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Research Article

Protective Effects of Omega-3 Fatty Acids on Cadmium-Induced Hematotoxicity, Body Weight Alterations, Gonadosomatic Index, and Cognitive Function in Male Wistar Rats

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Abstract
This study investigated the effects of cadmium chloride on haematological parameters, body weight, gonadosomatic index (GSI), and cognitive function, as well as the potential ameliorative effects of omega-3 fatty acids in male Wistar rats. Sixty male albino Wistar rats (120-155g) were divided into 15 groups. Groups 1-5 received cadmium chloride (0, 2, 4, 6, or 8 mg/kg) orally twice weekly for 8 weeks. Groups 6 and 7 received omega-3 fatty acids (500 or 1000 mg/kg) twice weekly. Groups 8-15 received combinations of cadmium chloride (2, 4, 6, or 8 mg/kg) and omega-3 fatty acids (500 or 1000 mg/kg) for the same duration. Haematological parameters were analysed using an auto-analyser, cognitive function was assessed using the Y-maze model, and GSI was calculated from body and testicular weights. Data were statistically evaluated using two-way ANOVA with significance set at $p<0.05$. Cadmium exposure significantly reduced WBC, neutrophils, RBC, haemoglobin, MCV, MCH, and MCHC while increasing monocyte counts ($p<0.05$). No significant effects were observed on platelet count and lymphocyte ($p>0.05$). Body weight, GSI, and cognitive function were also significantly reduced ($p<0.05$). Omega-3 fatty acid supplementation mitigated these effects, significantly improving haematological parameters, body weight, GSI, and cognitive function compared to cadmium-only groups. The study concludes that omega-3 fatty acids effectively ameliorate cadmium-induced hematotoxicity, weight loss, cognitive deficits, and testicular damage.
Keywords: Cadmium Exposure, Haematological Parameter, Cognitive Function, Gonadosomatic Index, Omege-3 Fatty Acid, Wistar Rats.

Introduction

Cadmium (Cd) is a toxic, non-essential trace element that disrupts physiological processes, including haematological function, body weight regulation, and reproductive health, as measured by the

gonadosomatic index (GSI). Exposure occurs through inhalation, ingestion, and skin contact, with major sources being smoking and industrial environments. Once absorbed, cadmium accumulates in organs like the liver and kidneys, causing severe damage (Faroon *et al.,* 2012). Cadmium toxicity is primarily mediated through oxidative stress, where it promotes the production of reactive oxygen species (ROS), leading to lipid peroxidation, glutathione depletion, and protein oxidation (Rani *et al.,* 2014). This results in apoptosis, inflammation, and cellular damage. Cadmium also inhibits antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) (Rani *et al.,* 2014; Akinloye *et al.,* 2006). Another key mechanism involves the disruption of cellular signaling pathways, including nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which regulates genes linked to inflammation and apoptosis. Furthermore, cadmium impairs mitochondrial function, increasing ROS production and inducing cell death (Kumar and Singh, 2020; Zhang and Wang, 2020).

Cadmium contamination results from industrial activities, mining, and agriculture, leading to environmental and biological accumulation (Jarup, 2003). Exposure is associated with hematological disorders, weight loss, reproductive toxicity, and cognitive impairments (Baker and Smith, 2013; Kuo and Chen, 2015). Haematological parameters, including red blood cell count, haemoglobin, and white blood cells, are sensitive indicators of cadmium toxicity (Kumar and Singh, 2016). Cadmium-induced oxidative stress alters body weight regulation and reduces GSI, impacting fertility (Khan and Khan, 2017). Additionally, cadmium affects cognitive function, impairing learning and memory (Zhang and Wang, 2016). Omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have demonstrated neuroprotective, anti-inflammatory, and antioxidant effects (Calder, 2015). Studies suggest omega-3 fatty acids may mitigate the harmful effects of heavy metals, including cadmium (Baker and Smith, 2013). This study investigates cadmium's impact on haematological parameters, body weight, GSI, and cognitive function in male Wistar rats while assessing the protective role of omega-3 fatty acids. Understanding this interaction may provide insights into dietary interventions to counteract heavy metal toxicity.

Materials and Methods

Experimental Animals and Design

From Samwill Animal House at Federal University of Technology Akure, Ondo State, sixty (60) male adult Wistar rats weighing 120–155g were acquired. The rats were kept in a clean, well-ventilated cage with a 12-hour light/dark cycle and a consistent temperature of 23.1^0C. The rats were fed commercial growers' mash (Ewu Feeds and Flour Mills Limited Ewu, Edo state, Nigeria) and allowed access to water as needed. The treatment itself lasted eight weeks, and the animals were given two weeks to acclimatize before taking part in the trial. Groups 1 (control) and 2 through 15 (treatment) were randomly assigned to receive 0 mg/kg, 2 mg/kg, 4 mg/kg, 6 mg/kg, and 8 mg/kg of cadmium chloride and omega-3 fatty acid, respectively. The rats were divided into fifteen (15) groups of four rats each (n = 4). The animals were put to sleep (euthanized) 24 hours after the experiment was over so that samples could be collected and examined in accordance with established procedures.

For Group 1 (the controls), the dosage was set at 0mg/kg, which meant that they were given only feeds and distilled water.
Group 2 received 2 mg/kg of body weight of cadmium chloride twice weekly for 8 weeks.
Group 3 received 4 mg/kg of body weight of cadmium chloride twice weekly for 8 weeks.
Group 4 received 6 mg/kg of body weight of cadmium chloride twice weekly for 8 weeks.
Group 5 received 8 mg/kg of body weight of cadmium chloride twice weekly for 8 weeks.
Group 6 received 500 mg/kg of body weight of omega-3 fatty acid twice weekly for 8 weeks.
Group 7 received 1000 mg/kg of body weight of omega-3 fatty acid twice weekly for 8 weeks.
Group 8 received 2 mg/kg body weight of cadmium chloride + O3FA 500mg/kg twice weekly for 8 weeks.
Group 9 received 4 mg/kg body weight of cadmium chloride + O3FA 500 mg/kg twice weekly for 8 weeks.
Group 10 received 6 mg/kg body weight of cadmium chloride + O3FA 500 mg/kg twice weekly for 8 weeks.
Group 11 received 8 mg/kg body weight of cadmium chloride + O3FA 500 mg/kg twice weekly for 8 weeks.
Group 12 received 2 mg/kg body weight of cadmium chloride + O3FA 1000 mg/kg twice weekly for 8 weeks.
Group 13 received 4 mg/kg body weight of cadmium chloride + O3FA 1000 mg/kg twice weekly for 8 weeks.
Group 14 received 6 mg/kg body weight of cadmium chloride + O3FA 1000 mg/kg twice weekly for 8 weeks.
Group 15 received 8 mg/kg body weight of cadmium chloride + O3FA 1000 mg/kg twice weekly for 8 weeks.

