



Evaluating the Efficacy of Black Cumin (*Nigella sativa*) as a Natural Remedy for Heavy Metal (Chromium) Toxicity in Broiler Chickens

Md. Rayhan¹, Ashrifa Akter Mukta¹, Md Shakil Islam¹, Md Eliusur Rahman Bhuiyan², Al Wasef³ and Mst Sharifa Jahan^{1*}

¹Department of Pharmacology and Toxicology, Sher-e-Bangla Agricultural University, Bangladesh

²Department of Physiology, Bangladesh Agricultural University, Bangladesh

³Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Bangladesh

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*Corresponding author: Dr. Mst. Sharifa Jahan, Department of Pharmacology and Toxicology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

Abstract

The poultry industry plays a significant role in the economy of Bangladesh and plays a great role in not only food security but also creating opportunities in employment. But most importantly, heavy metal contamination, with a very highlighted one as chromium (Cr) toxicity mainly due to industrial pollution sources, poses a very serious risk to sustainable poultry husbandry. This research work was carried out with the aim of assessing the possible ameliorative effects of black cumin (*Nigella sativa*) against Cr-induced toxicity in broiler chickens in terms of performance parameters such as body weight gain and liberation of hematological parameters, biochemical markers, and histopathological changes. The result demonstrates a decline in body weight of Cr treated broiler chickens, which indicates metabolic disturbances. On the contrary, it was found that supplementation with black cumin along with Cr markedly improved body weight. The hematological profile, however, showed marked reduction in total erythrocyte count (TEC), hemoglobin (Hb), and packed-cell-volume (PCV) in Cr-treated chickens, indicating anemia.

The ESR values decreased significantly ($p < 0.05$) with black cumin along with Cr treated group. The levels of liver enzyme activities, including ALT increased significantly ($p < 0.001$) in Cr-treated chickens which indicates that hepatocellular injury was caused. The black cumin with Cr treated groups significantly ($p < 0.05$) reduced such levels of ALT to nearly normal levels. Renal function markers, such as serum urea and creatinine, were increased in Cr-treated chickens, but the levels of creatinine decreased in the black cumin with Cr treated groups. Histopathological analysis shows cytoplasmic loss and micro-vacuolation in hepatocytes of Cr-treated chickens that signal oxidative stress. Health benefits of the black cumin supplement included the protection of liver and kidney architecture in these chickens indicating the hepato-protective and nephron-protective properties of the black cumin. Therefore, the current findings demonstrate the efficacy of black cumin in natural antioxidants to defend broiler chickens from heavy metal (Cr) injury and recommend black cumin in sustainable farming practices.

Keywords: Phytochemicals; Hemato-biochemical analysis; Heavy metal; Remedy; Broiler

Abbreviations: TEC: Total Erythrocyte Count; HB: Hemoglobin, PCV: Packed-Cell-Volume; ESR: Erythrocyte Sedimentation Rate; ALT: Alanine Transaminase

Introduction

Poultry farming has become a cornerstone of the agricultural economy in Bangladesh, contributing significantly to food safety, food security and rural livelihoods. As the population continues to grow and the demand for animal-derived protein rises, the poultry sector has witnessed remarkable growth, especially in

the last few decades. As an important sub-sector of livestock production, the poultry industry in Bangladesh plays a crucial role in economic growth and simultaneously creates numerous employment opportunities. The poultry industry, as a fundamental part of animal production, is committed to supplying the nation with a cheap source of good quality nutritious animal protein in

terms of meat and eggs Das et al. [1]. According to the Food and Agriculture Organization, poultry production is among the most dynamic agricultural subsectors globally, and in Bangladesh, its importance is further amplified by the adaptability of local and commercial breeds to varied climatic conditions and traditional farming systems Rahman et al. [2].

In addition to providing meat and eggs, poultry farming has become a vital instrument for poverty alleviation and socio-economic integration, particularly among rural women and marginalized populations Akinola & Essien [3]. Nutritionally, it offers high-quality protein and essential micronutrients, such as B-complex vitamins and phosphorus, while maintaining a relatively low-fat content, making it a favored option among health-conscious consumers Bordoni & Danesi [4]. However, the expansion of the poultry industry has not been without challenges. Among the most pressing concerns is the contamination of poultry feed and water sources with heavy metals such as chromium (Cr), particularly in regions affected by industrial activities like leather tanning, electroplating, and stainless-steel manufacturing Shanker et al. [5]. Chromium contamination-especially in its hexavalent form (Cr^{6+})-poses significant risks to poultry health. Cr^{6+} is known to penetrate biological membranes and induce oxidative stress, leading to lipid peroxidation, DNA damage, and protein oxidation Bagchi et al. [6]; Gutteridge & Halliwell [7]. In broiler chickens, chromium exposure has been linked to reduced growth performance, impaired immunity, and overall poor productivity Gabol et al. [8], which not only affects animal welfare but also reduces the economic value of poultry products.

