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DOXORUBICIN-INDUCED REPRODUCTIVE TOXICITY IN MALE WISTAR RATS: AMELIORATIVE EFFECT OF OMEGA-3 FATTY ACIDS

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ABSTRACT

Doxorubicin is a widely used antineoplastic agent for treatment of cancers. Its use is associated with side effects including reproductive impairment. Possible mitigative effect of a common antioxidant, Omega-3 was investigated on doxorubicin-induced male reproductive toxicity. Fifteen male adult Wistar rats were randomly divided into control, doxorubicin-only and doxorubicin+Omega-3 groups of 5 rats each. Doxorubicin was administered at a dose of 3mg/kg intraperitoneally on days 1, 7, 14 and 28 while Omega-3 (300mg/kg) was given orally, daily for 28 days. Results showed significantly decreased ($p<0.05$) sperm count, motility and viability in the doxorubicin-only and doxorubicin+Omega-3 groups compared with control, but significantly ($p<0.05$) higher in the doxorubicin+Omega-3 than in the doxorubicin-only groups. Teratozospermia was significantly ($p<0.05$) increased in the doxorubicin-only and doxorubicin+Omega-3 compared with control, though significantly ($p<0.05$) lower in the doxorubicin+Omega-3 than in doxorubicin-only groups. Serum testosterone and follicle-stimulating hormone were significantly ($p<0.05$) reduced in the doxorubicin-only compared with control and Doxorubicin+Omega-3 groups, though significantly increased in the doxorubicin+Omega-3 compared with the Doxorubicin--only groups. Serum luteinizing hormone was significantly ($p<0.05$) reduced in the doxorubicin-only and doxorubicin+Omega-3 compared with control, but significantly higher ($p<0.05$) in the doxorubicin+Omega-3 than in doxorubicin-only group. Testicular malondialdehyde was significantly (p<,0.05) raised in the doxorubicin-only and doxorubicin+Omega-3 groups compared with control ($p<0.05$), but significantly lower ($p<0.05$) in doxorubicin+Omega-3 than in doxorubicin-only groups. Testicular glutathione peroxidase, superoxide dismutase and catalase were significantly ($p<0.05$) elevated in the doxorubicin-only compared with the control and doxorubicin+Omega-3 groups, but significantly ($p<0.05$) higher in doxorubicin+Omega-3 than in doxorubicin-only groups. It is therefore concluded, doxorubicin-induced male reproductive toxicity and oxidative stress are mitigated by Omega-3.

KEYWORDS: Doxorubicin, male, reproductive toxicity, Omega-3, mitigate

INTRODUCTION

Doxorubicin (marketed as Adriamycin) is an antineoplastic agent used for the treatment of cancers. It is an anthracycline antitumour antibiotic derived from *Streptomyces peucetius* bacterium (Grimm *et al*. 1994). The antimitotic, chemotherapeutic agent is used in the treatment of many cancers including breast cancer, Karposi's sarcoma, lymphomas and leukemias (The America Society of Health System Pharmacy, 2016). Doxorubicin is widely used as a firstline, cornerstone chemotherapeutic agent for many solid and hematological malignancies due to its broad-spectrum efficacy (Tamzali and Kemp-Symnond, 2016).

The mechanism by which Doxorubicin (Dox) operates is through interaction with DNA by intercalation and inhibition of intracellular biosynthesis of macromolecules leading to apoptosis (Pommier *et al.,* 2010, Tacar *et al*. 2013). Doxorubicin also promotes formation of quinone-type free radicals leading to oxidation of DNA in the mitochondria and cell nucleus resulting in cytotoxicity (Rossi, 2013).

Though, doxorubicin is widely used as a first-line drug in the treatment of various cancers, its use is limited by the development of toxicities.

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Doxorubicin is reported to cause cardiotoxicity associated with down regulation of cardiac-specific genes, oxidative stress and cardiac myocytes apoptosis (Luu *et al*, 2018). The administration of doxorubicin has also been said to cause male reproductive toxicity (Prahalathan *et al*., 2005, Xin *et al*., 2012) possibly from oxidative stress and apoptosis (Trivedi *et al*., 2011). Doxorubicin also induces nephrotoxicity (El-Sawy *et al*., 2024) and neurotoxicity (Shokoohinia *et al.,* 2014).

Omega 3 fatty acids (OM3) are essential, heterogeneous polyunsaturated fatty acids which can only be taken exogenously. They are required as essential micronutrients for a variety of bodily functions including growth, reproduction, vision, and nervous system functions and skeletal health (Patted *et al,* 2024). Omega 3 fatty acids have intrinsic anabolic functions, anti-inflammatory Calder, 2010) and antioxidant (Heshmati *et al., 2019*, Mazza *et al,* 2007) properties. Omega-3 fatty acids improve cardiovascular, ocular and neurological health (Patted *et al,* 2024). Sources of Omega 3 fatty acids include flax seed, soybeans, Canola oil, walnuts, fish and black nuts (Patted *et al,* 2024). These acids lower serum triacyl glycerides and play key roles in energy metabolism.