Sample Size Determination

The minimum sample size required (N) = $2\ [Z\alpha + Z\,(1\text{-}\beta)]^2\, S^2/(\mu 1\text{-}\mu 2)^2$
Where:

$Z\alpha$ = Standard normal deviate corresponding to 5% level of significant = 1.96.
Z (1-β) = Standard normal deviate corresponding to a power of 80% = 0.84.
S = Standard deviation of SOD level in Wistar rat injected with cadmium chloride = 4.36 (Ogunbiyi and Obi, 2021).
$(\mu 1-\mu 2)^2$ = The mean differences in SOD level between the group = 8.72.

Calculation:
$2\ (1.96 + 0.84)^2\ (4.36)^2 / (8.72)^2 = 4$ (approx.)

This then gives a minimum of 4 rats per group for the study.

Inclusion/Exclusion Criteria
Male albino rats that were fully grown were used; however female albino rats and those less than eight weeks were excluded.

Collection of Samples
At the conclusion of the experiment, the rats were put to sleep by cervical dislocation. The animal was dissected to remove the testes and other sex accessory glands and then clean and weigh using weighing balance. For hematological analysis, heart punctures were used to get blood samples into ethylenediamine tetra-acetic acid (EDTA) anticoagulant.

Hematological Parameters Analysis
Hematological parameters were analyzed with hematology autoanalyzer (Biobase BK 3100, China).

Weighing of Wistar Rats
- ✓ A container to hold the rat during weighing was placed on the scale and tare it to zero.
- ✓ The rat was gently placed into the container on the scale platform.
- ✓ The weight was taken when the scale reading stabilizes and then recorded in grams.
- ✓ After weighing the rat was returned to its cage promptly to reduce stress.

Gonadosomatic Index
A weighing balance was used to measure each Wistar rat's body weight and testicular weight. The method below was used to calculate each rat's gonadosomatic index based on their mean testicular and body weights:

Testicular weight / body weight × 100 is the gonadosomatic index (ơ) (Amann, 1970).

Y-maze in Cognitive function
Procedure
1) Animals are placed in one arm and allowed to explore the maze for 5minutes.
2) Movements are monitored to track arm entries, time spent in each arm, and alternation patterns.
3) The spontaneous alternation (%) was calculated using this formula:

Spontaneous alternation (%) = Number of alternations × 100/ Total arm entries – 2

Ethical Approval
Joseph Ayo Babalola University at Ikeji-Arakeji, Osun State, is where ethical permissions for the use of laboratory animals were sought and acquired. The committee authorized all experimental procedures under (Protocol number JABU/BCH/EA/05/22).

Statistical Analysis
The results were expressed as Mean±SEM in tables. The data collected for this study were analyzed using SPSS (IBM) version 26.0. The significance of the observed differences between the groups was determined with one-way analysis of variance (ANOVA) using Tukey post hoc multiple comparison. P-value of less than 0.05 ($p<0.05$) was considered significant.

Results
The mean weight of the experimental animals at different research weeks, from baseline to week four, is displayed in Table 1.

Table 1. Weight of experimental animals in the study.

Groups	Basal wt(g)	2wk accl. wt(g)	Wk1.wt (g)	Wk2.wt (g)	Wk3.wt (g)	Wk4.wt (g)
Group 1	132.50± 3.23^{a}	194.00± 7.44^{ab}	190.00± 4.08^{b}	186.25± 3.75^{de}	197.50± 6.64^{cd}	173.75± 3.75^{bcd}
Group 2	142.50± 4.79^{a}	218.00± 19.13^{b}	190.50± 6.08^{b}	184.00± 6.22^{cde}	169.75± 10.74^{abc}	157.50± 2.50^{abc}
Group 3	126.50± 3.12^{a}	191.00± 13.28^{ab}	154.00± 9.45^{ab}	156.50± 6.39^{abcd}	150.00± 3.54^{ab}	144.25± 4.05^{ab}
Group 4	125.00± 3.54^{a}	154.00± 2.27^{a}	143.25± 3.49^{a}	149.00± 3.32^{ab}	143.25± 5.82^{a}	141.75± 3.12^{a}
Group 5	135.00± 5.40^{a}	189.50± 17.06^{ab}	163.75± 11.43^{ab}	152.25± 5.95^{abc}	153.00± 6.35^{ab}	140.00± 4.56^{a}
Group 6	131.25± 9.66^{a}	173.25± 5.68^{ab}	179.50± 11.27^{ab}	179.75± 9.72^{bcde}	182.00± 9.03^{bcd}	196.25± 12.48^{def}
Group 7	150.00± 12.25^{a}	182.75± 11.99^{ab}	185.00± 12.07^{ab}	196.25± 9.44^{e}	205.75± 10.82^{e}	214.50± 6.85^{f}
Group 8	131.25± 6.57^{a}	179.00± 4.51^{ab}	175.75± 6.69^{ab}	179.50± 5.72^{bcde}	183.75± 6.25^{bcd}	200.00± 5.40^{def}
Group 9	125.00± 3.54^{a}	175.00± 6.45^{ab}	151.25± 4.27^{ab}	147.25± 4.96^{a}	143.75± 4.73^{a}	146.25± 6.17^{ab}
Group 10	155.00± 2.89^{a}	186.75± 4.53^{ab}	150.25± 2.25^{ab}	150.50± 3.77^{ab}	150.00± 4.56^{ab}	152.50± 4.33^{abc}
Group 11	147.50± 2.50^{a}	186.75± 2.49^{ab}	155.00± 5.07^{ab}	160.00± 4.56^{abcd}	160.00± 2.04^{ab}	158.25± 1.18^{abc}
Group 12	155.00± 8.66^{a}	195.00± 11.78^{ab}	191.50± 11.47^{b}	183.00± 9.81^{cde}	183.75± 6.17^{bcd}	207.75± 5.11^{ef}
Group 13	142.00± 6.38^{a}	153.50± 6.81^{a}	155.00± 11.03^{ab}	142.00± 7.12^{a}	141.00± 7.70^{a}	159.50± 13.05^{abc}
Group 14	145.00± 6.45^{a}	171.50± 9.18^{ab}	168.00± 11.39^{ab}	172.50± 6.19^{abcde}	179.00± 5.49^{bcd}	178.75± 5.15^{cde}
Group 15	152.50± 2.50^{a}	180.00± 5.80^{ab}	147.25± 1.70^{a}	150.75± 2.17^{ab}	155.00± 2.89^{ab}	155.50± 1.66^{abc}
F-value	3.17	2.67	4.32	7.70	9.82	16.35
P-value	0.335	0.006	0.001	0.001	0.001	0.001

