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Ameliorative Potentials of Quercetin and Omega-3 Fatty Acid on Cadmium-Induced Alterations in Reproductive Parameters Inrats

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Abstract:- Infertility in animals is one of the most widespread problems with 48.5 million of the world population being infertile. Cadmium is a ubiquitous heavy metal and a toxic pollutant in the biosphere which has been implicated as one of the factors responsible for infertility. This work was therefore conducted to ascertain the effects of omega-3 and/or quercetin on cadmium- induced infertility in rats. 42 Wistar rats were assigned into 7 groups of 6 rats each and fed for 8 weeks with normal rat feed and drinking water. The treatment groups took either of cadmium chloride, omega-3 fatty acid and/or quercetin. The sham control groups 1 and 2 took olive oil (0/1mL/kg body wt. o.p) and DMSO (1mL/kg body wt. o.p) respectively. Semen was obtained from the epididymis and blood samples were collected for measurement of some sex hormones. Results obtained showed that the seminal parameters and sex hormones in the sham control groups were not significantly different from normal control. The LD_{50} for cadmium was 3.90mg/kg bw. Total sperm, motile and viable sperm counts reduced significantly ($p<0.05$) in Cd treated group compared with control (20.00 ±1.92 x106/; 75.00 ±9.49% and 85.00 ±6.32%), omega-3 and/or quercetin groups. Seminal fructose, serum LH and FSH concentrations reduced significantly in Cd groups compared with control, omega-3 and/or quercetin groups. Cytoarchitecture of testes in Cd group showed severe structural damage to the seminiferous tubules, sertoli cells, vacuolation and destruction of seminiferous epithelium. These were ameliorated in omega-3 and/or quercetin treated groups. In conclusion, administration of quercetin or omega-3 ameliorates the adverse effects of Cd on sperm function parameters and fertility hormones (FSH, LH, testosterone). Quercetin or omega-3 also prevents the destructive effects of Cd on testicular cells and sperm production. Quercetin or omega-3 had variable effects in ameliorating the adversity of Cadmium. However, combination of both quercetin and omega-3 produced a better ameliorating effect than when administered singly.

Keyword:- *Fertility, sex hormones, sperm, semen, cadmium, Quercetin, Omega-3 fatty acid.*

I. INTRODUCTION

Reproduction is one of the most important characteristics of all forms of life which enables species continuity and perpetuation. Several factors affect reproductive ability in humans, these include tubal factors, male factor; age factor, uterine factor; peritoneal factor infertility, ovulation factor and body weight[1]. Lifestyle factors as smoking, diet, exercise, psychological stress, caffeine consumption, alcohol consumption and exposure to environmental pollutants are implicated in male infertility. Environmental and occupational factors are encountered in everyday life in different forms such asexposure to biological (virus), physical (radiation), and toxic (chemical) sources[2,3].

Toxic heavy metal such as lead, mercury, cadmium and arsenic[4,5,6] may accumulate in the blood and consequenctly the reproductive organs causing male infertility.On the other hand, male infertility may result from low sperm production, abnormal sperm, blockages that prevent the delivery of sperm, illnesses, injuriesand chronic health problems, which in turn affect sperm parameter and characteristics[3].

Cadmium is a toxic heavy metal of both environmental and occupational concerns[7,8,9]. Cadmium ranks within the top 20 hazardous substances and the human activity has markedly increased the distribution of cadmium in the global environment[2]. This metal is present in the soil, water, air and food[10]. Cadmium enters water from industrial wastes and is found in soil by leaching of sewage, sludge through soil. Therefore, the general population may be exposed through food consumption, drinking water and incidental ingestion of contaminated soil and dust by cadmium[11].Cadmium is a recognized environmental toxicant and has been reported to reduce male fertility and altered sexual behavior in both humans and rodents[5,9]. Exposure to cadmium produces hepatic, pulmonary, testicular, renal, bone injury and cancer as well as toxicity to other organs.

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Cadmium has been reported to exert its toxic effects by inducing reactive oxygen species (ROS) generation through oxidative damage. These ROS, mainly O_2^-, H_2O_2 and OH^-initiate reactions with cellular biomolecules and consequently[10], results in lipid peroxidation, altering the antioxidants system, causing membrane protein damage, DNA damage and apoptosis[9].Omega-3 fatty acids are made up of polyunsaturated fatty acids; eicosapentaenoic acid (EPA) and decosahexaenoic (EHA). Omega-3 fatty acids are essential because humans, like all mammals cannot make them and must obtain them from their diets. They are found in the tissue of oily fish such as mackerel, sardines, salmon and mullet[12].

Protective effect of Omega-3 fatty acids is focused on decreasing triglycerides[13,14].The beneficial health effects of Omega-3 fatty acids have been related to brain development, coronary heart disease (CHD), cancer, inflammatory bowel disease, rheumatoid arthritis, psoriasis, mental health, and neurodegenerative diseases[15].

Quercetin, a plant antioxidant pigment flavonoid, is found mostly in onions, grapes, berries, cherries and citrus fruits. Quercetin has attracted much attention for its beneficial health effects due to its potential antioxidant property[16]. Different studies have suggested the risk of various chronic health disorders such as tumour development, diabetes, cardiovascular disease, neurodegenerative disease and stroke may be reduced by daily intake of this substance[17,18]. Mechanisms such as antioxidant activity, anti-inflammation, interaction with receptors and other proteins, modifications of signal transduction pathway have been attributed to the beneficial effect of quercetin[16,19].

Due to paucity of scientific information about the ameliorative effect of combining omega-3 and quercetin on cadmium-induced infertility and the dearth of information on the synergistic ameliorative effect of omega-3 and quercetin on cadmium-induced male infertility. This study is justifiable as it will provide a scientific clue on the synergistic effect of omega-3 and quercetin on cadmium-induced alterations in some reproductive parameters in rats.

II. MATERIALS AND METHODS

A. Chemcals

Cadmium chloride produced by Sigma-Aldrich, USA was obtained from the Department of Physiology, University of Calabar.

Omega-3 Fatty Acid manufactured by Nicholson Ltd, India. was purchased from Daphyl Pharmacy, Calabar.

Quercetin (BN: 1002181952) produced by Sigma-Aldrich (India) purchased at Eyamson Scientific Stores, Calabar, was used for the study.

The DMSO (BN: 20190422) produced by GHTECH, China, was obtained from Biochemistry Department, University of Calabar, Nigeria.

Olive oil was purchased from Bez pharmacy, Calabar, Nigeria.

B. Experimental animals

A total of sixty (60) male albino Wister rats weighting between 200-250g were obtained from the animal house of the Department of Physiology, University of Calabar, Calabar, Nigeria and were used for the study.

The animals were acclimatized for one week, then weighed and distributed into seven groups of six animals each as outline below. Ethical approval (No.: 071PHY3119; Ref.: FAREC/UC/FBMS/049; dated: 13/01/2020) was obtained from the Faculty of Basic Medical Sciencesanimal ethical committee.

III. METHODS

A. Preparation and administration of experimental drugs

Cadmium was administered at a dose of 2mg/kg boduy weight, i.p. once every 5 days. It was prepared (stock) by dissolving 0.1g in 50ml of distilled water, then 0.1ml was given to 100g rat.

