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Original article

Coral fossil: A potential adsorbent of natural source for cadmium removal in broilers

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ABSTRACT

Cadmium (Cd) is a pollutant that poses a health risk for humans and animals. Coral fossil (CF) acts as an adsorbent, yet limited knowledge is available on impacts of CF on Cd toxicities. The work was performed to figure out the effects of CF on hematobiochemical details and specific organs in Cd exposed broilers. The experiment was carried out with 45 broilers and were divided into three groups (15 in each). Group A was served as control. The birds in group B received Cd (75 mg /kg b. w.) orally. Whereas group C was orally supplemented with Cd (75 mg /kg b. w.) and CF (1 gm/kg b. w.). The trial was lasted for 30 days. For hematobiochemical analysis, blood samples were drawn, and sera were separated. Liver, kidney and muscle were collected to assess accumulation concentration. Brain, liver and kidney samples were also collected for histopathological study. The results showed that hematological parameters (TEC, Hb, PCV, MCV, MCH, MCHC and DLC) were altered by Cd but restored with CF supplementation. Liver (AST, ALT and ALP) and kidney (total protein and creatinine) biomarkers were increased significantly in Cd treated broilers while decreased significantly after CF supplementation. CF reduced accumulation concentration of Cd in liver, kidney and muscle. Cd intoxicated broilers showed degenerative changes in brain, hyperplastic bile duct and proliferation of renal tubular epithelium with focal degeneration and necrosis; and these were improved after CF supplementation. Therefore, it can be concluded that CF is a potential adsorbent against Cd toxicities.

1. Introduction

The chicken industry is one of the world's largest and fastest expanding agro-based sectors. It is important in producing animal proteins in the most efficient and cost-effective manner feasible in the shortest amount of time (Hosseinzadeh et al., 2010). Heavy metals occur naturally, and many of them are critical components of our ecosystems. Depending on their oxidation states, heavy metals can be very reactive and, as a result, hazardous to the majority of organisms even at low concentrations. Along with a few other heavy metals, cadmium (Cd) is regarded as the one that poses the most risk to poultry. This is because it is difficult to discharge and has a biological half-life of between ten and thirty lifetimes in living creatures, according to Shi et al. (2017). Nwude et al. (2010) identified food chain pollution as a common source of cadmium exposure in both humans and animals. Pollutants can transfer to animal products such as liver, meat, and milk when producing animals consume polluted feed (Paulien Adamse and Jacob de Jong, 2017). According to Waalkes (2000) and Gerberding (2005), cadmium causes cancer of the liver, kidney, and stomach. Fish exposed to CdCl2 experienced significant declines in RBC, Hb, PCV, and MCH levels while experiencing significant increases in WBC count as compared to the untreated group (Ali et al., 2018). Furthermore, Cd accumulates during the course of a person's lifetime because there is no effective excretory system and it has a half-life of 25 years for elimination in the body (Tai

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et al., 2022). According to recent research (Tokumoto et al., 2019; Fujiwara et al., 2012), Cd affects transcription factors and the regulation of their target genes, which results in the death of cells in a range of organs. Cadmium affects cell proliferation, differentiation, and apoptosis. These activities interact with DNA repair mechanism, the generation of reaction oxygen species (ROS) and the induction of apoptosis (Rani et al., 2014).

Certain materials are more effective at binding than others. Silicates, clays such as bentonite, and charcoal are examples of binder ingredients (Bhatti et al., 2018). The main component of coral fossil shells is amorphous silica, which dissolves readily in the body or system of the animal (Adeyemo, 2013). The exoskeletons of many different types of living things are used to create coral calcium (Laine et al., 2008). Coral fossil is a naturally occurring resource of saltwater calcium with a composition of 24% calcium, 12% magnesium, and>70 other minerals (Xu et al., 2020). The coral calcium hydrate (CCH) administration leads to gradual H_2 generation in gastrointestinal tract and promptly diffuse to whole body. CCH exerts antioxidant activity by significantly enhancing the basal endogenous antioxidant ability in the hippocampus (Hou et al., 2016). There are little or no more studies like the present study so this work does have the novelty in finding out the efficacy of coral fossil against cadmium toxicities. So, the investigation's goal was to assess the actions of coral fossil as an adsorbent for removing the harmful effects of cadmium in broiler.

