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Research Article

Omega-3 Fatty Acids as a Novel Antioxidant Strategy Against Cadmium-induced Testicular Dysfunction: A Proteomic Approach

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Abstract

Background: Cadmium-induced reproductive toxicity significantly threatens male fertility, primarily through oxidative stress and alterations in testicular proteins. *Objective:* This study investigates omega-3 fatty acids as a potential therapeutic strategy to counteract cadmium-induced testicular damage. Methods Adult male Wistar rats were exposed to cadmium chloride, followed by oral omega-3 fatty acid supplementation. Testicular tissues were analyzed for oxidative stress markers (malondialdehyde, MDA), antioxidant enzyme activities (Superoxide dismutase (SOD), catalase (CAT), gluthanione peroxidase (GPx), and key reproductive proteins (acrosin, clusterin, osteopontin, annexin-A2, kallikrein-1) using) enzyme linked immunoassay (ELISA and spectrophotometry. Data were analyzed using appropriate statistical tools. *Results:* Omega-3 supplementation significantly ($p < 0.05$) reduced MDA levels, restored antioxidant enzyme activities, and preserved testicular protein integrity. Proteomic analysis revealed modulation of proteins involved in oxidative stress response, apoptosis, and spermatogenesis. *Conclusion:* These findings suggest that omega-3 fatty acids may serve as a promising therapeutic agent for mitigating cadmium-induced reproductive toxicity and preserving male fertility. Incorporating omega-3 into dietary interventions may offer an effective strategy against heavy metal-induced testicular damage.

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Keywords

Cadmium Exposure, Sperm Dysfunction, Testis, Histomorphology, Wistar Rat

1. Introduction

Male infertility is a growing global health concern, with environmental toxicants such as cadmium emerging as significant risk factors [1]. Furthermore, discrepancies in experimental models, exposure dosages, and analytical techniques have led to inconsistencies in reported findings, complicating the establishment of definitive causal mechanisms [2]. Although oxidative stress is a well-established contributor to male infertility, the specific molecular pathways through which cadmium induces reactive oxygen species (ROS) overproduction and cellular damage remain inadequately explored.

Cadmium (Cd) is a pervasive environmental pollutant introduced into ecosystems mainly through industrial activities, agricultural practices, and tobacco smoke. It bioaccumulates in vital organs, particularly the testes, raising serious concerns about male reproductive health. The mechanisms underlying Cd-induced testicular toxicity are multifaceted. Cadmium induces oxidative stress by stimulating excessive ROS generation, resulting in lipid peroxidation, glutathione depletion, and protein oxidation [3]. This oxidative imbalance leads to inflammation, apoptosis, and cellular damage, while simultaneously impairing antioxidant defenses by inhibiting key enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) [3, 4]. Additionally, cadmium interferes with cellular signaling pathways such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), which are responsible for regulating genes related to inflammation and apoptosis.

Moreover, cadmium disrupts the blood-testis barrier, damages seminiferous tubules, and impairs the functional integrity of Sertoli and Leydig cells. These changes result in endocrine disruption, oxidative injury to spermatozoa, germ cell apoptosis, and arrested spermatogenesis [5, 6]. Cadmium also impairs mitochondrial function, contributing to increased ROS production and apoptosis [7, 8]. Collectively, these pathologies result in reduced sperm quality, motility, and overall male fertility [9, 10].

Given the profound implications of cadmium toxicity on reproductive function, there is an urgent need to explore effective therapeutic strategies. Omega-3 fatty acids are well-known for their antioxidant and anti-inflammatory properties and have gained attention for their potential to improve male fertility. Research suggests that omega-3 supplementation enhances sperm motility, morphology, and fertility outcomes. However, the mechanisms by which omega-3 fatty acids mitigate cadmium-induced testicular toxicity have not been fully elucidated [6].

This study aims to evaluate the therapeutic potential of omega-3 fatty acids in ameliorating cadmium-induced reproductive toxicity. Using proteomic analysis and oxidative stress biomarkers, the study investigates how omega-3 fatty acids may protect testicular function and preserve male fertility in the context of heavy metal exposure.

2. Materials and Methods

2.1. Experimental Animals and Design

This controlled experiment was conducted in the animal house of the Department of Biochemistry, Joseph Ayo Babalola University, Ikeji-Arakeji, Osun State, Nigeria. A total of sixty (60) adult male Wistar rats weighing 120-155 g was procured from Samwill Animal House, Federal University of Technology, Akure, Ondo State.

The animals were housed in clean, well-ventilated cages under a 12-hour light/dark cycle at a consistent temperature of 23.1 ℃. They were fed commercial grower mash (Ewu Feeds and Flour Mills Ltd., Ewu, Edo State, Nigeria) and had ad libitum access to water. Following a two-week acclimatization period, the treatment lasted for eight weeks.

The rats were randomly assigned into fifteen (15) groups (n = 4 per group):

1) Group 1: Control (0 mg/kg of cadmium chloride; received only feed and distilled water)
2) Groups 2-5: Received cadmium chloride at 2, 4, 6, and 8 mg/kg body weight, respectively, twice weekly
3) Groups 6-7: Received omega-3 fatty acids at 500 mg/kg and 1000 mg/kg, respectively, twice weekly
4) Groups 8-11: Received 2, 4, 6, and 8 mg/kg cadmium chloride plus 500 mg/kg omega-3 fatty acids
5) Groups 12-15: Received 2, 4, 6, and 8 mg/kg cadmium chloride plus 1000 mg/kg omega-3 fatty acids

Twenty-four hours after the final administration, the rats were euthanized, and tissues were harvested for analysis following standard procedures.

2.2. Sample Size Determination

The minimum sample size required $(N) = \frac{2\,(Z\alpha + Z\,(1-\beta))^2\,S^2}{(\mu 1-\mu 2)^2}$

Where:

Zα = 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 [11]

$(\mu 1-\mu 2)^2$ = the mean differences in SOD level between the group =8.72

Calculation:

$$\frac{2\,(1.96+0.84)^2(4.36)^2}{(8.72)^2}$$

= 4 (approx.)

This yields a minimum of 4 rats per group.

2.3. Inclusion and Exclusion Criteria

Only adult male albino rats were included. Female rats and males younger than eight weeks were excluded from the study.

2.4. Sample Collection

At the end of the study, animals were euthanized via cervical dislocation. The testes and accessory sex glands were excised, cleaned, and weighed. The right testis was fixed in Bouin's solution for histological processing, while the left testis was homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) for oxidative stress and proteomic assays.

2.5. Materials and Methods

2.5.1. Semen Analysis

Each rat's cauda epididymis was excised, and the semen was placed in a sterile Petri dish. A drop of semen was smeared onto a glass slide for evaluation of sperm morphology, count, and motility following WHO guidelines [12].

2.5.2. Sperm Motility

Progressive sperm motility was assessed using Zemjanis's method [13]. A semen drop was placed on a slide, covered with a coverslip, and allowed to rest at room temperature. Observations were made under 10x and 40x objectives, and motility was graded on a 0-4 scale based on randomly selected high-power fields.

2.5.3. Sperm Count

Sperm concentration was determined using a hemocytometer. The cauda epididymis was minced in normal saline, and a 10 µL aliquot was loaded into a Neubauer chamber. Counting was performed under a light microscope.

2.5.4. Sperm Morphology and Acrosome Index

Semen was stained with Papanicolaou stain, air-dried, and observed at 100× magnification. Sperm with normal morphology were recorded [14]. Acrosomal integrity was assessed based on size, shape, and staining. The acrosome index (AI) was calculated as:

$$\text{Acrosomal index (\%)} = \frac{\text{spermatozoa with normal acrosomes X 100}}{\text{total number of spermatozoa evaluated}}$$

2.5.5. Oxidative Stress Markers and Antioxidant Enzymes

Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were assayed using spectrophotometric methods as described by Sinha (1972), Rotruck (1973), Marklund (1974), and Ohkawa (1979), respectively [15-18].

2.5.6. Testicular Protein Assay

Levels of acrosin, clusterin (CLU), annexin A2 (ANXA2), kallikrein-1 (KLK-1), and osteopontin (OPN) were determined using ELISA kits (Sunlong Biotech Co. Ltd., China), in accordance with manufacturer protocols.

2.5.7. Sperm Abnormality Indices

1. The teratozospermic Index is calculated as follows: TZI = {(h + m + t)}/{x}

Where:

1) h= number of spermatozoa with head abnormalities
2) m= number of spermatozoa with midpiece abnormalities
3) t = number of spermatozoa with tail abnormalities
4) x = total number of abnormal spermatozoa counted [12]

2. Sperm Deformity Index (SDI):

The Sperm Deformity Index is calculated using the formula:

SDI = {(h + m + t)}/{n}

Where:

1) h = number of spermatozoa with head abnormalities
2) m = number of spermatozoa with midpiece abnormalities
3) t = number of spermatozoa with tail abnormalities
4) n = total number of spermatozoa counted [12]

2.5.8. Gonadosomatic Index (GSI)

Gonadosomatic index was measured following method described by Amann, 1970.

Gonadosomatic index (GSI): Testicular weight / body weight ×100.

2.6. Ethical Approval

All experimental protocols were approved by the Ethics Committee of Joseph Ayo Babalola University (Protocol No. JABU/BCH/EA/05/22). Procedures adhered to the NIH

Guide for the Care and Use of Laboratory Animals.

2.7. Statistical Analysis

Data were expressed as mean ± SEM. Statistical analyses were conducted using SPSS version 26.0. Group differences were evaluated using one-way ANOVA with Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

3. Results

Cadmium chloride exposure resulted in a dose-dependent decline in sperm quality. Rats administered 6 mg/kg and 8 mg/kg of cadmium showed significantly reduced sperm counts, motility, and viability compared to the control group ($p < 0.05$, Table 1). Sperm morphology was notably disrupted at higher doses, although differences in non-viable cells, sluggish motility, and morphology were not significant in lower-dose groups.

Table 1. *Levels of Seminal Indices in Preliminary Study.*

Groups	Sperm Count (10^6/ml)	Active Motility (%)	Sluggish Motility (%)	Non viable (%)	Viable sperm cells (%)	Normal Morphology (%)	Abnormal Morph (%)
Group 1	118.25 ± 10.94^{b}	80.00 ± 7.35^{c}	11.25 ± 4.73^{a}	8.75 ± 3.14^{a}	91.25 ± 3.15^{b}	72.50 ± 4.79^{b}	27.50 ± 4.78^{a}
Group 2	82.25 ± 26.51^{ab}	40.00 ± 9.35^{b}	33.75 ± 6.25^{a}	26.25 ± 3.75^{a}	62.50 ± 9.68^{ab}	53.75 ± 4.27^{b}	46.25 ± 4.26^{a}
Group 3	79.00 ± 28.48^{ab}	22.50 ± 8.50^{ab}	25.00 ± 9.35^{a}	27.50 ± 9.68^{a}	52.25 ± 18.87^{ab}	38.75 ± 14.49^{ab}	36.25 ± 13.75^{a}
Group 4	62.00 ± 22.37^{a}	18.75 ± 7.46^{ab}	26.25 ± 10.68^{a}	30.00 ± 13.38^{a}	28.75 ± 9.66^{a}	35.00 ± 11.73^{ab}	40.00 ± 13.38^{a}
Group 5	60.25 ± 36.30^{a}	6.25 ± 4.73^{a}	25.00 ± 14.43^{a}	18.75 ± 11.25^{a}	13.75 ± 8.50^{a}	6.25 ± 3.75^{a}	58.33 ± 29.20^{a}
F- value	4.13	14.13	0.701	0.880	7.25	7.46	0.640
P-value	0.019	0.001	0.603	0.499	0.012	0.002	0.640

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, Referance ranges: Sperm count- 100-200million/ml, Active motility- 70-80%, Viability- >70%, Normal morphology- 80-90%, Volume- 1.5- 2.5ml, WBC $<10^6$/ml.

