



## L-CARNITINE SUPPLEMENTATION MITIGATES DOXORUBICIN-INDUCED REPRODUCTIVE TOXICITY IN MALE WISTAR RATS

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### ABSTRACT

Doxorubicin is an effective antimitotic agent but whose use is limited by reported cases of reproductive toxicity attributed in part to oxidative stress. There is paucity of information on whether L-carnitine, a common and potent antioxidant mitigates doxorubicin-induced male reproductive impairment, hence this study. Fifteen (15) male Wistar rats used for the study were randomly divided into control, doxorubicin-only and doxorubicin+L-carnitine groups of 5 rats each. Duration of daily administration with L-carnitine and weekly with doxorubicin was 28 days after which animals were sacrificed and relevant samples obtained for determination of parameters. Results show significant ( $p<0.05$ ) decrease in relative testicular weight in the doxorubicin-only group compared with control, but significantly ( $p<0.001$ ) higher in the Doxorubicin + L-carnitine groups compared with doxorubicin-only group. Sperm count, motility, and viability were significantly ( $p<0.05$ ) reduced in the Doxorubicin-only compared with control group but significantly ( $p<0.05$ ) higher in the doxorubicin + L-carnitine compared with Doxorubicin-only group. Teratozotospermia was significantly higher in Doxorubicin-only than in the control ( $p<0.05$ ) but decreased in Doxorubicin+L-carnitine group ( $P<0.05$ ) compared with doxorubicin-only group. Serum testosterone, luteinizing hormone and follicle-stimulating hormone concentrations were significantly ( $p<0.05$ ) decreased in doxorubicin-only compared with control but significantly ( $p<0.05$ ) higher in doxorubicin + L-carnitine compared with the doxorubicin-only groups. We therefore conclude that L-carnitine ameliorates doxorubicin-induced male reproductive toxicity in Wistar rats probably via its anti-oxidant potentials.

**KEYWORDS:** Doxorubicin, L-carnitine, male Wistar rat, reproduction toxicity.

### INTRODUCTION

Doxorubicin (Adriamycin) is an anthracycline antitumor antibiotic which is widely used in the treatment of many cancers American Society of Health System Pharmacists, 2024; Rossi, 2013). Doxorubicin is in the list of WHO Essential Medicines (World Health Organization, 2019) and is also used in the treatment of solid organ tumors and hematological malignances. It is indicated in the treatment of breast, ovarian and bladder cancers as well as in the management of Kaposi's sarcoma (Rossi, 2013) and other malignancies (Brayfield, 2013, Sritharam and Sivalingam, 2021). It is administered alone or in combination with another antineoplastic agents (European Medicine Agency, 2024).

The mechanism of action of Doxorubicin is by intercalation with DNA and inhibiting topoisomerase 11, preventing transcription and replication of DNA strands (Takar *et al.*, 2013; Pommier *et al.*, 2013). Doxorubicin also acts by increasing quinone-type free radical production and oxidative stress secondary to Iron accumulation (Rossi, 2013). It induces P53-mediated apoptosis as well (Ichikawa *et al.*, 2014; Chatterje *et al.*, 2010).

Though doxorubicin is a widely used chemotherapeutic agent, its use or patronage is seriously hampered or limited by development of several side effects including those on male reproductive function, underscoring the need for effective and protective measures against its toxicity.

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Doxorubicin causes significant cardiotoxicity involving down regulation of cardiac specific genes, increased oxidative stress and cardiac myocyte apoptosis (Koleini *et al.*, 2019). Doxorubicin has been reported to cause nephrotoxicity (Ayla *et al.*, 2011). Treatment with doxorubicin has been associated with male reproductive toxicity causing testicular failure (Howell and Shalet. 2005, Xin *et al.*, 2012, Prahalathans *et al.*, 2025).