Since doxorubicin induces cytotoxicity via mechanisms which include oxidative stress, it became necessary to evaluate possible effect of a common and potent antioxidant, Omega-3 fatty acids on doxorubicin-induced male reproductive toxicity in rat model.

MATERIALS AND METHODS

Experimental animals

Fifteen male adult Wistar rats were used for the study. The rats were housed in the Animal house of the Department of Human Physiology, University of Calabar and handled in line with the 1985 guidelines of the National Institute of Health Publications for Laboratory animals. They were given free access to rat chow and portable water under a 12-hour night day cycle.

Ethical approval

The ethical clearance for this study was obtained from the Animal Ethics and Research Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar.

Experimental design

The rats were randomly divided into 3 groups namely, control, doxorubicin-only (Dox-only) and Doxorubicin+Omega-3 (Dox+OM3) groups, each having 5 rats. Doxorubicin was administered intraperitoneally at a once weekly dose of 3mg/kg (Adiyaman *et al.,* 2022) for 4 weeks. Omega 3 fatty acids was given by gavaging at a dose of 300mg/kg which based on allometric scaling, has been used by many researchers including Omole *et al*., (2025). The experimentation lasted for 28 days.

Collection of samples

At the end of 28 days, 60mg/kg of pentobarbital was administered to each rat intraperitoneally for anesthesia. Blood samples were collected by intracardiac puncture into pre-labelled plain capped sample bottles for evaluation of serum FSH, LH and testosterone levels while the testes and epididymis were dissected out for determination of sperm parameters and oxidative stress indices.

Measurement of gonadosomatic indices

An electronic weighing scale (Scout Pro Ohaus Corporation, USA) was used to measure testicular weight while body weight was measured using a standard weighing scale and body weight changes computed.

Evaluation of sperm parameters

Sperm count

This was done following the method of Raji et al. (2006). In brief, each cauda epididymis was pre-warmed in 2ml normal saline at 37°C and incisions made on it to enable the stored spermatozoa flow out from the epididymis into the fluid. Using a Pasteur pipette, 200µL of the sperm suspension was placed on both chambers of the improved Neubauer haemocytometer. With the aid of a microscope (Leica DM 750, Germany), the spermatozoa were counted in five large Thoma squares and made adjustment for volume of normal saline added.

Sperm motility

Sperm motility was assessed as described by Atashfaraz *et al.* (2013) 10µL of sperm suspension was placed on a clean pre-warmed glass slide and covered with a coverslip. It was examined at x100 magnification using a light microscope (Leica DM 750, Germany).

Sperm viability

The viability of sperms was evaluated as described by Wyrobek *et al.* (1983). In summary, 20µL of 0.05% eosin Y-nigrosin was added to equal volume of the sperm suspension on a glass slide and the mixture incubated for 2 min at room temperature. The slides were then viewed using a light microscope (Leica DM 750, Germany) and the percentages of viable (unstained) and non-viable (pink-stained) cells observed were computed.

Sperm morphology

Sperm morphology was determined using the method described by Narayana *et al*. (2005). A drop of the sperm suspension prepared for sperm count was smeared on a glass slide and stained with 1% eosin Y, air-dried, and viewed with a microscope (Leica DM 750, Germany) at ×400 magnification. Two hundred spermatozoa were screened for each rat and percentage of total abnormalities of head, middle piece and tail was determined.

Assay of serum male reproductive hormones

Testosterone

Serum testosterone was measured using ELISA kits, (BioAssay Systems, Hayward, CA, USA) and according to manufacturer protocol.

Luteinizing hormone

Serum LH was assayed by ELISA methods using ACCUBind LH ELISA kits manufactured by MonoBind Incorporated, U$A. The manufacturer' protocols were followed.

Follicle-stimulating hormone

Serum concentration of FSH was determined by ELISA method using FSH ELISA Kits (BioAssay Systems, USA) according to the manufacturer' instructions.

Determination of oxidative stress biomarkers in testes

Malondialdehyde

Lipid peroxidation, as concentration of MDA, was estimated in testicular homogenate by measuring spectrophotometerically (SP-V5100 Visible spectrophotometer, Scitek Global Co., Ltd, China.) the level of the lipid peroxidation product, malondialdehhyde (MDA) as described by Wallin et al. (1993). Lipid degradation occurs forming such products as malondialdehyde (from fatty acids with two or more double bonds), ethane and pentane (from the n – terminal carbons of 3 and 6 fatty acids, respectively). Malondialdehhyde reacts with thiobarbituric acid to form a red or pink coloured complex which in acid solution absorbs maximally at 532 nm.

