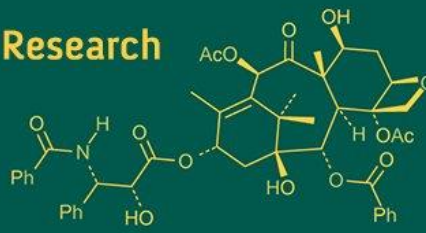
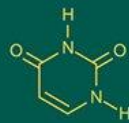
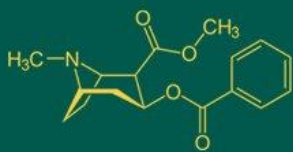


## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(7): 470-476

[www.biochemjournal.com](http://www.biochemjournal.com)

Received: 20-05-2026

Accepted: 23-06-2026

#### Nisarga MS

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Girish BC

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Shilpa VT

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Anjan Kumar KR

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Ravikaumar C, Hemanth Gowda K

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Pramod SN

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Akshatha P

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Borra Sruthi

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

#### Corresponding Author:

##### Nisarga MS

Department of Veterinary Pathology,  
Veterinary College, Hassan,  
Karnataka Veterinary, Animal and  
Fisheries Sciences University  
(KVAFSU), Bidar, Karnataka, India

## Protective effect of biorefined microalgal *spirulina* extract on haematological, biochemical and oxidative alterations in cadmium induced toxicity in Wistar rats

Nisarga MS, Girish BC, Shilpa VT, Anjan Kumar KR, Ravikaumar C, Hemanth Gowda K, Pramod SN, Akshatha P and Borra Sruthi

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7g.9060>

### Abstract

Cadmium (Cd) is a widespread environmental pollutant and a highly toxic heavy metal with a long biological half-life, causing bioaccumulation and oxidative damage in multiple organs, including the male reproductive system. Owing to its potent antioxidant and nutraceutical properties, *Spirulina* has gained considerable attention as a natural therapeutic agent against heavy metal-induced toxicity. The present study was undertaken to evaluate the ameliorative effects of biorefined microalgal *Spirulina* extract (BMSE) against cadmium-induced toxicity in Wistar rats. Forty-two male Wistar rats were randomly divided into seven groups (n=6 each): normal control, cadmium control (5 mg/kg bw), atenolol-treated (1.5 mg/kg bw), vitamin E (200 mg/kg bw) plus selenium (400 µg/kg bw)-treated, and BMSE-treated groups receiving 50, 100, and 200 mg/kg bw, respectively, along with cadmium chloride for 28 days. Cadmium exposure resulted in reduced body weight gain, altered hematological indices (Hb, TEC, TLC, and PCV), elevated serum biochemical markers indicative of hepatic and renal dysfunction, increased lipid peroxidation, and diminished antioxidant enzyme activities (SOD and CAT). BMSE supplementation significantly and dose-dependently ameliorated these cadmium-induced alterations by improving body weight, restoring hematological and biochemical parameters, reducing oxidative stress, and enhancing antioxidant defense. The highest dose of BMSE (200 mg/kg bw) exhibited the greatest protective effect. The findings suggest that BMSE possesses significant protective activity against cadmium-induced systemic toxicity, primarily through its antioxidant potential, and may serve as a promising natural therapeutic agent for mitigating heavy metal-induced oxidative damage.

**Keywords:** Cadmium, spirulina, oxidative stress, hematology and antioxidant activity

### Introduction

Cadmium (Cd) is a non-essential, highly toxic heavy metal widely distributed in the environment as a consequence of both natural processes and anthropogenic activities. Owing to its persistence, non-biodegradable nature, and exceptionally long biological half-life (20-30 years), Cd undergoes bioaccumulation and biomagnification through the food chain, posing a significant threat to human and animal health (Tchounwou *et al.*, 2012; Djukic-Cosic *et al.*, 2008). Environmental contamination arises primarily from mining, metal refining, industrial effluents, battery manufacturing, phosphate fertilizers, and fossil fuel combustion, while dietary intake, contaminated drinking water, cigarette smoke, and occupational exposure constitute the major routes of human exposure (Wilson, 2001; Charkiewicz *et al.*, 2023). Although naturally present in the Earth's crust at low concentrations (approximately 0.1 ppm), Cd is highly toxic even at trace levels because of its tendency to accumulate in vital organs such as the liver, kidneys, lungs, bones, brain, blood, and reproductive tissues (Jayamurali *et al.*, 2021). Consequently, Cd has been recognized as a global environmental toxicant associated with nephrotoxicity, hepatotoxicity, carcinogenicity, teratogenicity, endocrine disruption, and reproductive dysfunction (Nuli *et al.*, 2024).

The toxicological effects of cadmium are primarily mediated through oxidative stress. Cadmium indirectly promotes excessive generation of reactive oxygen species (ROS), impairs mitochondrial function, enhances lipid peroxidation, depletes endogenous antioxidant defenses, and causes oxidative damage to proteins, lipids, and DNA, ultimately leading to cellular dysfunction and apoptosis (Kolachi *et al.*, 2011) [37, 6, 38, 5, 16, 23, 19].

Because Cd lacks any known physiological role and affects multiple organ systems simultaneously, prolonged exposure results in systemic toxicity involving the liver, kidneys, cardiovascular system, nervous system, immune system, and reproductive organs. Among these, the male reproductive system is particularly susceptible, with Cd exposure impairing spermatogenesis, disrupting the blood-testis barrier, altering testicular architecture, reducing semen quality, inducing germ cell apoptosis, and ultimately compromising fertility (Ramachandran, 2014). These widespread toxic effects highlight the need for safe and effective therapeutic interventions capable of mitigating Cd-induced oxidative injury.