Conversely, omega-3 fatty acid supplementation significantly improved reproductive parameters. Rats treated with 500 mg/kg and 1000 mg/kg of omega-3 exhibited higher sperm counts and motility than cadmium-only groups ($p < 0.05$). Combined cadmium and omega-3 treatment showed partial to full restoration of sperm morphology and viability, indicating a protective role (Table 2).

Cadmium exposure notably increased head defects, particularly in rats exposed to 6 mg/kg and 8 mg/kg. These defects were significantly reduced in omega-3-treated groups, with the greatest improvement observed at 1000 mg/kg dosage (Table 3). No significant differences were found in midpiece and tail defects. The acrosomal index, which was reduced in cadmium-only groups, was restored in co-treated groups.

Sperm deformity index (SDI) and teratozoospermic index (TZI) were significantly elevated in cadmium groups, reflecting high abnormality rates. Both indices significantly decreased in omega-3 treated and co-treated animals, returning to near-normal values (Table 3).

Gonadosomatic index (GSI) was significantly reduced in all cadmium-treated groups, indicating testicular atrophy. Omega-3 treatment restored GSI values significantly, particularly in the co-treatment groups (Table 4).

MDA levels were elevated in cadmium groups, indicating increased lipid peroxidation. Antioxidant enzyme levels (SOD, GPx, CAT) were significantly decreased in these groups ($p < 0.05$). Omega-3 reversed these effects by lowering MDA and restoring enzyme activities, especially at higher doses or in co-treated animals (Table 5).

Cadmium exposure led to significant reductions in total protein, acrosin, clusterin, osteopontin, kallikrein-1, and annexin A2 levels. Omega-3 supplementation significantly upregulated the expression of these proteins, restoring them toward control levels. Co-treated groups showed more pronounced improvements than single-treatment groups (Table 6).

Positive correlations were observed between sperm quality indices (count, motility, viability, morphology) and reproductive proteins (acrosin, clusterin, osteopontin, KLK-1, ANXA2) ($p < 0.05$). These indices were negatively correlated with SDI and TZI, indicating a direct link between oxidative damage and sperm deformities (Tables 7-9).

Additionally, MDA negatively correlated with all repro-

ductive proteins, while antioxidant enzymes (SOD, GPx, CAT) positively correlated with sperm quality and proteomic expression. These relationships confirm the protective role of antioxidant defense systems in maintaining reproductive health (Table 10).

Table 2. *Levels of Seminal Indices in the Study Groups.*

Groups	SC (10^6/ml)	AM (%)	SM (%)	NVSC (%)	VSC (%)	NM (%)	ABM (%)
Group 1	254.50 ± 20.90^{bc}	68.75 ± 5.15^{bc}	13.75 ± 4.27^{ab}	17.50 ± 1.44^{b}	82.50 ± 1.44^{abcd}	61.25 ± 4.27^{b}	38.75 ± 4.27^{b}
Group 2	$100.00\pm8.16a^{b}$	28.75 ± 5.91^{a}	36.25 ± 5.54^{bcd}	32.50 ± 4.79^{c}	70.00 ± 9.13^{ab}	61.25 ± 5.15^{b}	38.75 ± 5.15^{b}
Group 3	114.00 ± 14.59^{ab}	25.00 ± 2.04^{a}	27.50 ± 4.79^{abcd}	47.50 ± 5.20^{d}	77.50 ± 7.50^{abcd}	32.50 ± 5.95^{b}	67.50 ± 5.95^{c}
Group 4	23.75 ± 5.54^{a}	17.50 ± 3.23^{a}	41.25 ± 4.27^{cd}	41.25 ± 1.25^{cd}	71.25 ± 5.15^{abc}	21.25 ± 3.75^{a}	78.75 ± 3.75^{c}
Group 5	23.75 ± 10.28^{a}	13.75 ± 2.39^{a}	45.00 ± 5.40^{c}	41.25 ± 4.73^{cd}	62.50 ± 4.79^{a}	13.75 ± 2.39^{a}	86.25 ± 2.39^{c}
Group 6	279.25 ± 21.75^{bc}	80.75 ± 4.15^{bc}	13.75 ± 5.15^{ab}	5.50 ± 1.66^{ab}	94.50 ± 1.66^{d}	72.50 ± 4.33^{bc}	27.50 ± 4.33^{ab}
Group 7	358.75 ± 44.18^{c}	90.00 ± 3.54^{c}	5.00 ± 2.04^{a}	5.00 ± 2.04^{ab}	95.00 ± 2.04^{d}	86.25 ± 5.54^{c}	13.75 ± 5.54^{a}
Group 8	173.00 ± 32.18^{abc}	80.00 ± 4.08^{bc}	11.25 ± 3.15^{ab}	8.76 ± 1.26^{ab}	88.75 ± 3.15^{bcd}	87.50 ± 3.23^{c}	12.50 ± 3.23^{a}
Group 9	69.25 ± 16.59^{ab}	68.75 ± 6.57^{bc}	22.50 ± 6.29^{abcd}	8.75 ± 1.24^{ab}	91.25 ± 1.25^{cd}	62.50 ± 4.79^{b}	37.50 ± 4.79^{b}
Group 10	219.00 ± 73.52^{abc}	76.25 ± 4.27^{bc}	15.00 ± 3.54^{ab}	8.77 ± 1.25^{ab}	91.25 ± 1.25^{cd}	65.00 ± 2.04^{b}	35.00 ± 2.04^{b}
group 11	171.00 ± 18.23^{abc}	63.75 ± 8.98^{b}	28.75 ± 9.66^{abcd}	7.50 ± 1.44^{ab}	92.50 ± 1.44^{d}	68.75 ± 3.75^{bc}	31.25 ± 3.75^{ab}
Group 12	366.58 ± 28.82^{c}	77.50 ± 4.33^{bc}	17.50 ± 4.33^{abc}	5.00 ± 0.01^{ab}	91.25 ± 3.75^{cd}	75.00 ± 2.89^{bc}	25.00 ± 2.89^{ab}
Group 13	122.00 ± 53.69^{ab}	75.00 ± 4.56^{bc}	17.50 ± 4.79^{abc}	7.50 ± 1.44^{ab}	92.45 ± 1.45^{d}	72.50 ± 3.23^{bc}	27.50 ± 3.23^{ab}
Group 14	192.50 ± 34.34^{abc}	83.75 ± 5.54^{bc}	8.75 ± 2.39^{a}	7.50 ± 3.23^{ab}	92.50 ± 3.23^{d}	72.50 ± 1.44^{bc}	27.50 ± 1.44^{ab}
Group 15	247.00 ± 97.69^{bc}	88.75 ± 6.25^{bc}	7.50 ± 4.33^{a}	2.50 ± 0.50^{a}	97.50 ± 2.50^{d}	87.50 ± 5.95^{c}	12.50 ± 5.95^{a}
F- value	17.24	28.59	6.26	34.05	7.03	30.38	30.39
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).

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, SC- Sperm count, AM- Active motility, SM-Sluggish motility, NVSC- Non viable sperm cell, VSC-Viable sperm cell, NM-Normal morphology, ABM- Abnormal morphology.

Table 3. *Levels of Structural Defect in Sperm Cells in the Study Groups.*

Groups	HD (%)	ND (%)	TD%)	AI (%)	SDI (%)	TZI	Volume (ml)
Group 1	17.50 ± 4.33^{abc}	11.25 ± 1.25^{abcd}	10.00 ± 0.01^{abc}	82.50 ± 4.33^{bc}	0.39 ± 0.04^{b}	0.22 ± 0.04^{ab}	3.00 ± 0.54^{ab}
Group 2	27.50 ± 3.23^{cd}	2.50 ± 1.44^{a}	8.75 ± 2.39^{abc}	72.50 ± 3.23^{bc}	0.38 ± 0.05^{b}	0.22 ± 0.04^{ab}	1.27 ± 0.26^{ab}
Group 3	37.50 ± 5.95^{d}	13.75 ± 2.39^{bcd}	16.25 ± 4.73^{bc}	62.50 ± 5.95^{abc}	0.68 ± 0.06^{c}	0.81 ± 0.21^{bc}	0.88 ± 0.24^{a}
Group 4	40.00 ± 4.08^{d}	18.75 ± 1.25^{d}	20.00 ± 2.89^{c}	60.00 ± 4.08^{ab}	0.79 ± 0.04^{c}	1.38 ± 0.29^{c}	0.80 ± 0.32^{a}
Group 5	67.50 ± 4.79^{e}	16.25 ± 3.75^{cd}	2.50 ± 1.44^{a}	32.50 ± 4.79^{a}	0.86 ± 0.02^{c}	2.30 ± 0.42^{d}	1.88 ± 0.77^{ab}
Group 6	12.50 ± 2.50^{abc}	$5.00\pm.01^{ab}$	7.50 ± 3.23^{ab}	87.50 ± 2.50^{bc}	0.28 ± 0.04^{ab}	0.13 ± 0.03^{ab}	3.50 ± 0.71^{b}