In response to such environmental challenges, conventional strategies have often relied on chemical additives and synthetic chelators to combat heavy metal toxicity. However, these methods are not always sustainable and may have unintended ecological and physiological consequences Veeraraghavan [9]. This has spurred interest in natural and eco-friendly alternatives. One such promising candidate is *Nigella sativa*, commonly known as black cumin. Rich in bioactive compounds-most notably thymoquinone-black cumin exhibits potent antioxidant, anti-inflammatory, and hepatoprotective effects Ahmad et al. [10]; Ali & Blunden [11].

Numerous animal studies have demonstrated black cumin's potential in mitigating oxidative stress and improving liver function. For instance, *Nigella sativa* has been shown to enhance antioxidant enzyme activity in rats suffering from carbon tetrachloride-induced hepatotoxicity El-Dakhakhny et al. [12], and to alleviate liver damage and oxidative stress in broilers exposed to lead Attia et al. [13]. Moreover, in poultry, black cumin supplementation has been associated with improved growth performance and immune function under various stress conditions Kumar & Patra [14]; Shewita & Taha [15]. While direct studies on black cumin's efficacy in counteracting chromium toxicity in broilers remain limited, existing evidence supports its potential as a natural protective agent in poultry exposed to environmental toxins

Seidavi et al. [16].

This study therefore aims to fill a crucial gap by investigating the protective role of black cumin in broiler chickens exposed to dietary chromium. It specifically seeks to evaluate the efficacy of black cumin in improving growth performance, restoring liver function, reducing oxidative stress, and ameliorating histopathological alterations induced by chromium exposure. Through this integrative approach, the research aspires to offer insights into natural, sustainable management strategies for enhancing poultry resilience in pollution-impacted regions, thereby contributing to safer food production and long-term agricultural sustainability.

Methodology

This experiment was conducted at the Sher-e-Bangla Agricultural University Poultry Farm, Dhaka, over a 28-day period (February 26 to March 24, 2024) using 80-day-old commercial broiler chicks to investigate the protective effects of black cumin (*Nigella sativa*) against chromium ($\text{K}_2\text{Cr}_2\text{O}_7$)-induced toxicity. After a 7-day brooding phase under standard management, chicks were randomly assigned to four treatment groups: control (C_1 & C_2), black cumin-treated (T_1), chromium-treated (T_2), and black cumin + chromium-treated (T_3), each with two replicates of 10 birds, except the controls which had single replicates of 10 birds each. Black cumin oil (5%) was mixed into the feed for T_1 and T_3 groups, while chromium was administered in drinking water at 0.037mg/kg for T_2 and T_3 . All birds were housed in disinfected pens at a stocking density of 10 birds/ m^2 , fed commercial starter and grower feeds (Kazi Feed), and given water ad libitum. Routine broiler management practices-including brooding, lighting (24h for first 2 weeks), ventilation, biosecurity, and vaccination (against ND, IB, and Gumboro)-were followed throughout. Body weights and feed consumption were recorded weekly. At day 28, blood samples were collected from the wing vein. Hematological parameters (Hb, PCV, ESR, TEC) were analyzed using standard protocols Nwaigwe et al. [17], and biochemical markers (ALT, AST, urea, creatinine) were evaluated from serum using an automated biochemistry analyzer. Liver and kidney tissues were preserved in 10% buffered formalin, sectioned at 4–6 μm , and stained with hematoxylin and eosin (H&E) for histopathological assessment Kumar & Balachandran [18]. All data were analyzed using GraphPad Prism v10.4.0. One-way ANOVA followed by Tukey's multiple comparison test was applied, with $p < 0.05$ considered statistically significant.

Results

Effects on body weight

The highest body weight was observed in the control group (2.0 ± 0.05 kg) and the lowest in the Cr treated group (1.648 ± 0.038 kg). Body weight in the black cumin with Cr-treated group were significantly increased ($p < 0.05$) than Cr-treated group indicating the beneficial effect of black cumin against Cr toxicity. Also, body weight in the Cr-treated group was significantly lower ($p < 0.05$) than both the control and black cumin-treated groups (Figure 1).