Natural products have long served as valuable sources of therapeutic agents owing to their abundance of bioactive phytochemicals and antioxidant compounds. Plant- and algae-derived antioxidants have attracted considerable interest because of their ability to neutralize free radicals, enhance endogenous antioxidant defense systems, and protect tissues against oxidative damage, making them promising alternatives or adjuncts to conventional therapies (Kumar *et al.*, 2023). Among these natural products, *Spirulina* (*Arthrospira* spp.) is a filamentous blue-green cyanobacterium widely cultivated in alkaline waters of tropical and subtropical regions. It is recognized as a nutrient-dense functional food rich in high-quality proteins, essential amino acids, vitamins, minerals, carotenoids, chlorophyll,  $\gamma$ -linolenic acid, and phycobiliproteins, particularly phycocyanin, which contribute to its potent antioxidant, anti-inflammatory, immunomodulatory, antiviral, and anticarcinogenic activities (Abdulqader *et al.*, 2000; Sanchez *et al.*, 2007; Amin *et al.*, 2006). These biological properties suggest that *Spirulina* may provide protection against oxidative stress-mediated toxicities induced by heavy metals. Despite increasing evidence supporting the antioxidant potential of *Spirulina*, studies investigating the efficacy of biorefined microalgal *Spirulina* extract (BMSE) against cadmium-induced systemic toxicity remain limited. Therefore, the present study was undertaken to evaluate the protective efficacy of BMSE in a rat model of sub-acute cadmium toxicity, with particular emphasis on body weight, hematological alterations, biochemical changes and oxidative stress parameters in target organs. The study further aimed to compare the protective effects of different doses of BMSE with standard therapeutic interventions, thereby assessing its potential as a natural therapeutic agent for mitigating cadmium-induced oxidative damage [30, 21, 1, 34, 3].

## Materials & methods

### Experimental Animals

The research was carried out on forty-two healthy adult male Wistar albino rats. Adult male Wistar rats, each weighing between 150 and 180 g, were obtained from Suraksheeth Life Sciences Private Limited, Gandasi, Hassan, a CPCSEA/CCSEA-approved laboratory animal breeding facility. All the rats were acclimatized to standard laboratory conditions at the Small Animal House Facility, Veterinary College, Hassan, for seven days prior to the initiation of the experiment and maintained under controlled environmental conditions with a housing temperature of  $22 \pm 3^\circ\text{C}$ , relative humidity of 50-70%, and a 12-hour light/dark cycle throughout the study period. The animals were provided with a standard pellet diet and free access to de-ionized drinking water ad libitum throughout the experimental period. All

experimental procedures were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee (IAEC), Veterinary College, Hassan (Approval No. HVC/IAEC/08/2024).

### Preparation of test items and mode of administration

Cadmium chloride ( $\text{CdCl}_2$ ) was used to induce toxicity and was administered orally at a dose of 5 mg/kg body weight daily for 28 days. Atenolol was used as Reference Control 1 (RC-1) and administered orally at a dose of 1.5 mg/kg body weight daily for 28 days. Vitamin E and selenium were used as Reference Control 2 (RC-2) and administered orally at doses of 200 mg/kg and 400  $\mu\text{g/kg}$  body weight, respectively, daily for 28 days. *Spirulina* extract was used as the test item and administered orally at dose levels of 50, 100, and 200 mg/kg body weight daily for 28 days and phosphate buffer saline was used as vehicle.

### Experimental protocol

This study consisted of seven groups (Group I to VII), comprising six rats in each group. Group I (normal control) rats received phosphate buffer saline orally daily for 28 days. Group II (cadmium control) rats received cadmium chloride ( $\text{CdCl}_2$ ) @ 5 mg/kg body weight orally daily for 28 days. In addition to cadmium chloride, Group III (Reference Control 1) rats received atenolol @ 1.5 mg/kg body weight orally daily for 28 days, while Group IV (Reference Control 2) rats received vitamin E @ 200 mg/kg body weight and selenium @ 400  $\mu\text{g/kg}$  body weight orally daily for 28 days. Groups V, VI, and VII received Biorefined microalgal *Spirulina* extract @ 50, 100, and 200 mg/kg body weight, respectively, along with cadmium chloride @ 5 mg/kg body weight orally daily for 28 days.

### Parameters assessed

Body weight (g) were assessed every week. All animals were examined twice daily for any signs of clinical abnormalities or symptoms. At the end of the study, all rats were ethically euthanized by administering an intramuscular overdose of Ketamine and Xylazine. Blood samples were drawn from the retro-orbital plexus and collected into EDTA vials for hematological analysis and serum vials for biochemical evaluation.

### Estimation of antioxidant enzymes

The representative tissue samples were dissected and washed with Normal saline to remove any tissue debris and blood clots. The collected samples were homogenized in a solution of ice-cold 0.1M Phosphate buffered saline (pH 7.4) at  $4^\circ\text{C}$  and centrifuged for 10 minutes at 15,000 rpm. The supernatants were stored at  $-80^\circ\text{C}$  for analysis of superoxide dismutase, catalase, and lipid peroxidation.

### Statistical analysis

Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Post-hoc test (Tukey's test) (Snedecor and Cochran, 1994) using the software SPSS, version-16.0.

## Results

**General observation:** Group I rats remained healthy and active throughout the experiment. Group II rats exhibited

clinical signs such as reduced feed intake, reduced body weight, ruffled hairs, dehydration, restlessness and were difficult to handle. The rats of Group III to VII manifested similar clinical signs as that of disease control rats, but with reduced intensity and frequency.