Groups	HD (%)	ND (%)	TD%	AI (%)	SDI (%)	TZI	Volume (ml)
Group 7	5.00 ± 2.04^{a}	3.75 ± 1.25^{a}	6.25 ± 2.39^{ab}	70.00 ± 23.36^{bc}	0.14 ± 0.06^{a}	0.06 ± 0.25^{a}	3.65 ± 0.75^{b}
Group 8	4.25 ± 0.75^{a}	2.50 ± 1.44^{a}	5.75 ± 2.17^{ab}	95.75 ± 0.75^{c}	0.13 ± 0.03^{a}	0.05 ± 0.01^{a}	2.40 ± 0.33^{ab}
Group 9	25.00 ± 6.45^{bcd}	6.25 ± 1.25^{ab}	6.25 ± 1.25^{ab}	75.00 ± 6.45^{bc}	0.38 ± 0.05^{b}	0.21 ± 0.04^{ab}	2.28 ± 0.30^{ab}
Group 10	16.25 ± 2.39^{abc}	10.00 ± 0.01^{abcd}	8.75 ± 1.25^{abc}	83.75 ± 2.39^{bc}	0.35 ± 0.02^{b}	0.18 ± 0.02^{ab}	3.13 ± 0.32^{ab}
Group 11	6.25 ± 1.25^{a}	13.75 ± 2.39^{bcd}	11.25 ± 3.15^{abc}	93.75 ± 1.25^{bc}	0.31 ± 0.04^{ab}	0.16 ± 0.03^{ab}	2.70 ± 0.12^{ab}
Group 12	7.50 ± 1.44^{ab}	10.00 ± 0.01^{abcd}	7.50 ± 1.44^{ab}	92.50 ± 1.44^{bc}	0.25 ± 0.03^{ab}	0.11 ± 0.02^{ab}	2.78 ± 0.48^{ab}
Group 13	8.75 ± 2.39^{ab}	11.25 ± 1.25^{abcd}	7.50 ± 1.46^{ab}	91.25 ± 2.39^{bc}	0.28 ± 0.03^{ab}	0.13 ± 0.02^{ab}	2.76 ± 0.47^{ab}
Group 14	8.75 ± 2.39^{ab}	8.75 ± 1.25^{abc}	10.00 ± 0.02^{ab}	91.25 ± 2.38^{bc}	0.28 ± 0.01^{ab}	0.13 ± 0.01^{ab}	3.15 ± 0.65^{ab}
Group 15	5.00 ± 2.04^{a}	7.50 ± 3.23^{abc}	2.50 ± 1.45^{a}	95.00 ± 2.04^{bc}	0.13 ± 0.06^{a}	0.06 ± 0.03^{a}	3.35 ± 0.39^{b}
F- value	25.98	7.37	4.04	6.34	30.38	19.14	3.56
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 ($P>0.05$).

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, HD- Head defect, ND-Neck defect, TD-Tail defect, AI-Acrosomal index, SDI-Sperm deformity index, TZI-Teratozospemic index.

Table 4. *Levels of Gonadosomatic Index in the Study Groups.*

Groups	Gonadosomatic index (%)
Group 1	6.10 ± 0.09^{bc}
Group 2	3.72 ± 0.25^{ab}
Group 3	2.49 ± 0.38^{a}
Group 4	1.44 ± 0.26^{a}
Group 5	1.18 ± 0.13^{a}
Group 6	6.01 ± 1.04^{bc}
Group 7	5.97 ± 0.33^{bc}
Group 8	10.63 ± 1.53^{d}
Group 9	3.11 ± 0.22^{ab}
Group 10	2.89 ± 0.39^{ab}
group 11	3.18 ± 0.71^{ab}
Group 12	8.55 ± 1.36^{cd}
Group 13	3.22 ± 0.27^{ab}
Group 14	3.29 ± 0.20^{ab}
Group 15	2.56 ± 0.26^{a}
F- value	16.04

Groups	Gonadosomatic index (%)
P-value	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, 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.

Table 5. *Levels of Oxidative Stress Marker and Antioxidant Enzymes in the Study Group.*

Groups	MDA (Umol/gProtein	SOD (U/mg protein)	GPX (U/mg protein)	CAT (Umol/H202/min/mg protein
Group 1	0.33 ± 0.19^{ab}	20.24 ± 5.19^{c}	209.50 ± 59.73^{bc}	1245.45 ± 349.39^{c}
Group 2	3.18 ± 0.38^{c}	1.90 ± 0.25^{a}	30.39 ± 10.71^{a}	92.08 ± 8.04^{ab}
Group 3	2.55 ± 0.51^{c}	1.17 ± 0.12^{a}	13.39 ± 1.22^{a}	56.23 ± 17.14^{ab}
Group 4	2.39 ± 0.82^{bc}	0.80 ± 0.25^{a}	13.93 ± 3.16^{a}	25.19 ± 7.30^{a}
Group 5	5.99 ± 0.82^{d}	0.60 ± 0.19^{a}	12.56 ± 3.72^{a}	28.50 ± 6.26^{a}
Group 6	0.15 ± 0.09^{a}	11.14 ± 0.72^{abc}	184.59 ± 53.58^{abc}	1047.95 ± 318.23^{bcd}
Group 7	0.09 ± 0.05^{a}	19.22 ± 5.89^{c}	188.41 ± 36.96^{abc}	1229.98 ± 343.91^{c}
Group 8	0.14 ± 0.07^{a}	18.58 ± 2.76^{bc}	275.60 ± 46.99^{c}	1086.39 ± 309.51^{cd}
Group 9	1.28 ± 0.55^{abc}	13.21 ± 4.49^{abc}	179.04 ± 74.55^{abc}	$130.43\pm28.15ab^{c}$
Group 10	1.23 ± 0.19^{abc}	10.80 ± 6.40^{abc}	119.37 ± 19.66^{abc}	68.28 ± 12.18^{ab}
group 11	1.60 ± 0.46^{abc}	2.45 ± 1.06^{ab}	54.36 ± 25.29^{ab}	77.81 ± 16.88^{ab}
Group 12	0.19 ± 0.09^{a}	15.81 ± 1.79^{abc}	277.99 ± 26.12^{c}	1273.62 ± 315.53^{c}
Group 13	1.07 ± 0.24^{abc}	10.43 ± 2.77^{abc}	133.73 ± 13.15^{abc}	628.03 ± 120.96^{abcd}
Group 14	1.23 ± 0.32^{abc}	8.69 ± 2.68^{abc}	133.84 ± 22.58^{abc}	700.61 ± 74.54^{abcd}
Group 15	1.57 ± 0.32^{abc}	7.38 ± 3.26^{abc}	125.80 ± 14.06^{abc}	430.51 ± 129.67^{abcd}
F- value	13.81	4.58	6.73	6.85
P-value	0.001	0.001	0.001SS	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 Cdcl2 + 500mg/kg omega-3 fatty acid, Group 9- 4mg/kg Cdcl2 + 500mg/kg omega-3 fatty acid, Group 10- 6mg/kg Cdcl2 + 500mg/kg omega- 3 fatty acid, Group 11- 8mg/kg Cdcl2 + 500mg/kg omega-3 fatty acid, Group 12- 2mg/kg Cdcl2 + 1000mg/kg omega-3 fatty acid, Group 13- 4mg/kg Cdcl2 + 1000mg/kg omega-3 fatty acid, Group 14- 6mg/kg Cdcl2 + 1000mg/kg omega-3 fatty acid, Group 15- 8mg/kg Cdcl2 + 1000mg/kg omega-3 fatty acid,), MDA- Malondialdehye, SOD- Superoxide dismutase, GPX-Glutathione peroxidase, CAT-Catalase.

Table 6. *Levels of Protein and Selected Seminal Proteomes.*

Groups	Protein (mg)	Acrosin (pg/ml)	CLU (ng/ml)	KLK-1 (pg/ml)	OPN (ng/ml)	ANXA2 (pg/ml)
Group 1	5.84±1.05^{cde}	1200.00±204.12^{cd}	90.50±2.90^{bcd}	987.50±82.60^{c}	31.93±2.36^{cd}	307.28±52.73^{cde}
Group 2	0.94±0.13^{ab}	587.50±106.80^{ab}	29.25±4.03^{ab}	375.00±52.04^{ab}	10.76±2.08a	98.98±20.95^{abcd}
Group 3	0.16±0.09^{a}	212.50±77.39^{a}	15.00±2.04^{a}	120.00±40.82^{a}	15.44±2.04^{ab}	50.18±17.69^{a}
Group 4	0.74±0.22^{a}	81.25±46.99^{a}	10.00±1.87^{a}	50.00±18.71^{a}	13.73±3.63^{ab}	50.05±4.07^{a}
Group 5	0.32±0.09^{a}	40.25±24.68^{a}	3.00±1.78^{a}	8.75±5.09^{a}	10.24±3.49^{a}	44.18±13.03^{a}
Group 6	6.40±1.19^{de}	1500.00±204.12^{de}	152.63±12.20^{de}	1412.50±82.60^{d}	47.58±3.78^{e}	325.10±66.40^{de}
Group 7	8.76±0.76^{e}	1900.00±212.13^{e}	212.75±31.90^{e}	1862.50±224.88^{e}	48.87±3.99^{e}	332.75±102.46^{de}
Group 8	6.82±0.67^{de}	875.00±52.04^{bc}	130.44±26.25^{d}	625.00±87.79^{bc}	43.99±4.48^{de}	358.82±69.87^{e}
Group 9	1.14±0.41^{ab}	600.00±45.64^{ab}	35.00±6.77^{ab}	205.00±69.10^{a}	16.57±3.04^{ab}	296.95±57.62^{bcde}
Group 10	2.09±0.93^{ab}	89.50±40.58^{a}	13.00±2.65^{a}	53.75±19.83^{a}	9.09±3.11^{a}	84.38±7.35^{abc}
group 11	2.36±0.98^{abc}	39.50±20.84^{a}	7.00±0.91^{a}	11.25±6.57^{a}	9.23±1.55^{a}	65.84±10.36^{ab}
Group 12	6.72±1.02^{de}	1375.00±125.00^{cde}	106.75±14.64^{cd}	912.50±59.07^{c}	45.99±2.59^{de}	334.19±71.68^{de}
Group 13	2.72±0.53^{abc}	912.50±42.69^{bc}	66.25±13.13^{abc}	362.50±121.41^{ab}	21.52±2.41^{abc}	73.37±23.48^{abc}
Group 14	4.47±0.63^{bcd}	235.00±114.35^{a}	17.00±2.79^{a}	65.00±22.17^{a}	26.86±1.06^{bc}	65.17±16.19^{ab}
Group 15	2.33±0.67^{abc}	37.75±16.11^{a}	9.50±0.96^{a}	13.75±8.00^{a}	19.64±2.76^{abc}	81.45±11.90^{abc}
F- value	15.18	30.84	26.91	49.74	25.78	7.90
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).

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, CLU-Clusterin, KLK-1-Kallikrein-1, OPN-Osteopontin, ANXA2- Annexin A2.