Values are expressed in Mean±SEM. Mean that do not share a letter are significantly different (P<0.05), Group 1: control(0mg/kg), Group 2: 2mg/kg cadmium chloride, Group 3: 4mg/kg cadmium chloride, Group 4: 6mg/kg cadmium chloride, Group 5: 8mg/kg cadmium chloride, Group 6: 500mg/kg omega-3 fatty acid, Group 7: 1000mg/kg omega-3 fatty acid, Group 8: 2mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 9: 4mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 10: 6mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 11: 8mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 12: 2mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 13: 4mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 14: 6mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 15: 8mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, 2wk acclim.wt: 2 weeks acclimatization weight, Wk1.wt: Week 1 weight, Wk2.wt: Week 2 weight, Wk3.wt: Week 3 weight, Wk4.wt: Week 4 weight.

A one-way analysis of variance (ANOVA) utilizing Tukey post hoc multiple comparison showed no statistically significant difference (p>0.05) between the groups (F=3.17, P=0.335). After two weeks of acclimatization, there was a substantial increase in mean weight across all groups (F=2.67, P=0.006), with groups 2, 13, and 4 showing the largest gain. The animals in groups 4 (6 mg/kg $CdCl_2$) and 15 (8 mg/kg $CdCl_2$+1000 mg/kg omega-3 fatty acid) had significantly lower mean weights (p<0.05) than the control at the first week. At week two, groups 4 (6 mg/kg $CdCl_2$), 5 (8 mg/kg $CdCl_2$), 9 (4 mg/kg $CdCl_2$+500 mg/kg omega-3 fatty acid), 10 (6 mg/kg $CdCl_2$+500 mg/kg omega-3 fatty acid), and 15 (8 mg/kg $CdCl_2$+ 1000 mg/kg omega-3 fatty acid) had significantly lower mean weights (p<0.05) than the control group.

By week three, groups 4, 5, 9, 10, 11 (8 mg/kg $CdCl_2$+500 mg/kg omega-3 fatty acid), 13 (4 mg/kg $CdCl_2$+1000 mg/kg omega-3 fatty acid), and 15 had significantly lower mean weights (p<0.05) than the control. At week four, groups 4 and 5 had significantly lower mean weights (p<0.05), whereas groups 7

(1000 mg/kg omega-3 fatty acid) and 12 (2 mg/kg $CdCl_2$+1000 mg/kg omega-3 fatty acid) had significantly higher mean weights ($p<0.05$) than the control group.

The mean weight of experimental animals from week five to week eight is displayed in Table 2.

Table 2. Weight of experimental animals in the study.

Groups	**Basal wt (g)**	**2wk accl. wt (g)**	**Wk5.wt (g)**	**Wk6.wt (g)**	**Wk 7.wt (g)**	**Wk8.wt (g)**
Group 1	132.50± 3.23^{a}	194.00± 7.44^{ab}	156.25± 3.75^{abc}	156.50± 5.61^{ab}	182.50± 6.29^{bcde}	180.75± 6.10^{bcde}
Group 2	142.50± 4.79^{a}	218.00± 19.13^{b}	151.00± 4.51^{abc}	162.00± 3.39^{ab}	154.25± 4.05^{abc}	134.25± 2.17^{a}
Group 3	126.50± 3.12^{a}	191.00± 13.28^{ab}	155.50± 7.03^{abc}	148.75± 7.18^{a}	132.50± 6.29^{a}	136.25± 5.15^{a}
Group 4	125.00± 3.54^{a}	154.00± 2.27^{a}	132.50± 1.44^{a}	140.00± 2.04^{a}	142.00± 2.12^{a}	133.75± 2.39^{a}
Group 5	135.00± 5.40^{a}	189.50± 17.06^{ab}	141.75± 3.82^{ab}	137.50± 3.23^{a}	132.75± 2.75^{a}	132.25± 1.55^{a}
Group 6	131.25± 9.66^{a}	173.25± 5.68^{ab}	201.00± 14.15^{de}	201.75± 13.03^{cd}	208.75± 12.48^{ef}	211.25± 12.48^{ef}
Group 7	150.00± 12.25^{a}	182.75± 11.99^{ab}	212.75± 9.31^{e}	213.75± 8.75^{d}	217.50± 9.68^{f}	221.00± 8.78^{f}
Group 8	131.25± 6.57^{a}	179.00± 4.51^{ab}	205.75± 5.75^{de}	205.00± 5.00^{d}	204.25± 3.61^{ef}	198.25± 3.12^{ef}
Group 9	125.00± 3.54^{a}	175.00± 6.45^{ab}	147.50± 6.61^{ab}	147.25± 4.78^{a}	148.25± 3.71^{ab}	147.00± 2.38^{a}
Group 10	155.00± 2.89^{a}	186.75± 4.53^{ab}	153.00± 3.85^{abc}	151.25± 5.15^{a}	149.50± 4.94^{abc}	151.25± 4.53^{ab}
Group 11	147.50± 2.50^{a}	186.75± 2.49^{ab}	175.00± 8.50^{bcde}	165.25± 13.09^{abc}	161.25± 12.54^{abcd}	157.00± 11.21^{abc}
Group 12	155.00± 8.66^{a}	195.00± 11.78^{ab}	198.25± 1.18^{def}	190.75± 2.69^{bcd}	183.75± 2.39^{cdef}	189.75± 7.28^{cdef}
Group 13	142.00± 6.38^{a}	153.50± 6.81^{a}	164.50± 8.77^{abcd}	163.50± 9.74^{ab}	155.50± 3.33^{abc}	152.50± 2.63^{ab}
Group 14	145.00± 6.45^{a}	171.50± 9.18^{ab}	184.75± 7.85^{cdef}	190.75± 8.84^{bcd}	194.50± 8.58^{def}	192.75± 8.38^{def}
Group 15	152.50± 2.50^{a}	180.00± 5.80^{ab}	155.00± 4.08^{abc}	157.50± 4.79^{ab}	157.50± 6.91^{abc}	162.50± 6.61^{abcd}
F-value	3.17	2.67	13.78	11.63	16.74	20.81
P-value	0.335	0.006	0.001	0.001	0.001	0.001

Values are expressed in Mean±SEM. Mean that do not share a letter are significantly different ($P<0.05$), Group 1: control(0mg/kg), Group 2: 2mg/kg cadmium chloride, Group 3: 4mg/kg cadmium chloride, Group 4: 6mg/kg cadmium chloride, Group 5: 8mg/kg cadmium chloride, Group 6: 500mg/kg omega-3 fatty acid, Group 7: 1000mg/kg omega-3 fatty acid, Group 8: 2mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 9: 4mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 10: 6mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 11: 8mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 12: 2mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 13: 4mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 14: 6mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 15: 8mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, 2wk acclim.wt: 2 weeks acclimatization weight, Wk5.wt: Week 5 weight, Wk6.wt: Week 6 weight, Wk7.wt: Week 7 weight, Wk8.wt: Week 8 weight.

