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Research Article

Quercetin and Omega-3 fatty acid averts the deleterious effects of Cadmium on NO, NOS, anti-oxidants and MDA levels in rats

Gekpe C. G. [1], Adie P. U. [1], Chukwu John AO. [2], Ofem O. E. [1]*

[1] *Physiology Department, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria.*

[2] *Physiology Department, Faculty of Basic Medical Sciences, College of Medical Sciences, Gregory University, Uturu, Abia State, Nigeria*

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*Address for Correspondence:

Prof. Ofem E. Ofem, Physiology Department, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria

Abstract

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. Infertility in animals is one of the most widespread problems with 48.5 million of the world population being infertile. This study aimed to investigate the effects of omega-3 and/or quercetin on cadmium- induced alterations in nitric oxide (NO), nitric oxide synthase (NOS), and anti-oxidants levels. 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. Serum was collected for measurement of the biochemical assay. Results obtained showed no significant differences were observed in all the parameters assayed between the sham control groups and the normal control. The LD_{50} for cadmium was 3.90mg/kg bw. Concentrations of NO, NOS reduced significantly in Cd groups compared with control, omega-3 and/or quercetin groups. SOD, CAT, GPx reduced significantly ($p<0.05$) while MDA increased significantly in Cd groups compared with control, omega-3 and/or quercetin groups. In conclusion, administration of quercetin or omega-3 ameliorates the adverse effects of Cd on NO, NOS, antioxidants (SOD, CAT, GPx), and MDA levels. A combination of both quercetin and omega-3 produced a better ameliorating effect than when given singly.

Keywords: Cadmium, Omega-3, quercetin, NO, NOS, anti-oxidants, MDA, rats.

INTRODUCTION

Infertility is one of the most widespread problems with 48.5 million of the world population being infertile[1]. It has become the main concern in medicine due to its pressure on reproduction. Several factors are held responsible for this problem and cadmium is implicated as one of the major toxic pollutants in the biosphere[2]. Cadmium exposure to the environment leads to great health threats and has been found to create series of biological and physiological dysfunction in both humans and laboratory animals[3,4,5]. Hence, it becomes pertinent to investigate the therapeutic effects of omega-3 and quercetin on cadmium- induced infertility in rats as a model for human representative.

Cadmium is a toxic heavy metal of both environmental and occupational concerns[6,7]. Cadmium ranks within the top 20 hazardous substances and the human activity has markedly increased the distribution of cadmium in the global environment[1]. This metal is present in the soil, water, air and food[8]. 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[9].

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, results in lipid peroxidation, altering the antioxidants system, causing membrane protein damage, DNA damage and apoptosis[10,7]. It becomes pertinent that potent antioxidants could retard or inhibit the basic mechanism of Cd-induced deleterious alterations and possibly ameliorate its toxic effects in biological systems particularly the reproductive system[7]. Omega-3 fatty acid is a potent anti-oxidant.

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[11]. Omega-3 fatty acids are represented by linoleic acid (LA; 18:2*w*6). Linoleic acid is plentiful in nature and is found in the seeds of most plants except for coconut, cocoa and palm[2].

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[12].

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[13]. 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[14,15,16,17]. 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[13,18].

There is paucity of scientific literatures on the impact of omega-3 and / or quercetin on cadmium induced alterations in nitric oxide, nitric oxide synthase, anti-oxidants levels and lipid peroxidation product, which are some indices to sperm functions and male fertility.

This study therefore was aimed to ascertain the effects of omega-3 and/or quercetin on cadmium-induced alterations in nitric oxide, nitric oxide synthase, anti-oxidants and lipid peroxidation product (malondialdehyde) levels in rats.

MATERIALS AND METHODS

Chemicals

Chemicals used in this study were of analytical grade, 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. The Olive oil used was purchased from Bez pharmacy, Calabar, Nigeria.

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 both the LD_{50} study and the main work.

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 Sciences animal ethical committee.

Preparation and administration of experimental drugs

Cadmium was administered at a dose of 2mg/kg body 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% dimethyl sulfoxide (DMSO) solvent, then given at 0.1ml/100g bw. subcutaneously once daily[19].

DSMO (2%) was given at the dose of 1mL/kg orally and once daily to 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.

Acute toxicity study

Eighteen (18) albino Wistar rats were used for the determination of LD_{50}. 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[20].

Experimental design

A total of forty-eight (48) albino Wistar rats were randomly 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 + Quercetin): 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/kg o.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 utilized euthanized.

Collection of experimental samples

After the 56 days (8 weeks) feeding regimens, the animals were then fasted overnight, weighed and anaesthetized with 5% chloroform. Blood samples were be collected via cardiac puncture into plain caped sample bottles for serum extraction from clotted blood samples[21]. 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.