2. Methodology

2.1. Ethical statement

The animal handling, administration, and experimentation procedures received acceptance by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh. AWEEC/BAU/2021 (41) is the reference number.

2.2. Chicks

About 45 broilers day old chicks were selected for monitoring the effects of cadmium (Cd) and coral fossil and was reared in an open house system for 30 days with timely vaccination.

2.3. Diets and experimental protocol

The research was conducted with 45 broilers which was divided into three groups randomly with 15 broilers in each group. Only daily doses of the poultry feed were administered to Group A. The broilers in Group B received daily doses of Cd (75 mg /kg BW) orally. Whereas group C received daily doses of Cd (75 mg / kg BW) + coral fossil (1 gm /kg BW) orally. The dose of cadmium and coral fossil were selected according to the study by Subhan et al. (2011) and Xu et al. (2020). Both the Cd and coral fossil were given with water. Cd was collected from KURI & COMPANY (PVT.) LTD., BANGLADESH whereas Coral fossil was imported as a powder from CORAL INTERNATIONAL CO., LTD., JAPAN. The trial lasted for 30 days.

2.4. Hematobiochemical studies

5 ml of blood was collected in a vacutainer for hematology and 5 ml blood was allowed to clot. Serum was separated from the clot and will be centrifuged to get the clear serum. The Hematological parameters- total erythrocyte count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count (DLC) were analyzed in physiology department and biochemical parameters- serum aspartate aminotransferase (AST), alanine transaminase (ALT), creatinine, total protein (TP) and albumin were executed.

2.5. Histopathology

After full blood removal with the use of phosphate-buffer saline perfusion, selected organs (brain, liver, and kidney) each set of birds was collected and kept for 15 days in 10% neutral buffered formalin. In conjunction with the Department of Pathology, Bangladesh Agricultural University, Mymensingh-2202, the properly treated tissues were subsequently prepared, sectioned off, and stained. For a better presentation of the histological findings, a photomicroscope (Model: CX43, Olympus Corporation, Tokyo, Japan) was utilized to acquire histology images at various magnifications.

2.6. Determination of Cd contents

At the time of necropsy, the targeted organs—muscle, liver, and kidney were obtained from each group of broilers. ICP-MS (Agilent 7500c, Japan) was then used to measure the accumulation of Cd in the muscle, liver, and kidney (Mazarakioti et al.,2022).

2.7. Statistical analysis

All data was gathered and documented in Microsoft Excel 2016 before being transferred to GraphPad Prism 5.0 for analysis with the one-way ANOVA with Bonferroni multiple comparison test and 95% confidence intervals ($p \leq 0.05$).

3. Results

3.1. Effect of coral fossil on hematological parameters in cadmium treated broilers

The hematological analysis revealed that the total RBC count, Hb conc. and PCV decreased significantly ($p < 0.05$) in Cd treated broilers than the control broilers. Supplementation of coral fossil in the Cd treated broilers substantially ($P < 0.05$) increased these values (Table. 1). The mean corpuscular volume (MCV) increased significantly in Cd treated group as compared to control groups (Table. 1). Whereas, supplementation of coral fossil significantly ($P < 0.05$) decreased MCV value. The mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)

Table 1
Effect of coral fossil on hematological parameters in cadmium exposed broilers.