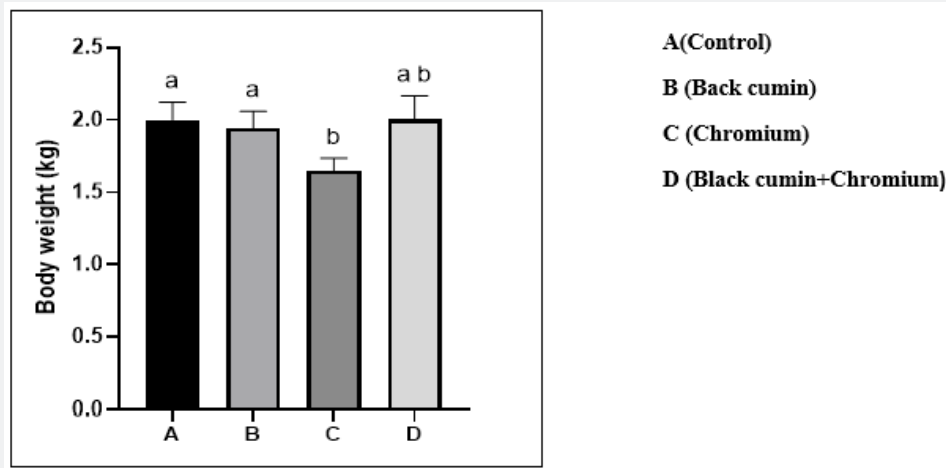


Figure 1: Body weight of broiler chicken after treatment with chromium and black cumin. Values are represented as Mean \pm SE. Values with different superscript letters above bar differ significantly ($p < 0.01$).

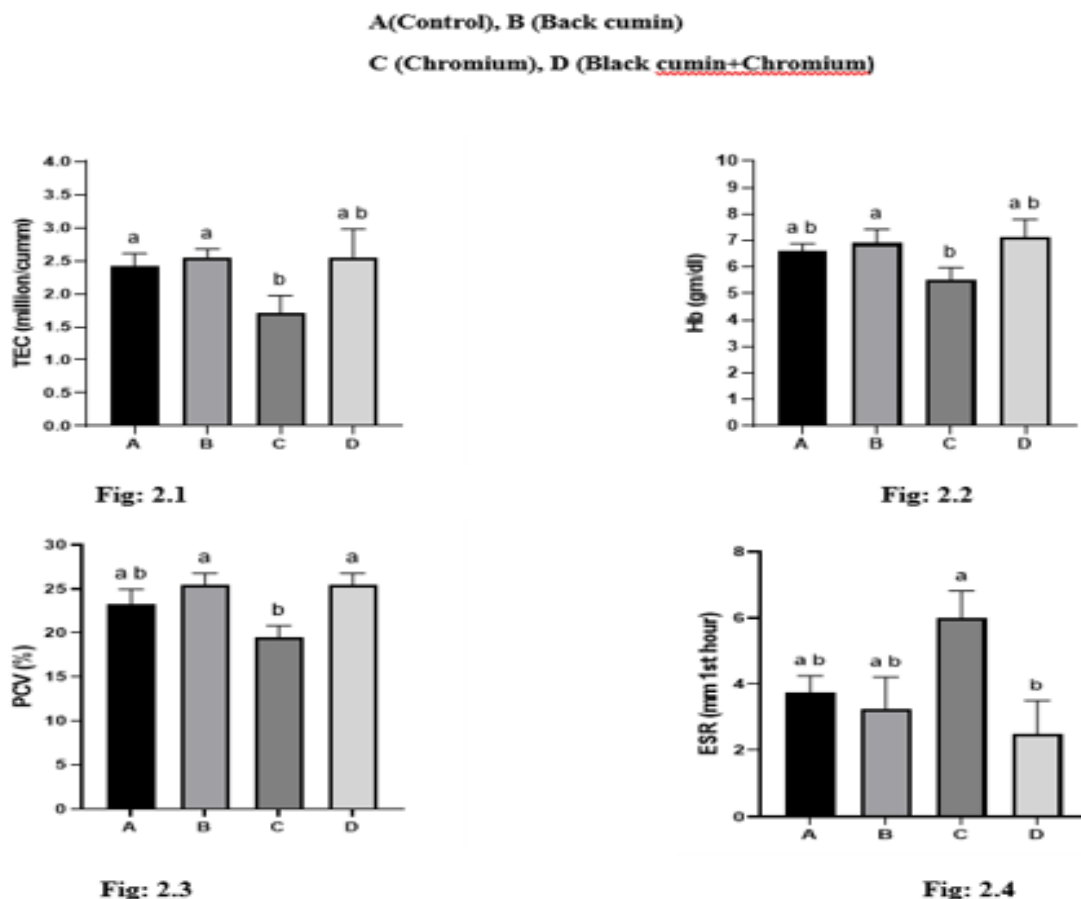


Figure 2.1-2.4: Hematological parameters of broiler chicken (2.1) Total Erythrocyte Count (2.2) Hemoglobin (2.3) Packed Cell Volume (2.4) Erythrocyte Sedimentation Rate after treatment with chromium and black cumin. Values are represented as Mean \pm SE. Values with different superscript letters above bar differ significantly ($p < 0.05$).