Antioxidants assay

Determination of superoxide dismutase (SOD)

Principles: Superoxide dismutase uses the phosphochemical reduction of riboflavin as oxygen generating sustain and catalyzed the inhibition of NB reduction and can be assayed spectrophotometrically[22]. .

Test Procedure: Exactly 0.5g of the sample was ground with 3.0 mL of phosphate buffer (pH 7.8), centrifuge at 2000 RPM for 5 minutes and the supernatant was used for the assay. The incubation contains a final volume of potassium phosphate buffer (pH 7.8), methione and riboflavin and potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure sufficient accuracy. The tube was placed in an aluminium foil for lined box maintain at 25^{o} C and equipped with 15W florescent lamps. Reduced nitroblue tetrazalium (NBT) was measured spectrophotometrically at 600nm after exposure to the light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of the enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were calculated as units/mg proteins.

Determination of malondialdehyde (MDA)

Into the test tubes labelled serum test and blank were added 5.0µl of serum and distilled water respectively. This was

followed by addition of 0.5ml of 25% TCA (trichloroacetic acid) and 0.5ml of the TBA (Thiobarbituric acid) in 0.3% NaoH. The mixture was boiled for 40 minutes in water bath and cooled in cold water. 0.1ml of 20% sodium dodecyl sulfate (SDS) was then added to the cooled solution and mixed properly. The absorbance was taken at wave lengths 532 and 600nm against the blank[23].

$$MDA = \frac{ABs1 - ABs2}{0.00693}$$

Estimation of Glutathione (GPx)

Exactly 20% homogenate was obtained by homogenizing 0.5g of samples in 2.5ml of 5% TCA. Precipitating of the protein was effected by addition of 125µl of 25% TCA in 0.5ml of the tissue homogenate. The precipitated protein was centrifuged at 1000rpm for 10 minutes. The homogenate was cooled on ice and 0.1ml of supernatant was taken for the estimation. The supernatant was made up to 1ml with 0.2m sodium phosphate buffer (pH 8.0). 2.0ml of freshly prepared 5,5-dithiobis- (2-nitrobenzoic acid). (DTNB) solution was added to the tubes containing the supernatant and the intensity of yellow colour formed was read at 4.2nm in a spectrophotometer after 10 minutes[24].

Measurement of catalase activity

The frozen samples were homogenized in 25mM, Na-phosphate buffer (pH 6.8) using a mortar and pestle and centrifuge at 12000 xg for 20 minutes at 4°C. The supernatant obtained was used as the crude enzyme solution. A 3ml reaction mixture consisted of crude extract, Na-phosphate buffer (pH 6.8) and 10ml MH_2O_2 diluted in buffer, initiated the reaction. The decrease in absorbance was recorded followed by the decomposition of H_2O_2 240nm per minute mg protein[25].

After wash, wash buffer was aspirated or decanted. The plate was inverted and blotted against clean paper towels. 100µl of HRP-covidin (IX) was added to each well and microliter plate covered with a new adhesive strip. Then, the microliter plate was incubated for 1 hour at 37°C. A repeat of the aspiration/wash process was carried out for 5 times at 37°C. 10µl of TMB substrate was added to each well and the plate was gently tapped to ensure thorough mixing. The optical density of each well was determined within 5 minutes, using a microplate reader set to 450nm. The readings at 540nm were subtracted from the readings at 450nm. This subtraction was for correction of optical imperfections in the plate.

Determination of NO and NOS concentrations (Using Griess reagent)

NO and NOS were assayed based on measurement of total sample nitrites. The method employed nitrate reductase for reduction of nitrate to nitrite using purified plant nitrate reductase with NADH helps prevent NADPH interference with the Griess reaction. Nitrites are converted to nitrous acid (HNO_2) in sulfanilamide, HCl solution. HNO_2 subsequently reacts with sulfanilamide to form sulfanilamide diazonium salt. Diaazonium salt was then reacted with N-(1-Napthyl) – ethylenediamine (NED) to produce a chromophore directly measurable at 540m.

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.

RESULTS

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.

Nitric oxide (NO) concentration of the different experimental groups

NO activity (µmol/L) in NC, SC-1 and SC-2 were 34.05 ± 0.45, 35.21 ±1.48 and 34.63 ± 0.70 respectively. No significant differences were recorded among the different control groups. Significant (p<0.05) reductions in NO were observed in Cd groups with activity of 27.09 ± 0.90 compared with the controls and other experimental groups, FIG. 19.