Table 7. *Correlation Between Selected Proteomes and Seminal Indices.*

Parameters	r- value	p-value	Parameters	r- value	p-value
Acrosin/sperm count	0.502	0.001	Osteopontin/sperm count	0.579	0.001
Acrosin/Active motility	0.422	0.001	Osteopontin/Active motility	0.516	0.001
Acrosin/viable sperm cells	0.250	0.054	Osteopontin/viable sperm cells	0.328	0.010
Acrosin/normal morphology	0.441	0.001	Osteopontin/normal morphology	0.496	0.001
Acrosin/acrosomal index	0.202	0.121	Osteopontin/acrosomal index	0.301	0.020
Acrosin/sperm deformity index	-0.441	0.001	Osteopontin/sperm deformity index	-0.496	0.001
Acrosin/Teratozoospermic index	-0.388	0.002	Osteopontin/Teratozoospermic index	-0.348	0.006
Clusterin/sperm count	0.565	0.001	Annexin A2 /sperm count	0.414	0.001
Clusterin/Active motility	0.476	0.001	Annexin A2/Active motility	0.354	0.005
Clusterin/viable sperm cells	0.325	0.011	Annexin A2/viable sperm cells	0.236	0.070

Parameters	r- value	p-value	Parameters	r- value	p-value
Clusterin/normal morphology	0.485	0.001	Annexin A2/normal morphology	0.380	0.003
Clusterin/acrosomal index	0.143	0.276	Annexin A2/acrosomal index	0.275	0.034
Clusterin/sperm deformity index	-0.485	0.001	Annexin A2/sperm deformity index	-0.380	0.003
Clusterin/Teratozoospermic index	-0.361	0.005	Annexin A2/Teratozoospermic index	-0.556	0.001
Kallikrein-1/sperm count	0.566	0.001			
Kallikrein-1/Active motility	0.401	0.001			
Kallikrein-1/viable sperm cells	0.252	0.052			
Kallikrein-1/normal morphology	0.407	0.001			
Kallikrein -1/acrosomal index	0.172	0.189			
Kallikrein-1/sperm deformity index	-0.407	0.001			
Kallikrein-1/Teratozoospermic index	-0.330	0.010			

r- Pearson correlation, P<0.05- Significant, P>0.05- Non-Significant.

Table 8. *Correlation Between Oxidative Stress Marker, Antioxidant Enzymes and Seminal Indices.*

Parameters	r- value	p-value	Parameters	r- value	p-value
MDA/sperm count	-0.591	0.001	GPX/sperm count	0.444	0.001
MDA/Active motility	-0.680	0.001	GPX/Active motility	0.594	0.001
MDA/viable sperm cells	-0.622	0.001	GPX/viable sperm cells	0.421	0.001
MDA/normal morphology	-0.667	0.001	GPX/normal morphology	0.582	0.001
MDA/acrosomal index	-0.554	0.001	GPX/acrosomal index	0.359	0.005
MDA/sperm deformity index	0.667	0.001	GPX/sperm deformity index	-0.582	0.001
MDA/Teratozoospermic index	0.687	0.001	GPX/Teratozoospermic index	-0.475	0.001
SOD/sperm count	0.418	0.001	CAT/sperm count	0.516	0.001
SOD/Active motility	0.513	0.001	CAT/Active motility	0.540	0.001
SOD/viable sperm cells	0.381	0.003	CAT/viable sperm cells	0.341	0.008
SOD/normal morphology	0.478	0.001	CAT/normal morphology	0.519	0.001
SOD/acrosomal index	0.092	0.486	CAT/acrosomal index	0.247	0.057
SOD/sperm deformity index	-0.478	0.001	CAT/sperm deformity index	-0.519	0.001
SOD/Teratozoospermic index	-0.410	0.001	CAT/Teratozoospermic index	-0.386	0.002

r- Pearson correlation, P<0.05- Significant, P>0.05- Non-Significant, MDA- Malonnaldehye, SOD- Superoxide dismutase, GPX-Glutathione peroxidase, CAT-Catalase.

Table 9. *Correlation Between Spermogenic Index and Seminal Indices.*

Parameters	r- value	p-value
Acrosomal index/sperm count	0.408	0.001
Acrosomal index/Active motility	0.640	0.001

Parameters	r- value	p-value
Acrosomal index/viable sperm cells	0.574	0.001
Acrosmal index/normal morphology	0.667	0.001
Sperm deformity index/sperm count	-0.606	0.001
Sperm deformity index/Active motility	-0.874	0.001
Sperm deformity index/viable sperm cells	-0.715	0.001
Sperm deformity index/normal morphology	-1.000	0.001
Teratozospermic index/sperm count	-0.493	0.001
Teratozospermic index/Active motility	-0.733	0.003
Teratozospermic index/viable sperm cells	-0.628	0.001
Teratozospermic index/normal morphology	-0.862	0.001

r-Pearson's correlation, P<0.05- Significant, P>0.05- Non-Significant.

Table 10. *Correlation Between Oxidative Stress Marker, Antioxidant Enzymes and Selected Proteomes.*

Parameters	r- value	p-value
MDA/acrosin	-0.530	0.001
MDA/clusterin	-0.535	0.001
MDA/kallikrein-1	-0.507	0.001
MDA/osteopontin	-0.602	0.001
GPX/acrosin	0.492	0.001
GPX/clusterin	0.561	0.001
GPX/kallikrein-1	0.452	0.001
GPX/osteopontin	0.639	0.001
SOD/acrosin	0.543	0.001
SOD/clusterin	0.572	0.001
SOD/kallikrein-1	0.491	0.001
SOD/osteopontin	0.536	0.001
CAT/acrosin	0.716	0.001
CAT/clusterin	0.708	0.001
CAT/kallikrein-1	0.634	0.001
CAT/osteopontin	0.686	0.001

r- Pearson's correlation, P<0.05- Significant, P>0.05- Non-Significant.

4. Discussion

Infertility is a major global health concern, often associated with impaired semen quality [12]. Among various environmental pollutants, cadmium (Cd), a heavy metal, is recognized for its adverse effects on male reproductive health through oxidative stress and testicular toxicity [19]. Conversely, omega-3 fatty acids-particularly EPA and DHA-possess established antioxidant and anti-inflammatory properties and have been proposed as potential therapeutic agents against toxicants like cadmium [20, 21]. This study investigated the protective effects of omega-3 fatty acids against Cd-induced testicular toxicity in adult male Wistar rats.

Cadmium exposure significantly decreased the gonadosomatic index (GSI), indicating testicular atrophy. Histopathological findings revealed severe testicular damage, including necrosis, hemorrhage, and disrupted seminiferous tubules. These results corroborate previous studies showing that cadmium disrupts testicular morphology and reduces organ weight [22-24]. The dose-dependent impact of cadmium further supports its deleterious role in spermatogenesis [19, 25].

Biochemical assays revealed that cadmium elevated malondialdehyde (MDA) levels and suppressed antioxidant enzymes-SOD, GPx, and CAT-thereby disrupting redox homeostasis. This oxidative imbalance contributes to apoptosis, impaired spermatogenesis, and cellular damage [1, 26]. Such enzymatic reductions are also common in infertile males [27, 28].

Rats supplemented with omega-3 fatty acids showed marked improvement in sperm count, motility, morphology, and viability. These findings align with previous reports showing that omega-3 enhances male fertility [29-31]. The improvement may be attributed to reduced lipid peroxidation, restored antioxidant enzymes, and improved testicular function [32]. Histological evidence supported these results, showing reduced tissue damage and restored spermatogenesis in omega-3 treated rats.

Proteomic markers were also evaluated. Clusterin, a pro-

tein critical for sperm maturation and antioxidative defense, was significantly reduced in cadmium-treated rats but restored with omega-3 supplementation. Similar patterns were observed for annexin A2 (ANXA2), a marker of sperm motility and calcium regulation [33-35]. Omega-3 treatment enhanced ANXA2 expression, supporting its role in spermatogenesis.

Osteopontin (OPN), involved in acrosome reaction and sperm-egg binding, was downregulated by cadmium but restored by omega-3, suggesting improved fertilization potential [36-38]. Kallikrein-1 (KLK-1), which aids in sperm capacitation and seminal liquefaction, was similarly improved by omega-3 [39, 40]. Lastly, acrosin, essential for sperm penetration of the zona pellucida, was significantly increased following omega-3 supplementation, reinforcing its therapeutic role [41].

5. Conclusion

This study demonstrates that cadmium chloride has significant detrimental effects on male reproductive health, including oxidative stress, reduced sperm quality, testicular degeneration, and altered reproductive protein expression. Omega-3 fatty acid supplementation significantly mitigated these effects, enhancing antioxidant enzyme activity, improving sperm quality, and restoring testicular protein integrity. The observed restoration of key molecular markers-clusterin, ANXA2, osteopontin, KLK-1, and acrosin-underscores the protective effect of omega-3 fatty acids. These findings support the use of omega-3 supplementation as a potential therapeutic strategy for mitigating heavy metal-induced male infertility.

Abbreviations

SC	Sperm Count
AM	Active motility
SM	Sluggish Motility
NVSC	Non Viable Sperm Cell
VSC	Viable Sperm Cell
NM	Normal Morphology
ABM	Abnormal Morphology
SOD	Superoxide Dismutase
CAT	Catalase
GPx	Gluthanione Peroxidase
CLU	Clusterin
OPN	Osteopontin
ANXA2	Annexin-a2
KLK-1	Kallikrein-1
Cd	Cadmium
NF-κB	Nuclear Factor-kappa b
AP-1	Activator Protein-1
ELISA	Enzyme Linked Immunoassay
TZI	Teratozospermic Index
SDI	Sperm Deformity Index
Sem	Standard Error of Mean
ANOVA	Analysis of Variance

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Author Contributions

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Babatunde Ishola Gabriel Adejumo: Methodology, investigation, data collection

Kelly Iria Esezobor: Methodology, investigation, data collection

Emmanuel Onosetale Afeikhena: Methodology, investigation, data collection

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Juliana Edusola Olaniyan: Validation, formal analysis, and data curation

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Aigbokhan Akhere Caleb: Writing-review and editing

All authors read and approved the final manuscript.