Effect on hematological parameters

Calculation of hematological parameters can be used to determine the extent of lethal effect of Cr on blood components of an animal. The values of Total Erythrocyte Counts (TEC million/ mm^3), Hemoglobin Concentration (Hb, gm/dl), Packed Cell Volume (PCV%) and Erythrocyte Sedimentation Rate (ESR, mm in first hour) of all treated broiler were presented in (Figure 2.1 – 2.4). The total number of TEC, PCV and Hb were suggestively ($p < 0.05$) declined in all cases in Cr treated broiler (1.71 ± 0.13 , 19.5 ± 0.64 and 5.5 ± 0.24 respectively) compared to the control (6.6 ± 0.14 , 23.25 ± 0.85 and 2.41 ± 0.01 respectively). Conversely, ESR values was significantly increased ($p < 0.01$) in Cr treated broiler (6 ± 0.40 mm in first hour) compared to black cumin with Cr treated group (2.5 ± 0.5). The values of TEC and PCV in black cumin treated groups (2.55 ± 0.06 & 25.5 ± 0.64 respectively) were significantly ($p < 0.01$) increased than Cr treated group. Moreover, TEC & PCV value in Cr with black cumin treated group also significantly increased than Cr treated also and the level were more or less similar to control.

Effects on liver function

Assessing enzyme activities in the liver plays a crucial role in examining and diagnosing liver damage. Levels of AST, ALT bio-marker enzymes in the liver are commonly utilized as biochemical parameters for evaluation (Figure 3.1-3.2). In our investigation, serum levels of ALT exhibited significant ($p < 0.001$) elevation ($16.18 \pm 0.72 \text{ U/L}$) following chromium induction compared to the control group ($9.12 \pm 1.80 \text{ U/L}$), indicating aberrations in liver cell function Figure 4. Histology of liver section showed also loss of cytoplasm in hepatocytes (white arrowhead) (Figure 5). The serum ALT concentration in the black cumin treated group ($8.04 \pm 0.92 \text{ U/L}$) was significantly decreased ($p < 0.01$) than Cr treated group ($16.18 \pm 0.7 \text{ U/L}$) and also the ALT level in black cumin with Cr treated group ($10.73 \pm 0.52 \text{ U/L}$) was significantly decreased ($p < 0.05$) than Cr alone treated group ($16.18 \pm 0.7 \text{ U/L}$). However, AST serum concentration was insignificantly lower ($237.23 \pm 16.94 \text{ U/L}$) in group black cumin with Cr treated group compared to control ($268.02 \pm 10.32 \text{ U/L}$) and also insignificantly lower than Cr treated group ($280.54 \pm 4.60 \text{ U/L}$).