Doxorubicin causes cytotoxicity via several mechanisms. These mechanisms include free radicals-mediated oxidative damage, cellular apoptosis, autophagy, ferroptosis and immunomodulation (Johnson-Arbor and Dubey, 2023). Other side effects of doxorubicin include typhilitis, myelosuppression, hepatic impairment, reactivation of hepatitis B virus and skin pigmentation (Kaczmarek *et al.*, 2012; Dillon *et al.*, 2012). Oxidative stress is a major pathway for many substances and drugs to affect body systems including the central nervous system, cardiovascular system, gastrointestinal system and this may explain some loss of function associated with exposures to different substances and environmental pollutants which may be used to explain functional impairments (Urom *et al.* 2015, Gharibi *et al.* 2022, Ayla *et al.*, 2011, Davies *et al.*, 2013).

L-carnitine is a stereoisomer of carnitine and occurs naturally in animals (Sawicka *et al.*, 2020). L-Carnitine is also a quaternary Ammonium compound derived from leucine, methionine and glycine and is involved in metabolism in most mammals, plants and some bacteria (Vidal-Casariago *et al.*, 2020; Cox and Hoppel 1973).

The major sources of L-carnitine are animal products, beef, pork, milk and codfish. Its biosynthesis occurs in the liver, kidney and brain (Rebouche, 2006; Durazzo *et al.*; 2020, National Institutes of Health, 2023; Vas and Wanders, 2002).

L-carnitine has several biological roles. It transports long chain fatty acids from the cytosol into the mitochondria across the mitochondrial membrane for oxidation of the fatty acid and production of energy (Vidal-Casariago *et al.*, 2020). It regulates cellular metabolism by enabling cells to switch between carbohydrates and fatty acids metabolism and also detoxifies acyl groups by preventing accumulation of potentially toxic acyl intermediates and also has antioxidant activity, protecting cells from oxidative stress (Sawicka *et al.*, 2020).

Medically, L-carnitine is used as an adjuvant product in the treatment of several diseases including type II diabetes mellitus (Vidal-Casariago *et al.*, 2020), myocardial infarction, angina pectoris, Alzheimer's disease and peripheral arterial disease among others (Meister *et al.*, 2016). The study therefore sought to investigate the effect of L-carnitine, a common and potent antioxidant on doxorubicin-induced reproductive impairment which at least in part has been blamed on oxidative stress.

## MATERIALS AND METHODS

### Experimental animals

Fifteen male Wistar rats aged 10 – 12 weeks were used for this study. They were kept in well-ventilated wooden cages in the Animal House of the Department of Physiology, University of Port Harcourt, Port Harcourt, Nigeria, under a 12/12hour light/dark cycle. They were acclimatized for 4 weeks and handled in line with the 1985 guidelines of the National Institute of Health publication for laboratory animals. They were given access to water and animal feed *ad libitum*.

### Experimental design

The rats were randomly divided into 3 groups of 5 rats each. The groups were control, doxorubicin-only and doxorubicin+L-carnitine groups. Doxorubicin was administered intraperitoneally at a once-weekly dose of 3 mg/kg (Adiyaman *et al.*, 2023) for four weeks. L-carnitine (Monola Pharmaceuticals, India) was given at a daily dose of 10 mg/kg (Ahmed *et al.*, 2014). The duration of experimentation was 28 days.

### Collection of samples

At the end of 4 weeks or 28 days the animals were anaesthetized with intra-peritoneal pentobarbital 60mg/kg and blood samples collected via cardiac puncture into pre-labeled plain sample bottles for determination of serum concentrations of testosterone, FSH and LH. The testes and epididymis were harvested for assessment of sperm indices and oxidative stress biomarkers.

### Measurement of gonadosomatic indices

Body weight was measured with an electronic weighing scale (Scout Pro, Ohaus Corporation, USA). Testicular and epididymal weights were measured with an electronic weighting balance while body weight changes and relative weights were computed.

### Evaluation of sperm parameters

#### Sperm count

The method used by Raji *et al.*, (2006), was followed for determination of sperm count. In brief, small incisions were made on cauda epididymis, prewarmed in 2ml of normal saline at 37°C to release spermatozoa stored therein. Two hundred microliter (200µL) of the sperm suspension was transferred to both chambers of the improved Neubauer hemocytometer. Sperms were viewed using a microscope (Leica DM, 750, Germany) and counted in five large Thema squares, making adjustment for volume of normal saline.