Omega-3 was given at dose of 14.29 mg/kg (extrapolated from human dose of 1000 mg / 70 kg) orally, once daily by dissolving 1 capsule in 5mL of Olive oil and 0.01mL was given to 100g rat.

Quercetin was administered at a dose of 20mg/kg body weight to the rats subcutaneously once daily. 0.1g of quercetin was dissolve in 5ml of 2% dimethly sulfoxide (DMSO) solvent, then given at 0.1ml/100g bw.subcutaneously once daily20.

DSMO (2%) was given at the dose of 1mL/kg orally and once dailyto the sham control-1 group while olive oil was given at Olive oil (0.1 mL/kg orally and once daily) to the sham control-2 group.

B. Acute toxicity study

Eighteen (18) albino Wistar rats were used for the determination of LD_{50} following the method described previously[21]. The animals were randomly assigned into 6 groups of 3 rats each, group 1 was control, while groups 2 to 6 took graded doses of the cadmium chloride.

C. Experimental design

A total of forty two (42) albino Wistar rats were ramdonly assigned into 7 groups of 6 rats each and were treated thus:

- Group 1 (control): Received normal rat chow + drinking water
- Group 2 (Sham control-1): DMSO (2%) 1mL/kg orally and once daily.
- Group 3 (Sham control-2): Olive oil (0.1 mL/kg orally and once daily)
- Group 4: (Cadmium group): Cadmium (2mg/kg, i.p)
- Group 5 (Cd + Quercetine): Cadmium (2mg/kg, i.p) + Quercetin (20mg/kg s.c)
- Group 6 (Cd + Omega-3): Took Cadmium (2mg/kg, intraperitoneally) + Omega-3 (14.29mg/kg orally)

- Group 7: (Cd + Quercetin + Omega-3): Cadmium (2mg/kg, intraperitoneally) + Omega-3 (14.29mg/kgo.p) + Quercetin (20mg/kg, subcutaneously)

All the animals had free access to normal rat feed and drinking water. The feeding regimens lasted for 56 days. The animals were utenized euthanized.

D. Collection of experimental samples

Afterthe 56 days (8 weeks) feeding period,the animals were then fasted overnight, weighed and anaesthetized with 5% chloroform. Blood samples were be collected via cardiac puncture[22] into plain caped sample bottles for serum extraction from clotted blood samples. The samples bottles were left to stand for 2 hours for proper clotting, thereafter, there were spun at 1,000 rev / min for 5 minutes. The supernatants (serum) were extracted with needled syringes into other capped plain bottles for biochemical assay.

Semen samples were also collected from the epididymis into 9% NaCl solution in sample bottles. Testes were isolated for histological study.

E. Semen Analysis

➢ *Determination of sperm count*

The epididymal content was obtained with forcept, weighed and placed on a petri-dish containing physiological saline. The suspension was separated into fragments by fattening through 80 micronmeter stainless mesh. A tissue – free aliquot obtained was loaded into the Neubauer haemocytometer (deep 1/10 Labart. Germany). Different sperm counts weredone using microscope[23]. Mean count for each rat was calculated using the formula:

$$Sperm\ count = \frac{Total\ No.of\ sperm\ cells\ in\ the\ cytometer}{Mean\ value}$$

➢ *Determination of sperm viability*

The improved one step eosin-nigrosin staining techniquewas adopted for the determination of sperm viability24.A fraction of each suspension of sperm sample was mixed with equal volume of eosin-nigrosin stain. Smears were made on slides and air-dried. The air dried smears were then prepared on a slide for each sample accordingly. The slides were coated randomly and examine under the microscope for viability.

The percentage viability was calculated based on the number of viable (live) sperm cells divided by the number of sperm cells within 30 minutes multiply by 100.

➢ *Determination of sperm motility and Ph*

Semen samples from different treated groups were collected and dropped on a glass slide and viewed under the (Olympus, Japan) microscope at x400 magnification. The percentage of sperm was analysed for progressive motile sperm (PMS), non-progressive – motile sperm (NPMS), and distinguished – by the movement of the sperm.

The pH meter was used to determine the pH of the semen samples.

➢ *Determinationof sperm morphology*

One drop of suspension of each semen sample was placed on a glass slide. The slidewas air dried and stained with 1% eosin. The morphological abnormalities of the sperm were evaluated from a total of two hundred sperm per animal. Following the standard criteria[25,26], the results were recorded as percentage of the abnormal sperms.

F. Hormonal Assay

➢ *Determination of Testosterone*

Tetosterone concentration was asssayedusing ELISA kit in Zone-3 diagnostic laboratory[27], Akpandem Street, Calabar, Nigeria**.**A working solution of the testosterone – HRP conjugate and wash buffer were prepared. The required numberof microwell strips were displaced.100µl of the conjugate working solution was placed in each well. This was followed by addition of 50µl of each calibrator, control and specimen sample into corresponding labelled wells. Duplicates of the wells were made.

All wells were incubated on a plate shaker approximately 200rpm for 1 hr at room temperature. Each well was washed 3 times with 300µl wash buffer per well and the plate was tap firmly against absorbent paper to ensure that it was dry. Thereafter, 150µl of 5,5-tetrameltylbenzidine (TMB) substrate was pipette into each well at time intervals and re-incubated on a plate shaker 10 – 15 minutes at room temperature (or until calibrator attain a dark) blue color for desired OD. At the expiration of the incubation, 5µl of stop solution was added to each well at the same time intervals. Finally, reading of the plates was on a microwell plate reader at 450nm within 20 minutes.

➢ *Determination of leutenizing hormone (LH) and follicle stimulatinghormone (FSH)*

ELISA kitwas adopted for the determination of leutenizing hormone (LH) and Follicle stimulating hormone[27]. LH- conjugate and wash buffer solutions were prepared. 25µl of each calibrator, control and specimen samples was pipetted into correspondingly labelled wells in duplicate. The plate shaker was incubated for 200rpm for 30 minutes at room temperature. The wells were then incubated on the plate shaker for 15 – 20 minutes at room temperature (or until calibrator attain dark blue color for desired OD). Thereafter, 50µl of stop solution wastransferred into each well at the same time interval.Reading of the plate on a micro well plate reader was at 450nm within 20 minutes after dilution of the stop solution.

Similar procedures were followed for the determination of follicle stimulating hormone. Howerever, FSH binds to anti β-FSH receptors and required a volume of 300ml of Mela and 2 diluent buffers in the buffer bottle to properly process the assay run.

G. Statistical analysis

The data obtained were presented as mean ±SEM, data were analyzed using one way analysis of variance followed with Tukey post hoc test. This was done with the aid of a statistical package, SPSS Version 25.0 for windows. $P<0.05$ was considered significant.

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IV. RESULTS

A. Lethality study

The LD_{50} of cadmium was 3.90 mg/kg body weight, extrapolated from the log dose probits (percentage mortalities) plot. A test dose of 2.0 mg/kg was then chosen for the experiment, Fig. 1.

B. Semen pH of the different experimental groups

The semen pH for the normal control (NC) group was 6.97 ±0.04, while the sham control-1 (SC-1) and sham control-2 (SC-2) had semen pH of 7.03 ±0.02 and 7.03 ±0.06 respectively. It was 6.90 ±0.04 for the cadmium (Cd) only treated group. The group treated with Cadmium + Quercetin (Cd + QT), Cadmium + Omega-3 (Cd + OM-3) and Cadmium + Quercetin + Omega-3 (Cd + QT + OM-3) had semen pH of 6.90 ± 0.10, 6.80 ±0.10 and 6.93 ± 0.08 respectively. No significant differences were observed in the semen pH among the different experimental groups, Table 1.