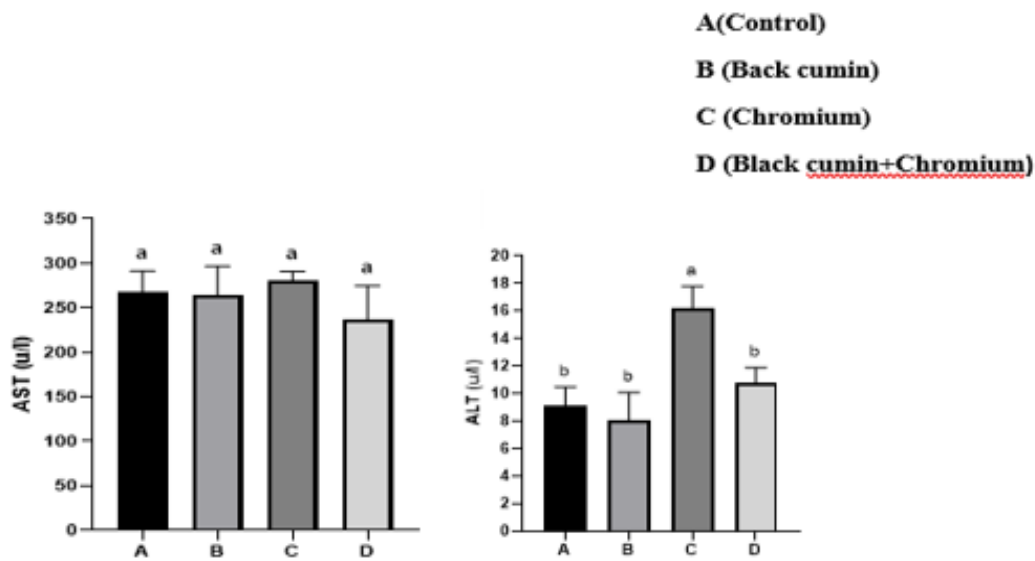


Fig: 3.1

Fig: 3.2

Figure 3.1-3.2: Liver function test of broiler chickens after treatment with chromium and black cumin. Values are represented as Mean \pm SE. Values with different superscript letters above bar differ significantly ($p < 0.05$).

Effects on kidney function

The value of urea in black cumin with Cr treated group was insignificantly lower after black cumin treatment ($20.06 \pm 1.07 \text{ mg/dl}$) than Cr treated group ($21.09 \pm 0.63 \text{ mg/dl}$) group. Serum creatinine levels were insignificantly higher ($0.21 \pm 0.01 \text{ mg/dl}$) in Cr treated group compared to control ($0.18 \pm 0.01 \text{ mg/dl}$). On the

other hand, creatinine level was similar in black cumin treatment group ($0.17 \pm 0.01 \text{ mg/dl}$) and treatment with black cumin with Cr group ($0.17 \pm 0.01 \text{ mg/dl}$) indicates the amelioration of black cumin seed oil in kidney tissue. Histopathological analysis also showed that no abnormal histological changes in black cumin with Cr treated group in kidney tissues (Figure 6).

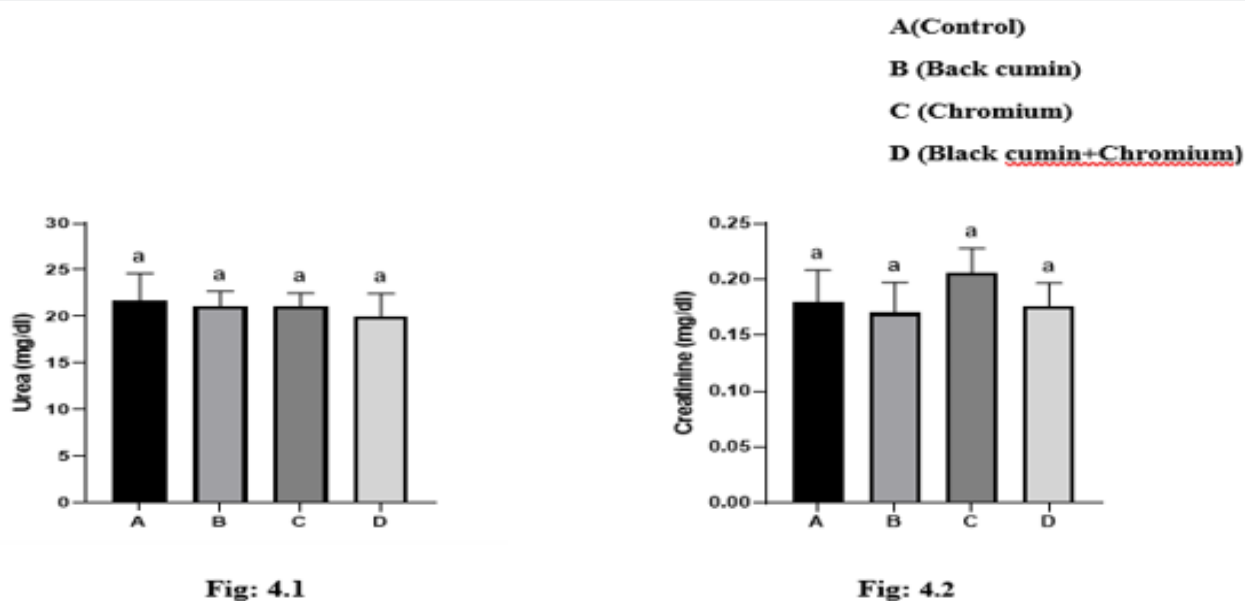


Figure 4.1-4.2: Kidney function test of broiler chickens after treatment with chromium and black cumin. Values are represented as Mean \pm SE. Values with different superscript letters above bar differ significantly ($p < 0.05$).