Nitric oxide synthase (NOS) activity in the different experimental groups

Nitric oxide synthase activity (µmol/L) in NC, SC-1 and SC-2 were 15.92 ± 0.14, 16.03 ± 0.48 and 15.60 ± 0.56 respectively, showing no significant differences among the controls. However, the Cd treated groups showed significant (p<0.05) reduction in NOS concentration (11.69 ± 0.46) compared with controls and other experimental groups. It was also significantly (p<0.05) lower in the Cd groups treated with quercetin and/or omega-3 compared with controls, FIG. 20.

Superoxide dismutase (SOD) activity in the different experimental groups

SOD activity in the NC, SC-1 and SC-2 groups were 9.24 ± 0.12, 9.33 ± 0.10 and 9.43 ± 0.10 ng/mL respectively, showing no significant differences among the control groups. The Cd treated group had significant (p<0.05) reduction in SOD (5.95 ± 0.39) compared with controls and other treatment groups, FIG. 21.

Catalase (CAT) activity in the different experimental groups

No significant differences were observed in CAT activity (pg/mL) among the control groups (NC 80.99 ± 0.65, SC-1 78.33 ±1.70 and SC-2 79.40 ±1.25). CAT levels decreased significantly (p<0.05) in Cd treated group (59.16 ±1.11) compared with controls, Cd +QT (60.94 ± 0.30), Cd +OM-3 (66.15 ±1.46) and Cd + QT + OM-3 (66.38 ±1.24), FIG. 22.

Glutathione peroxidase (GPx) activity in the different experimental groups

As illustrated in FIG. 23, no significant differences were recorded in the GPx levels (ng/mL) among the control groups (NC 3.66 ± 0.27; SC-1 4.08 ± 0.19 and SC-2 3.95 ± 0.16). However, significant (p<0.05) reductions were seen in the Cd treated group (5.95 ± 0.39) compared with controls, and significantly (p<0.05) higher in other treatment groups compared with Cd treated.

Malondialdehyde (MDA) activity in the different experimental groups

There were no significant differences were observed in MDA concentrations (nmol/mL) among the control groups. MDA concentration in the NC was 12.75 ± 0.08 while SC-1 and SC-2 had MDA of 12.34 ± 0.19 and 12.50 ± 0.21 respectively. It increased significantly (p<0.05) in Cd treated group control with control, significant (p<0.05) elevations were seen in other treatment groups compared with Cd treated groups. Values obtained for Cd + QT was 13.80 ± 0.13, 21, while SC-1 and SC-2 had values of 74 ± 1.17 and 11.60 ± 0.32 respectively, FIG. 24.

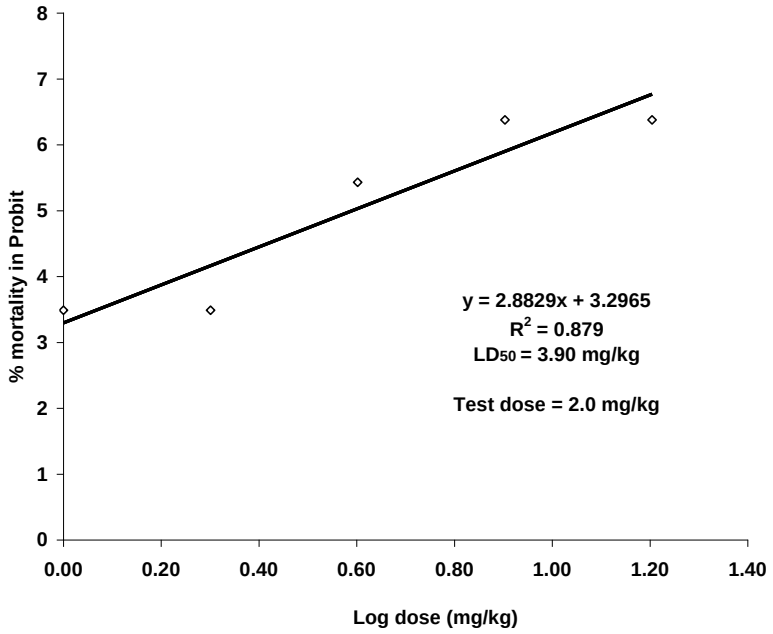

FIG. 1: Acute toxicity study for determination of LD_{50} of cadmium chloride in mice. (LD_{50} = 3.90 mg/kg).