Glutathione peroxidase

Glutathione peroxidase activity was assayed using the method described by Ellman (1959) with reagents from Sigma Aldrich, USA. The measurement was based on its reaction with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) to give a compound that absorbs at 412 nm.

Superoxide dismutase (SOD)

Superoxide dismutase activity was determined by the method of Kakkar et al. (1984). Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyzes the inhibition of NBT (Nitro blue Tetrazolium) reduction, the extent of which can be assayed spectrophotometrically.

Catalase (CAT) activity

Catalase activity was assayed spectrophotometrically following the method of Luck (1974). In this procedure, UV light absorption of hydrogen peroxide can be easily measured between 230 – 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity can be arrived at from this decrease.

Statistical analysis

Results were presented as mean ± SEM. All data were analyzed using Statistical Package for Social Sciences (SPSS), version 25, IBM Incorporation, US. Normally distributed data (from Kolmogorov Smirnov test) were analyzed using one way analysis of variance (ANOVA) followed by Tukey post hoc test (after homogeneity of variance was confirmed using Levene test) to make comparisons. Values of $p<0.05$ were considered statistically significant.

RESULTS

Comparison of body weight changes, relative testicular weight

The mean body weight changes (g) were 27.8 ± 5.31 for the control, - 17.6 ± 4.44 for Dox-only and 2.60 ± 5.86 for Dox+OM3 groups. It was significantly ($p<0.05$) reduced in the Dox-only group compared with control. It was however significantly ($P<0.05$) higher in the Dox+OM3 group than in the Dox-only group as shown in Table 1.

Relative testicular weights (g) of the Dox-only (1.01 ± 0.12) was significantly ($p<0.05$) reduced compared to the control (1.43 ± 0.21), but significantly ($p<0.05$) higher in the Dox+OM3 (1.01 ± 0.12) than in the Dox-only groups as shown in Table 1.

TABLE 1: Body weight change and relative testicular weight of the different experimental groups

	Body weight change (g)	Relative testicular weight
Control	27.8 ±5.31	1.43 ±0.21
Dox-only	-17.6 ±4.44[a]	1.01 ±0.12[a]
Dox + OM3	2.6 ±5.86[a,b]	1.01 ±0.12

Values are expressed as mean ±SEM, n = 5.
a = $p<0.05$ vs control
b = $p<0.05$ vs DOX-only