### Body Weight

The mean body weights (g)  $\pm$  standard error of the mean (SEM) at day 0 and at the end of the 1st, 2nd, 3rd, and 4th weeks of the experiment are presented in Table 1. Rats in Group I (normal control) remained healthy and active throughout the experimental period and exhibited a steady and progressive increase in body weight. In contrast, Group II rats showed a significant ( $p < 0.05$ ) reduction in body weight from the 2nd to the 4th week compared with Group I. Rats in Groups III and IV demonstrated a improvement in body weight, with a consistent increase observed from the 2nd to the 4th week compared with Group II. Similarly, Spirulina-treated rats (Groups V-VII) exhibited a significant ( $p < 0.05$ ) and sustained increase in body weight throughout the experimental period relative to Group II. Among the treatment groups, Group VII showed the greatest improvement, with body weight values comparable to those of the normal control (Group I) by the end of the study.

### Hematology parameters

The mean values of the hematological parameters with standard error of mean on the 28th day of the experiment are presented in Table 2. The mean values of all hematological parameters, namely Total Erythrocyte Count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV), Total Leucocyte Count (TLC), and Mean Corpuscular Haemoglobin Concentration (MCHC), of Group II rats were significantly ( $p < 0.05$ ) decreased when compared to Group I rats, whereas Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were significantly ( $p < 0.05$ ) increased in Group II rats compared to Group I. Group III rats showed significantly ( $p < 0.05$ ) lower Hb, TEC, TLC, MCHC, and higher MCV and MCH values compared to Group I, with values remaining comparable to Group II for PCV, MCV, MCH, and MCHC. Group IV rats exhibited significant ( $p < 0.05$ ) improvement in Hb, TEC, TLC, PCV, MCV, and MCHC compared to Group II, with values comparable to those of Group I, while MCH showed a non-significant reduction compared to Group II. The treatment groups (Groups V-VII) demonstrated significant ( $p < 0.05$ ) improvement in TEC, TLC, PCV, and MCV compared to Group II. Hb values were significantly ( $p < 0.05$ ) increased in Groups VI and VII, whereas Group V showed a non-significant increase. MCHC and MCH values were significantly ( $p < 0.05$ ) improved only in Group VII, with values comparable to those of Group I. Overall, Group VII showed the greatest restoration of hematological parameters, followed by Groups VI, V, and IV, with values approaching those of the normal control group.

### Serum Biochemistry parameters

The mean values with standard error of mean on 28th day of the experiment is presented in Table 3. The mean values of the serum biochemical parameters with standard error of mean on the 28th day of the experiment are presented in Table 3. The mean values of Serum Total Protein (TP) of Group II

rats were significantly ( $p < 0.05$ ) decreased, whereas Serum Alkaline Phosphatase (ALP), Alanine Transaminase (ALT), Serum Creatinine, and Blood Urea Nitrogen (BUN) were significantly ( $p < 0.05$ ) increased when compared to Group I rats. Group III rats showed significantly ( $p < 0.05$ ) lower Total Protein and higher ALP, ALT, Creatinine, and BUN values compared to Group I rats, with values remaining comparable to those of Group II rats. Group IV rats exhibited significant ( $p < 0.05$ ) improvement in all biochemical parameters compared to Group II rats, with ALP, Total Protein, and Creatinine values becoming comparable to those of Group I rats. The treatment groups (Groups V-VII) showed significant ( $p < 0.05$ ) improvement in all serum biochemical parameters compared to Group II rats, with decreased ALP, ALT, Creatinine, and BUN levels and increased Total Protein levels. Group VII exhibited the greatest improvement, with most parameters approaching or becoming comparable to those of the normal control group.

### Lipid peroxidation assay as an oxidative stress marker

The mean values of testicular lipid peroxidation (LPO) levels expressed as nmol malondialdehyde (MDA)/mg protein, with standard error of mean on the 28th day of the experiment, are presented in Table 4. There was a significant ( $p < 0.05$ ) increase in testicular LPO levels in Group II rats compared to Group I rats. Group III and Group IV rats showed a significant ( $p < 0.05$ ) reduction in LPO levels compared to Group II rats. Similarly, all treatment groups (Groups V-VII) exhibited a significant ( $p < 0.05$ ) decrease in LPO levels in comparison to Group II rats. Among the treatment groups, Group VII showed the greatest reduction in LPO levels, with values comparable to those of Group I and Group IV rats.

### Assessment of Oxidative Stress

Portions of testis were stored at  $-20^{\circ}\text{C}$ . Homogenates (30% w/v) were prepared in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged at 3000 rpm for 10 min. The supernatant was used for analysis.

### Catalase (CAT)

There was a significant ( $p < 0.05$ ) decrease in testicular catalase enzyme activity of Group II rats compared to Group I rats. Group III and Group IV showed a significant ( $p < 0.05$ ) increase in catalase activity compared to Group II rats. Among the treatment groups (Groups V-VII), Groups VI and VII exhibited a significant ( $p < 0.05$ ) increase in catalase activity compared to Group II rats, whereas the increase in Group V was non-significant. Furthermore, Group VII showed the highest catalase activity and was comparable to that of Groups I and IV.

### Superoxide dismutase (SOD)

There was a significant ( $p < 0.05$ ) decrease in testicular superoxide dismutase enzyme activity of Group II rats compared to Group I rats. Group III and Group IV showed a significant ( $p < 0.05$ ) increase in SOD activity compared to Group II rats. Similarly, all treatment groups (Groups V-VII) exhibited significantly ( $p < 0.05$ ) higher SOD activity than Group II rats. Among the treatment groups, Group VII showed the greatest improvement, with SOD activity comparable to that of Group I rats.