Hematological data (Mean ± SEM)	Treatment		
	Control	Cadmium	Cadmium + Coral fossil
TEC(million/mm^3)	2.02 ± 0.09^a	1.01 ± 0.05^b	1.84 ± 0.07^a
Hb (gm%)	8.33 ± 0.43^a	4.40 ± 0.30^b	5.93 ± 0.52 ^{a}
PCV (%)	29 ± 0.57^a	21.33 ± 0.33^b	24.00 ± 1.00 ^{a}
MCV (cubic micron)	151.95 ± 2.60^a	213.34 ± 1.02^b	130.81 ± 1.75 ^{a}
MCH (pg)	35.11 ± 2.11 ^{a}	44.18 ± 1.85 ^{a}	32.08 ± 1.68 ^{a}
MCHC (%)	23.07 ± 0.53 ^{a}	20.62 ± 1.38 ^{a}	24.79 ± 2.41 ^{a}
Heterophil (%)	30.00 ± 3.00^a	13.50 ± 0.50^b	27.50 ± 0.50 ^{a}
Lymphocyte (%)	56.50 ± 1.50^a	66.00 ± 2.00^b	58.50 ± 1.50 ^{a}
Monocyte (%)	5.50 ± 0.50^a	8.00 ± 1.00 ^{a}	7.00 ± 1.00 ^{a}
Eosinophil (%)	6.50 ± 0.50^a	11.50 ± 1.50 ^{a}	7.50 ± 0.50 ^{a}
Basophil (%)	1.50 ± 0.50^a	3.00 ± 1.00 ^{a}	0.50 ± 0.50 ^{a}

Data indicate mean ± SEM. Values with different superscript letters in a same column differ significantly ($p < 0.05$).

value were altered non-significantly ($P > 0.05$) by Cd and coral fossil treatment. There was a significant ($P < 0.05$) decrease in the number of heterophil whereas lymphocytes were found to increase significantly ($P < 0.05$) in Cd treated group as compared to control groups (Table. 1). The decrease in heterophil was 13.50 ± 0.50 % and the increase in lymphocytes was 66.00 ± 2.00 % in Cd treated group respectively which were corrected by coral fossil supplementation. Additionally, no statistically significant ($P > 0.05$) changes were found in the percentages of monocyte, eosinophil and basophil after Cd and coral fossil supplementation (Table. 1).

3.2. Effect of coral fossil on serum AST and ALT in cadmium treated broilers

The serum AST (Fig. 1a) and ALT (Fig. 1b) level were 238 ± 6.37 U/L and 22.63 ± 0.93 U/L in control group which raised greatly ($p < 0.05$) after Cd treatment having the value 425.5 ± 8.63 U/L and 34.81 ± 2.37 U/L respectively. Supplementation of coral fossil considerably lowered these values in the cadmium treated broilers.

3.3. Effect of coral fossil on serum creatinine, total protein (TP) and albumin in cadmium treated broilers

The kidney function test revealed that serum creatinine level (Fig. 2a) increased in Cd treated broilers (1.33 ± 0.05 mg/dl) compared to the control (0.69 ± 0.02 mg/dl). Whereas, the serum creatinine level declined substantially ($p < 0.05$) in Cd exposed broilers supplemented with coral fossil (0.77 ± 0.08 mg/dl). But, serum total protein (TP) (Fig. 2b) and albumin (Fig. 2c) were decreased in Cd treated broilers compared to control group. Whereas, Supplementation of coral fossil along with Cd significantly ($p < 0.05$) normalized serum total protein and albumin.

3.4. Cd contents of muscle, liver and kidney

The Cd level of muscle rose considerably in Cd-treated broilers compared to controls. Whereas, muscle's Cd content decreased significantly ($p < 0.05$) after coral fossil supplementation (Fig. 3a). Similarly, Cd contents of liver (Fig. 3b) and kidney (Fig. 3c) were comparable between the control and Cd treated broilers. Supplementation of coral fossil in Cd treated broilers partly decreased Cd contents of liver and kidney.