C. Fructose concentration of the different experimental groups

As shown in Table 1, the mean fructose concentration for NC was 3.47 ± 0.33, SC-1 and SC-2 had mean fructose concentrations of 3.66 ± 0.52 and 3.91 ± 0.51 mmol/L respectively. The Cd group had significant ($p<0.05$) decrease in fructose (2.76 ± 0.31 mmol/L) compared with other experimental groups except with NC and Cd + QT + OM-3 groups.

D. Total sperm count of the different experimental groups

The control groups had a mean total sperm count ($x10^6$/L) of 20.00 ± 1.92, while SC-1 and SC-2 had counts of 22.50 ± 0.76 and 21.18 ± 3.27 respectively. No significant differences were observed among the control groups. it was significantly ($p<0.05$) lower in the Cd alone treated group (18.83 ± 0.60) compared with controls and other experimental groups, Table 1.

E. Motile sperm count in the different experimental groups

The results obtained for motile and non- motile sperm count is shown in Table 1. The motile sperm count for the NC was 75.00 ±9.49 %, it was 76.17 ±1.68 % and 70.83 ±3.52 % respectively for the SC-1 and SC-2 groups. No significant differences were observed among the control groups. It was significantly ($p<0.05$) lower in the Cd only treated group (23.33 ±2.11 %) compared with the control groups. It was significantly ($p<0.05$) raised in the Cd + QT (36.67 ±4.94 %), Cd + OM-3 (40.83 ±6.11 % and Cd + QT + OM-3 (54.17 ±3.75 %) compared with Cd alone, Table 1.

F. Viable sperm count in the different experimental groups

As shown in Table 1, the viable sperm count (%) in the NC was 85.00 ±6.32, SC-1 (86.33 ±1.38), SC-2 (83.33 ± 0.71). No significant differences were observed among the control groups. Treatment with Cd significantly ($p<0.05$) reduced the viable sperm count (46.67 ±1.05) compared with the other experimental groups.

G. Percentage count of sperm with rapid progressive movement

No significant differences were observed in the percentage of sperms exhibiting rapid progressive forward movement among the different control groups. it was 58.33 ±10.70 for NC, 56.17 ± 1.33 and 57.67 ± 0.67 % for SC-1 and SC-2 groups respectively. Significant ($p<0.05$) reductions were observed in the Cd alone treated group (6.67 ± 1.05 %), Cd + QT (6.67 ± 1.05 %) and Cd + OM-3 (1.67 ± 1.05 %) compared with control groups. It increased significantly ($p<0.05$) in Cd + QT + OM-3 group compared with Cd alone treated group, Table 2.

H. Percentage sperm count with slow progressive movement

As shown in Table 2, the percentage count of sperm with slow progressive movement was not significantly different among the control groups (NC, 8.33 ± 2.11; SC-1, 7.67 ± 0.88; SC-2, 9.67 ± 2.26). it increased significantly ($p<0.05$) in the Cd only treated group (21.67 ± 1.05 compared controls and other experimental groups.

I. Percentage count of sperm having forward progressive movement (FPM)

In the control groups, no significant differences were recorded in FPM. It was 8.33 ±2.11, 9.00 ± 0.26 and 8.67 ±1.25 % respectively for NC, SC-1 and SC-2 groups. it increased significantly ($p<0.05$) in the Cd, Cd + OM-3 and Cd + QT groups compared with the control and Cd + QT + OM-3 groups, Table 2.

J. Percentage count of sperm with residual movement (RM)

As shown illustrated in Table 2, no significant differences were observed in the sperms with residual movement among the different experimental groups. For the controls, mean values of sperms with RM were 6.67 ±1.05 for NC, 6.67 ±1.05 and 8.33 ±1.05 for SC-1 and SC-2 respectively.

K. Assessment of sperm morphology of the different experimental groups

Results obtained for sperm morphology are shown in Table 2 No significant differences were observed in the percentage count of sperm with tail defect, head defect and total defects among the different experimental groups.

The mean percentage count for tail defect was 1.33 ± 0.21, 1.67 ± 0.21 and 1.33 ± 0.21 respectively for NC, SC-1 and SC-2 groups respectively. Values obtained for head defect for these groups were 0.33 ± 0.21, 0.67 ± 0.33 and 0.33 ± 0.21 respectively and 1.67 ± 0.21, 2.33 ± 0.33 and 1.67 ± 0.33 respectively for total defects.

L. Follicle stimulating hormone (FSH) concentration in the different groups

In fig. 2, there was significant ($p<0.05$) decrease in FSH concentration (IU/L) in the Cd treated group (0.38 ± 0.02) compared with controls (NC 1.44 ± 0.19, SC-1 1.33 ± 0.22 and SC-2 1.44 ± 0.21). FSH concentrations increased significantly ($p<0.05$) in Cd + QT (1.26 ± 0.46), Cd + OM-3 (1.38 ± 0.43 and CD + QT + OM-3 (1.47 ± 0.36) compared with Cd treated group, fig. 2.

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M. Luteinizing hormone (LH) concentration in the different experimental groups

The LH concentration (IU/L) reduced significantly ($p<0.05$) in Cd treated group (2.19 ± 0.48) compared with NC (3.60 ± 0.17), SC-1 (3.88 ± 0.20) and SC-2 (3.80 ± 0.16). No significant differences were seen among the control groups. FSH increased significantly ($p<0.05$) in the CD + QT (4.60 ± 1.07), CD + OM-3 (4.72 ± 1.00) and Cd + QT + OM-3 (5.64 ± 0.20) compared with Cd only group, fig. 3.

N. Testosterone concentration in the different experimental groups

The testosterone concentration (ng/L) in the NC, SC-1 and SC-2 were 3.74 ± 0.14, 3.46 ± 0.20 and 3.76 ± 0.77 resepctively. Testosterone levels reduced significantly ($p<0.05$) in the Cd group which had a mean value of 2.24 ± 0.57 compared with other experimental groups, fig. 4.

O. Histological studies

Results obtained for the cytoarchitexture of the testes for the different experimental groups are shown in PLATE 1 to 7.

A cross section of testes for the NC group shows the presence of testicular seminiferous tubules with thick walls, plump testicular stroma cells and abundant cytoplasm extending from the basement membrane to the lumen.

The cytoarchitexture of a cross section of testes in SC-1 shows testicular cells with normal, well organized architecture and mature spermatozoa, lumen is filled and seminiferous tubules compact with less interstitial space.

SC-2 presented with normal architecture with spermatogonia and sertoli cells resting on intact basement membrane. Primary spermatocytes were seen during different stages of meiotic division

In the Cd treated group, severe structural damage to the seminiferous tubules, and sertoli cells were seen with vacuolation and destruction of seminiferous epithelium. The Cd + QT group showed testes with decreased spermatogenesis with a thin germinal epithelium, seminiferous tubules with abberrant morphology abnormalities of the testicular stroma.

Cd + OM-3 group presented with normal arrangement of spermatogonia and sertoli cells with decreased thickness of the testicular seminiferous tubule walls, less apparent swirling contours.