**Table 1:** The Mean ( $\pm$ SE) animal body weight (g) of treatment groups at different intervals of time

| Groups               | Day 0                          | Week 1                          | Week 2                          | Week 3                           | Week 4                          |
|----------------------|--------------------------------|---------------------------------|---------------------------------|----------------------------------|---------------------------------|
| Group I (NC)         | 156.67 $\pm$ 2.11 <sup>A</sup> | 174.83 $\pm$ 3.34 <sup>A</sup>  | 209.167 $\pm$ 2.71 <sup>B</sup> | 238.33 $\pm$ 3.57 <sup>Ba</sup>  | 273.33 $\pm$ 4.41 <sup>Ca</sup> |
| Group II (DC)        | 158.33 $\pm$ 3.07 <sup>A</sup> | 171.33 $\pm$ 4.05 <sup>A</sup>  | 183.16 $\pm$ 4.47 <sup>AB</sup> | 191.66 $\pm$ 3.50 <sup>Bb</sup>  | 205.83 $\pm$ 2.00 <sup>Bb</sup> |
| Group III (RC-1)     | 159.50 $\pm$ 3.30 <sup>A</sup> | 172.16 $\pm$ 3.60 <sup>AB</sup> | 184.83 $\pm$ 4.58 <sup>AB</sup> | 195.50 $\pm$ 3.12 <sup>Bcb</sup> | 218.33 $\pm$ 3.07 <sup>Cb</sup> |
| Group IV (RC-2)      | 157.83 $\pm$ 2.77 <sup>A</sup> | 171.83 $\pm$ 3.06 <sup>A</sup>  | 212.17 $\pm$ 2.77 <sup>B</sup>  | 222.83 $\pm$ 3.06 <sup>Ba</sup>  | 250.83 $\pm$ 4.17 <sup>Ca</sup> |
| Group V (BMSE 50)    | 158.33 $\pm$ 2.79 <sup>A</sup> | 177.50 $\pm$ 2.14 <sup>AB</sup> | 205.50 $\pm$ 2.75 <sup>B</sup>  | 234.50 $\pm$ 3.69 <sup>Ca</sup>  | 263.83 $\pm$ 2.89 <sup>Ca</sup> |
| Group VI (BMSE 100)  | 160.50 $\pm$ 3.69 <sup>A</sup> | 180.66 $\pm$ 3.33 <sup>AB</sup> | 212.50 $\pm$ 2.81 <sup>BC</sup> | 239.83 $\pm$ 3.42 <sup>Ca</sup>  | 268.16 $\pm$ 2.52 <sup>Da</sup> |
| Group VII (BMSE 200) | 157.50 $\pm$ 4.78 <sup>A</sup> | 179.16 $\pm$ 3.52 <sup>AB</sup> | 219.83 $\pm$ 4.09 <sup>BC</sup> | 247.83 $\pm$ 3.56 <sup>Ca</sup>  | 276.17 $\pm$ 3.00 <sup>Da</sup> |

**Note:** Values bearing different superscripts in capital letters between rows within a column vary significantly ( $p \leq 0.05$ ).

Values bearing different superscript in small letters between the row vary significantly ( $p \leq 0.05$ ).

**Table 2:** The mean ( $\pm$  SE) values of various haematological parameters of rats in different groups on final day (28<sup>th</sup> day) of the study

| Groups               | Hb (g/dl)                      | TEC (10 <sup>6</sup> /μl)     | TLC (10 <sup>3</sup> /μl)     | PCV (%)                       | MCV (fL)                      | MCH (pg)                       | MCHC (%)                        |
|----------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|---------------------------------|
| Group I (NC)         | 14.05 $\pm$ 0.42 <sup>a</sup>  | 7.45 $\pm$ 0.11 <sup>a</sup>  | 8.70 $\pm$ 0.11 <sup>a</sup>  | 41.28 $\pm$ 0.25 <sup>a</sup> | 53.17 $\pm$ 0.57 <sup>a</sup> | 17.90 $\pm$ 0.52 <sup>b</sup>  | 33.74 $\pm$ 0.50 <sup>a</sup>   |
| Group II (DC)        | 10.98 $\pm$ 0.53 <sup>c</sup>  | 5.62 $\pm$ 0.15 <sup>c</sup>  | 5.28 $\pm$ 0.20 <sup>d</sup>  | 30.84 $\pm$ 0.13 <sup>d</sup> | 63.82 $\pm$ 1.17 <sup>b</sup> | 23.21 $\pm$ 1.10 <sup>a</sup>  | 27.15 $\pm$ 0.80 <sup>c</sup>   |
| Group III (RC-1)     | 10.86 $\pm$ 0.33 <sup>c</sup>  | 6.22 $\pm$ 0.18 <sup>bc</sup> | 5.98 $\pm$ 0.28 <sup>d</sup>  | 31.90 $\pm$ 0.23 <sup>d</sup> | 61.53 $\pm$ 0.72 <sup>b</sup> | 24.28 $\pm$ 1.16 <sup>a</sup>  | 27.93 $\pm$ 0.99 <sup>c</sup>   |
| Group IV (RC-2)      | 13.50 $\pm$ 0.56 <sup>ab</sup> | 7.29 $\pm$ 0.18 <sup>a</sup>  | 7.30 $\pm$ 0.12 <sup>a</sup>  | 36.43 $\pm$ 0.13 <sup>c</sup> | 54.11 $\pm$ 0.97 <sup>a</sup> | 20.39 $\pm$ 1.08 <sup>ab</sup> | 32.73 $\pm$ 0.83 <sup>ab</sup>  |
| Group V (BMSE 50)    | 11.93 $\pm$ 0.60 <sup>bc</sup> | 6.80 $\pm$ 0.18 <sup>ab</sup> | 6.78 $\pm$ 0.10 <sup>c</sup>  | 36.11 $\pm$ 0.14 <sup>c</sup> | 56.29 $\pm$ 1.12 <sup>a</sup> | 21.15 $\pm$ 0.86 <sup>ab</sup> | 29.68 $\pm$ 0.85 <sup>bc</sup>  |
| Group VI (BMSE 100)  | 13.64 $\pm$ 0.39 <sup>ab</sup> | 7.12 $\pm$ 0.13 <sup>a</sup>  | 7.61 $\pm$ 0.13 <sup>b</sup>  | 38.52 $\pm$ 0.13 <sup>b</sup> | 55.44 $\pm$ 0.79 <sup>a</sup> | 20.35 $\pm$ 1.09 <sup>ab</sup> | 30.31 $\pm$ 0.86 <sup>abc</sup> |
| Group VII (BMSE 200) | 14.02 $\pm$ 0.22 <sup>a</sup>  | 7.22 $\pm$ 0.14 <sup>a</sup>  | 8.30 $\pm$ 0.20 <sup>ab</sup> | 39.17 $\pm$ 0.12 <sup>a</sup> | 53.68 $\pm$ 0.69 <sup>a</sup> | 18.46 $\pm$ 0.86 <sup>b</sup>  | 33.02 $\pm$ 0.54 <sup>ab</sup>  |