A one-way ANOVA with Tukey post hoc multiple comparison showed no significant differences between the groups ($p>0.05$, $F=3.17$, $P=0.335$). After two weeks of acclimatization, all groups showed a significant mean weight increase ($F=2.67$, $P=0.006$), with groups 2, 13, and 4 showing the largest gain. At week five, groups 6 (500 mg/kg omega-3 fatty acid), 7, 8 (2 mg/kg $CdCl_2$+500 mg/kg omega-3 fatty acid), and 12 had significantly higher mean weights than the control group ($p<0.05$). At week six, groups 6, 7, and 8 maintained significantly higher mean weights than the control ($p<0.05$). At week seven, groups 3 (4 mg/kg

$CdCl_2$), 4, and 5 had significantly lower mean weights than the control group ($p<0.05$). By week eight, groups 2, 3, 4, 5, and 9 had significantly lower mean weights ($p<0.05$) than the control.

Hematological parameter levels are displayed in Table 3.

Table 3. Haematological parameters of the study groups.

Groups	WBC ($x10^9$/L)	NEUT (%)	LYMP (%)	MON (%)	RBC (10^{12}/L)	HCT (%)	Hb (g/dl)
Group 1	7.43± 0.67^{c}	46.25± 2.39^{b}	48.88± 2.42^{abcd}	8.50± 1.19^{a}	4.63± 0.43^{cd}	47.50± 0.96^{e}	141.25± 4.15^{ef}
Group 2	2.08± 0.38^{a}	9.31± 4.49^{a}	52.93± 5.31^{bcd}	38.00± 1.41^{d}	2.48± 0.39^{abc}	24.00± 5.72^{abc}	85.00± 6.45^{bc}
Group 3	1.38± 0.63^{a}	4.53± 2.60^{a}	54.69± 2.09^{cde}	41.03± 1.76^{de}	0.99± 0.06^{ab}	16.25± 2.39^{ab}	82.50± 11.09^{abc}
Group 4	2.04± 0.42^{a}	6.18± 2.82^{a}	48.25± 1.49^{abcd}	45.75± 2.016^{e}	0.70± 0.12^{a}	16.25± 3.75^{ab}	60.00± 9.13^{ab}
Group 5	1.00± 0.61^{a}	3.53± 1.11^{a}	34.25± 2.33^{ab}	62.38± 1.63^{f}	0.85± 0.33^{ab}	11.25± 1.25^{a}	33.75± 3.75^{a}
Group 6	5.63± 0.89^{bc}	65.25± 3.47^{c}	37.43± 0.90^{abc}	7.50± 1.04^{a}	4.90± 0.39^{cd}	42.57± 2.69^{de}	108.75± 4.27^{bcde}
Group 7	8.45± 0.68^{c}	85.75± 2.29^{d}	35.84± 2.56^{abc}	9.85± 0.98^{ab}	5.75± 0.52^{cd}	49.00± 2.27^{e}	167.25± 4.15^{f}
Group 8	7.23± 1.09^{c}	52.00± 3.39^{bc}	31.25± 5.15^{a}	16.80± 2.00^{bc}	4.77± 0.49^{cd}	43.50± 1.55^{de}	100.00± 4.08^{bcde}
Group 9	0.95± 0.66^{a}	8.38± 2.67^{a}	75.83± 2.52^{f}	16.23± 0.66^{bc}	4.90± 0.42^{cd}	42.00± 0.82^{de}	125.00± 6.45^{cdef}
Group 10	1.70± 0.62^{a}	12.15± 3.13^{a}	73.93± 4.64^{ef}	14.10± 1.64^{abc}	3.97± 1.24^{abcd}	35.50± 5.87^{cde}	91.8± 7.95^{bcd}
Group 11	2.60± 0.96^{ab}	6.30± 0.73^{a}	76.25± 1.65^{f}	17.15± 1.04^{bc}	6.52± 1.90^{d}	29.70± 1.53^{bcd}	104.50± 20.37^{bcde}
Group 12	7.09± 0.65^{c}	55.10± 8.09^{bc}	80.00± 3.08^{f}	14.43± 1.02^{abc}	6.39± 0.87^{d}	44.75± 2.49^{de}	148.50± 16.68^{ef}
Group 13	2.00± 0.39^{a}	8.23± 2.62^{a}	75.73± 3.76^{f}	16.05± 1.19^{bc}	4.40± 0.42^{bcd}	45.50± 1.55^{cde}	137.06± 4.57^{def}
Group 14	1.65± 0.19^{a}	12.18± 3.96^{a}	73.25± 6.22^{ef}	14.09± 2.42^{abc}	4.42± 0.34^{bcd}	43.13± 4.94^{de}	129.53± 14.88^{cdef}
Group 15	2.00± 0.38^{a}	13.34± 5.38^{a}	63.74± 6.95^{def}	18.45± 0.46^{f}	2.67± 0.23^{abc}	35.84± 4.09^{cde}	168.75± 4.15^{f}
F-value	17.24	53.46	21.18	122.65	7.35	15.15	15.89
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

Values are expressed in Mean±SEM. Mean that do not share a letter are significantly different ($P<0.05$), Means that share a letter are non-significantly different ($P>0.05$), WBC: White blood cells, NEUT: Neutrophil, LYMP: Lymphocyte, RBC: Red blood cells, HCT: Hematocrit, Hb: Hemoglobin, MON: Monocyte, Group 1: control(0mg/kg), Group 2: 2mg/kg cadmium chloride, Group 3: 4mg/kg cadmium chloride, Group 4: 6mg/kg cadmium chloride, Group 5: 8mg/kg cadmium chloride, Group 6: 500mg/kg omega-3 fatty acid, Group 7: 1000mg/kg omega-3 fatty acid, Group 8: 2mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 9: 4mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 10: 6mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 11: 8mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 12: 2mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 13: 4mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 14: 6mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 15: 8mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid.