3.5. Histopathology

Section of brain of control grouped broiler birds showed normal histological structure whereas cadmium treated broiler birds showed focal degenerative changes around the blood vessel. In case of coral fossil supplementation, there was less edema around blood vessel (Fig. 4). Section of liver of control grouped broilers showed normal histological structure whereas cadmium treated broilers showed hyperplastic bile duct. In case of coral fossil supplementation, liver also showed normal histological structure (Fig. 5). Cadmium altered kidney's histology having proliferation of tubular epithelium, focal tubular degeneration, focal necrosis compared to control. Kidney showed slight regenerative changes, slight degenerative changes and focal interstitial necrosis after coral fossil supplementation (Fig. 6).

4. Discussion

Our current study revealed that the hematological parameters significantly altered in Cd treated broilers compared to the controlled broilers. Supplementation of coral fossil in those treated broilers significantly restored these values. Our findings are in line with those of Yilmaz et al. (2012), who discovered that the hematological values were significantly lower in the group exposed to heavy metals. Heavy metals may alter hemoglobin properties by lowering its preference for oxygen holding ability, causing blood cells to become more fragile and leakier, resulting in cell expansion, deformation, as well as destruction (Witeska and Kosciuk, 2003). The prior study has demonstrated a considerable decline in RBC, Hb and PCV in aquatic fish exposed to toxic metals (Shalaby, 2001). The addition of calcium montmorillonite clay to toxin-exposed Red Drum meals led in a safeguarding impact as shown by a significant improvement in hematobiochemical parameters (Zychowski et al., 2013). Previously, antioxidants were found to be helpful in restoring TEC and Hb in heavy metal-treated broilers (Jaiswal et al., 2017). The use of coral fossil in the current study appears to have restored these parameters in Cd-exposed broilers. These could be attributable to the antioxidant capabilities of coral fossils, but the specific mechanism is unknown to us.

According to Pollack et al. (2015), low levels of cadmium are linked to changes in a few biomarkers for kidney and liver function. Cadmium mostly inhibits hepatic function via its interaction with Cd binding molecules known as metallothioneins. According to Habeebu et al. (2000), liver cell regeneration, granulomatous inflammation, apoptosis, and non-specific chronic inflammation have all been shown in mice models of chronic cadmium exposure. Our findings show that supplementing with coral fossil significantly reduces serum AST and ALT in Cd-treated broiler chickens, which agrees with the outcomes of Azadbakht et al. (2017), who found that dietary supplementation with bentonite clay, a

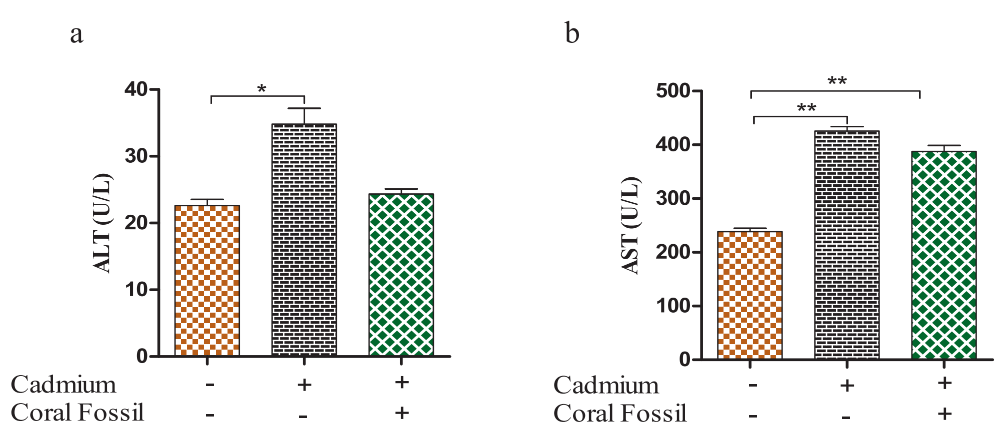

Fig. 1. Effect of coral fossil on serum AST and ALT in cadmium treated broilers. Data indicate mean ± SEM. One-way ANOVA with Bonferroni multiple comparison test was performed. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