Cd + QT + OM-3 group shows architecture that appears disoriented, with the wide lumen of the seminiferous tubules containing many spermatozoa. spermatocytes seen during different stages of meiotic division .The seminiferous tubule with elongating spermatids with a little degeneration in interstitial spaces and less sloughing in epithelial layer of cells.

Table 1: Spermfunction parameters in the different experimental groups

	pH	Fructose (mmol/L)	Sperm count ($x10^6/mm^3$)	Motile sperm (%)	Viable sperm (%)
Control	6.97	3.47	20.00	75.00	85.00
	±0.04	±0.33	±1.92	±9.49	±6.32
Sham control-1	7.03	3.66	22.50	76.17	86.33
	±0.02	±0.52	±0.76	±1.68	±1.38
Sham control-2	7.03	3.91	21.18	70.83	83.33
	±0.06	±0.45	±3.27	±3.52	±0.71
Cd	6.90	2.76	18.83	23.33	46.67
	±0.04	±0.31[a]	±0.80*[,a,b]	±2.11*[,a,b]	±1.05*[,a,b]
Cd + Quercetin	6.90	4.67	21.95	36.67	55.83
	±0.10	±0.79[c]	±0.89[c]	±4.94*[,a,b,c]	±2.39*[,a,b]
Cd + Omega-3	6.80	4.43	21.23	40.83	60.00
	±0.10	±0.56[c]	±0.83[c]	±6.11*[,a,b,c]	±4.65*[,a,b,c]
	6.93	3.74	21.47	54.17	62.50
Cd + Omega-3 + Quercetin	±0.08	±0.28	±1.07[c]	±3.75*[,a,b,c]	5.28*[,a,b,c]

Values are expressed as mean +SEM, n = 6.

* = $p<0.05$ vs control
a = $p<0.05$ vs sham control-1
b = $p<0.05$ vs sham control-2
c = $p<0.05$ vs Cd

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Table 2: Sperm-function parameters in the different experimental groups

	RPM (%)	SPM (%)	FPM (%)	RM (%)	TD (%)	HD (%)	TDS (%)
Control	71.67	8.33	8.33	6.67	1.33	0.33	1.67
	±2.79	±2.11	±2.11	±1.05	±0.21	±0.21	±0.21
Sham control-1	56.17	7.67	9.00	6.67	1.67	0.67	2.33
	±1.33	±0.88	±0.26	±1.05	±0.21	±0.33	±0.33
Sham control-2	57.67	9.67	8.67	8.33	1.33	0.33	1.67
	±0.67	±2.26	±1.23	±1.05	±0.21	±0.21	±0.33
Cd	6.67	21.67	10.00	8.33	1.67	0.67	2.33
	±1.05*,a,b	±1.05*,a,b	±1.83	±1.05	±0.21	±0.21	±0.42
Cd + Quercetin	6.67	6.67	13.33	8.35	2.00	0.33	2.33
	±1.05*,a,b	±1.05*,a,c	±1.05*,a,b	±1.83	±0.00	±0.21	±0.21
Cd + Omega-3	1.67	5.00	13.33	10.00	2.33	0.00	2.67
	±1.05*,a,b	±0.00*,a,c	±1.05*,a,b	±1.05	±0.21	±0.00	±0.42
	8.33	6.67	10.00	6.67	1.67	0.33	2.00
Cd + Omega-3 + Quercetin	±1.05*,a,b	±1.05*,a,c	±0.00d,e	±1.05	±0.21	±0.21	±0.00

Values are expressed as mean +SEM, n = 6.

* = p<0.05 vs control
a = p<0.05 vs sham control-1
b = p<0.05 vs sham control-2
c = p<0.05 vs Cd
d = p<0.05 vs Cd + quercetin
e = p<0.05 vs Cd + Omega-3

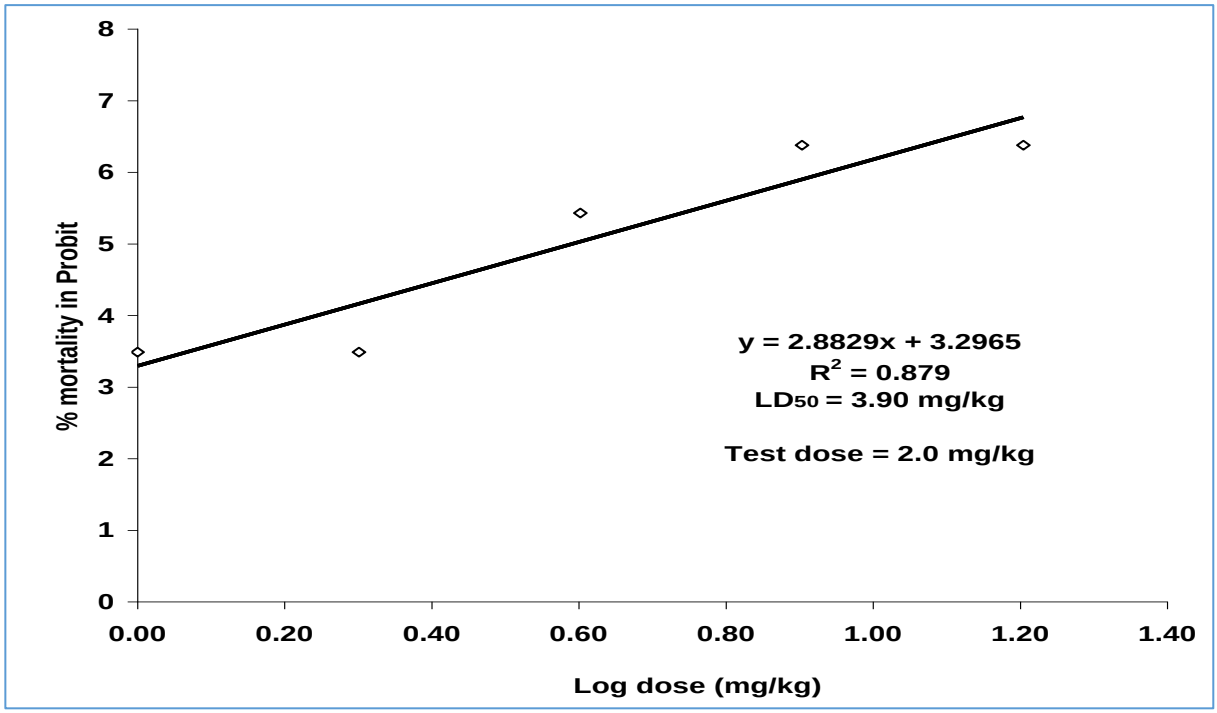

Fig. 1: Acute toxicity study for determination of LD50 of Cadmium Chloride in mice (LD50=3.90 mg/kg)
RPM = rapid progressive movement; SPM = Slow progressive movement
FPM = forward progressive movement; RM = Residual movement
TD = Tail defect; HD = Head defect
TDS = Total defective sperm