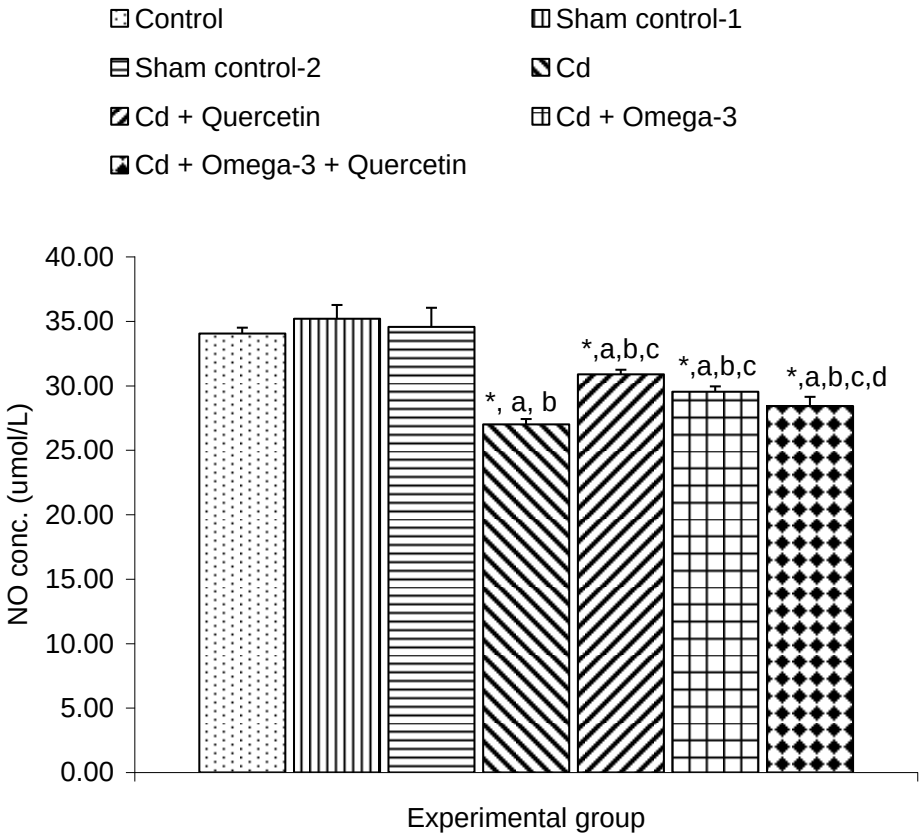

FIG. 19: Nitric oxide activity in the different groups.

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
d = p<0.05 vs Cd + quercetin