ANOVA with Tukey post hoc multiple comparison showed statistically significant differences ($p<0.05$). Groups 2, 3, 4, and 5 had significantly lower white blood cell (WBC) levels than the control, while groups 6, 7, 8, and 12 had significantly higher WBC levels ($p<0.05$) than groups 2–5. Neutrophil counts in groups 2–5 were significantly lower ($p<0.05$) than the control, whereas groups 6 and 7 had significantly higher neutrophil counts ($p<0.05$). Compared to groups 2–5, groups 8 and 12 had significantly higher neutrophil levels ($p<0.05$). Lymphocyte counts in groups 2–5 did not significantly differ ($p>0.05$) from the control, but

groups 9–14 showed a significant increase ($p<0.05$). Monocyte levels were significantly higher in groups 2–5 than in the control ($p<0.05$), but significantly lower in groups 6–14 compared to groups 2–5 ($p<0.05$). Groups 3–5 had significantly lower red blood cell (RBC) levels ($p<0.05$) than the control, while groups 6–9, 11, and 12 had significantly higher RBC levels than groups 3–5 ($p<0.05$). Haematocrit levels in groups 2–5 were significantly lower than the control ($p<0.05$), while groups 6–9, 12, and 14 had significantly higher haematocrit levels than groups 2–5 ($p<0.05$). Groups 2–5 had significantly lower haemoglobin levels than the control ($p<0.05$), while groups 7, 12, 13, and 15 had significantly higher haemoglobin levels than groups treated with cadmium chloride ($p<0.05$).

Table 4 displays additional hematological parameters.

Table 4. Haematological parameters of the study groups.

Groups	PLT (10^9/L)	MCH (pg)	MCHC (g/l)	MCV (fL)	RDWCV (%)	MPV (fL)
Group 1	112.24± 5.29^{ab}	41.00± 1.35^{d}	242.72± 21.03^{cd}	74.75± 9.14^{de}	15.00± 1.08^{a}	9.00± 0.91^{ab}
Group 2	320.00± 33.91^{a}	16.00± 2.16^{a}	335.00± 6.45^{bc}	76.50± 10.56^{e}	19.00± 3.19^{abc}	12.25± 2.78^{ab}
Group 3	85.00± 6.45^{a}	13.75± 1.75^{a}	277.50± 10.31^{a}	26.00± 2.27^{ab}	22.50± 1.76^{abc}	15.00± 2.61^{b}
Group 4	57.50± 15.48^{a}	11.63± 1.14^{a}	140.00± 15.81^{a}	22.75± 5.49^{ab}	35.75± 4.05^{c}	12.50± 1.94^{ab}
Group 5	85.00± 11.90^{a}	14.25± 0.85^{a}	125.00± 11.90^{a}	21.25± 4.27^{a}	33.25± 2.66^{bc}	9.25± 1.11^{ab}
Group 6	67.50± 20.16a^{b}	36.25± 2.39^{cd}	124.25± 9.20^{cd}	15.00± 4.36^{a}	14.50± 1.04^{a}	10.00± 0.41^{ab}
Group 7	282.50± 54.52^{ab}	40.50± 2.10^{d}	346.25± 7.47^{cd}	25.75± 2.17^{ab}	17.50± 1.04^{ab}	10.25± 0.85^{ab}
Group 8	271.25± 33.44^{ab}	23.25± 0.75^{abc}	355.00± 8.66^{bc}	22.50± 3.22^{a}	18.75± 0.48^{abc}	10.75± 1.89^{ab}
Group 9	325.00± 16.58^{ab}	20.25± 1.70^{ab}	301.25± 6.57^{b}	50.75± 0.48^{c}	19.00± 1.35^{abc}	7.75± 0.47^{a}
Group 10	195.00± 35.24^{ab}	23.00± 1.22^{ab}	250.00± 24.83^{bc}	51.25± 0.48^{cd}	18.50± 0.29^{abc}	10.75± 0.48^{ab}
Group 11	200.00± 17.79^{ab}	13.98± 4.04^{a}	283.75± 11.43^{cd}	51.73± 0.44^{cd}	24.25± 0.75^{abc}	6.97± 0.25^{a}
Group 12	338.00± 64.09^{b}	31.25± 4.73^{bcd}	358.00± 13.30^{d}	24.00± 0.91^{ab}	22.25± 1.25^{abc}	7.47± 0.22^{a}
Group 13	474.25± 134.96^{b}	26.05± 7.99^{abcd}	392.50± 17.25^{cd}	46.50± 6.76^{bc}	23.50± 1.71^{abc}	7.04± 0.10^{a}
Group 14	462.25± 78.87^{ab}	18.98± 0.79^{ab}	350.75± 14.35^{bc}	54.25± 1.25^{cde}	34.50± 11.51^{bc}	6.82± 0.30^{a}
Group 15	237.25± 86.68^{ab}	20.00± 0.99^{ab}	282.50± 11.81^{d}	51.00± 1.46^{cd}	29.25± 2.95^{abc}	6.67± 0.11^{a}
F-value	5.37	10.94	32.82	17.69	3.86	3.61
P-value	0.001	0.001	0.001	0.001	0.001	0.001

Values are expressed in Mean±SEM. Mean that do not share a letter are significantly different ($P<0.05$), Means that share a letter are non-significantly different ($P>0.05$), PLT: Platelets, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume, RDWCV: Red cell distribution coefficient of variation, MPV: Mean platelets volume, Group 1: control(0mg/kg), Group 2: 2mg/kg cadmium chloride, Group 3: 4mg/kg cadmium chloride, Group 4: 6mg/kg cadmium chloride, Group 5: 8mg/kg cadmium chloride, Group 6: 500mg/kg omega-3 fatty acid, Group 7: 1000mg/kg omega-3 fatty acid, Group 8: 2mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 9: 4mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 10: 6mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 11: 8mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 12: 2mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 13: 4mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 14: 6mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 15: 8mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid.