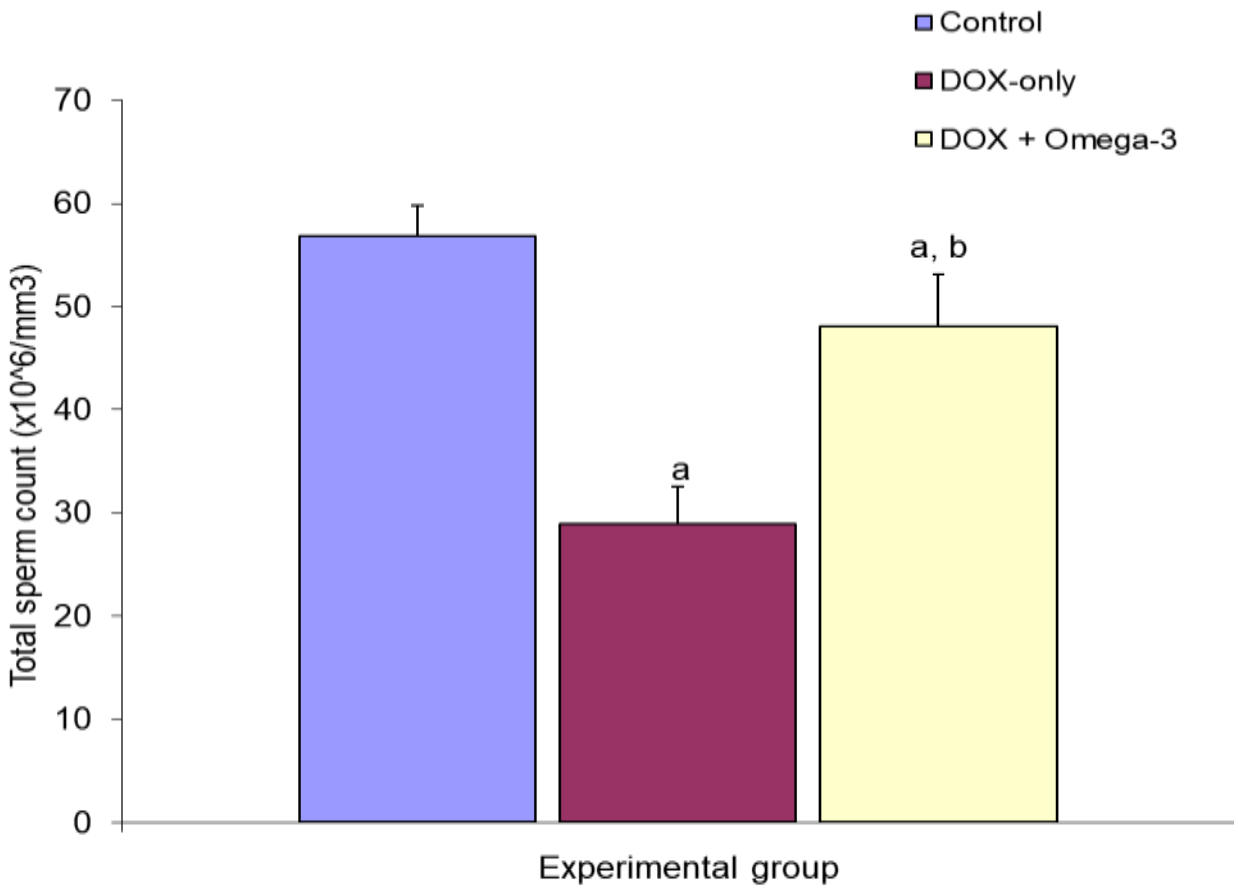

Fig. 1: Total sperm cell count in the different experimental groups.

Values are expressed as mean+SEM, n = 5.
a = p<0.05 vs control
b = p<0.05 vs DOX-only

Comparison of sperm parameters

Sperm count

Sperm count (x10^6/mL) in the control, Dox-only and Dox+OM3 groups were 56.86 ± 5.46, 28.96 ± 4.16 and 48.08 ± 4.09 respectively. It was significantly reduced ($p<0.05$) in the Dox-only and Dox+OM3 groups compared with control though higher ($p<0.05$) in the Dox+OM3 than in the Dox-only group as shown in Fig 1.

Sperm motility

The sperm motility (%) was significantly ($P<0.05$) reduced in Dox-only (50.60 ± 3.64) and Dox+OM3 (68.00 ± 5.04) groups compared with the control (79.00 ± 2.91) but significantly higher ($p<0.05$) in the Dox+OM3 than in the Dox-only group as shown in Fig 2.

Sperm Viability

Sperm viability (%) in the control, Dox-only and Dox+OM3 groups were 72.80 ± 2.77, 52.47 ± 2.97 and 73.40 ± 3.97 respectively. It was significantly reduced ($p<0.05$) in the Dox-only group compared with control but significantly higher ($p<0.05$) in the Dox+OM3 group than in the Dox-only group, Fig 2.

Sperm morphology

The Percentages (%) of abnormal Sperm Morphology were 10.80 ± 1.70, for the control, 30.20 ± 2.38 for Dox-only and 18.20 ± 2.14 for Dox+OM3 groups. It was significantly higher ($p<0.05$) in both Dox-treated groups than in the control though significantly reduced in the Dox+OM3 compared with Dox-only groups as shown in Fig 2.