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Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Appendix: Histological Photomicrograph

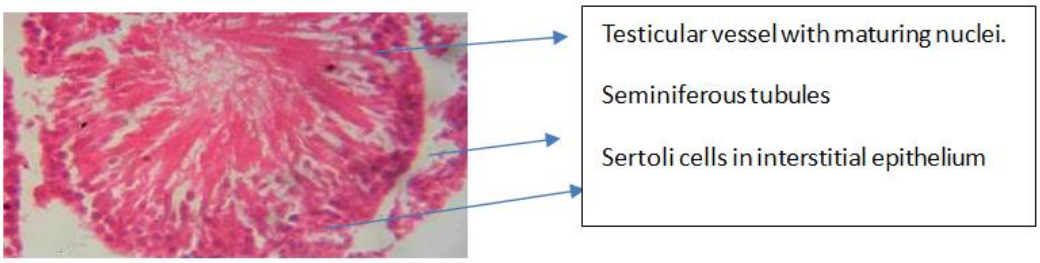


Figure 1. *Testes of Rat (Control). A well-formed testicular vessel consisting of numerous seminiferous tubules embedded in matured interstitial tissues. The tissue is well lined by columnar epithelium of the Sertoli cells consisting of almost visible germ cells lined up in the tubular epithelium. The interstitial tissues consist of matured clusters of Leydig cells (H&E x 100).*

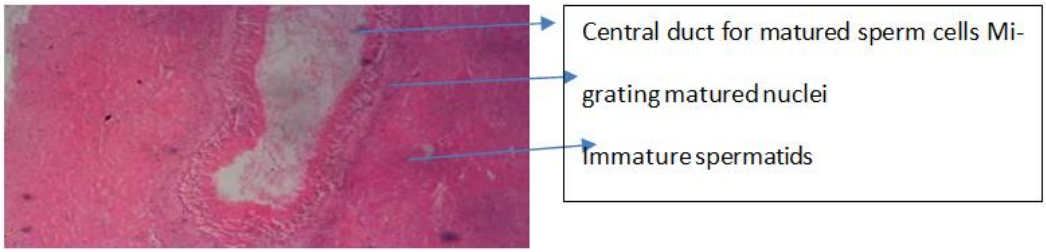


Figure 2. *Epididymis of Rat (Control): Showing full single epididymis with a central duct canal and a lumen with clusters of spermatozoa. The small dense head tapers into the vas deferens (H&E x 100).*

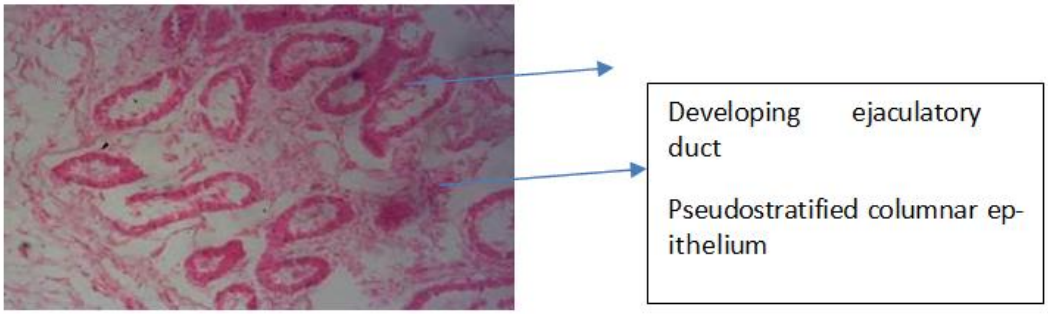


Figure 3. *Vas deferens of Rat (Control): Showing forming ejaculatory ducts lined by lamina propria terminating in the pseudostratified columnar epithelium (H&E x 100).*

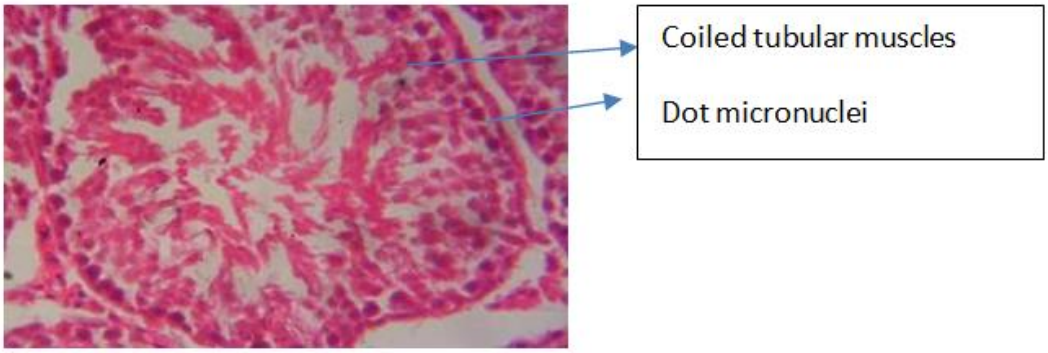


Figure 4. *Seminal vesicle of Rat (Control): Showing well formed with coiled tubular muscle walls. Maturing sperm cells appear with dot micronuclei (H&E x 100).*

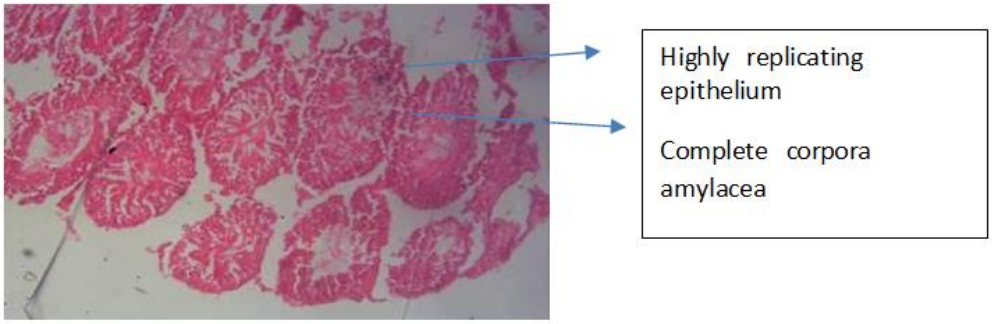


Figure 5. *Prostate of Rat (Control): Showing well-formed highly replicating prostate epithelium that is highly lined columnar and secretory cells. Also, glandular lumen contains adequate corpora amylacea (H&E x 100).*

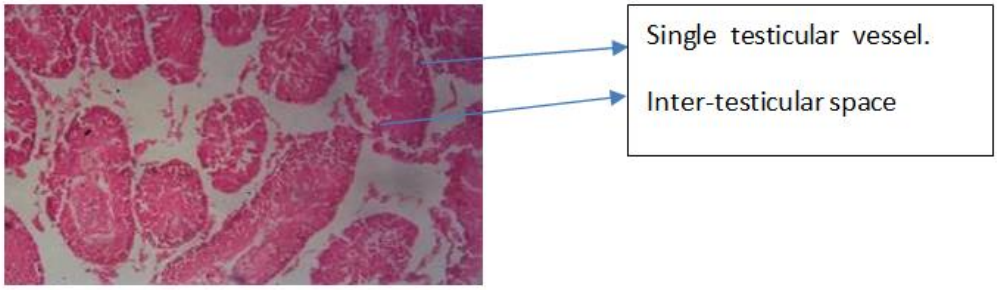


Figure 6. *Testes of Rat given 2mg/kg of cadmium chloride. It consists of several columnar epithelium separated from each other and they are less visible in maturity and function. Sertoli are poorly lined and interspersed in the individual rete testis. (H&E x 100).*

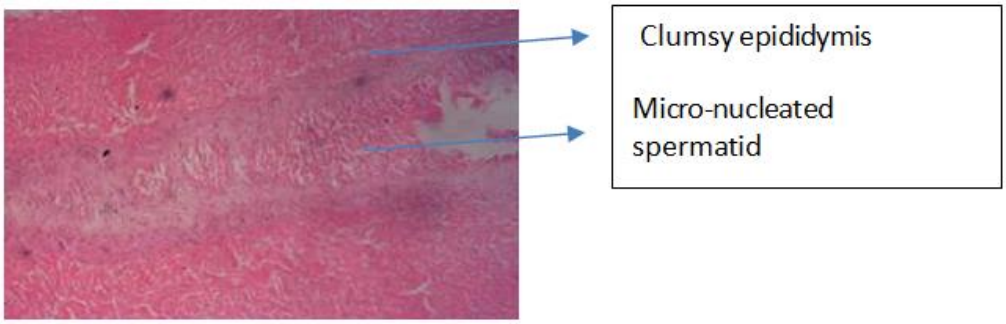
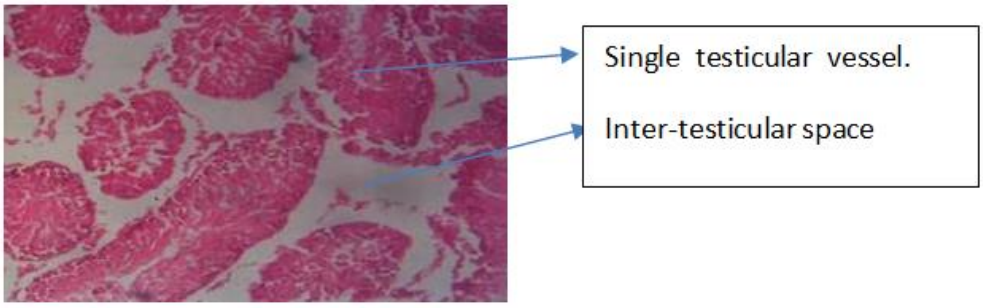


Figure 7. *Epididymis of Rat given 2mg/kg of cadmium chloride. It shows clumsy head with vacuolated tail surrounded by unstriated smooth muscles (H&E x 100).*



Sertoli are poorly formed in pseudostratified columnar epithelium in the individual rete testis (H&E x 100)

Figure 8. *Testes of Rat given 4mg/kg of cadmium chloride. Showing several columnar epithelia that are widely separated from each other and they are less visible in maturity and function.*

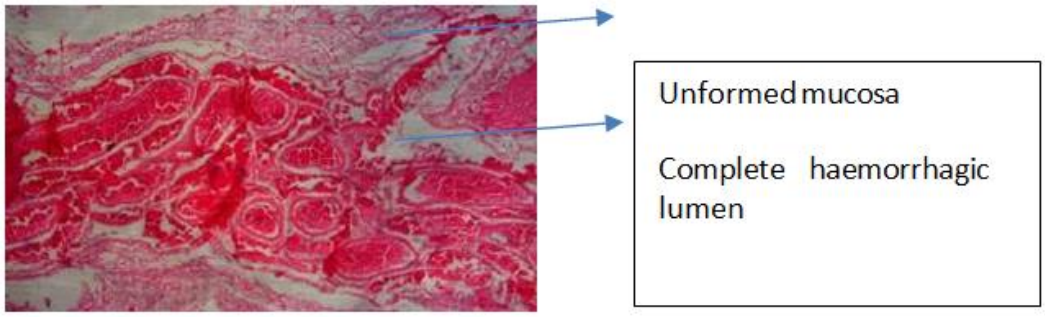


Figure 9. *Vas deferens of Rat given 4mg/kg of cadmium chloride. showing haemorrhagic lamina propria. The entire lumen mucosa is unformed (H&E x 100).*

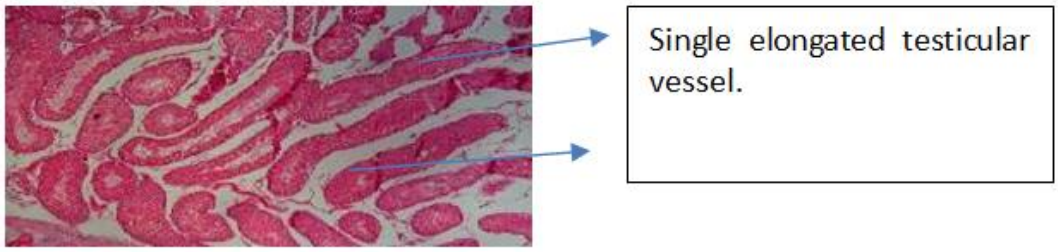


Figure 10. *Testes of Rat given 6mg/kg of cadmium chloride. Rete testis is becoming smaller and elongated with few haemorrhages at the central spot. Epididymal interstitial ducts are poorly formed (H&E x 100).*