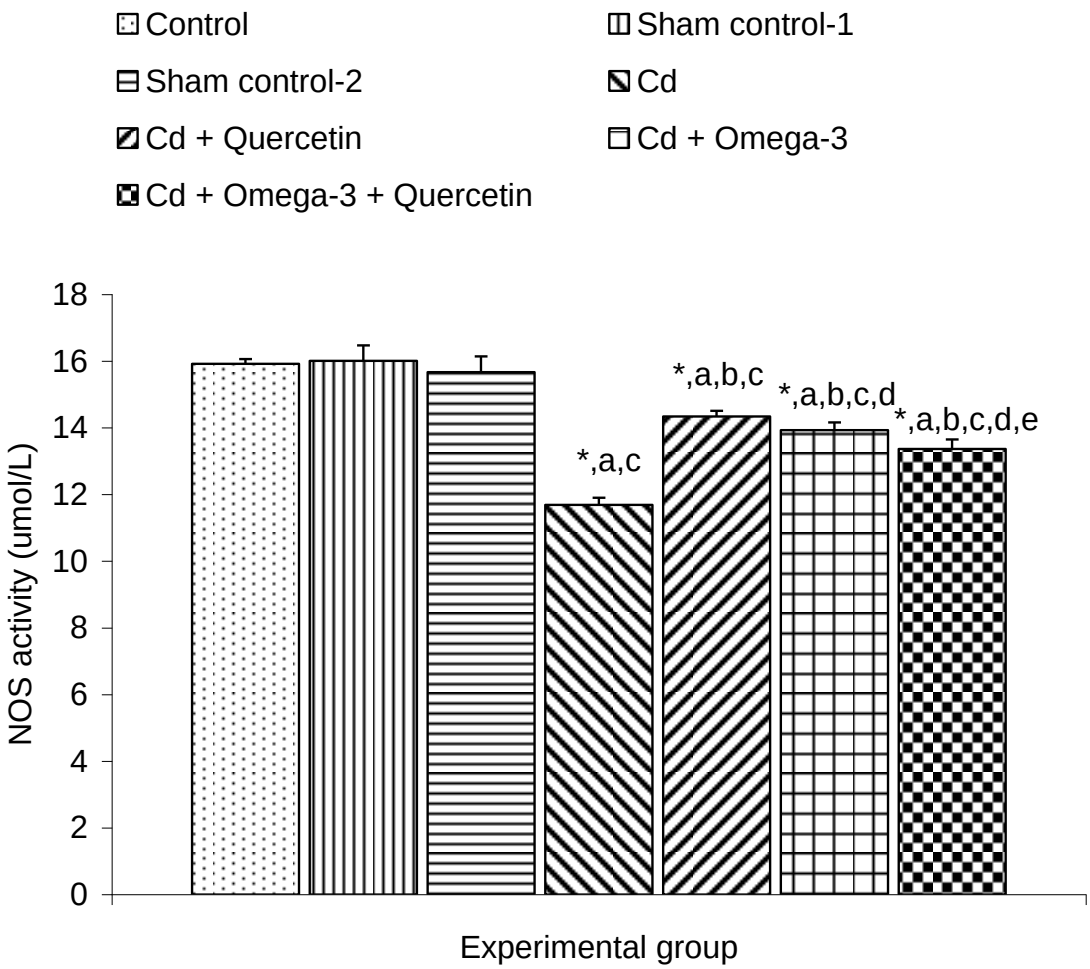

FIG. 20: Nitric oxide synthase activity in the different experimental groups.

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
d = p<0.05 vs Cd + quercetin
e = p<0.05 vs Omega-3

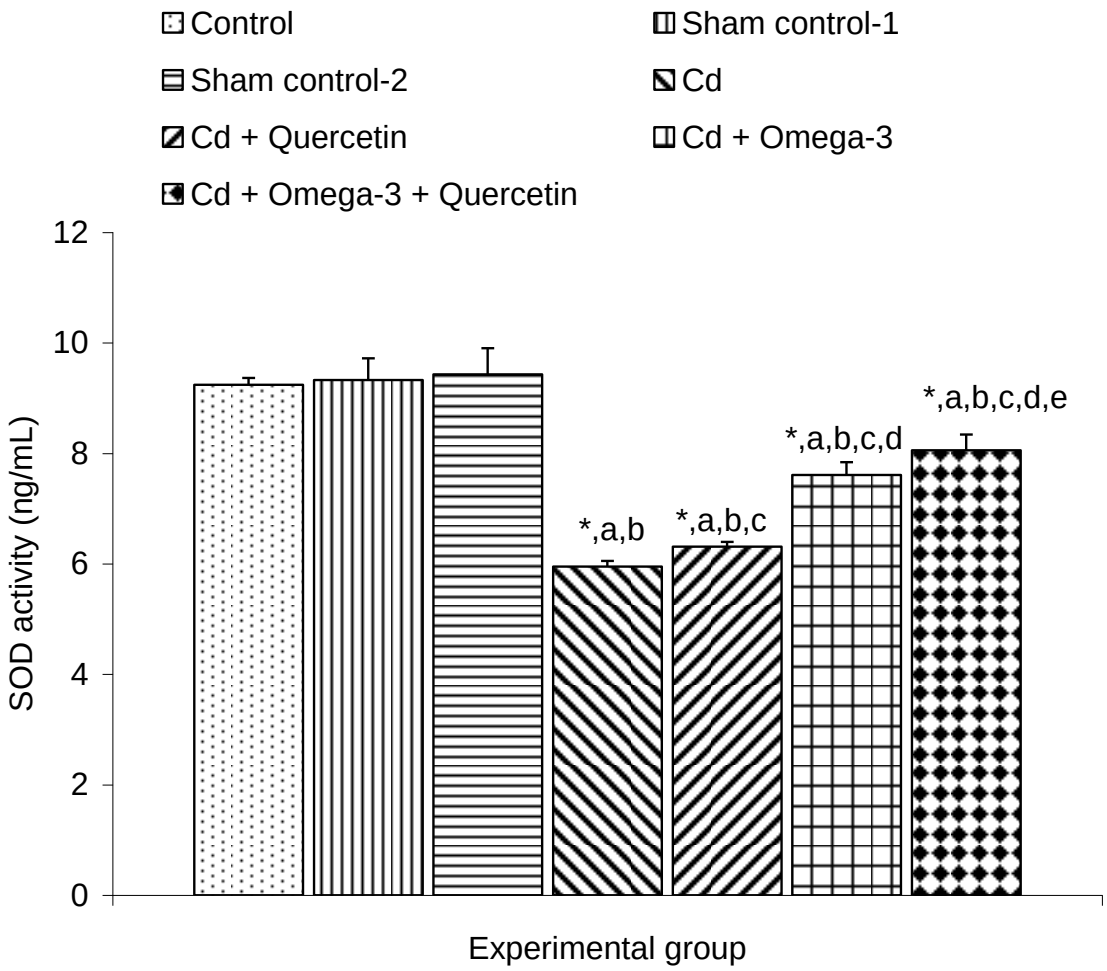

FIG. 21: Superoxide dismutase level in the different experimental groups.