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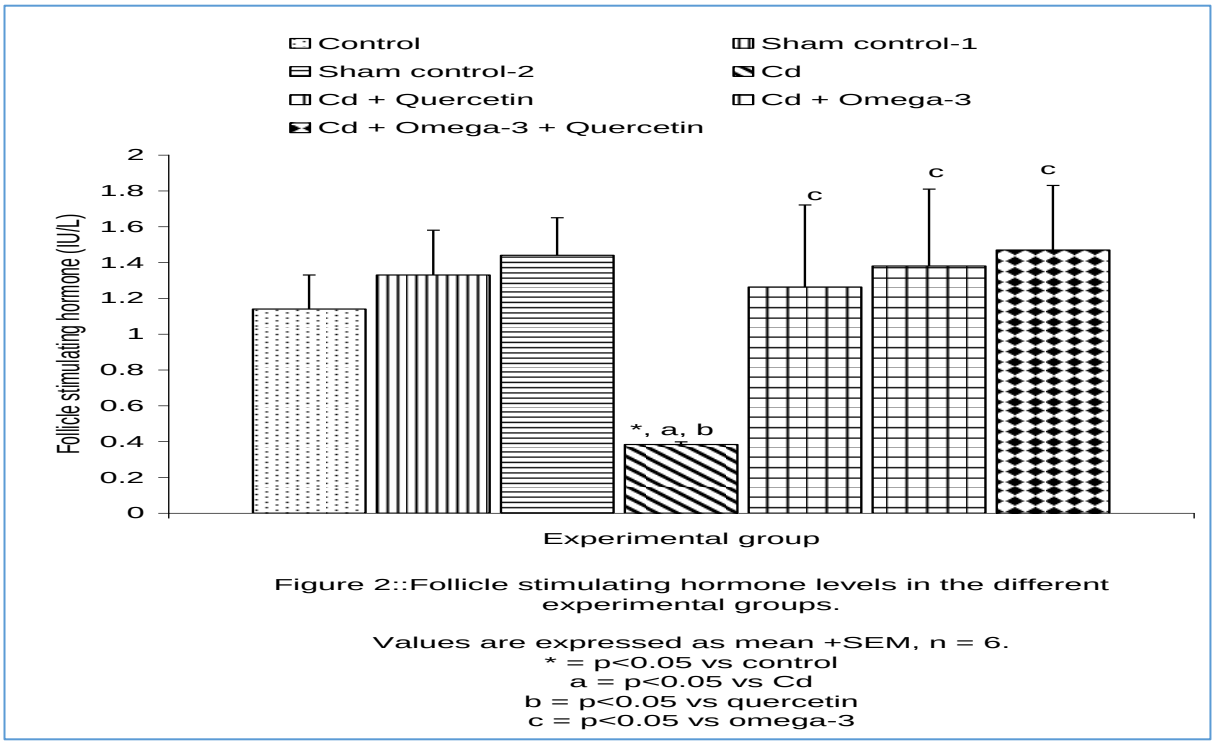

Figure 2::Follicle stimulating hormone levels in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = p<0.05 vs control
a = p<0.05 vs Cd
b = p<0.05 vs quercetin
c = p<0.05 vs omega-3

Fig. 2: Follicle Stimulating hormone levels in the different experimental groups

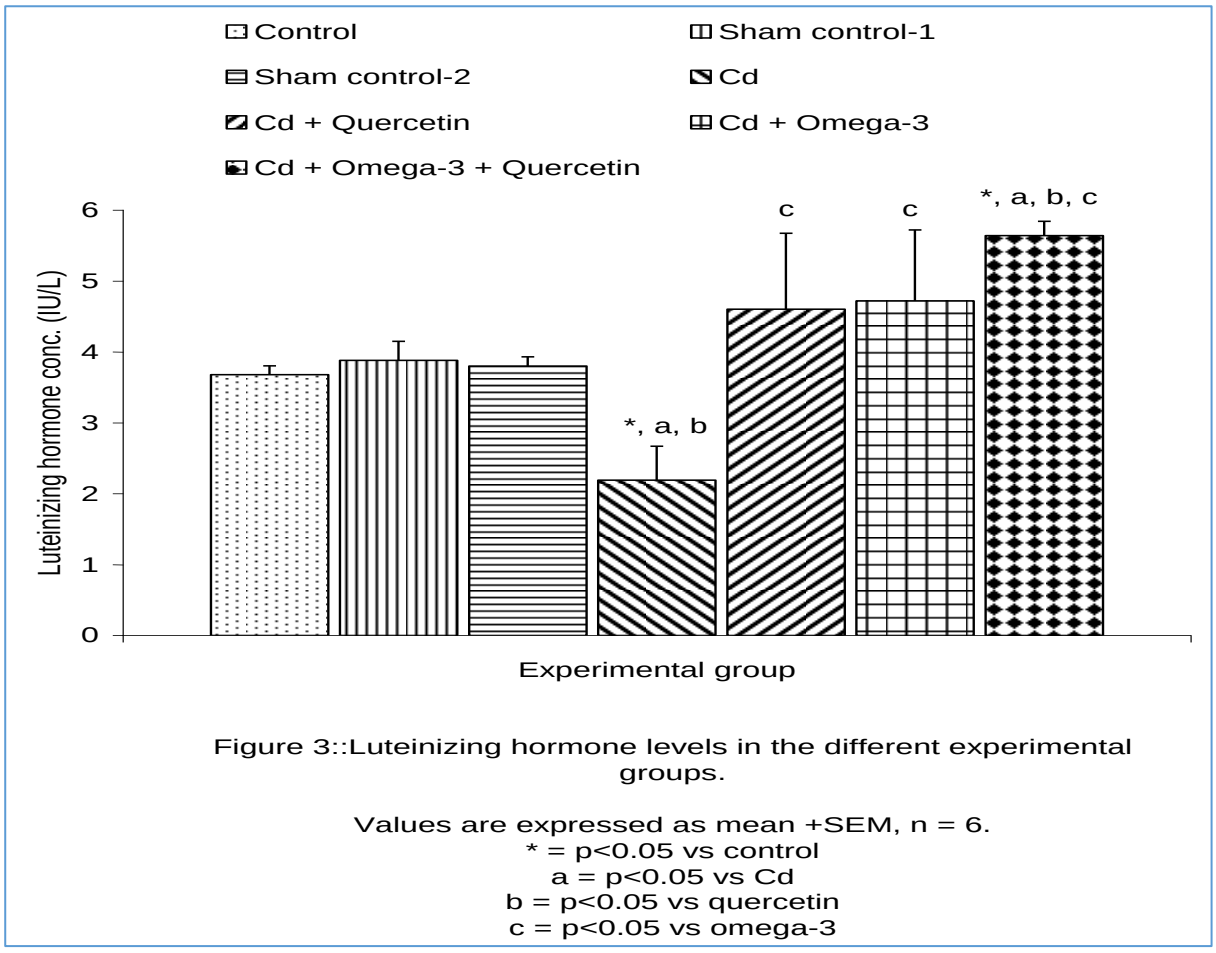

Figure 3::Luteinizing hormone levels in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = p<0.05 vs control
a = p<0.05 vs Cd
b = p<0.05 vs quercetin
c = p<0.05 vs omega-3