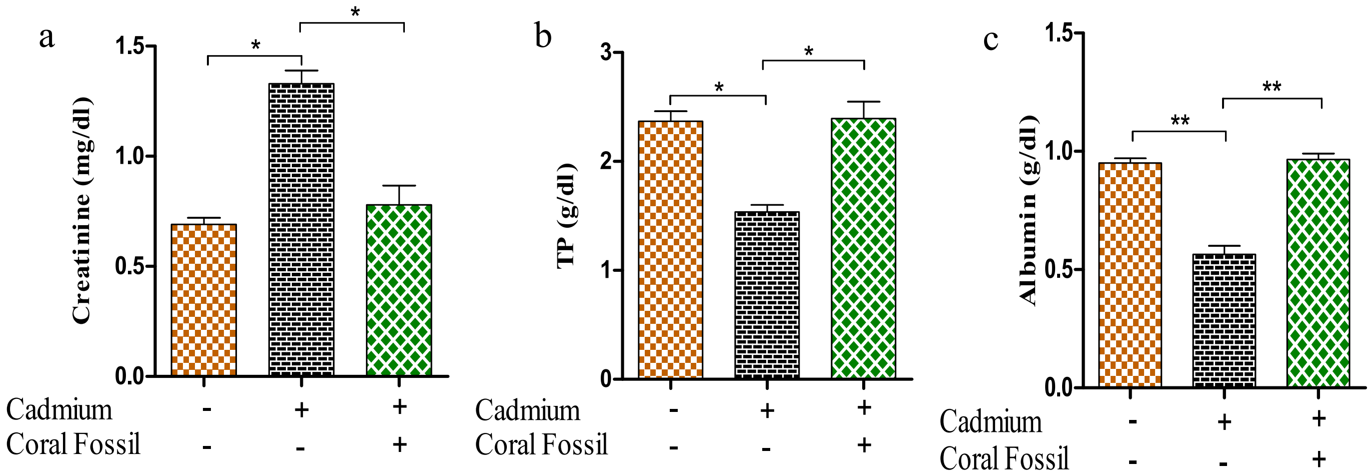

Fig. 2. Effect of coral fossil on serum creatinine, total protein (TP) and albumin in cadmium treated broilers. Data indicate mean ± SEM. One-way ANOVA with Bonferroni multiple comparison test was performed. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