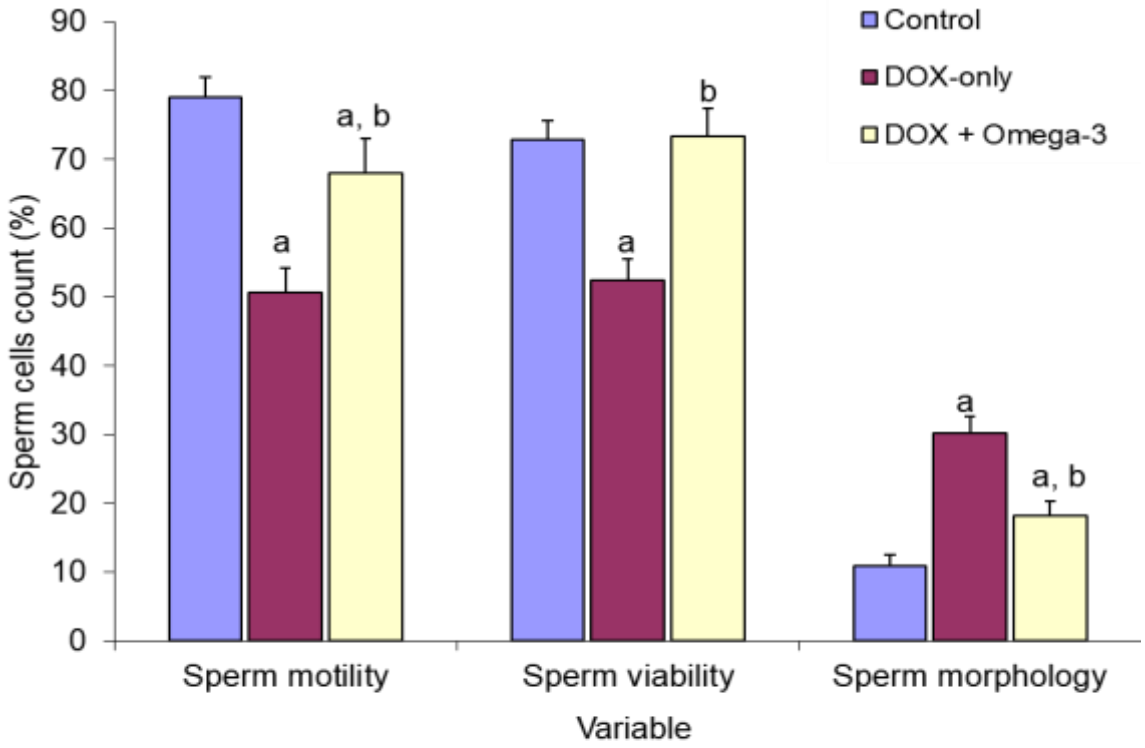

Fig. 2: Sperm cells motility, vaibility and morphology among the different experimental groups.

Values are expressed as mean+SEM, n = 5.
a = p<0.05 vs control
b = p<0.05 vs DOX-only

Comparisons of male serum reproductive hormones

Testosterone

The concentrations of serum testosterone (ng/mL) were 5.11 ± 0.85, 2.03 ± 0.52 and 4.04 ± 0.57 for control, Dox-only and Dox+OM3 groups respectively. It was significantly ($p<0.05$) decreased in the Dox-only group compared with control but significantly higher ($p<0.05$) in the Dox+OM3 than in the Dox-only groups, Fig 3.

Luteinizing hormone (LH)

The serum concentrations (ng/mL) of LH for control, Dox-only, OM3-only and Dox+OM3 groups were 5.31 ± 0.73, 1.68 ± 0.21 and 4.74 ± 0.07 respectively. It was significantly ($p<0.05$) decreased in the Dox-only compared with control groups, though significantly higher ($p<0.05$) in the Dox+OM3 than in the Dox-only groups as shown in Fig 3.

Follicle-stimulating hormone (FSH)

The concentrations (ng/mL) of FSH were 6.59 ± 0.45, 1.66 ± 1.00 and 5.04 ± 1.08 for the control, Dox-only and Dox+OM3 groups respectively. It was significantly ($p<0.05$) decreased in the Dox-only group compared with control though significantly higher ($p<0.05$) in the Dox + Omega-3 than in the Dox-only group as seen in Fig 3.

