elevated levels of ALT, AST, GGT enzymes in the sera of Cd intoxicated chickens might be due to the damage in hepatocytes induced by Cd causing outflow of these enzymes from the liver cytosol into the circulation (Ibrahim et al., 2018). The reduction in the levels of serum total proteins and albumin could be attributed to hepatic and renal tubular insufficiency, alterations in protein synthesis and/or metabolism or drop in feed consumption (Singh et al., 2016).

Spirulina platensis significantly improved liver histological picture and function in this study reflected by null to mild degeneration in hepatocytes and restoration of all serum biochemical indicators. This improvement could be attributed to the antioxidant and scavenging properties of some natural constituents of Spirulina i.e. carotene, linolenic acid, tocopherols, and phenolic compounds which may act individually or in combination to offer hepatoprotection against Cd toxicity (García-Martínez et al., 2007). In addition, chlorophyll pigment as a constituent of Spirulina was found to play a vital role in toxin removal while phycocyanin was shown to support the absorption of protein and stimulates the immune function (Aladaileh et al., 2020). Previous studies have shown that Cd causes oxidative stress by activating lipid peroxidation (LPO) and suppressing intracellular antioxidant function (Fonad et al., 2009). Cd increases the generation of ROS in different cell types and compromises the antioxidant protection system in vivo (Ferramola et al., 2012). In this study, Cd induced an elevation in the level of MDA (15.88 ± 0.66 mmol/ml), and this may be due to degradation of polysaturated lipids by the action of ROS. Marked decline in SOD level (22.80 ± 1.93 mmol/ml) was recorded in the sera of Cd intoxicated chickens. It has been shown that SOD has a critical antioxidant role including scavenging free radicals and damage to cells as well as repairing damaged cells (Nampoothiri et al., 2007). The decline in TAC level (397.40 ± 4.60 mmol/ml) in Cd intoxicated chickens may indicate that the total capacity of different antioxidants to scavenge oxygen free radicals in both enzymatic and non-enzymatic systems was compromised (Dai et al., 2018) (Zhu et al., 2020). On contrary, Spirulina co-treated chickens exhibited great improvement in antioxidant levels with marked reduction in MDA (0.22 ± 0.35 mmol/ml) and elevation in SOD (47.20 ± 2.03 mmol/ml) and TAC (689.60 ± 15.25 mmol/ml) levels. In accordance, many studies have shown that Spirulina improves the activity of antioxidant enzymes such as SOD, CAT, GST, GSH-PX and reduces lipid peroxidation (MDA) and has powerful scavenging activity against free radicals (Ibrahim and Abdel Daim, 2015; Ibrahim et al., 2018). Spirulina significantly decreased Cd residue in the tissues of chickens and this could be due to its detoxification effect. The chelation of Cd with antioxidant herbs like Spirulina was proposed to speed up Cd removal and prevents Cd bioaccumulation in liver and kidneys (Bharavi et al., 2010). Spirulina also protected against Cd nephrotoxicity in this study thus improving the elimination of Cd from the body.

5. CONCLUSION

In conclusion, the cyanobacteria Spirulina platensis promoted antioxidant defenses that halted cd-induced toxicity in chickens. Spirulina restored the altered biochemical parameters, oxidative parameters, and growth performance parameters in Cd-intoxicated chickens. In addition, Spirulina improved the histological structure of liver and kidney tissues and protected against Cd-induced hepatic and renal damage. It also decreased Cd residual concentration in muscle, liver, and kidney tissues. Spirulina could be a beneficial supplement in chicken ration to prevent potential Cd chloride toxicity.

6. REFERENCES

15. Dua, T.K., Dewanjee, S., Khanra, R., Bhattacharya, N., Bhaskar, B., Zia-Ul-Haq, M., De Feo, V. 2015. The effects of two common edible herbs, Ipomoea aquatica and Enhydra fluctuans, on cadmium-induced pathophysiology: a focus on oxidative metabolism or drop in feed consumption (Singh et al., 2016).