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
d = p<0.05 vs Cd + quercetin
e = p<0.05 vs Omega-3

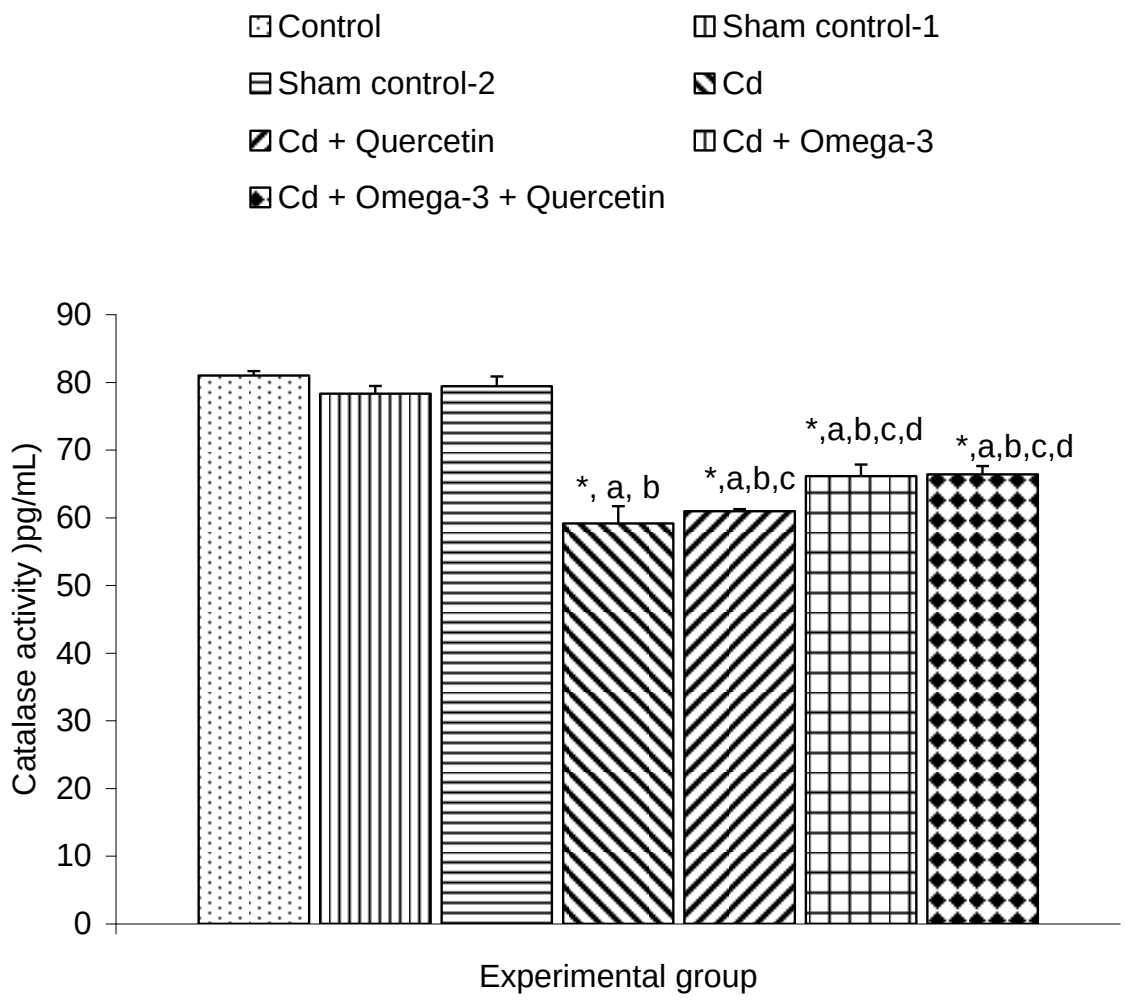

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FIG. 22: Catalase activity in the different groups.

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
d = p<0.05 vs Cd + quercetin