#### Sperm motility

In brief, 10 µL of sperm suspension was obtained from the cauda epididymis and then placed on a prewarmed slide which was then covered with a cover slip and examined at x100 magnification using a light microscope (Leica DM 750, Germany).

### Sperm morphology

Sperm morphology was determined by the method used by Narayana *et al.*; (2005). Briefly, 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 light microscope (Leica DM 750, Germany) at x 400 magnification. Two hundred spermatozoa were screened per rat and percentage of abnormalities in head, mid piece and tail was determined.

### Sperm viability

Sperm viability was determined as described by Wyrobek *et al.*, (1983). In summary, 2ml of 0.05% eosin Y-nigrosin was mixed with equal volume of sperm suspension on a slide and incubated for 2 minutes at room temperature. The slides were then viewed with a light microscope (Leica DM 750, Germany) at x 100.

### Determination of serum levels of male reproductive hormones

#### Testosterone

This was determined using ELISA method with ELISA Kits by BioAssay Systems, Hayward, CA, USA). The procedure outlined by the manufacturers was followed. Absorbance was read at 450 nm and the concentration calculated using the formula,

$$\text{Concentration (ng/ml)} = \frac{\text{Absorbance of Sample} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

#### Luteinizing hormone

This was evaluated using the Accubind ELISA microwells for LH (Monobind Inc; CA, USA). The procedure was carried out following manufacturers protocol. The absorbance was read at 450nm and the concentration of LH in serum (ng/ml) calculated using above formula.

#### Assay of FSH

Concentration of serum FSH was determined using FSH ELISA kits from BioAssay Systems, CA, USA. The procedure was carried out according to the protocol outlined by the manufacturers. The absorbance was read at 450nm and the concentration calculated using the formula above.

### Assay of oxidative stress biomarkers

#### Lipid peroxidation (Malondialdehyde)

Lipid peroxidation was estimated by measuring spectrophotometrically (SP-V5100 visible spectrophotometer, Scitek Global Co., Ltd, China.) the level of the lipid peroxidation product, malondialdehyde (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). Malondialdehyde 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 (1957) 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 catalyses 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  $\pm$  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 and epididymal weight.

Mean body weight changes (g) in the control, doxorubicin-only and doxorubicin + L-carnitine groups were  $27.8 \pm 5.31$ ,  $-17.8 \pm 4.44$  and  $7.0 \pm 2.12$  respectively. There was a significant weight loss in the doxorubicin-only compared with control groups ( $p < 0.001$ ). It was also significantly ( $p < 0.05$ ) lower in the doxorubicin + L-carnitine than in the control groups ( $p < 0.05$ ) as shown in Table 1.

Relative testicular weights (g) were  $1.43 \pm 0.21$ ,  $0.93 \pm 0.09$  and  $1.00 \pm 0.09$  for control, doxorubicin-only and doxorubicin-only + L-carnitine respectively. It was significantly ( $p < 0.05$ ) reduced in the doxorubicin-only and doxorubicin + L-carnitine groups compared with control but significantly ( $p < 0.05$ )

higher in the doxorubicin + L-carnitine compared with the doxorubicin-only groups as shown in Table 1.

Epididymal weights were  $1.58 \pm 0.19$ ,  $1.12 \pm 0.13$  and  $1.44 \pm 0.15$  for control, doxorubicin-only and doxorubicin + L-carnitine groups respectively. It was significantly lower in Doxorubicin-only and doxorubicin + L-carnitine compared with the control group, but was significantly ( $p < 0.05$ ) higher in the doxorubicin + L-carnitine than in the doxorubicin-only groups as shown in Table 1.

### Comparison of sperm parameters in different experimented groups

#### Sperm count

The mean sperm counts ( $\times 10^6/\text{ml}$ ) were  $56.86 \pm 5.46$ ,  $28.96 \pm 4.16$  and  $48.94 \pm 3.81$  for control, doxorubicin-only and doxorubicin + L-carnitine groups respectively. The count was significantly ( $p < 0.05$ ) decreased in the doxorubicin-only compared with the control groups. It was significantly ( $p < 0.05$ ) higher in the doxorubicin + L-carnitine groups than in doxorubicin-only group, Fig 1.