Fig. 3: Luteinizing hormone Levels in the different experimental groups

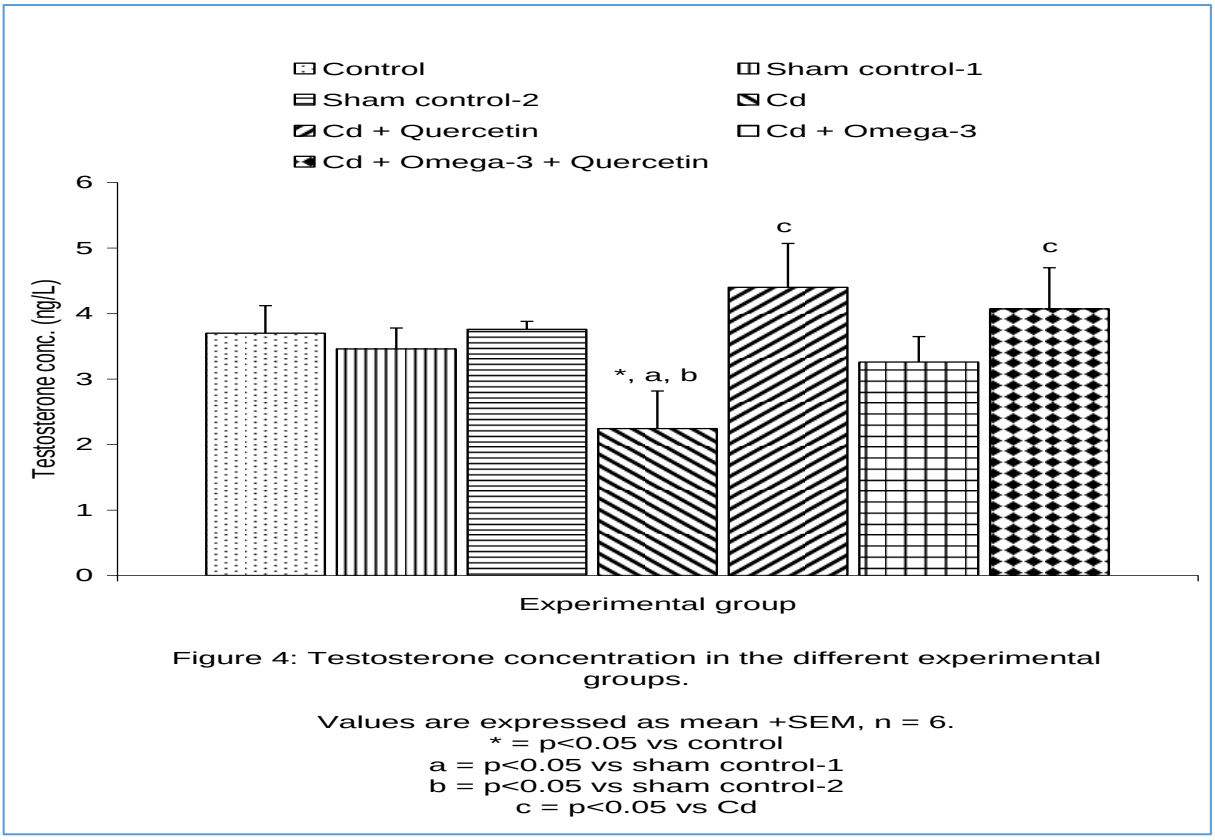

Figure 4: Testosterone concentration in the different experimental groups.

Values are expressed as mean +SEM, n = 6.
* = p<0.05 vs control
a = p<0.05 vs sham control-1
b = p<0.05 vs sham control-2
c = p<0.05 vs Cd

Fig. 4: Testosterone Concentration in the different experimental

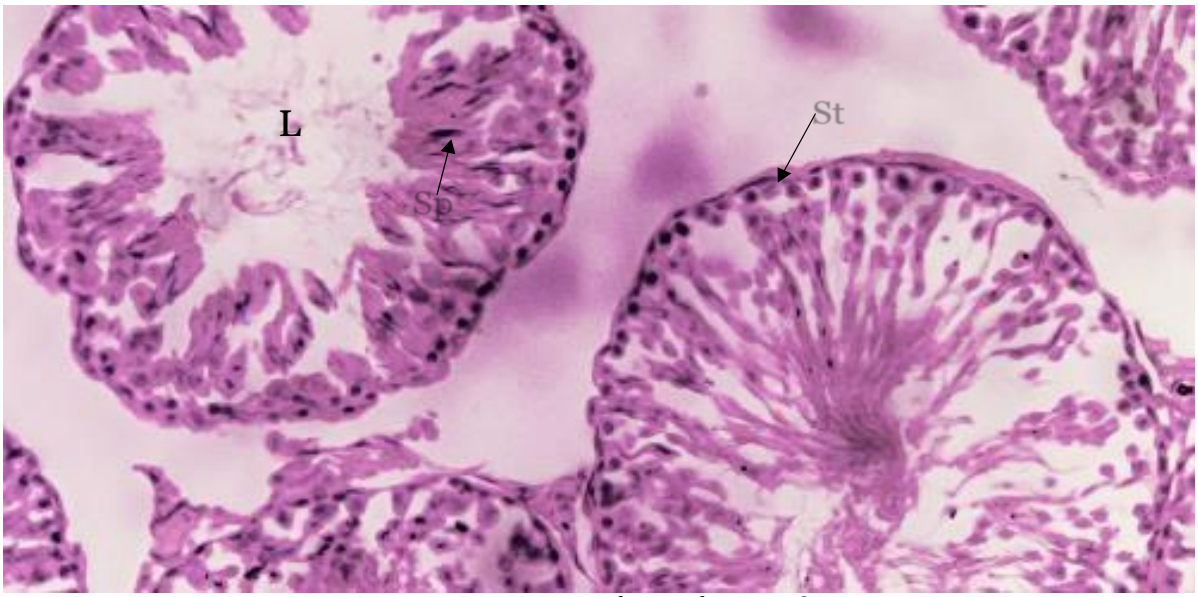

PLATE 1a**:** Cross section of testes for control group
Stained with H and E and Magnification x 400. L= Lumen

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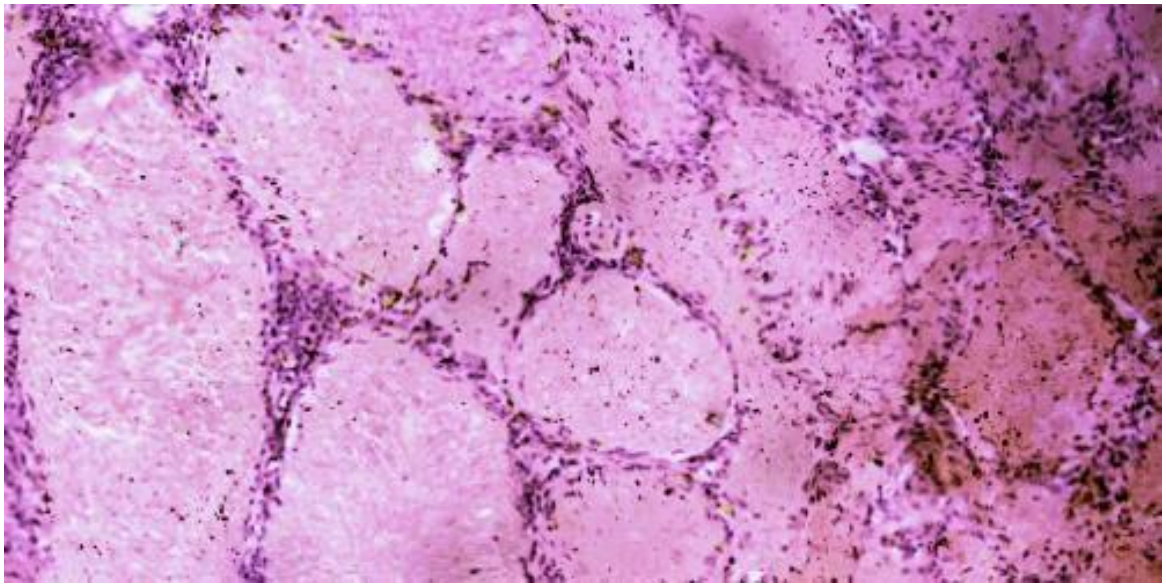