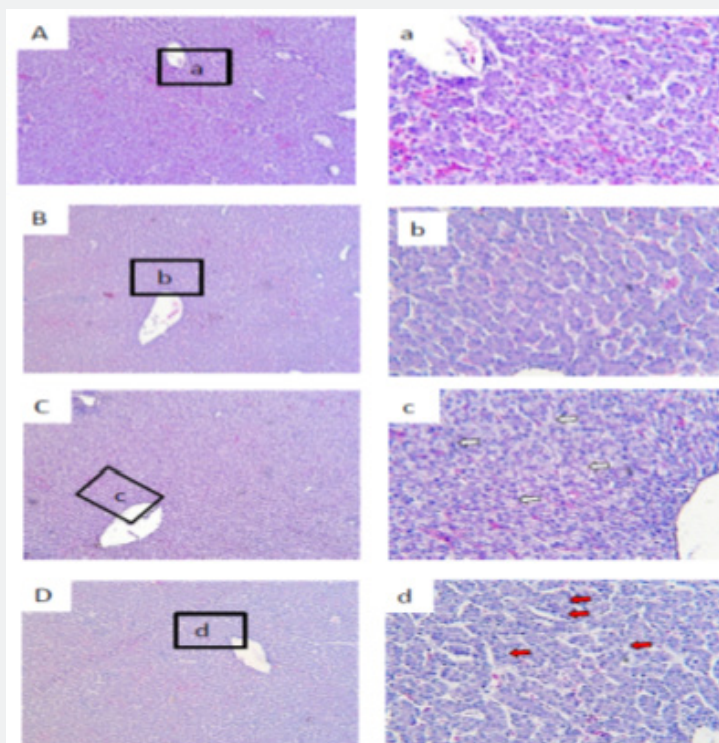


Figure 5: Histological changes of liver. H & E staining (4x & 40x objectives). A) Liver of control group had normal structure B) Liver of black cumin treated group showed normal structure C) Liver of Cr treated showed loss of cytoplasm in hepatocytes (white arrowhead) D) Black cumin with Cr treated group's liver had micro-vacuoles in hepatocytes (red arrowhead).

Histological analysis of the liver

In the control group the liver tissue does not show any architectural changes, with hepatocytes having an intact cytoplasm and centrally located nuclei (Figure 5A). The black cumin treated group is similar to the control, no significant changes were observed in the liver of this group and normal histological characteristics were maintained (Figure 5B). In the Chromium treated group, there is an indication of cytoplasm loss in the hepatocytes, marked by the white arrowheads. Such changes indicate injury cell (Figure 5C). The black cumin with Cr treated group showed micro-vacuoles in hepatocytes symbolized by red arrowheads (Figure 5D).

Histological analysis of the kidney

The results of the histological analysis of kidney tissues were similar among the various groups. A control group section of kidney showed a normal glomerular and tubulointerstitial structure (Figure 6A). Just like a control group, no significant changes have been observed in black cumin treated group (Figure 6B). In Cr treated group the overlying glomeruli and tubulointerstitial structures did not show any pathological changes (Figure 6C). In black cumin with Cr treated group the kidney has normal architecture with no signs of damage (Figure 6D).

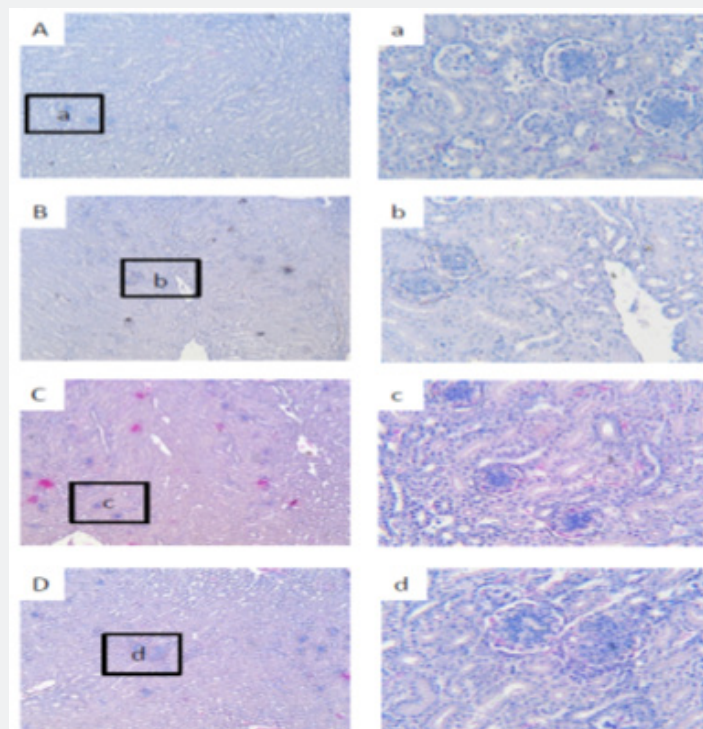


Figure 6: Histology of kidney. H & E staining (4x & 40x objectives). (A-D) Denotes kidney of control, black cumin treated, Cr treated and black cumin with Cr treated respectively. All groups had normal glomerulus and tubulointerstitial.