ANOVA with Tukey post hoc comparison showed significant differences ($p<0.05$). Platelet levels in groups 2–5 did not significantly differ from the control ($p>0.05$), but groups 12 and 13 showed a significant increase compared to groups 2–5 ($p<0.05$). Mean corpuscular haemoglobin (MCH) levels were significantly lower in groups 2–5 than in the control ($p<0.05$), while groups 6, 7, and 12 showed significantly higher MCH levels than groups 2–5 ($p<0.05$). Mean corpuscular haemoglobin concentration (MCHC) was significantly lower in groups 4 and 5 than in the control ($p<0.05$), but significantly higher in groups 7–9 and 12–15 than in groups 4 and 5 ($p<0.05$).

Table 5 presents percentage alternation and gonadosomatic index (GSI).

Table 5. Levels of gonadosomatic index and percentage alternation the study groups.

Groups	Gonadosomatic index (%)	Alternation (%)
Group 1	6.10 ± 0.09^{bc}	70.23 ± 1.19^{d}
Group 2	3.72 ± 0.25^{ab}	48.33 ± 5.53^{abcd}
Group 3	2.49 ± 0.38^{a}	32.44 ± 3.87^{abc}
Group 4	1.44 ± 0.26^{a}	24.99 ± 3.57^{ab}
Group 5	1.18 ± 0.13^{a}	22.17 ± 5.17^{a}
Group 6	6.01 ± 1.04^{bc}	59.52 ± 2.38^{cd}
Group 7	5.97 ± 0.33^{bc}	58.48 ± 5.22^{cd}
Group 8	10.63 ± 1.53^{d}	54.91 ± 4.21^{bcd}
Group 9	3.11 ± 0.22^{ab}	37.92 ± 10.21^{abc}
Group 10	2.89 ± 0.39^{ab}	37.49 ± 4.17^{abc}
Group 11	3.18 ± 0.71^{ab}	26.79 ± 3.56^{ab}
Group 12	8.55 ± 1.36^{cd}	69.99 ± 4.74^{d}
Group 13	3.22 ± 0.27^{ab}	20.83 ± 12.49^{a}
Group 14	3.29 ± 0.20^{ab}	29.16 ± 4.17^{abc}
Group 15	2.56 ± 0.26^{a}	45.50 ± 10.53^{abcd}
F-value	16.04	7.46
P-value	0.001	0.001

Values are expressed in Mean±SEM. Mean that do not share a letter are significantly different ($P<0.05$), Means that share a letter are non-significantly different ($P>0.05$), Group 1: control(0mg/kg), Group 2: 2mg/kg cadmium chloride, Group 3: 4mg/kg cadmium chloride, Group 4: 6mg/kg cadmium chloride, Group 5: 8mg/kg cadmium chloride, Group 6: 500mg/kg omega-3 fatty acid, Group 7: 1000mg/kg omega-3 fatty acid, Group 8: 2mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 9: 4mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group10: 6mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 11: 8mg/kg $CdCl_2$+500mg/kg omega-3 fatty acid, Group 12: 2mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 13: 4mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 14: 6mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid, Group 15: 8mg/kg $CdCl_2$+1000mg/kg omega-3 fatty acid.

ANOVA with Tukey post hoc comparison showed significant differences ($p<0.05$). Groups 3–5 had significantly lower GSI levels than the control ($p<0.05$), whereas groups 6–8 and 12 showed significantly higher GSI levels than groups 3–5 ($p<0.05$). Alternation levels in groups 3–5 was significantly lower than in the control ($p<0.05$), while group 12 showed significantly higher alternation levels than groups 3–5 ($p<0.05$).

Discussion

Cadmium (Cd) is a toxic, non-essential trace element that disrupts various physiological processes, including haematological parameters, body weight regulation, and reproductive health as measured by the gonadosomatic index (GSI). Cadmium chloride, a major environmental contaminant found in cigarette smoke, industrial processes, and food, is linked to health issues such as reproductive disorders, lung cancer, haematological disorders, and renal impairment (Wang, 2020). Its toxicity is due to its ability to accumulate in biological systems and its long half-life. Studies suggest that a diet rich in omega-3 fatty acids improves health and reduces chronic disease risk (Stanhiser *et al.,* 2022).

In this study, cadmium chloride exposure significantly ($p<0.05$) reduced body weight, GSI levels, haematological parameters (white blood cells, neutrophils, haemoglobin, and red blood cells), and cognitive function due to increased lipid peroxidation (MDA). Rats treated with cadmium chloride exhibited

substantial weight loss across study weeks, attributed to decreased food intake and increased energy expenditure, aligning with Zhang (2015). This weight loss was linked to cadmium-induced damage to vital organs such as the kidneys, liver, and endocrine glands. Cadmium reduces thyroid hormone synthesis, which regulates metabolism and energy balance (Wang, 2017), leading to metabolic dysfunction and weight loss. Additionally, cadmium crosses the blood-brain barrier, accumulates in the brain, and disrupts neurotransmitter signaling, affecting appetite control.

Cadmium exposure also induces oxidative stress by increasing reactive oxygen species (ROS) production and weakening antioxidant defenses, leading to damage in the kidneys, liver, and adipose tissue (Liu, 2019). It disrupts lipid metabolism by inhibiting lipogenesis and promoting lipolysis, causing body weight and adipose tissue mass reduction (Zhang, 2018). These findings align with prior research on cadmium's effects on weight (Abdo and Abdulla, 2013; Zhang, 2015). Conversely, omega-3 fatty acid treatment during weeks 4-6 led to significant weight gain ($p<0.05$). Omega-3 fatty acids have anti-inflammatory and anti-obesity properties, which aid in weight management. Wistar rats supplemented with omega-3 fatty acids exhibited significant weight gain compared to untreated animals, indicating a potential role in counteracting cadmium-induced weight loss (Jones, 2019). The weight gain mechanism may involve enhanced nutrient absorption and metabolism (Brown, 2018). Omega-3 fatty acids also modulate inflammation, reducing blood inflammatory markers linked to metabolic dysfunction (White, 2020). Their role in improving weight gain aligns with previous studies (Smith *et al.,* 2021; Brown, 2018; Jones, 2019; White, 2020).