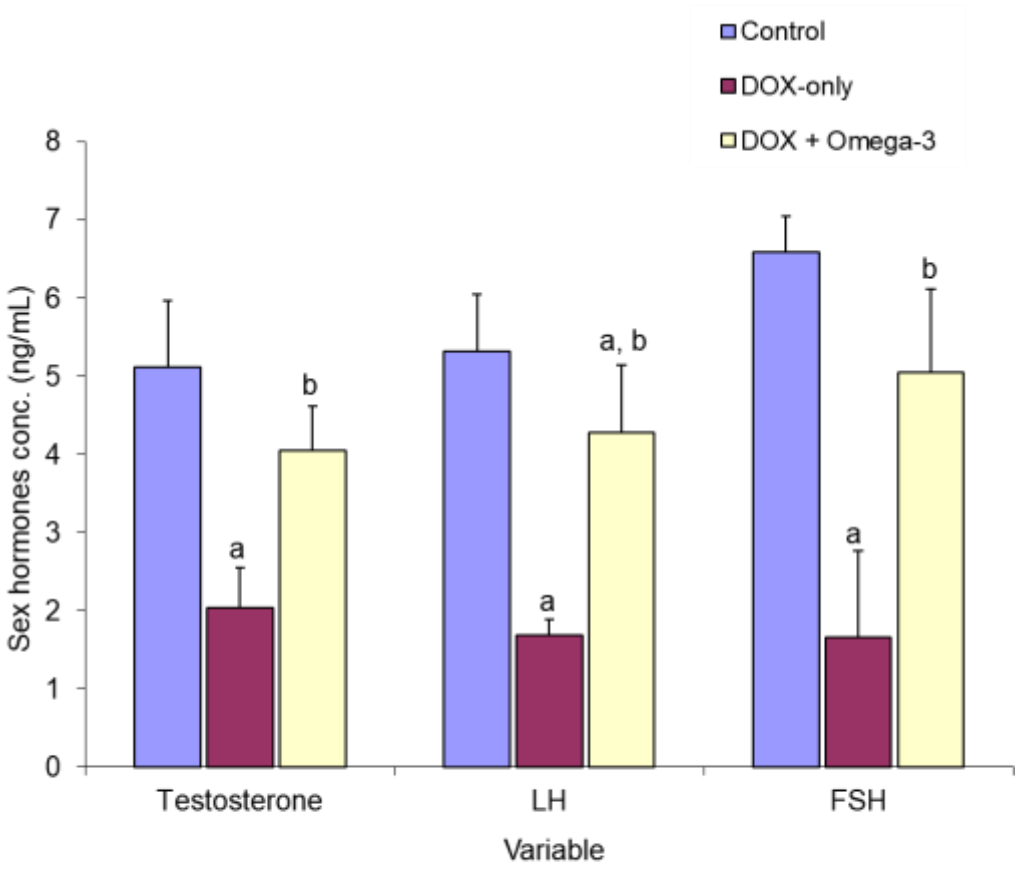

Fig. 3: Serum testosterone, lutenizing and follicle stimulating hormones in the different experimental groups.

Values are expressed as mean+SEM, n = 5.
a = p<0.05 vs control
b = p<0.05 vs DOX-only

Comparison of testicular oxidative stress biomarkers in different experimental groups

Superoxide dismutase (SOD)

The activities of SOD (U/mg Protein) were 33.09 ± 1.09, 19.37 ± 1.08 and 29.75 ± 1.71 for the control, Dox-only and Dox+OM3 groups respectively. It was significantly decreased (p<0.05) in the Dox-only group compared with control and significantly (p<0.05) elevated in the Dox+OM3 compared with the Dox–only group as shown in Fig 4.

Glutathione Peroxidase (GPx)

The activities of GPx (U/mg Protein) in were 22.43 ± 1.34 for the control, 12.21± 0.89 for Dox-only and 18.28±2.31 for the Dox+OM3 groups. It was significantly (p<0.05) reduced in the Dox-only and Doxx+Om3 groups compared with the control, though significantly higher (p<0.05) in the DOX+OM3 than in the Dox–only groups as shown in Fig 4.

Catalase (CAT)

The activity of testicular catalase (U/mg Protein) was significantly (p<0.05) reduced in the Dox-only (5.15± 1.25) and Dox+OM3 (9.46 ± 0.90) compared with control (12.99 ± 1.46), though it was significantly higher (p<0.05) in the Dox+OM3 than in the Dox-only groups, Fig 4.

Malondialdehyde (MDA)

The testicular concentrations of MDA (nmol/mg Protein) for the control, Dox-only and Dox+OM3 groups were 87.30 ± 0.53, 16.79 ± 1.05 and 11.09 ± 0.66 respectively. It was significantly (p<0.05) increased in Dox-only group compared with control but significantly (p<0.05) higher in the Dox+OM3 compared with the Dox-only groups, Fig 5.