PLATE 1b: Cross section of testes of cadmium group:
Stained with H and E and Magnification x 400

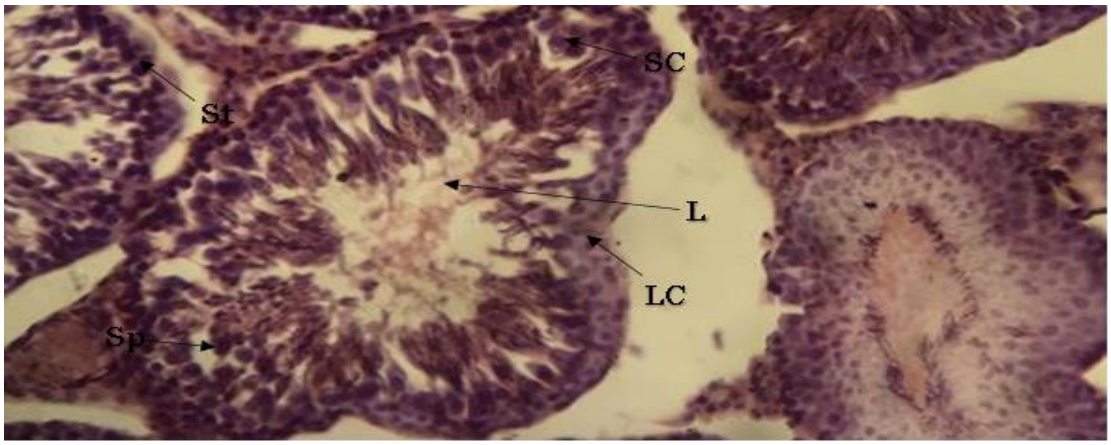

PLATE 2a: Cross section of testes of Sham control-1 group:

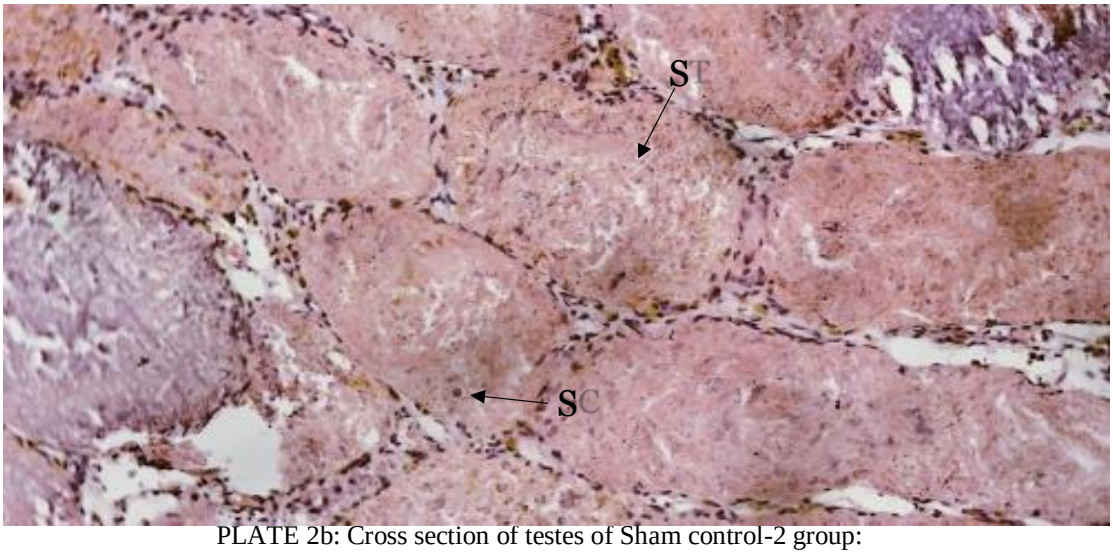

PLATE 2b: Cross section of testes of Sham control-2 group:
Stained with H and E and Magnification x 400. SC = Sperm cells, ST = Seminiferous tubule

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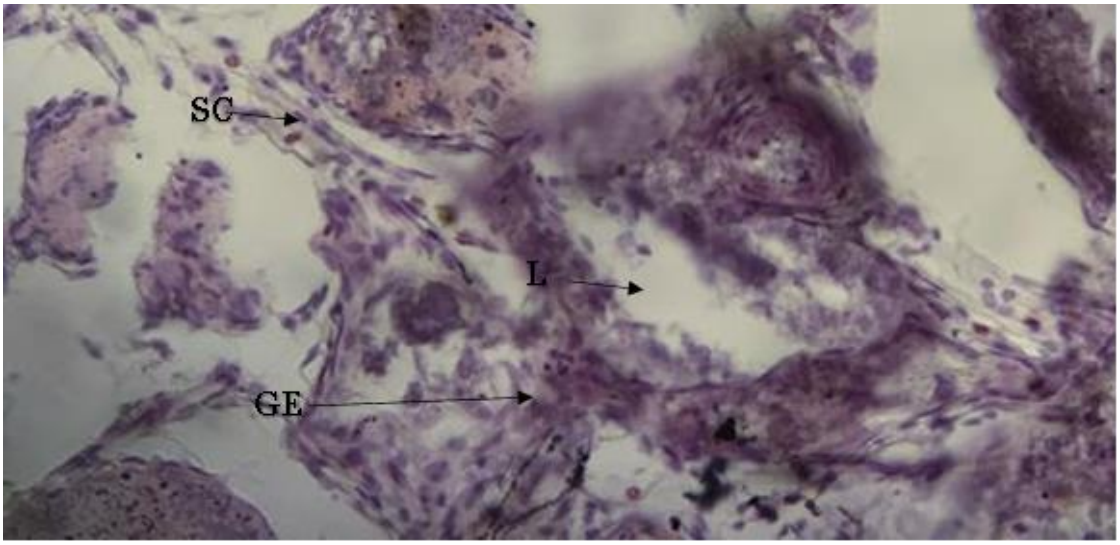

PLATE 3a: Cross section of testes of Cd + QT group:
Stained with H and E and Magnification x 400.
GE = Germinal epithelium. SG = spermatogonia