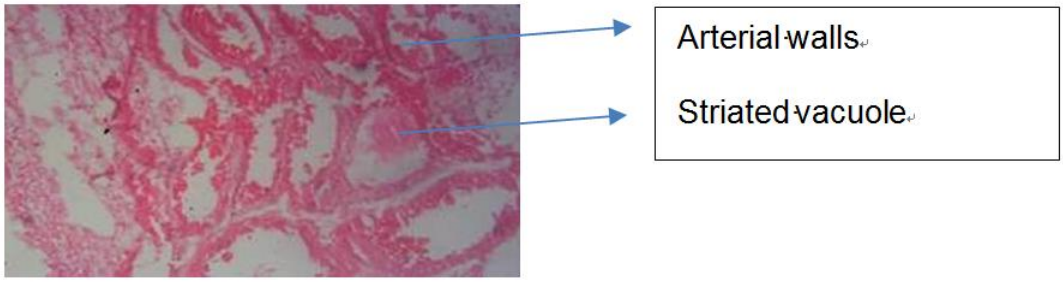
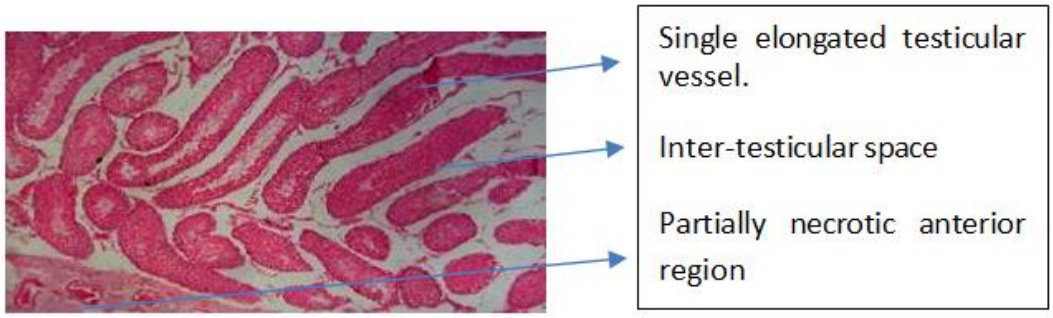


Figure 11. *Rat seminal vesicle of Rat given 6mg/kg of cadmium chloride. Seminal vesicle battles proper formation of muscular walls with partial necrotic wall populated with arterial supply (H&E x 100).*



Epididymal interstitial ducts are poorly formed. (H&E x 100)

Figure 12. *Testes of Rat given 8mg/kg of cadmium chloride. Rete testis is becoming smaller and elongated with few haemorrhages and partially necrotic at the anterior and posterior end.*

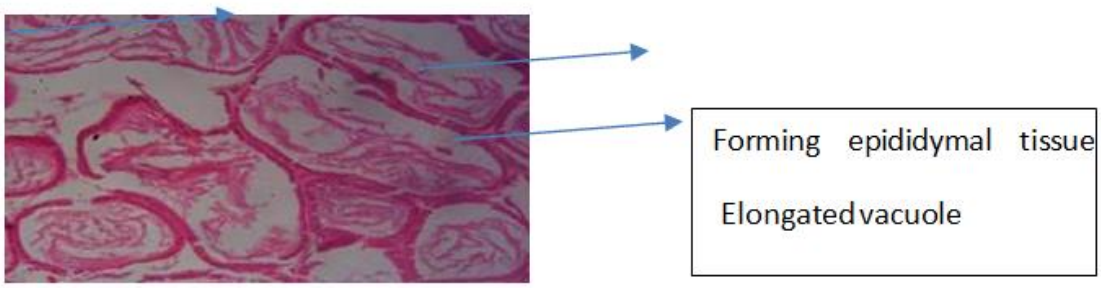


Figure 13. *Epididymis of Rat given 500mg/kg of omega 3 fatty acid. Epididymis is well-formed with no negative pathological features detected. Each consist of nearly visible epididymal duct, a head, a tail and well-formed epididymal lumen (H&E x 100).*

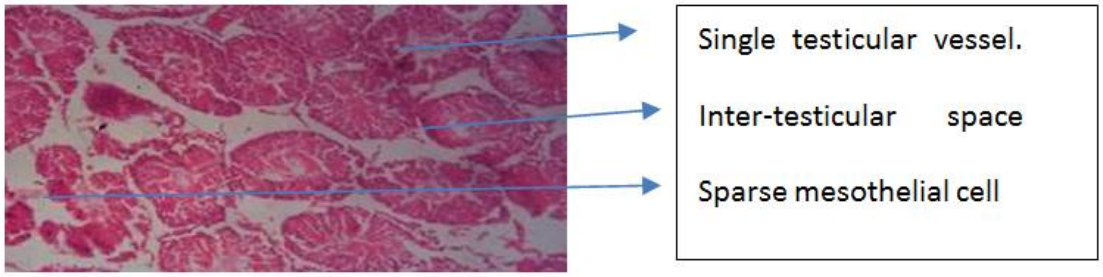


Figure 14. *Testes of Rat given 1000mg/kg of omega- 3 fatty acid. Showing several well-formed testicular vessels consisting of maturing seminiferous tubules embedded in interstitial tissues, but all having fatty fibromuscular stroma. The tissues of individual rete testis are sparsely lined by columnar epithelium of the Sertoli cells (H&E x 100).*

Figure 15. *Testes of Rat given 2mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. Several un-formed testicular vessels are seen with little fatty fibromuscular stroma. The peritoneal serosa tissues of rete testis are sparsely lined by mesothelial cells (H&E x 100).*

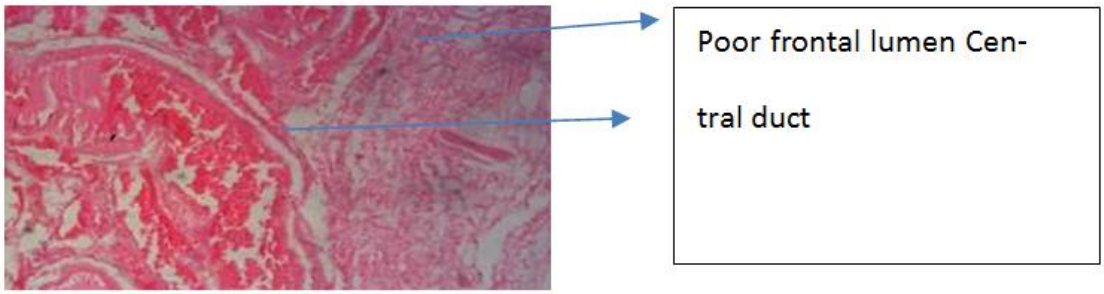


Figure 16. *Epididymis of Rat given 2mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. It shows poorly formed posterior epididymal lumen, while the anterior lumen is formed but has haemorrhagic appearance around the central duct (H&E x 100).*

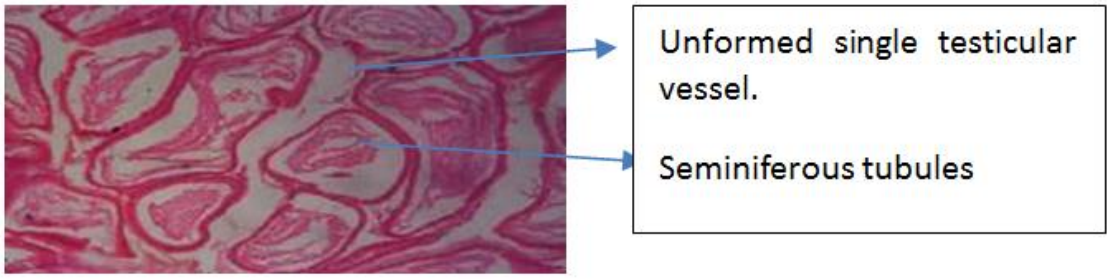


Figure 17. *Testes of Rat given 4mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. It shows a slight washing away of the thickened surface of the tunica albuginea of the entire rete testis leading to poor formation of the tunica vaginalis and visceral peritoneum (H&E x 100).*

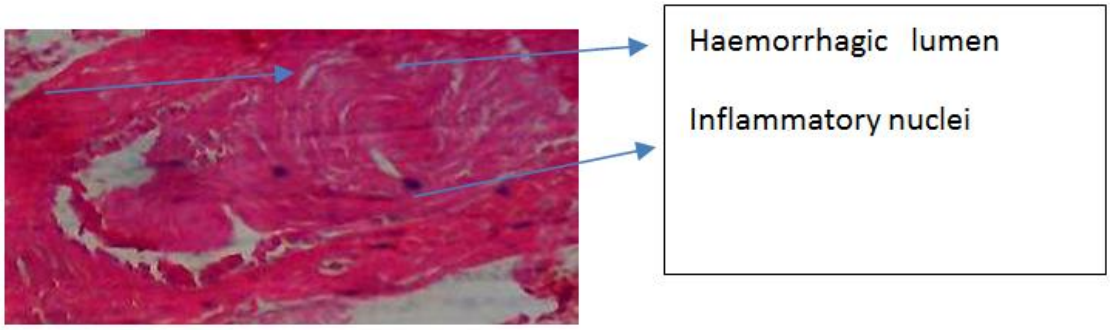


Figure 18. *Vas deferens of Rat given 4mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. It shows complete haemorrhagic lumen and lamina propria is poorly formed (H&E x 100).*

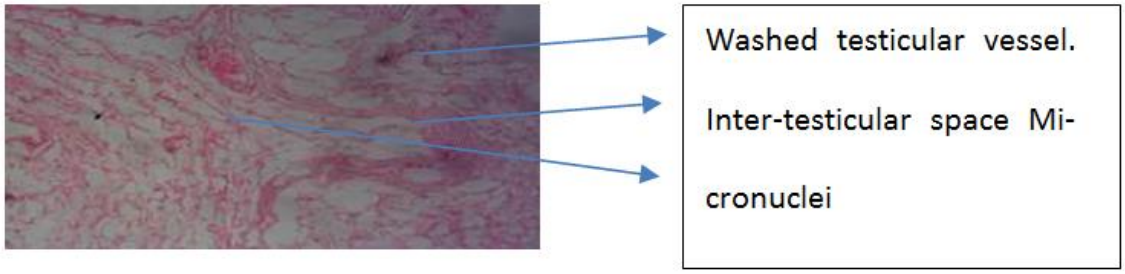


Figure 19. *Testes of Rat given 6mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. It shows complete washing away of the thickened surface of the tunica albuginea of the entire rete testis leading to poor formation of the fibrous septa with complete haemorrhagic tissue of the entire lumen having specific micronucleus of the stereocilia (H&E x 100).*