One-way ANOVA with Tukey's post hoc test (IBM SPSS)

Mean  $\pm$  SE values with different superscript differ significantly at  $p \leq 0.05$  (n=6).

**Table 3:** The mean ( $\pm$  SE) values of various serum biochemical parameters of rats in different groups on final day (28<sup>th</sup> day) of the study

| Groups               | ALP (IU/L)                     | ALT (IU/L)                     | TP (g/dl)                     | CRT (mg/dl)                   | BUN (mg/dl)                    |
|----------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|--------------------------------|
| Group I (NC)         | 109.62 $\pm$ 1.19 <sup>e</sup> | 41.04 $\pm$ 0.46 <sup>d</sup>  | 8.14 $\pm$ 0.17 <sup>a</sup>  | 0.56 $\pm$ 0.02 <sup>c</sup>  | 16.16 $\pm$ 0.20 <sup>e</sup>  |
| Group II (DC)        | 181.95 $\pm$ 1.35 <sup>a</sup> | 68.73 $\pm$ 0.76 <sup>a</sup>  | 5.70 $\pm$ 0.17 <sup>d</sup>  | 1.39 $\pm$ 0.07 <sup>a</sup>  | 24.03 $\pm$ 0.25 <sup>a</sup>  |
| Group III (RC-1)     | 178.25 $\pm$ 1.03 <sup>a</sup> | 65.66 $\pm$ 0.94 <sup>a</sup>  | 5.82 $\pm$ 0.44 <sup>cd</sup> | 1.28 $\pm$ 0.03 <sup>a</sup>  | 23.06 $\pm$ 0.53 <sup>ab</sup> |
| Group IV (RC-2)      | 112.38 $\pm$ 0.72 <sup>d</sup> | 47.29 $\pm$ 0.71 <sup>c</sup>  | 7.51 $\pm$ 0.20 <sup>ab</sup> | 0.63 $\pm$ 0.02 <sup>bc</sup> | 21.00 $\pm$ 0.41 <sup>c</sup>  |
| Group V (BMSE 50)    | 124.88 $\pm$ 1.40 <sup>b</sup> | 53.02 $\pm$ 1.14 <sup>b</sup>  | 6.80 $\pm$ 0.21 <sup>bc</sup> | 0.73 $\pm$ 0.02 <sup>b</sup>  | 22.19 $\pm$ 0.23 <sup>bc</sup> |
| Group VI (BMSE 100)  | 119.15 $\pm$ 0.89 <sup>c</sup> | 49.84 $\pm$ 1.36 <sup>bc</sup> | 6.92 $\pm$ 0.22 <sup>b</sup>  | 0.68 $\pm$ 0.01 <sup>bc</sup> | 19.36 $\pm$ 0.25 <sup>d</sup>  |
| Group VII (BMSE 200) | 112.94 $\pm$ 0.86 <sup>d</sup> | 46.45 $\pm$ 0.83 <sup>c</sup>  | 7.86 $\pm$ 0.16 <sup>ab</sup> | 0.66 $\pm$ 0.02 <sup>bc</sup> | 18.17 $\pm$ 0.24 <sup>d</sup>  |

One way ANOVA with Tukey's post hoc test (IBM SPSS)

Mean values with different superscript differ significantly at  $p \leq 0.05$  (n=6).

**Table 4:** The mean ( $\pm$  SE) values of various serum oxidative stress parameters of testicular tissue of rats in different groups on final day (28<sup>th</sup> day) of the study

| Groups               | LPO (nmol MDA/mg of protein)  | CAT ( $\mu$ /mg protein)       | SOD (U/mg of protein)          |
|----------------------|-------------------------------|--------------------------------|--------------------------------|
| Group I (NC)         | 0.31 $\pm$ 0.01 <sup>d</sup>  | 15.15 $\pm$ 0.23 <sup>a</sup>  | 44.35 $\pm$ 0.35 <sup>a</sup>  |
| Group II (DC)        | 2.45 $\pm$ 0.04 <sup>a</sup>  | 8.83 $\pm$ 0.22 <sup>e</sup>   | 17.89 $\pm$ 0.42 <sup>e</sup>  |
| Group III (RC-1)     | 1.75 $\pm$ 0.01 <sup>b</sup>  | 9.73 $\pm$ 0.23 <sup>d</sup>   | 23.43 $\pm$ 0.44 <sup>bc</sup> |
| Group IV (RC-2)      | 0.44 $\pm$ 0.01 <sup>c</sup>  | 13.27 $\pm$ 0.28 <sup>bc</sup> | 41.42 $\pm$ 0.47 <sup>b</sup>  |
| Group V (BMSE 50)    | 0.91 $\pm$ 0.02 <sup>b</sup>  | 9.17 $\pm$ 0.19 <sup>e</sup>   | 31.46 $\pm$ 0.32 <sup>d</sup>  |
| Group VI (BMSE 100)  | 0.75 $\pm$ 0.01 <sup>b</sup>  | 11.56 $\pm$ 0.18 <sup>c</sup>  | 36.02 $\pm$ 0.46 <sup>c</sup>  |
| Group VII (BMSE 200) | 0.43 $\pm$ 0.01 <sup>cd</sup> | 12.85 $\pm$ 0.19 <sup>ab</sup> | 38.21 $\pm$ 0.39 <sup>a</sup>  |

One way ANOVA with Tukey's post hoc test (IBM SPSS), Mean values with different superscript differ significantly at  $p \leq 0.05$  (n=6).