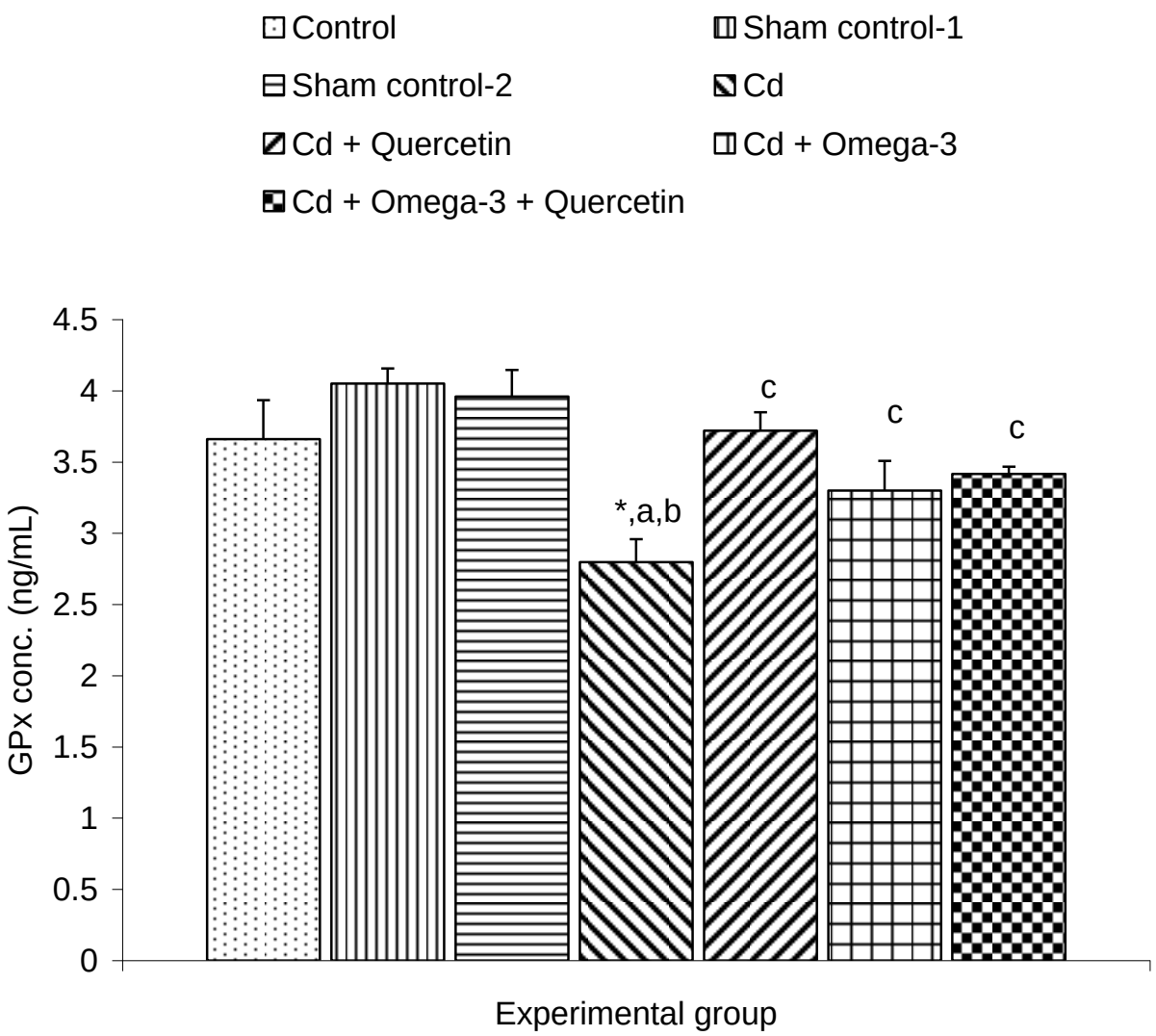

FIG. 23: Glutathion activity in the different experimental groups.