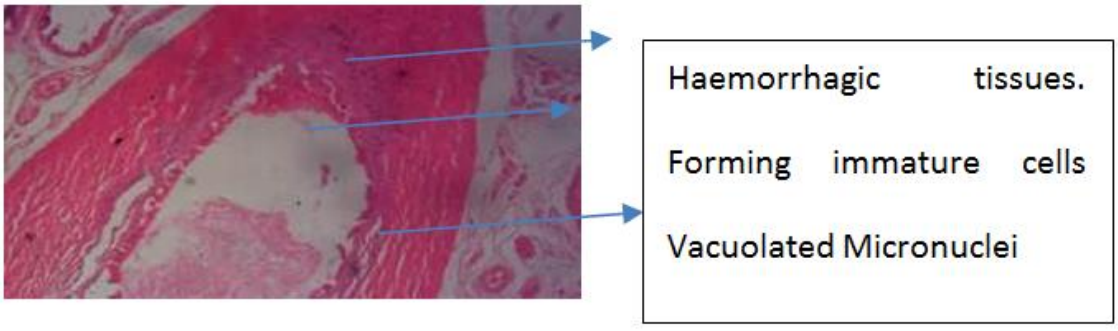


Figure 20. *Seminal vesicle of Rat given 6mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. Seminal vesicle wall is haemorrhagic with necrotic displasia and centrally vacuolated with arterial supply (H&E x 100).*

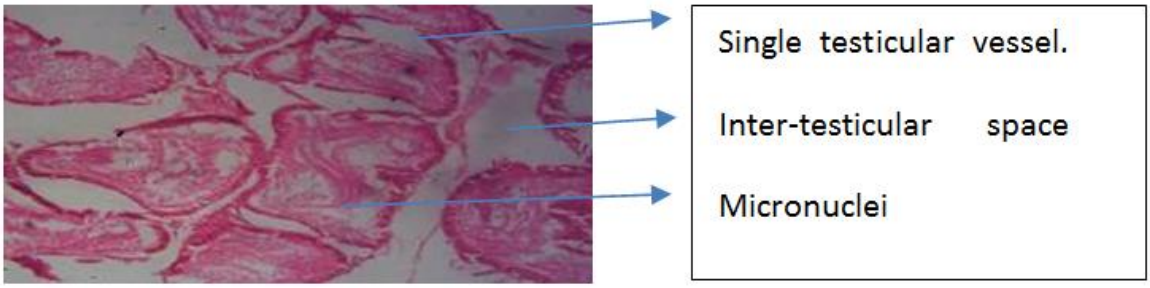


Figure 21. *Testes of Rat given 8mg/kg cadmium chloride + 500mg/kg of omega-3 fatty acid. Each peritoneal cavity shows a very poor formation of mesothelial cells lining the tunica of the seminiferous tubules and epididymis.*

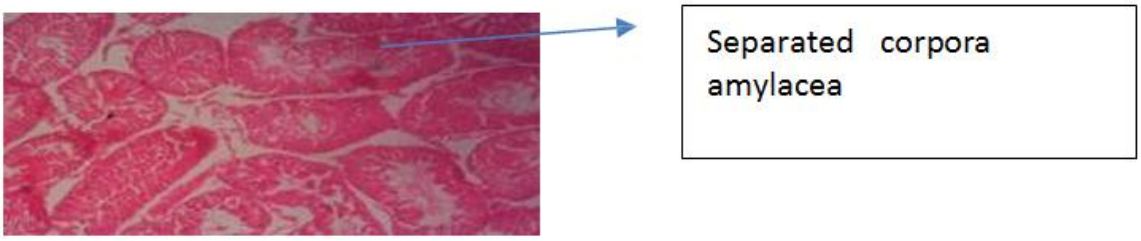


Figure 22. *Prostate of Rat given 8mg/kg cadmium + 500mg/kg of omega-3 fatty acid. It shows a highly haemorrhagic replicating prostate epithelium that is lined with few columnar and secretory cells. The glandular lumen has adequate corpora amylacea separations (H&E x 100).*

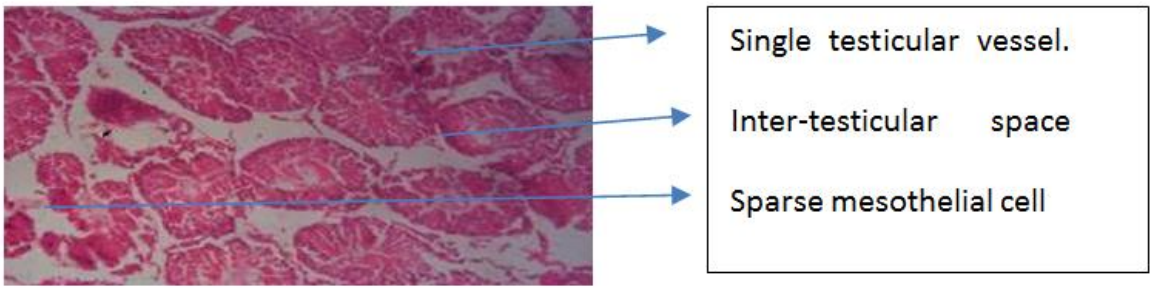


Figure 23. *Testes of Rat given 2mg/kg cadmium chloride + 1000mg/kg of omega-3 fatty acid. Several well-formed testicular vessels consisting of maturing seminiferous tubules embedded in interstitial tissues, but all having fatty fibromuscular stroma. The tissues of individual rete testis are sparsely lined by columnar epithelium of the Sertoli cells (H&E x 100).*

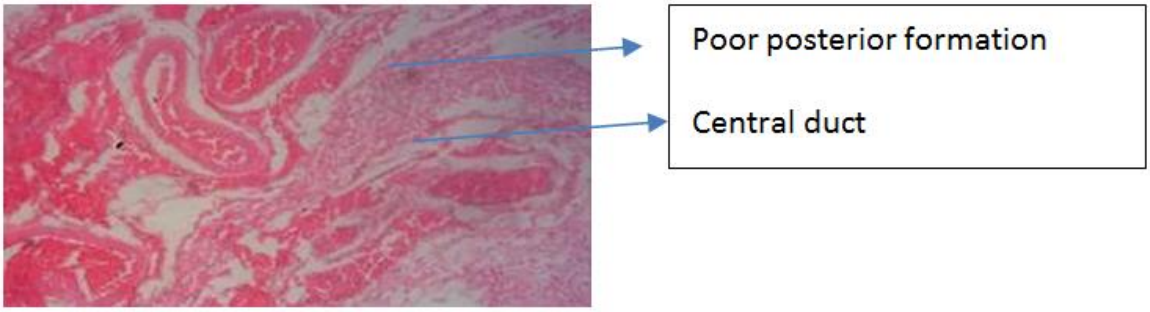


Figure 24. *Epididymis given 2mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. It shows formed and forming epididymal tissues consisting of ducts and seminiferous tubules of the apical stereocilia (H&E x 40).*

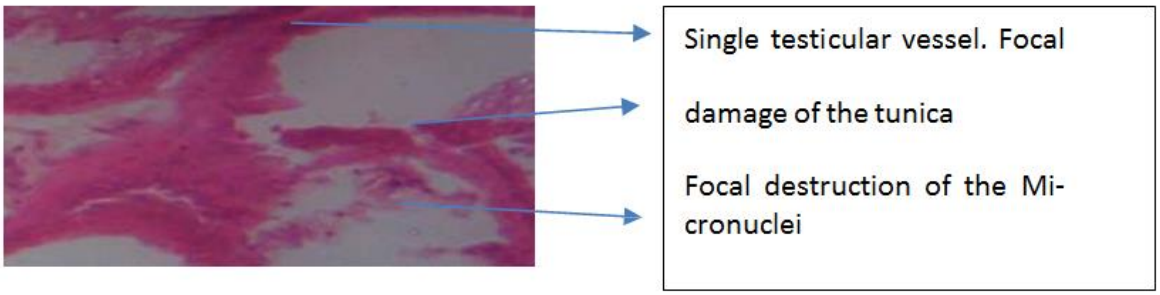


Figure 25. *Testes of Rat given 4mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. Photomicrograph shows complete focal destruction of rete testis, tunica vaginalis and tunica albuginea leading to very poor mesothelial cells of the serosa (H&E x 100).*

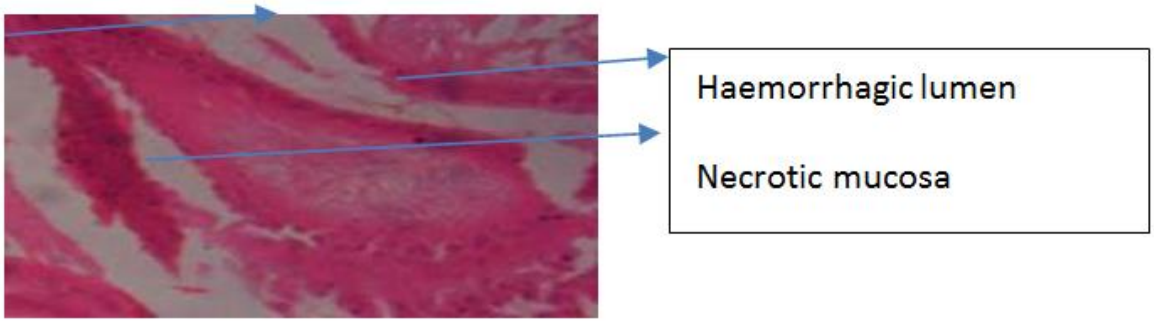


Figure 26. *Vas deferens given 4mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. It shows entirely diffused interstial haemorrhagic lumen mucosa and dysplasia (H&E x 100).*

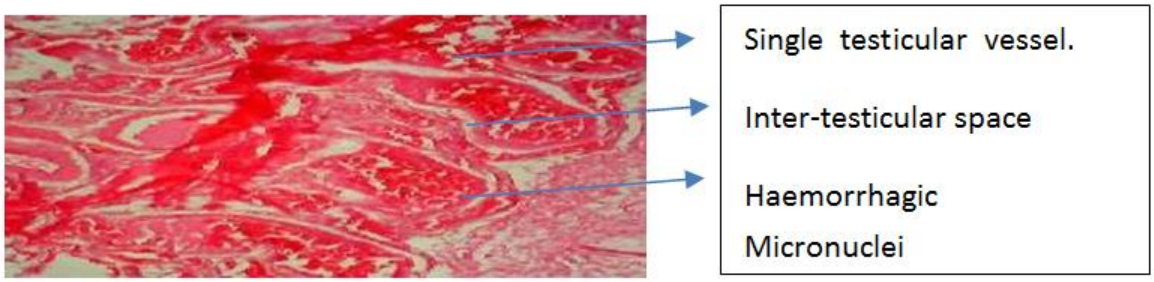


Figure 27. *Testes of Rat given 6mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. It shows complete focal destruction of rete testis, tunica vaginalis and tunica albuginea leading to central haemorrhage of the mesothelial cells of the serosa (H&E x 100).*