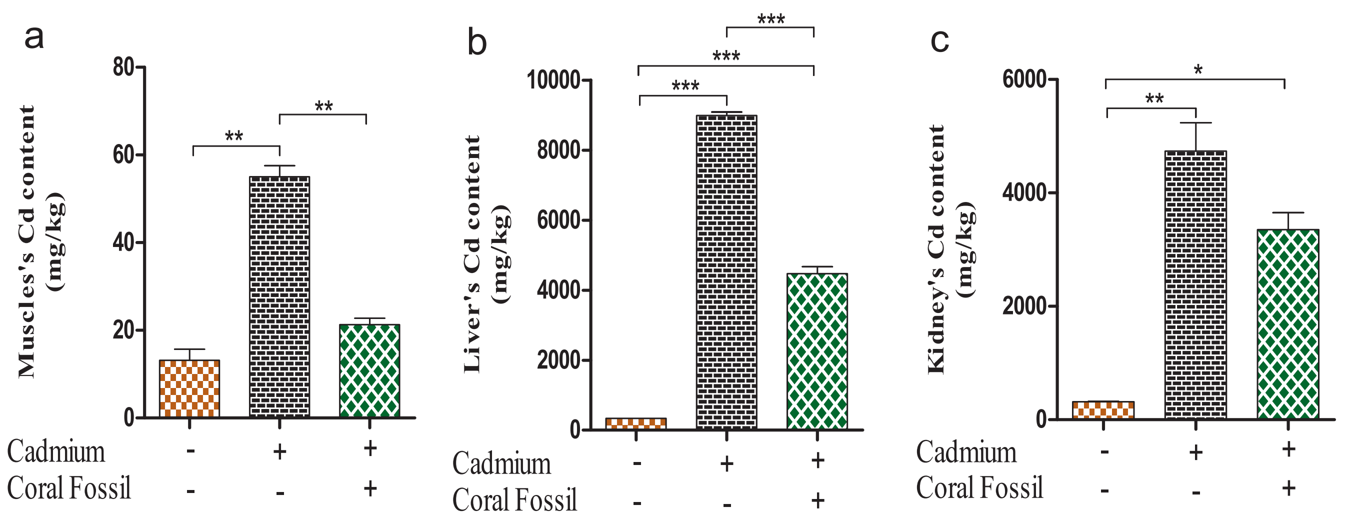

Fig. 3. Effect of coral fossil on cadmium contents of muscle, liver and kidney in Cd intoxicated broilers. Data indicate mean ± SEM. One-way ANOVA with Bonferroni multiple comparison test was performed. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

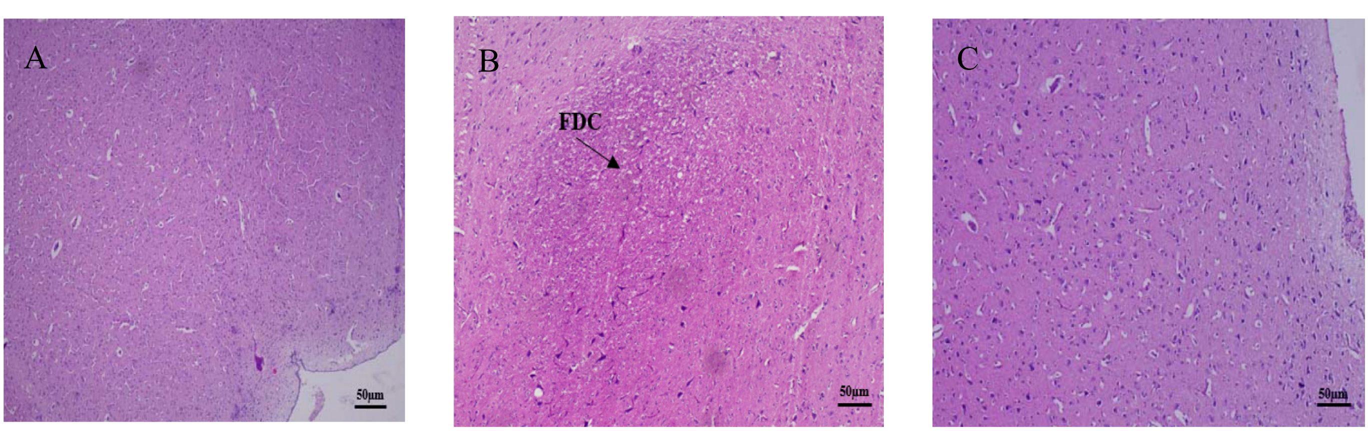

Fig. 4. Effect of coral fossil on brain in cadmium exposed broiler. Photomicrograph of brain in broiler of control (A); cadmium @75 mg/kg BW (B); cadmium @ 75 mg/kg BW + Coral Fossil 1 gm/kg BW (C) at 100x magnification. FDC - Focal Degenerative Changes.