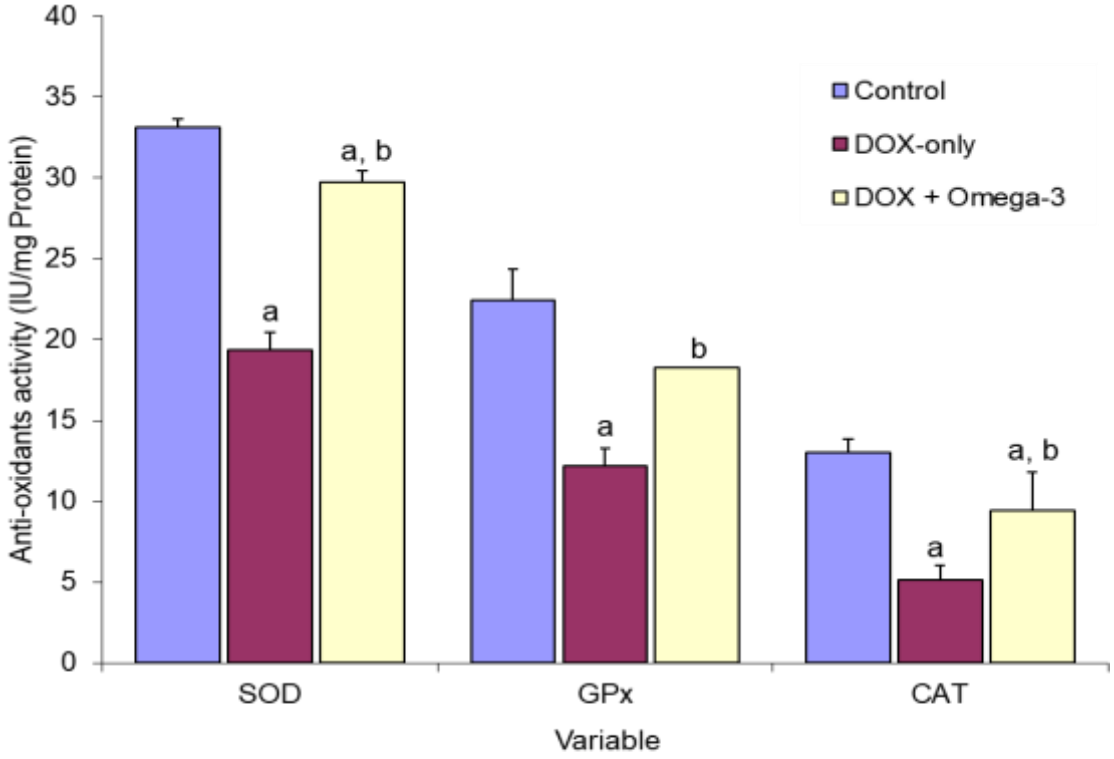

Fig. 4: Serum superoxide dismutase, glutathion and catalase activity in the different experimental groups.

Values are expressed as mean+SEM, n = 5.
a = p<0.05 vs control
b = p<0.05 vs DOX-only

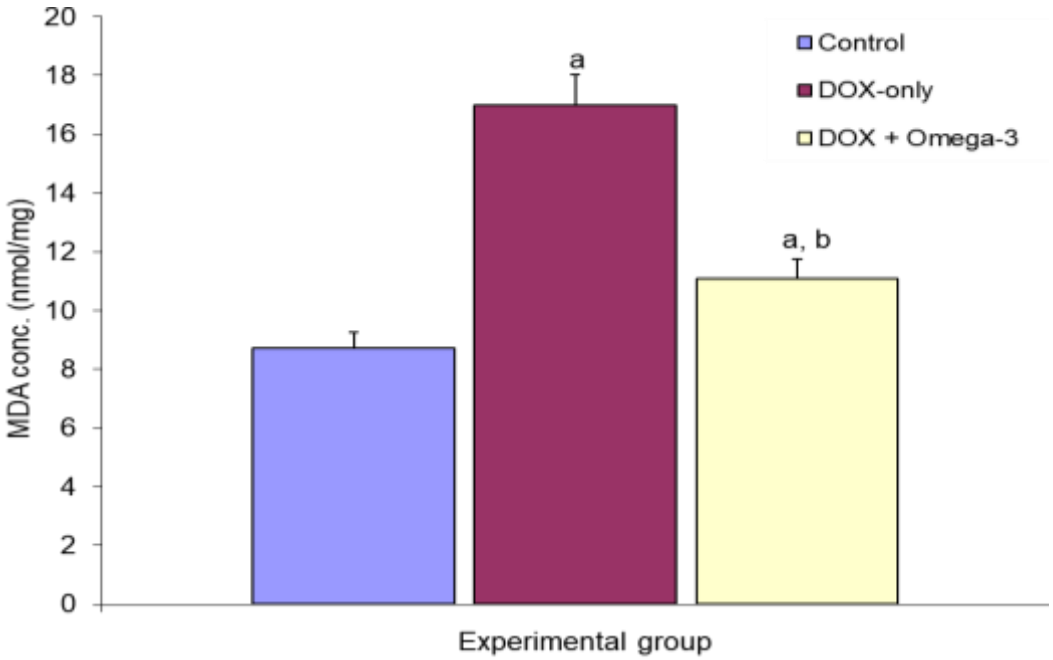

Fig. 5: Malondialdehyde concentration in the different experimental groups.

Values are expressed as mean+SEM, n = 5.
a = p<0.05 vs control
b = p<0.05 vs DOX-only

DISCUSSION

In this study, we investigated the mitigating effect of Omega-3 fatty acids on Doxorubicin-induced reproductive toxicity in male Wistar rats. Our findings are discussed.

The decrease in relative body weight in the Dox-only rats observed in this study is similar to the finding by de Lima *et al*., (2016). This might have resulted from Doxorubicin-induced cytotoxicity (Rossi, 2013) causing cell death (Tacar *et al*. 2013) and eventual reduction in cell mass. Doxorubicin induces cytotoxicity at least in part via oxidative stress (Rossi, 2013). Supplementation with Omega-3 fatty acid ameliorated this effect probably via it's antioxidant potential (Heshmati *et al.,* 2019, Mazza et al., 2008).