## Discussion

In the present investigation, cadmium exposure produced clinical signs including weakness, dullness, anorexia, lethargy, ruffled hair coat, mild alopecia and progressive reduction in body weight, indicating the development of systemic toxicity. These observations are in agreement with previous reports of cadmium-induced toxicity in rats (Nordberg *et al.*, 2007; Prozialeck *et al.*, 2016; Genchi *et al.*, 2020). The significant decline in body weight observed following cadmium administration was consistent with earlier studies (Rencuzogullari and Erdogan, 2007; Shagirtha

*et al.*, 2011; Olufemi *et al.*, 2014; Wongmekiat *et al.*, 2018). Cadmium-induced loss of body weight has been attributed to reduced feed intake associated with anorexia, gastrointestinal irritation, impaired nutrient absorption and altered protein metabolism. In addition, cadmium-induced mitochondrial dysfunction disrupts oxidative phosphorylation, resulting in reduced ATP production, impaired energy metabolism and growth retardation (Shagirtha *et al.*, 2011; Wongmekiat *et al.*, 2018). Administration of vitamin E and selenium (Group IV) significantly improved body weight compared with disease control rats. This beneficial effect may be attributed to their

ability to suppress lipid peroxidation, restore glutathione homeostasis, normalize antioxidant enzyme activities and inhibit inflammatory mediators including NF- $\kappa$ B, TNF- $\alpha$ , iNOS and COX-2, thereby preserving mitochondrial integrity and normal cellular metabolism (Olaniyan *et al.*, 2021) [22, 29, 13, 32, 35, 24, 39, 35, 39].

Cadmium exposure also caused significant alterations in hematological parameters, characterized by reductions in TEC, Hb, PCV, TLC and MCHC, accompanied by increases in MCV and MCH. These findings are consistent with previous reports describing cadmium-induced hematotoxicity (El-Demerdash *et al.*, 2004; Badr *et al.*, 2019; Donmez *et al.*, 2019; Kısadere *et al.*, 2022). The decrease in Hb, TEC and PCV may be attributed to inhibition of  $\delta$ -aminolevulinic acid dehydratase (ALAD), an essential enzyme involved in heme biosynthesis, leading to impaired hemoglobin synthesis and erythropoiesis. Cadmium also induces oxidative damage to erythrocyte membranes through excessive generation of reactive oxygen species (ROS), resulting in increased membrane fragility, hemolysis and reduced erythrocyte lifespan (Stohs and Bagchi, 1995; Flora *et al.*, 2008). Furthermore, cadmium accumulation within the bone marrow suppresses hematopoietic stem cell proliferation and interferes with erythropoietin signaling, thereby reducing erythrocyte production. The increase in MCV and MCH observed in cadmium-treated rats suggests the development of macrocytic anemia, which may result from oxidative membrane injury, compensatory release of immature erythrocytes and impaired DNA synthesis during erythroid maturation (Kısadere *et al.*, 2022). The significant reduction in TLC observed in the present study may be attributed to cadmium-induced oxidative stress, apoptosis of hematopoietic progenitor cells and suppression of leukopoiesis, ultimately leading to immunosuppression (Badr *et al.*, 2019) [9, 4, 8, 18, 36, 12, 18, 36, 12, 18, 4].

Cadmium intoxication significantly increased serum ALT, ALP, BUN and creatinine concentrations, while decreasing total protein, indicating hepatic and renal dysfunction. Similar observations have been reported previously (Pari and Murugavel, 2005; Renugadevi and Prabu, 2010; Djurasevic *et al.*, 2017; Kumar *et al.*, 2021; Poli *et al.*, 2022). Cadmium accumulates in hepatocytes and renal tubular epithelial cells, where it promotes mitochondrial dysfunction, excessive ROS generation and lipid peroxidation, resulting in disruption of cellular membrane integrity and leakage of intracellular enzymes into the circulation. In addition, cadmium activates inflammatory pathways including MAPK and NF- $\kappa$ B, resulting in increased production of pro-inflammatory cytokines and nitric oxide, thereby aggravating hepatic injury and suppressing protein synthesis. Renal dysfunction is primarily associated with cadmium-metallothionein complex accumulation within proximal tubular cells, causing oxidative stress, tubular degeneration and impaired glomerular filtration, ultimately leading to increased serum creatinine and blood urea nitrogen concentrations (Flora *et al.*, 2008; Kumar *et al.*, 2021; Poli *et al.*, 2022) [36, 33, 7, 20, 28].

The present study also demonstrated a significant increase in lipid peroxidation together with marked reductions in SOD and CAT activities following cadmium exposure, indicating severe oxidative stress. These findings are consistent with previous investigations (El-Sharakly *et al.*, 2007; Othman *et al.*, 2014; Rehman *et al.*, 2020; Yi *et al.*, 2021). Although cadmium is not a redox-active metal, it indirectly generates excessive ROS by disrupting mitochondrial electron

transport, depleting intracellular glutathione and inhibiting endogenous antioxidant enzymes. The resulting oxidative stress causes membrane lipid peroxidation, protein oxidation and DNA damage, ultimately leading to cellular dysfunction (Flora *et al.*, 2008). Cadmium also suppresses Nrf2-mediated antioxidant signaling while activating NF- $\kappa$ B-dependent inflammatory pathways, thereby amplifying oxidative injury and reducing antioxidant enzyme expression (Jahan *et al.*, 2014; Yi *et al.*, 2021) [10, 25, 31, 40, 12, 15, 40].