Discussion

Previous study suggested that decreased body weight is due to metabolic deregulations consequent to Cr toxic effects over the liver Ray [19]. Researcher have noted that loss of body weight might involve changes in liver glycogen and triglycerides as well as disturbances some metabolic enzymes that lead to weight loss Jabeen et al. [20]. Feed intake reduction might be caused by chromium inhibiting the appetite center and reducing food interest Asma et al. [21]. Irregularities in metabolism introduced by chromium-induced liver damage also contribute to body weight loss Saxena & Tripathi [22]. On the other hand, the addition of black

cumin in the diet increased the body weight due to black cumin as a feed additive stimulates the activity of the digestive system as well as enhanced the liver enzyme activity Ahmad et al. [10].

Previous research has documented reduced hematological parameters in rats following Cr treatment Ahmad et al. [10] and broilers Jeevana et al. [23]; Lee et al. [24] respectively. A decline in erythrocyte count, hemoglobin concentration, signifies the onset of anemia, likely stemming from diminished iron availability for hemoglobin synthesis due to exposure to heavy metals Ray [19]. Another contributing factor may involve Cr ability to penetrate the red blood cell membrane, forming DNA-protein crosslinks and

precipitating anemia. Additionally, chromium binding to hemoglobin chains could impede heme synthesis, exacerbating the anemic condition Ścibior et al. [25] Upon uptake by erythrocytes, chromium undergoes reduction to its trivalent form with the assistance of reduced glutathione Lopotych et al. [26]. The cytopathic effects of chromium involve disruption of erythrocyte energy production pathways, resulting in ATP production interference and mitochondrial enzyme dysfunction, ultimately leading to elevated erythrocyte sedimentation rate Mashkooor et al. [27]. On the other hand, black cumin seed has been reported to stimulates bone marrow and immune cell sand thus increased both the Packed Cell Volume, Hemoglobin Al-Ankari [28] as similar to our study.

Previous research noted elevated concentrations of ALT and AST in chromium- intoxicated rats. Elevated ALT and AST levels may signify hepatocyte damage due to heavy metal exposure, as these enzymes leak into circulation. Moreover, increased levels may result from Cr biotransformation in hepatocytes, leading to cellular injury Mashkooor et al. [29]. Heavy metals are recognized for generating reactive oxygen species (ROS) in the body Mansoor et al. [30]. Hepatocytes employ multiple defense mechanisms against ROS to prevent cellular damage and subsequent elevation of serum enzyme levels. In another study, Cr produced increase level of AST, ALT. Eman & Farag [31]. This shows agreement with studies of other researchers. Soudani et al. [32]. They explained this due to the liberation of these molecules from the cytoplasm signals liver tissue damage. Furthermore, alternative research links this phenomenon to oxidative stress and depletion of hepatic glutathione (GSH), a vital component of the body's antioxidant defenses Gunaratnam & Grant [33]. Yet another study clarified that the plasma concentration of ALT surpasses AST in the cytoplasm, resulting in elevation during inflammation or infection, in cases of infiltrative diseases affecting both cytoplasmic and mitochondrial membranes, AST levels exceed those of ALT Akila et al. [34].

In comparison with the normal control, black cumin treat-

ment was able to restore ALT to normal levels. The result therefore indicates the black cumin extract has shown significant potential in mitigating the toxicity caused by heavy metals (Cr) by their antioxidant as well as potential chelators activities. In another study, the levels of AST and ALT were notably elevated in rats treated with Cr, suggesting liver cell abnormalities Mera et al. [35]. However, broiler chickens therapeutically treated with black cumin exhibited a significant reduction in Cr toxicity effects, as evidenced by the gradual decrease in ALT and AST levels. Previous studies have demonstrated an increase creatinine levels in rats exposed to chromium toxicity Mashkooor et al. [29]. The decline in creatinine levels could be attributed to the generation of reactive oxygen species (ROS), which subsequently induce lipid peroxidation. These lipid droplets precipitate in the glomerular endothelium, impairing the glomerular filtration rate (GFR) and ultimately causing damage to membrane components and necrosis, resulting in elevated creatinine levels Mashkooor et al. [29].