The gonadosomatic index (GSI), an indicator of sexual maturity (Barber and Blake, 2006), was significantly lower ($p<0.05$) in cadmium-treated groups (3, 4, and 5) compared to the control. This reduction was attributed to cadmium-induced testicular damage. These findings align with previous studies showing that cadmium chloride causes testicular degeneration and weight loss (Ige *et al.,* 2012; Ekhoye *et al.,* 2013). Research has consistently shown cadmium's detrimental effects on testicular tissues, contributing to male infertility (Mendiola *et al.,* 2011; Akunna *et al.,* 2013; Khanna *et al.,* 2016). The testis is particularly vulnerable to cadmium toxicity, even at low doses (Blanco *et al.,* 2007). Both low and high doses of cadmium have been shown to impact testicular weight and morphology. Acute cadmium exposure reduces testicular weight due to necrotic and degenerative changes (Blanco *et al.,* 2007). Cadmium also impairs the tunica tissues of the testes, which are essential for healthy sperm production (Baker *et al.,* 2016). These findings are consistent with earlier studies (Biswas *et al.,* 2001; Yang *et al.,* 2006; Blanco *et al.,* 2007). Morphometric analysis showed that lower cadmium doses caused minor changes, while higher doses significantly reduced testis and epididymis weight, GSI, and seminiferous tubule (ST) length after 7 and 56 days (de Souza Predes *et al.,* 2010).

The current study observed that groups treated with cadmium chloride had significantly lower levels of white blood cells, neutrophils, red blood cells, haematocrit, haemoglobin, mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) ($p<0.05$) compared to the control group. Cadmium-induced oxidative stress, which can cause red blood cell death and hinder bone marrow function, was attributed to this decline in haematological markers. These findings are consistent with previous studies (Liu *et al.,* 2015), where Wistar rats exposed to cadmium showed a reduction in haemoglobin, haematocrit, and red blood cell counts. Beyond oxidative stress, cadmium exposure can disrupt the body's balance of essential minerals such as iron and zinc, which are necessary for red blood cell synthesis (Liu *et al.,* 2015; Wang, 2017). This disturbance in iron metabolism may worsen the decline in haematological markers in cadmium-exposed rats. Additionally, cadmium has been shown to impair the immune system, leading to immune dysfunction and inflammation. Li *et al.,* (2019) reported that rats exposed to cadmium had higher levels of pro-inflammatory cytokines and lower levels of anti-inflammatory cytokines, disrupting immune balance and further reducing haematological parameters. Inflammation linked to cadmium exposure can also impair red blood cell synthesis and function, exacerbating the decline in haematological markers.

The toxic effects of cadmium on haematopoietic organs such as the kidney, spleen, and liver may also contribute to these declines, as these organs are critical for blood cell production. Some researchers have suggested that anaemia due to cadmium exposure results from increased red blood cell destruction caused by altered membrane permeability, impaired erythropoiesis from direct metal toxicity, and defective iron metabolism due to mucosal lesions in the intestine (Kassebaum, 2020). This multifactorial explanation highlights the complex interplay between environmental factors, nutritional deficiencies, and physiological responses to cadmium exposure. Cadmium toxicity has also been linked to leukopenia, particularly in cases of severe liver failure. Similar effects were noted in lead-treated mice (Lee, 2004; Veena *et al.,* 2011; Lodia and Kansala, 2012). The marked reduction in haemoglobin levels, associated with a decline in MCHC,

suggests that red blood cells in cadmium-exposed rats are prone to microcytosis and hypochromia, characteristic of impaired haematopoiesis. High cadmium concentrations may inhibit haem production, further reducing MCH and MCHC levels (Veena *et al.,* 2011; Lodia and Kansala, 2012). This study's findings align with Angmo *et al.,* (2015), who reported a significant ($p<0.05$) decrease in MCH and MCHC, although Abdo and Abdulla, (2013) found no significant changes in cadmium-treated chickens. However, omega-3 fatty acid treatment significantly increased ($p<0.05$) red blood cell count, haemoglobin, and haematocrit in cadmium-exposed groups. Omega-3 fatty acids directly influence bone marrow function and stimulate erythropoietin, a hormone responsible for red blood cell production. This results in increased haemoglobin levels and improved red blood cell count. Additionally, omega-3 fatty acids possess anti-inflammatory properties, which help mitigate cadmium-induced oxidative stress and inflammation. Since inflammation and oxidative stress contribute to increased red blood cell destruction, omega-3 fatty acids may protect red blood cells and maintain higher haemoglobin levels (Kaur and Meena, 2019).

Regarding immune function, this study found a significant ($p<0.05$) decrease in neutrophil levels in cadmium-treated groups. This decline coincided with increased oxidative stress and inflammation, suggesting that cadmium disrupts immune cell balance. Angmo *et al.,* (2015) observed contrasting results, reporting a significant increase in neutrophils and a decrease in lymphocytes, while this study found a drop in neutrophils, a rise in monocytes, and no significant change in lymphocytes. The mechanism underlying these variations remains unclear, but physiological responses to cadmium toxicity may be involved. Cadmium reduces neutrophil counts by activating the NF-κB signaling pathway, which regulates genes associated with inflammation and immune response (Wang, 2017). NF-κB activation leads to upregulation of pro-inflammatory cytokines such as TNF-α and IL-6, which suppress neutrophil production and function. Cadmium also induces neutrophil apoptosis by activating caspase-3, a key enzyme in the apoptotic pathway. Additionally, cadmium alters the bone marrow microenvironment, where neutrophils are produced. It disrupts essential regulatory factors such as G-CSF and GM-CSF, which are necessary for neutrophil differentiation and maturation (Li *et al.,* 2019; Zhang and Wang, 2020). This alteration results in reduced neutrophil production, consistent with earlier research by Wang (2017).

Lymphocyte counts did not differ significantly between cadmium-treated groups and controls ($p>0.05$), possibly due to compensatory mechanisms. The body may counteract cadmium's effects by upregulating detoxifying proteins such as metallothionein and antioxidant enzymes (Jin *et al.,* 2019). Another explanation is that cytokine signalling maintains immune balance despite cadmium exposure. Cytokines regulate lymphocyte function, and cadmium-induced changes in cytokine production might trigger compensatory mechanisms that preserve lymphocyte count (Pathak and Khandelwal, 2008). The thymus, which plays a crucial role in T lymphocyte development, may also contribute to maintaining lymphocyte levels. Cadmium exposure has been shown to cause thymic atrophy, reducing T lymphocyte production (Wang, 2017). However, increased lymphocyte production in other immune organs, such as the spleen and lymph nodes, may compensate for this reduction. This could explain why some studies report cadmium-induced lymphocyte decline (Angmo *et al.,* 2015), while this study found no significant changes.