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

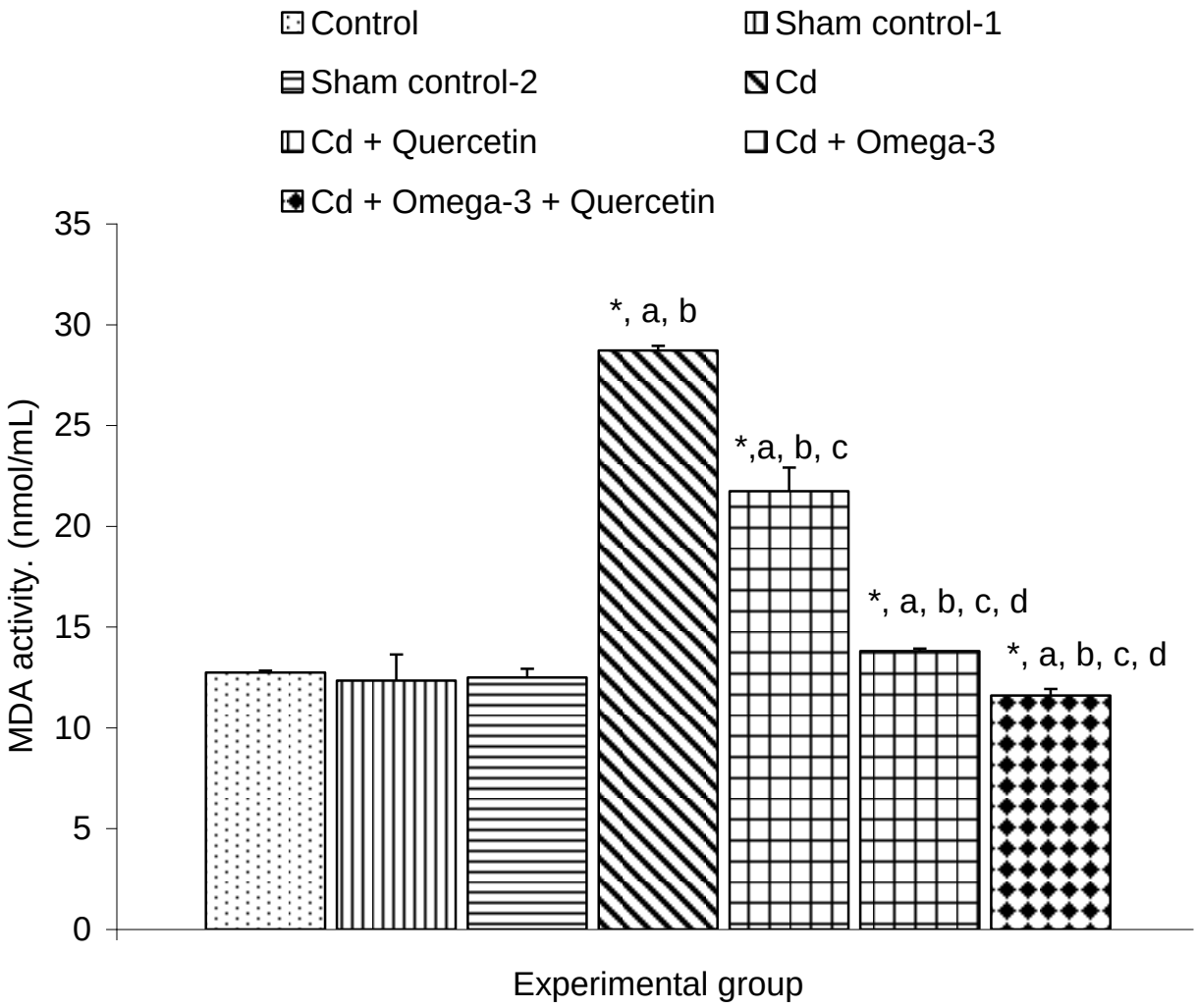

FIG. 24: Malondialdehyde activity in the different experimental groups.

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
d = p<0.05 vs Cd + quercetin

DISCUSSION

This study evaluated the ameliorative effect of quercetin and / or omega-3 on cadmium induced alterations in nitric oxide, nitric oxide synthase and antioxidant levels in rats. Cadmium exposure is implicated among other factors for infertility which has affected about 48.5 million of the world population, this has pose a major concerned in families & medicine. Cadmium induces generation of free radicals which lead to the production of oxidative stress resulting in cellular damage[26,27,28].

This present study also added to wealth of knowledge on the adverse effect of cadmium on NO and NOS concentration. NO and NOS levels were significantly reduced by Cd administration. Administration of quercetin and/or omega-3 ameliorated this abnormally. NO has both positive and negative effects on sperm functions and semen parameters depending on its level. Physiological levels of NO are essential for sperm functions such as capacitation, acrosomal reaction, zona pellucida and binding[29].

More so, NO contributes to normal sperm morphology, which has been shown to facilitate the accurate prediction of fertility status and pregnancy outcomes during invitro fertilization. Nevertheless, similar to sperm motility, it has been shown that in high concentrations NO induces atypical sperm morphology. Sperm viability is also affected by NO[29].

Serum levels of SOD, CAT and GPx were significantly reduced while MDA was significantly elevated following Cd administration is this present study. Previous studies have shown that cadmium binds with thiol groups of enzymatic antioxidants; SOD, catalase (CAT) and glutathione peroxidase (GB) leading into decrease of intracellular GSH content. This activity of Cd on the antioxidants inhibits the antioxidant mechanism, hence oxidative stress may occur[30].

Administration of quercetin and omega-3 ameliorated the adverse effect of Cd on serum antioxidant and lipid peroxidation product. A better effect was observed in the combined therapy group than in quercetin or omega-3 group.

In conclusion, administration of quercetin and/or omega-3 ameliorates the adverse effects of Cd on NO, NOS, antioxidants (SOD, CAT, GPx) and MDA. This could improve sperm quality and enhance male fertility. Quercetin and/or omega-3 had variable effects in ameliorating the adversity of Cadmium. Administration of both quercetin and omega-3 produced a better ameliorating effect than when administered singly.

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