The histology finding of the liver indicates different degrees of damage to the hepatic cells as a consequence of the treatments. While it was noted that no damage could be observed due to control group and black cumin treated caused evidence for cytoplasmic loss in hepatocytes which could be attributed to the oxidative stress or very mild inflammatory response (based on the finding of previous studies that were performed on similar chemicals Parola & Robino [36]. Cr treated group was responsible for inducing the generation of micro vacuoles, which are very much characteristic of steatosis, or stress induced in the cell. This was consistent with previous research associating these types of histological observations with defect-induced metabolism or with hepatotoxic agent exposures Ramachandran & Kakar [37]. Thus, it can be inferred that such treatments are not nephrotoxic by the experimental conditions employed. The observation was further supported by the well-preserved glomerular and tubulointerstitial structures, in agreement with findings in studies that assessed the effects of such low-dose or short duration of exposure Marini et al. [38].

Table 1: All groups were observed over the experimental period for changes in body weight, hematological indices, and selected biochemical markers. The dosage and duration were maintained uniformly across all treatment groups.

Group Name	Description
Control Group C1 and C2 (n=20)	Normal feed + drinking water
Black cumin Treated Group T1R1, and T1R2 (n=20)	Normal feed mixed with Black cumin seed oil (5%) + drinking water
Cr Treated Group T2R1 and T2R2 (n=20)	Normal feed + drinking water + $K_2Cr_2O_7$ (0.037mg/kg) mixed with drinking water.
Black cumin+ Cr Treated Group T3R1, and T3R2 (n=20)	Normal feed mixed with Black cumin seed oil (5%) + drinking water + $K_2Cr_2O_7$ (0.037mg/kg) mixed with drinking water.

Summary

Black cumin (*Nigella sativa*) is known for its antioxidant, anti-inflammatory, and hepatoprotective effects, making it a natural feed additive in terms of heavy meal toxicity mitigation. Poultry

serves a meaningful purpose for Bangladesh's economy-by and large, though the poultry industry faces radically environmental Contamination like hexavalent chromium (Cr) (V_1) through industrial waste from manufactured poultry feed. Exposure to Cr (VI)-based compounds leads to lifetime health repercussions for

both humans and animals. It has a bearing on physiological parameters, organ systems, and productivity overall in poultry. This study aimed to assess the toxic effects of Cr (VI) contamination in broiler chickens, focusing on growth performance, hematological and biochemical markers, as well as histopathological changes. Indications of anemia and systemic oxidative stress were seen as Chromium treated chickens showed decreased body weight and reduction in hematological indices such as TEC, Hb, and PCV with an increase of ESR levels.

Activities of liver enzymes (ALT), markers of kidney function (urea, creatinine), were significantly elevated ($p < 0.001$) as indications for causing damage. Supplements with black cumin alongside chromium showed impressive protective effects. It significantly brought to near-normal levels body weight, hematologic indices (TEC, Hb, and PCV; $p < 0.05$), and organ function markers. Significant evident decrease was observed in ESR values ($p < 0.01$) and it also showed a remarkable decline of its activities ($p < 0.05$) when compared with the Cr-treated group. Additionally, black cumin-treated groups exhibited creatinine levels similar to the control group, indicating its nephroprotective role. The results affirm black cumin's efficacy in mitigating Cr-induced toxicity through its bioactive compounds, which enhance antioxidant defense mechanisms and support organ recovery. These findings underscore the potential of black cumin as a sustainable, natural solution for improving poultry health and productivity in chromium-contaminated environments, this study investigated the therapeutic effect of black cumin on Chromium intoxicated broiler chickens, on growth performance, hematological parameters and histopathological changes of liver and kidney.

Conclusion

The findings of our study indicate that chromium (Cr) is detrimental to certain physiological parameters of broilers including body weight, hematological parameters, and liver and kidney function markers. The marked reduction in erythrocyte parameters with an increased erythrocyte sedimentation rate indicates the development of anemia and intoxication in the experimental broiler. The severe decrease in ALT liver enzymes further substantiates the adverse effect of Cr exposure on these vital organs. Supplementation of black cumin with Cr caused significant protection restoring body weight, hematological indices such as TEC, PCV, and ESR. Compared to Cr-alone treatment, the effects of black cumin therapy were significantly reduced ($p < 0.05$) in liver enzyme activities (ALT), showing improvement of liver function. The creatinine levels in black cumin treated and Cr with black cumin treated group were similar to that of the control group and demonstrated that black cumin offered some degree of nephroprotective. The results confirm that black cumin has efficacy in alleviating Cr induced toxicity by its bioactive components acting potentially in the function enhancement of hematopoietic organs, enhancing antioxidant defense mechanisms and recovery support of the affected organs. Findings support that black cumin has a

future in the sustainable natural prophylaxis for health welfare to humans and animal households.

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