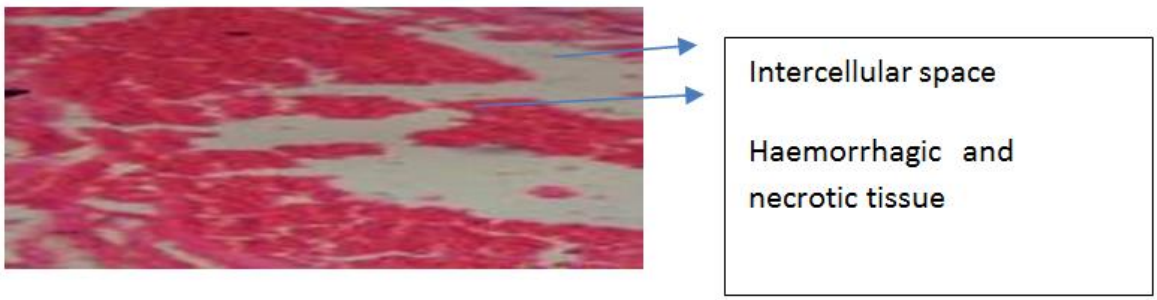


Figure 28. *Seminal vesicle of Rat given 6mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. It shows completely damaged with no proper formation of muscular walls and having partial necrotic wall populated with arterial supply (H&E x 40).*

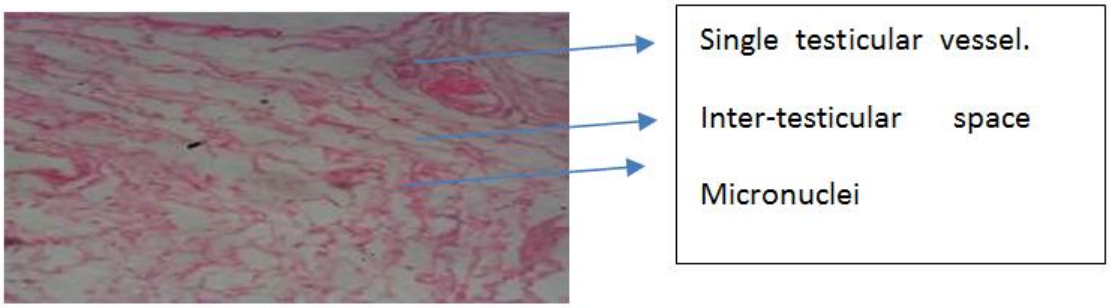


Figure 29. *Testes given 8mg/kg cadmium chloride + 1000mg/kg of omega-3 fatty acid. It shows complete washing away of the thickened surface of the tunica albuginea of the entire rete testis leading to poor formation of the fibrous septa (H&E x 40).*

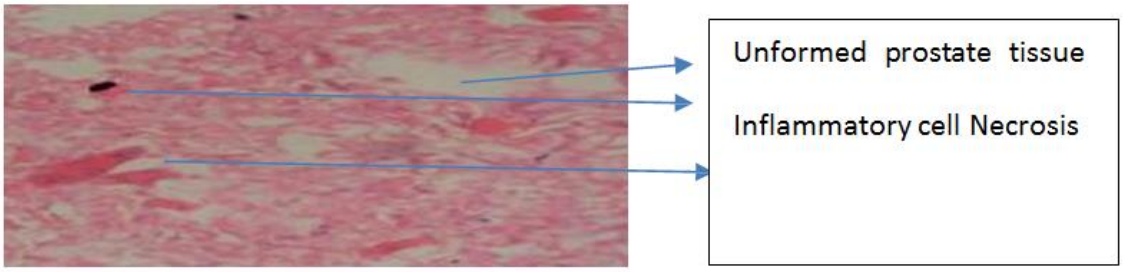


Figure 30. *Prostate of Rat given 8mg/kg cadmium chloride + 1000mg/Kg of omega-3 fatty acid. It shows completely unformed with necrosis. There is no distinct prostate epithelium and glandular lumen has no adequate corpora amylacea separations (H&E x 100).*

References

[1] Abarikwu SO. Toxic effects of cadmium on mammalian reproduction and the male reproductive system: A review. Toxicology Research and Application. 2013; 2: 1-14.

[2] Chen Q, Jin J, He J, Wang S, Li M, Wang H. Mechanisms of cadmium-induced testicular toxicity: Oxidative stress, apoptosis, and autophagy. Environ Pollut. 2021; 273: 116462.

[3] Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. Int J Environ Health Res. 2014; 24(4): 378-399.

[4] Akinloye O, Arowojolu AO, Shittu OB, Anetor JI. Cadmium toxicity: a possible cause of male infertility in Nigeria. Reprod Biol. 2006; 6(1): 17-30.

[5] Ikokide EJ, Oyagbemi AA, Oyeyemi MO. Impacts of cadmium on male fertility: Lessons learnt so far. Andrologia. 2022; 54(9): e14516.

[6] Bhardwaj JK, Siwach A, Sachdeva D, Sachdeva SN. Revisiting cadmium-induced toxicity in the male reproductive system: An update. Arch Toxicol. 2024; 98(11): 3619-3639.

[7] Kumar S, Singh A. Genetic causes of male infertility: A review. Hum Reprod Update. 2020; 28(1): 15-30.

[8] Zhang Y, Wang H. The role of reactive oxygen species in cadmium-induced cell death: A review. Toxicol Lett. 2020; 323: 1-10.

[9] Zhao X, Cheng Z, Zhu YI, Li S, Zhang L, Luo Y. Effects of paternal cadmium exposure on the sperm quality of male rats and the neurobehavioral system of their offspring. Exp Ther Med. 2015; 10(6): 2356-2360.

[10] He Y, Zou L, Luo W, Yi Z, Yang P, Yu S, Liu N, Ji J, Guo Y, Liu P, He X, Lv Z, Huang S. Heavy metal exposure, oxidative stress and semen quality: Exploring associations and mediation effects in reproductive-aged men. Chemosphere. 2020; 244: 125498.

[11] Ogunbiyi OJ, Obi FO. Evaluation of cadmium toxicity and its association with iron on the gonads of female rats. Niger Soc Exp Biol. 2021; 33(3): 795-808.

[12] World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO; 2021.

[13] Zemjanis R. Collection and evaluation of semen. In: Zemjanis R, editor. Diagnostic and therapeutic techniques in animal reproduction. Baltimore: William and Wilkins; 1977. p. 242.

[14] Narayana K, Prashanthi N, Nayanatara A, Kumar HH, Abhilash K, Bairy KL. Effects of methyl parathion (o,o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutat Res. 2005; 588: 28-34.

[15] Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47: 389-394.

[16] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973; 179: 588-590.

[17] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47: 469-474.

[18] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-358.

[19] Blanco A, Moyano R, Vivo J. Quantitative changes in the testicular structure in mice exposed to low doses of cadmium. Environ Toxicol Pharmacol. 2007; 23: 96-101.

[20] Shivtia TS, Michael G, Yehudith A, Rachel KL, Eliyahu MH. Effect of omega-3 supplements or diets on fertility in women: A meta-analysis. Heliyon. 2024; 10(8): e29324.

[21] Stanhiser J, Jukic AMZ, McConnaughey DR, Steiner AZ. Omega-3 fatty acid supplementation and fecundability. Hum Reprod. 2022; 37(5): 1037-1046.

[22] Ige SF, Olaleye SB, Akhigbe RE, Akanbi TA, Oyekunle OA, Udoh UA. Testicular toxicity and sperm quality following cadmium exposure in rats: Ameliorative potentials of Allium cepa. J Hum Reprod Sci. 2012; 5: 37-42.

[23] Ekhoye EI, Nwangwa EK, Aloamaka CP. Changes in some testicular biometric parameters and testicular function in cadmium chloride administered Wistar rats. Br J Med Health Res. 2013; 3: 2031-2041.

[24] Akunna GG, Saalu LC, Ogunlade B, Enye LA. Spermatotoxicity in animal models exposed to fragrance components. J Med Sci. 2014; 14: 46-50.

[25] Yang HS, Han DK, Kim JR, Sim JC. Effects of α-Tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. J Korean Med Sci. 2006; 21: 445-455.

[26] Ali W, Bian Y, Ali H, Sun J, Zhu J, Ma Y, Liu Z, Zou H. Cadmium-induced impairment of spermatozoa development by reducing exosomal-MVBs secretion: A novel pathway. Aging (Albany NY). 2023; 15(10): 4096-4107.

[27] Zelen I, Mitrovic M, Jurisic-Skevin A, Arsenijevic S. Activity of superoxide dismutase and catalase and content of malondialdehyde in seminal plasma of infertile patients. Med Pregl. 2010; 63: 234-245.

[28] Otamere HO, Akpamu U, Adisa WA, Shelu OJ, Imhantabhunu ES. Oxidative stress in testis of rats exposed to cadmium. Afr J Biomed Res. 2023; 26(1): 96-104.

[29] Motahareh B, Haidar A, Seyed EH, Saeed CA. Effects of walnut oil on plasma levels of testosterone pre and post puberty in male rats. Am J Ethnomed. 2014; 4: 266-275.

[30] Safarinejad MR. The effect of omega-3 fatty acids on semen quality and fertility in men: A systematic review and meta-analysis. J Diet Suppl. 2015; 12(3): 245-256.

[31] Agarwal A. Oxidative stress and its implications in male infertility: A systematic review. World J Mens Health. 2016; 34(1): 1-12.

[32] Gonzalez CA, Rojas J. Omega-3 fatty acids and their role in male fertility: A review. Reprod Biol Endocrinol. 2018; 16(1): 1-10.

[33] Milardi D, Pirotta M, Gallo A. Clusterin: A key player in male fertility and semen liquefaction. Sci Rep. 2022; 12(1): 1-12.

[34] Frapsauce C, Pionneau C, Bouley J, Delarouziere V, Berthaut I, Ravel C, Soubrier F. Proteomic identification of target proteins in normal but nonfertilizing sperm. Fertil Steril. 2014; 102(2): 372-380.

[35] Ghosh P, Dutta S, Mukherjee S. Cadmium exposure and male reproductive health: A review of epidemiological studies. Environ Res. 2020; 182: 109071.

[36] Zhu Q, Li X, Ge RS. Toxicological effects of cadmium on mammalian testis. Front Genet. 2020; 11: 527.

[37] Zhao Y. Effects of environmental pollutants on reproductive health: A study on coiled tail anomalies in Wistar rats. Environ Toxicol Pharmacol. 2021; 82: 103511.

[38] Abedin SN, Leela V, Devendran P, Suganya G, Rangasamy S, Loganathasamy K. Seminal plasma osteopontin: A marker for potential fertility in dogs. Indian J Anim Res. 2021; 55: 758-762.

[39] de Franciscis P. Cadmium as a potential factor of male infertility. Minerva Urol Nefrol. 2015; 67(3): 241-246.

[40] Sinha R, Kumar S, Sharma R. Impacts of cadmium on male fertility: Lessons learnt so far. Environ Toxicol Pharmacol. 2020; 79: 103371.

[41] sJones B, Brown C, Smith A. Omega-3 fatty acids modulate immune function and inflammation in response to cadmium exposure. J Nutr Biochem. 2017; 30(2): 89-95.