Treatment with biorefined microalgal Spirulina extract (BMSE) produced significant dose-dependent improvement in body weight, hematological indices, serum biochemical parameters and antioxidant status. These beneficial effects may be attributed to the rich composition of Spirulina, including C-phycocyanin,  $\beta$ -carotene, chlorophyll, tocopherols, phenolic compounds, vitamins, minerals and essential amino acids, which collectively exert potent antioxidant, anti-inflammatory and metal-chelating activities (Parikh *et al.*, 2001; Khan *et al.*, 2005; Ambati *et al.*, 2019). C-phycocyanin effectively scavenges reactive oxygen species, inhibits lipid peroxidation and suppresses inflammatory signaling through inhibition of NF- $\kappa$ B and MAPK pathways while activating Nrf2-mediated antioxidant responses. Spirulina has also been reported to enhance erythropoiesis through its high iron, vitamin B12 and folic acid content and to protect cells against apoptosis by upregulating Bcl-2 expression and inhibiting cytochrome-c release (Hayashi *et al.*, 2009; Finamore *et al.*, 2017). In addition, its metal-chelating properties may reduce cadmium bioaccumulation and facilitate its elimination, thereby limiting tissue injury and preserving hepatic and renal function. The highest dose of BMSE exhibited the greatest protective effect, with most hematological, biochemical and antioxidant parameters approaching normal values [27, 17, 2, 14, 11].

Overall, the present study demonstrates that BMSE provides significant dose-dependent protection against cadmium-induced toxicity through its antioxidant, anti-inflammatory, anti-apoptotic and metal-chelating properties. The marked improvement in body weight, hematological profile, liver and kidney function and antioxidant defense system suggests that BMSE has considerable potential as a natural therapeutic agent for mitigating cadmium-induced toxicity. Further mechanistic studies and clinical investigations are warranted to validate its therapeutic efficacy and translational applicability.

## Conclusion

Biorefined microalgal Spirulina extract (BMSE) demonstrated significant protective effects against cadmium chloride-induced systemic toxicity in male Wistar rats by ameliorating hematological and biochemical alterations and restoring antioxidant status. Cadmium exposure resulted in anemia, altered erythrocytic indices, elevated hepatic and renal biomarkers, and increased oxidative stress, whereas BMSE supplementation significantly improved these parameters by reducing lipid peroxidation and enhancing the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT). The protective effects were dose dependent, with the 200 mg/kg body weight dose exhibiting the greatest ameliorative efficacy compared with the 50 and 100 mg/kg doses, highlighting the potential of BMSE as a natural antioxidant and cytoprotective agent against cadmium-induced toxicity.

Future studies should focus on elucidating the molecular mechanisms underlying the protective effects of BMSE, particularly its role in regulating oxidative stress, inflammation, apoptosis, and heavy metal detoxification pathways. Further investigations aimed at identifying the principal bioactive constituents responsible for its therapeutic activity, optimizing dosage regimens, evaluating long-term safety, and conducting well-designed preclinical and clinical studies are warranted to establish its efficacy and facilitate its development as a promising natural therapeutic intervention for heavy metal-induced toxicity.