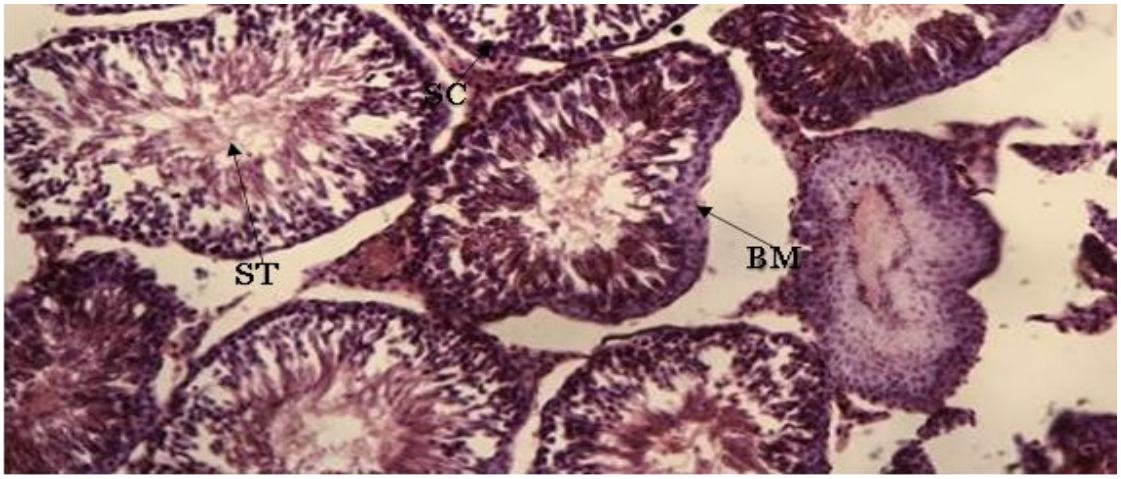

PLATE 3b: Cross section of testes of Cd + Omega-3 group:
Stained with H and E and Magnification x 400
ST = semiferous tubule, BM = basement membrane

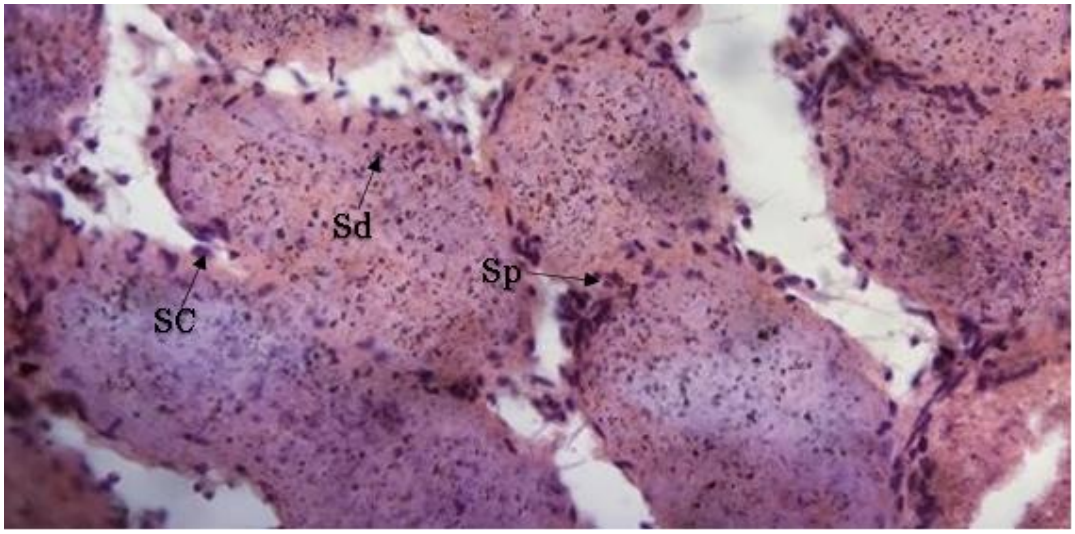

PLATE 4: Cross section of testes of Cd + QT + OM-3 group:
Stained with H and E and Magnification x 400
SC = Sperm cells, Sd = spermatid, Sp = spermatocytes

V. DISCUSSION

This study was conducted to evaluate the ameliorative effect of quercetin and / or omega-3 on cadmium induced alterations in reproductive parameters in male Wistar rats. Infertility is one of the most widespread problems with 48.5 million of the world population being infertile, a major concerned in families & medicine due to its pressure on reproduction. Cadmium exposure is implicated among other factors for infertility. Cadmium induces generation of free radicals which lead to the production of oxidative stress resulting in cellular damage3,4,5.

Results obtained from this study shows that there were no significant changes in the semen pH of the different experimental groups. Optimal semen pH is essential for effective function of the sperm and low values of pH are associated with low seminal vesicle function and the absence of ejaculatory ducts that affect sperm quality and fertility28,29.

Other sperm function parameters recorded in this present study were adversely affected by administration of Cd. The sperm motility, viability and total sperm count were significantly reduced in Cd treated group compared with the controls and groups treated with quercetin and/or omega-3. This is consistent with previous reports on the adverse effect of Cd exposure on sperm motility, viability and count30,31. Sperm motility is the movement and swimming of sperm. Poor motility means the sperms do not swim properly which may lead to male infertility. Sperm viability refers to the percentage of live sperms in the semen sample. It is also a determinant of fertility. Reduction in the number of viable sperm could lead to infertility. Abnormalities in sperm morphology might affect sperm motility, sperm fertilize ability and conception. Sperm cells with slow pregressive movement were significantly raised following cadmium administration while forward progressive movement was impared by Cd administration. These effects also could contribute to Cd induced infertility.

These adverse effects of Cd on sperm motility (including slow and forward progressive movements), viability and total sperm count were ameliorated by the administration of quercetin and omega-3. Previous study had indicated that quercetin found in various products possesses antioxidant, anti-proliferative, anti-inflammatory and anti-histamine properties. Several reports have indicated that quercetin exerts protective effects on various cells including myocytes, testes, renal cells and liver cells in ischemia and reperfusion injury32.

Indeed, significant reductions in sperm count, sperm motility, and the percentage of sperm with abnormal morphology were studied in Cd exposure-induced mice, and supplementation with omega-3 could largely alleviate the effects of Cd on semen quality33. Researchers in another study thought that this improvement might be mediated by the antioxidant properties of omega-332,34.

Cadmium administration caused significant reduction in semen fructose concentration. Fructose is an essential sugar for metabolism and sperm motility, the main energy source for spermatozoa35. Fructose is produced by the seminal vesicles with some contribution from the ampulla of the ductus deferens36. The adverse effect on Cd was also ameliorated by the adminstration of quercetin and/or omega-3.

Cadmium adminstration was also observed to significantly reduce the serum sex hormone levels below the control values. This is also consistent with earlier reports on the deleterious effect of cadmium on testosterone, LH and FSH concentrations. However, many studies suggested that cadmium increased testosterone level. Still others showed that cadmium administration attenuates it37,38.

Similarly, gonadal damage has been shown to develop following administration of cadmium to adult male rats either orally or subcutaneously. Cadmium enters the seminiferous tubules through a branch of the blood testis barriers and causes focal testicular necrosis and dystrophy with consequent reduction in germ cell numbers, leading to infertility37.

Omega-3 and quercetin administration in this present study reversed the adverse effect of Cd on testosterone, LH and FHS concentrations. These beneficial effects were more in the group that received the combined therapy of omega-3 and quercetin39.

In conclusion, administration of quercetin and/or omega-3 ameliorates the adverse effects of Cd on sperm function parameters. Quercetin and/or omega-3 also prevent destructive effects of Cd on testicular cells and sperm production. Quercetin and/or omega-3 had variable effects in ameliorating the adversity of Cadmium. However, combination of both quercetin and omega-3 produced a better ameliorating effect than when administered singly.

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