Interestingly, rats exposed to cadmium and treated with omega-3 fatty acids exhibited a significant increase in lymphocyte counts. The exact mechanism remains unclear, but omega-3 fatty acids are known to enhance immune function. They modulate cytokine production, counteract the immunosuppressive effects of cadmium, and promote the formation of immune cells, including lymphocytes (Jones, 2015). These effects likely contributed to the observed increase in lymphocyte count in omega-3-treated rats. This study found that Wistar rats exposed to cadmium chloride had significantly higher monocyte levels ($p<0.05$) than the control group. Several factors likely contributed to this increase, including oxidative stress, cadmium's toxic effects on the bone marrow, and immune activation. Cadmium disrupts immune function, leading to excessive monocyte production in response to inflammation or infection (Liu *et al.,* 2015). Due to its immunosuppressive properties, cadmium upsets immune cell balance, promoting monocyte proliferation (Smith *et al.,* 2018; Johnson and Smith, 2020).

Additionally, cadmium's pro-inflammatory properties may have influenced monocyte elevation. However, previous research suggests cadmium exposure reduces monocyte counts, a key immune cell type (Smith *et al.,* 2018). Interestingly, this study observed a more pronounced monocyte decline in rats treated with both cadmium and omega-3 fatty acids compared to cadmium-exposed rats alone. The interaction between omega-3 fatty acids and cadmium explains this effect. Cadmium induces oxidative stress and inflammation, which weaken immune responses (Jones *et al.,* 2017), while omega-3 fatty acids counteract these effects through their anti-inflammatory properties (Brown *et al.,* 2019). Omega-3 fatty acids may compete with

cadmium for binding sites on cells and enzymes, reducing cadmium toxicity (Smith *et al.,* 2018). Cadmium disrupts protein and enzyme function by binding to them, but omega-3 fatty acids may occupy these sites, preventing damage. Moreover, omega-3 fatty acids enhance the activity of antioxidant enzymes like catalase and superoxide dismutase, which protect cells from cadmium-induced oxidative damage (Brown *et al.,* 2019). This mechanism may explain why monocyte levels decreased more significantly in the omega-3-treated group than in cadmium-exposed rats alone.

Additionally, omega-3 fatty acids regulate inflammation-related gene expression (Jones *et al.,* 2017). They reduce the production of pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which increase during cadmium toxicity (Artiach, 2020). By suppressing these cytokines, omega-3 fatty acids prevent excessive monocyte activation and migration to inflammatory sites. This modulation of inflammation helps maintain immune homeostasis and has broad implications for reducing inflammation-related conditions.

Platelet levels were significantly higher ($p<0.05$) in groups treated with omega-3 fatty acids compared to cadmium-exposed groups, but there was no significant difference ($p>0.05$) between the cadmium-exposed and control groups. These findings suggest that cadmium alone does not drastically alter platelet counts. However, the combination of cadmium exposure and omega-3 fatty acids resulted in increased platelet levels. One explanation is omega-3 fatty acids' role in maintaining platelet function under cadmium-induced stress. Cadmium exposure leads to inflammation, which can elevate platelet activation and aggregation (Calder, 2017). It also triggers the production of reactive oxygen species (ROS), which cause oxidative damage to platelets. Omega-3 fatty acids neutralize ROS and protect platelets from oxidative stress (Mori, 2020).

Dietary omega-3 polyunsaturated fatty acids (PUFAs) enhance endogenous antioxidant enzyme activity, such as superoxide dismutase and glutathione peroxidase, thereby reducing oxidative stress (Mori and Beilin, 2020; Thorsdottir, 2020). These protective effects may explain why platelet counts were higher in rats treated with omega-3 fatty acids and cadmium compared to the control group. In addition to haematological effects, cadmium exposure significantly impaired learning and memory in Wistar rats. Rats treated with cadmium chloride (groups 3–5) exhibited significantly lower ($p<0.05$) percentage alternation scores in the Y-maze test, which measures cognitive function, compared to the control group. The primary causes of cognitive decline in cadmium-exposed rats include neuronal damage due to oxidative stress, structural brain abnormalities, and altered neurotransmitter levels. Cadmium-induced oxidative stress leads to increased malondialdehyde (MDA) levels and reduced antioxidant defenses, such as glutathione (GSH) and superoxide dismutase (SOD) (Adebiyi *et al,* 2022). This imbalance causes lipid peroxidation, which damages neuronal cell membranes and impairs neural function (Ojo *et al.,* 2023).

Cognitive deficits in cadmium-exposed rats were evident in longer escape latencies in the Y-maze test, indicating impaired spatial learning and memory retention. These results align with previous research showing that cadmium exposure disrupts hippocampal function, a brain region essential for memory (El-Tarras *et al.,* 2016). Furthermore, cadmium alters neurotransmitter levels, particularly dopamine and serotonin, which regulate mood and cognitive functions (Ojo *et al.,* 2023). Rats exposed to cadmium displayed behavioral changes, including anxiety and depression-like symptoms. In contrast, omega-3 fatty acids exert neuroprotective effects by enhancing antioxidant defenses, promoting neurogenesis, and improving synaptic plasticity. Docosahexaenoic acid (DHA), a key omega-3 component, supports neuronal health by facilitating neurite growth and synapse formation, essential for cognitive function (Zhao *et al.,* 2024).

Research suggests DHA enhances synaptic plasticity, leading to better memory retention (Zhou *et al.,* 2022). Omega-3 supplementation has been shown to improve cognitive performance following neurotoxic damage. Omega-3 fatty acids mitigate oxidative stress by lowering lipid peroxidation markers like MDA while increasing antioxidant enzyme activity (Sarikaya *et al.,* 2020). By restoring oxidative balance, omega-3 fatty acids preserve neuronal integrity and function. Rats treated with omega-3 fatty acids and cadmium showed improved cognitive performance in memory tests, including shorter escape latencies in the Morris Water Maze (MWM) test compared to cadmium-exposed rats alone (El-Tarras *et al.,* 2016). Additionally, omega-3 fatty acids positively influence neurotransmitter systems. They increase serotonin levels, which may reduce depressive behaviors linked to cadmium exposure (Ojo et al, 2023). This dual action-enhancing cognitive function and stabilizing mood-suggests that omega-3 supplementation could be a promising intervention for mitigating heavy metal neurotoxicity.

Conclusion

The results of this study indicated a significant risk of cadmium injury and omega-3 fatty acid supplementation could ameliorate this toxicity. It is reasonable to assume that the omega-3 fatty acid ameliorated the cadmium-induced impairment of hematological disorder, body weight alteration, gonadosomatic index and enhance spatial memory hence, possess gonadoprotective, neuroprotective, and haematoprotective properties. In order to avoid chronic illness brought on by exposure to cadmium, it is suggested that omega-3 fatty acids be included in the diet.

Declarations

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