## References

1. Abdulqader G, Barsanti L, Tredici MR. Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. *J Appl Phycol*. 2000;12(3):493-498.
2. Ambati RR, Gogisetty D, Aswathanarayana RG, Ravi S, Bikkina PN, Bo L, Yuepeng S. Industrial potential of carotenoid pigments from microalgae: Current trends and future prospects. *Crit Rev Food Sci Nutr*. 2019;59(12):1880-1902.
3. Amin A, Hamza AA, Daoud S, Hamza W. *Spirulina* protects against cadmium-induced hepatotoxicity in rats. *Am J Pharmacol Toxicol*. 2006;1(2):21-25.
4. Badr GM, Elsayy H, Sedky A, Eid R, Ali A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM. Protective effects of quercetin supplementation against short-term toxicity of cadmium-induced hematological impairment, hypothyroidism, and testicular disturbances in albino rats. *Environ Sci Pollut Res*. 2019;26(8):8202-8211.
5. Charkiewicz AE, Omeljaniuk WJ, Nowak K, Garley M, Niklinski J. Cadmium toxicity and health effects: A brief summary. *Molecules*. 2023;28(18):6620-6636.
6. Djukic-Cosic D, Jovanovic MC, Bulat ZP, Ninkovic M, Malicevic Z, Matovic V. Relation between lipid peroxidation and iron concentration in mouse liver after acute and subacute cadmium intoxication. *J Trace Elem Med Biol*. 2008;22(1):66-72.
7. Djurasevic S, Jama A, Jasnic N, Vujovic P, Jovanovic M, Mitic-Culafic D, Knezevic-Vukcevic J, Cakic-Milosevic M, Ilijevic K, Djordjevic J. The protective effects of probiotic bacteria on cadmium toxicity in rats. *J Med Food*. 2017;20(2):189-196.
8. Donmez HH, Donmez N, Kisadere I, Undag I. Protective effect of quercetin on some hematological parameters in rats exposed to cadmium. *Biotech Histochem*. 2019;94(5):381-386.
9. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: Protective role of vitamin E and  $\beta$ -carotene. *Food Chem Toxicol*. 2004;42(10):1563-1571.
10. El-Sharaky AS, Newairy AA, Badreldeen MM, Eweda SM, Sheweita SA. Protective role of selenium against renal toxicity induced by cadmium in rats. *Toxicology*. 2007;235(3):185-193.
11. Finamore A, Palmery M, Bensehaila S, Peluso I. Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly *Spirulina*. *Oxid Med Cell Longev*. 2017;2017(1):3247528-3247545.
12. Flora SJS, Mittal M, Mehta A. Heavy metal-induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res*. 2008;128(4):501-523.
13. Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. *Int J Environ Res Public Health*. 2020;17(11):3782-3796.
14. Hayashi K, Surani MA. Self-renewing epiblast stem cells exhibit continual delineation of germ cells with epigenetic reprogramming *in vitro*. *Development*. 2009;136(21):3549-3556.
15. Jahan S, Zahra A, Irum U, Iftikhar N, Ullah H. Protective effects of different antioxidants against cadmium-induced oxidative damage in rat testis and prostate tissues. *Syst Biol Reprod Med*. 2014;60(4):199-205.
16. Jayamurali D, Varier KM, Liu W, Raman J, Ben-David Y, Shen X, Gajendran B. An overview of heavy metal toxicity. In: *Metal, Metal Oxides and Metal Sulphides for Biomedical Applications*. Vol. 15. 2021. p. 323-342.
17. Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of *Spirulina*. *Curr Pharm Biotechnol*. 2005;6(5):373-379.
18. Kisadere I, Aydın MF, Undag I. Partial protective effects of melatonin on cadmium-induced changes in hematological characteristics in rats. *Biotech Histochem*. 2022;97(3):192-198.
19. Kolachi NF, Kazi TG, Afridi HI, Kazi N, Khan S, Kandhro GA, Shah AQ, Baig JA, Wadhwa SK, Shah F, Jamali MK. Status of toxic metals in biological samples of diabetic mothers and their neonates. *Biol Trace Elem Res*. 2011;143(1):196-212.
20. Kumar A, Siddiqi NJ, Alrashood ST, Khan HA, Dubey A, Sharma B. Protective effect of eugenol on hepatic inflammation and oxidative stress induced by cadmium in male rats. *Biomed Pharmacother*. 2021;139:111588-111598.
21. Kumar R, Sharma V, Das S, Patial V, Srivatsan V. *Arthrospira platensis* (*Spirulina*)-fortified functional foods ameliorate iron and protein malnutrition by improving growth and modulating oxidative stress and gut microbiota in rats. *Food Funct*. 2023;14(2):1160-1178.
22. Nordberg GF, Fowler BA, Nordberg M, editors. *Handbook on the Toxicology of Metals*. 3rd ed. Amsterdam: Elsevier Academic Press; 2007. p. 445-486.
23. Nuli M, Kumar JP, Korn R, Putta S. Cadmium toxicity: Unveiling the threat to human health. *Indian J Pharm Sci*. 2024;86(5):31-39.
24. Olufemi MV, Tams GE, Dolapo IA. The effect of methanolic extract of *Dissotis rotundifolia* on cadmium-induced testicular damage in Wistar rats. *IOSR J Pharm*. 2014;4(7):56-65.
25. Othman MS, Nada A, Zaki HS, Abdel Moneim AE. Effect of *Physalis peruviana* L. on cadmium-induced testicular toxicity in rats. *Biol Trace Elem Res*. 2014;159(1-3):278-287.
26. Pari L, Murugavel P. Role of diallyl tetrasulfide in ameliorating the cadmium-induced biochemical changes in rats. *Environ Toxicol Pharmacol*. 2005;20(3):493-500.
27. Parikh P, Mani U, Iyer U. Role of *Spirulina* in the control of glycemia and lipidemia in type 2 diabetes mellitus. *J Med Food*. 2001;4(4):193-199.
28. Poli V, Madduru R, Aparna Y, Kandukuri V, Motireddy SR. Amelioration of cadmium-induced oxidative

- damage in Wistar rats by vitamin C, zinc and N-acetylcysteine. *Med Sci.* 2022;10(1):7-21.
29. Prozialeck WC, VanDreel A, Ackerman CD, Stock I, Papaeliou A, Yasmine C, Wilson K, Lamar PC, Sears VL, Gasiorowski JZ, DiNovo KM. Evaluation of cystatin C as an early biomarker of cadmium nephrotoxicity in the rat. *Biometals.* 2016;29(1):131-146.
30. Ramachandran R. Cadmium-induced inflammatory response and alteration of inflammatory markers in testicular tissue. *Int J Adv Res.* 2014;2(9):606-611.
31. Rehman NU, Ansari MN, Ganaie MA, Madkhali HA, Saeedan AS, Imam F, Hamad AM. Cadmium-induced hepatotoxicity and oxidative stress in rats: Protection by roflumilast via NF- $\kappa$ B and HO-1 pathway. *Int J Pharmacol.* 2020;16(2):154-163.
32. Rencuzogullari N, Erdogan S. Oral administration of lycopene reverses cadmium-suppressed body weight loss and lipid peroxidation in rats. *Biol Trace Elem Res.* 2007;118(2):175-183.
33. Renugadevi J, Prabu SM. Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Exp Toxicol Pathol.* 2010;62(2):171-181.
34. Sanchez M, Bernal-Castillo J, Rozo C, Rodríguez I. *Spirulina (Arthrospira)*: An edible microorganism. A review. *Univ Sci.* 2007;12(1):7-24.
35. Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium-induced oxidative stress-related neurotoxicity in rats. *Eur Rev Med Pharmacol Sci.* 2011;15(9):1039-1050.
36. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med.* 1995;18(2):321-336.
37. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. *Mol Clin Environ Toxicol.* 2012;3(2):133-164.
38. Wilson B. Environmental aspects of cadmium production and use. *J Environ Monit.* 2001;3(1):14-18.
39. Wongmekiat O, Peerapanyasut W, Kobroob A. Catechin supplementation prevents kidney damage in rats repeatedly exposed to cadmium through mitochondrial protection. *Naunyn Schmiedeberg's Arch Pharmacol.* 2018;391(4):385-394.
40. Yi L, Dai J, Chen Y, Tong Y, Li Y, Fu G, *et al.* Reproductive toxicity of cadmium in pubertal male rats induced by cell apoptosis.