The observed decrease in relative testicular weight in rats treated with only doxorubiciin, is similar to the report by (Brithante *et al.* (2012). This might have been due to testicular injury from doxorubicin (Rossi, 2013). The attenuation of this effect following administration of Omega-3 fatty acids could also be due to its anti-oxidant effect effect (Heshmati *et al.,* 2019)

Earlier reports have shown that doxorubicin impairs spermatogenesis (Brithante *et al.* 2012, Sangweri *et al*., 2022) and causes decreased sperm count. This could explain the decrease in sperm count observed in the Dox-only group, and is also in line with the report by Silva et al, (2018) and Ijaz *et al*. (2024). Co-administration of Omega-3 with Doxorubicin mitigated the effect. This might have been due to the antioxidant effect of Omega-3 on doxorubicin-induced impairment of spermatogenesis or the direct enhancing effect of Omega-3 on spermatogenesis.

The decrease in the percentage of viable spermatozoa of the doxorubicin- only group seen in this study agrees with the study by Al-Garaghuli (2023) and Ijaz *et al.* (2024). As observed, the negative trend on sperm viability was attenuated by administration of Omega-3 which could have been due to its natural ability to reduce DNA sperm fragmentation (Safarinejad and Safarinejad, 2023).

The decrease in sperm motility in the Dox-only compared with control groups agrees with the studies by (Ijaz *et al.* 2024). This might have been due to oxidative damage to the sperm motility apparatus. The ability of Omega-3 to ameliorate this negative effect might have been due to its natural tendency to increase sperm motility, improves sperm tail flexibility or serve as an extra energy source for the spermatozoa (Safarinejad and Safarinejad, 2023).

Similar to the report by Ijaz et al. (2024) on the teratozospermic effect of doxorubicin, we observed a significant increase in the percentage of morphological abnormalities in the Dox–only group compared with control. Supplementation with Omega-3 mitigated this effect, which among other possible mechanisms might have been its natural ability to maintain a normal sperm morphology and reduce sperm fragmentation (Al-Qarahuli, 2023). The increase in the percentage of morphologically abnormal sperm cells in the Dox–only treated rats might have contributed to the decrease in sperm count observed in the group administered only doxorubicin (Hall and Hall, 2021).

In this study we noted a decrease in the plasma concentration of testosterone in the Dox-only rats treated with only doxorubicin compared with the control. This is in tandem with the observation by Ijaz *et al.* (2024. This observation might have been caused by doxorubicin-induced testicular toxicity on the Leydig cells which affected testosterone synthesis. The decrease in serum testosterone might have contributed to the reduction in sperm count in the Dox-only group since testosterone is essential for spermatogenesis (Moustafa, 2021). Supplementation with Omega-3 mitigated the negative effect of doxorubicin, likely by its natural ability to improve testicular function.

Similar to the findings by Agan *et al.* (2024), we found a significant decrease in the level of LH in the doxorubicin-treated groups suggesting LH receptors attenuation in the testes and this might have contributed to the low testosterone concentrations observed in the doxorubicin-treated rats. LH stimulates Leydig cells to produce testosterone (Moustafa, 2021).

Follicle-stimulating hormone, (FSH) secreted by the anterior pituitary, is an important primary hormone that stimulates spermatogenesis (Moustafa, 2021, Agan et al, 2024). We also observed a decrease in the serum level of FSH of doxorubicin-only treated rats which could have been due to a primary anterior pituitary toxicity from doxorubicin and may have contributed to the reduction in sperm count in the Dox-only group. Supplementation with Omega-3 mitigated the negative effect on serum FSH level.

The increase in testicular level of MDA, an end product of lipid peroxidation observed in the doxorubicin-only treated rats is in tandem with observation by Ijaz *et al*. (2024). Malondialdehyde is often used as biomarker of lipid peroxidation and oxidative stress) Del Rio *et al*., 2005). Administration of Omega-3 mitigated the increased lipid peroxidation induced by doxorubicin.

The decreases in testicular activities of testicular GPx, SOD and CAT in doxorubicin-only group observed is this study are similar to reports by Angustaraux *et al*. (2015). These endogenous antioxidant enzymes are consumed in elevated oxidative stress states, and result in cellular injury. Supplementation with Omega-3 ameliorated these effects indicating that, administration of Omega-3 could prevent oxidative damage to tissues doxorubicin-induced oxidative stress. The mechanism by which Omega-3 inhibits or protects tissues from oxidative damage in part via their antioxidant effect (Innocent *et al*., 2021, Aribo *et al*., 2025, Philip *et al*., 2026).

CONCLUSION: Omega-3 supplementation mitigates doxorubicin-induced impairment in testicular function, male reproductive hormones and oxidative stress.

Compliance with ethical standards

Disclosure of conflicts of interest: The authors declare that there are no conflict of interest.

Statement of ethical approval: The approval for this study which was a PhD thesis, was granted by the Animal Ethics and Research Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria,

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