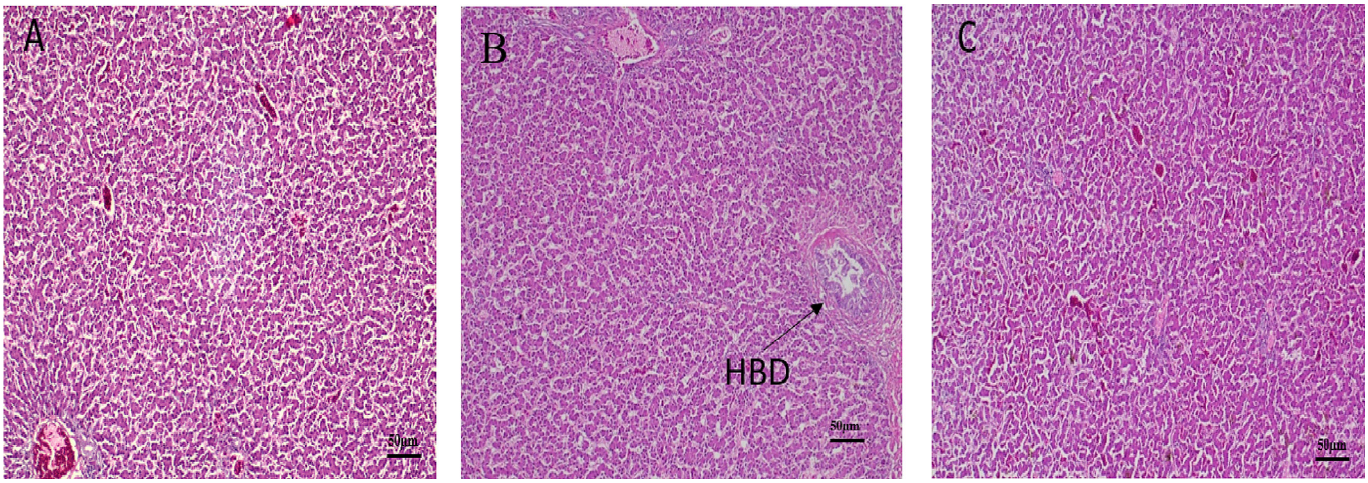

Fig. 5. Effect of coral fossil on liver in cadmium exposed broiler. Photomicrograph of liver in broiler of control (A); cadmium @75 mg/kg BW (B); cadmium @ 75 mg/kg BW + Coral Fossil 1 gm/kg BW (C) at 100x magnification. HBD- Hyperplastic bile duct.

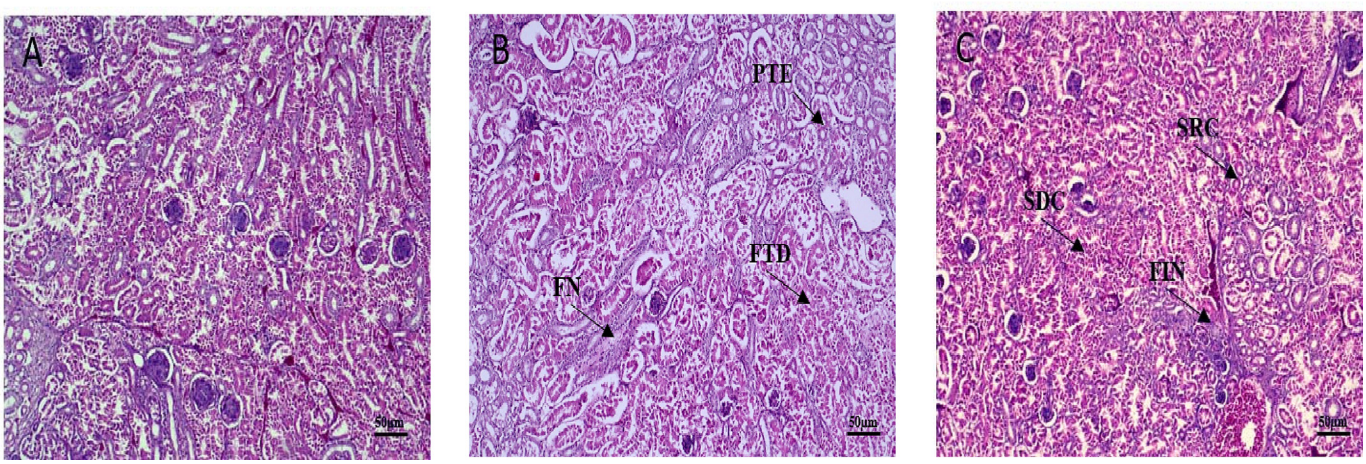

Fig. 6. Effect of coral fossil on kidney in cadmium exposed broiler. Photomicrograph of kidney in broiler of control (A); cadmium @75 mg/kg BW (B); cadmium @ 75 mg/kg BW + Coral Fossil 1 gm/kg BW (C) at 100x magnification. PTE- Proliferation of Tubular Epithelium, FTD- Focal Tubular Degeneration, FN- Focal Necrosis, SRC- Slight Regenerative Changes, SDC- Slight Degenerative changes and FIN- Focal Interstitial Necrosis.

calcium-rich toxin binder, can protect lambs against heavy metal toxicity by reducing heavy metal absorption and elimination from the gastro-intestinal tract (GIT). Coral fossils may be associated with these enzymes in order to eliminate heavy metals since they contain a lot of calcium.

According to earlier research, renal disorders are linked to exposure to heavy metals (Alissa et al., 2011). Nephrotoxic heavy metals have also been linked to the development of renal illnesses or renal injury (Navas-Acien et al., 2009; Wang et al., 2011). The involvement of hydrogen-rich coral calcium in oxygen transport that raises eGFR, a measure of renal function in individuals with autoimmune illness (Shen et al., 2022). In order to restore the functional state of the kidneys in both healthy and damaged tubules of the nephron, water treated with the coral-mine mixture may be advised as a therapy for recovery (Zaliavska et al., 2021). The kidney function is enhanced by coral fossils because they are a potential source of calcium that is hydrogen-rich.

The findings of the current study, feeding coral fossil with Cd lowers Cd absorption in broiler muscle, liver, and kidney. Organ affinity for metals is extremely specific, varying with metal chemical properties and organ physiological parameters (Storelli et al., 2006). According to Iwegbue et al. (2008), heavy metals are also present in meat naturally and their accumulation can influence the quality of the meat. According to Klaassen et al. (2009), the primary mechanism by which cadmium binds to sulfhydryl groups in the kidney's metallothionein protein and causes the highest concentration of cadmium in kidney samples is long-term exposure. In addition to binding heavy metals in the stomach, calcium can also compete with metals for absorption, modify intestinal cell activity, and change the affinity of heavy metals in target tissues (Moussa et al., 2001).

Cd treated broilers showed focal degenerative changes in the brain, hyperplastic bile duct and proliferation of tubular epithelium, focal tubular degeneration with necrosis in kidney. Coral fossil supplementation recovered all cadmium-induced histological changes. Renal histopathology explains edema, degeneration, necrosis, and apoptic changes in tubular epithelium after cadmium treatment (Hesaraki et al., 2010). Heavy metals produce perineuronal and perivascular edema with brain malacia, as well as hyperplastic nodules with intranuclear inclusion bodies within hepatocytes that ameliorate with calcium (Al-Naimi et al., 2011). Because coral fossil is a potential supply of calcium, it may interact with Cd absorption, promote urine excretions of Cd, and reduce the detrimental effects of Cd. So, coral fossil may be used as a potential therapy to reduce cadmium toxicities in broilers. There was a limitation of previous data in broilers regarding to coral fossil that's why we didn't find the actual mechanism of action. To ascertain the precise mechanism, further investigation is required.

5. Conclusion

Coral fossil supplementation in the water greatly reduce the harmful effects of Cd on hematobiochemical parameters and internal organs of broiler. As a result, coral fossil supplementation may be an effective strategy to combat Cd induced detrimental effects. However, more investigation is required to pinpoint the precise mechanism through which coral fossils mitigate the negative effects in broilers exposed to Cd.

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CRediT authorship contribution statement

Afrina Mustari: Conceptualization, Funding acquisition, Writing – original draft. **Mahabub Alam:** Data curation, Formal analysis, Investigation, Writing – original draft. **Murshida Khatun:** Data curation, Writing – original draft. **Md. Rockybul Alam:** Data curation, Writing – original draft. **Mohammad Alam Miah:** Validation. **Emdadul Haque Chowdhury:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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