#### Sperm motility

Mean sperm motility (%) were  $77.00 \pm 2.91$ ,  $50.60 \pm 3.64$  and  $69.4 \pm 6.58$  for control, doxorubicin-only and doxorubicin+L-carnitine groups respectively. It was significantly reduced ( $p < 0.05$ ) in the doxorubicin-only compared with control groups but higher ( $p < 0.05$ ) in the doxorubicin+L-carnitine than in the doxorubicin-only groups as shown in Fig 2.

#### Sperm viability

Sperm viability (%) was significantly ( $p < 0.05$ ) lower in the doxorubicin-only ( $52.47 \pm 2.97$ ) compared with control ( $72.80 \pm 2.77$ ) groups. It was however significantly ( $p < 0.05$ ) higher in the doxorubicin+L-carnitine ( $73.80 \pm 4.02$ ) compared with doxorubicin-only groups as shown in Fig 3.

#### Sperm morphology

The mean percentage of abnormal sperm cells were  $10.80 \pm 1.70$ ,  $30.20 \pm 2.38$  and  $16.80 \pm 2.16$  for control, doxorubicin-only and doxorubicin + L-carnitine groups respectively. It was significantly ( $p < 0.05$ ) higher in the doxorubicin-only compared with the control groups. It was significantly ( $p < 0.05$ ) lower in the L-carnitine-only and doxorubicin + L-carnitine compared with the doxorubicin-only groups and higher in the doxorubicin + L-carnitine than in the doxorubicin-only groups, Fig.

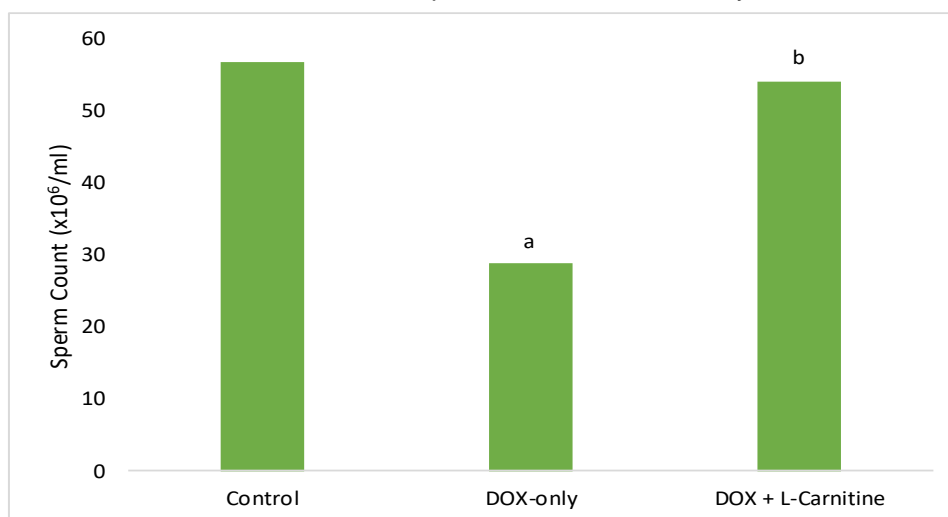
**Table 1: Comparison of Body Weight Changes, Relative Testicular Weight and epididymal Weights**

Parameters	Control group	Doxorubicin-only	Doxorubicin + L-Carnitine
Body Weight Changes (g)	$27.80 \pm 5.31$	$17.80 \pm 4.44^a$	$7.00 \pm 2.12^{a,b}$
Relative Testicular Weight (g)	$1.43 \pm 0.21$	$0.93 \pm 0.09^a$	$1.01 \pm 0.09^a$
Epididymal Weight (g)	$1.58 \pm 0.19$	$1.12 \pm 0.13^a$	$1.44 \pm 0.15$

Values are expressed as mean  $\pm$  SEM, n = 5

a =  $p < 0.05$  vs control

b =  $p < 0.05$  vs doxorubicin-only



**Fig 1: Comparison of sperm count in the different experimental groups.**

Values are expressed as Mean  $\pm$  SEM, n = 5.

a =  $p < 0.05$  vs Control

b =  $p < 0.05$  vs DOX-only

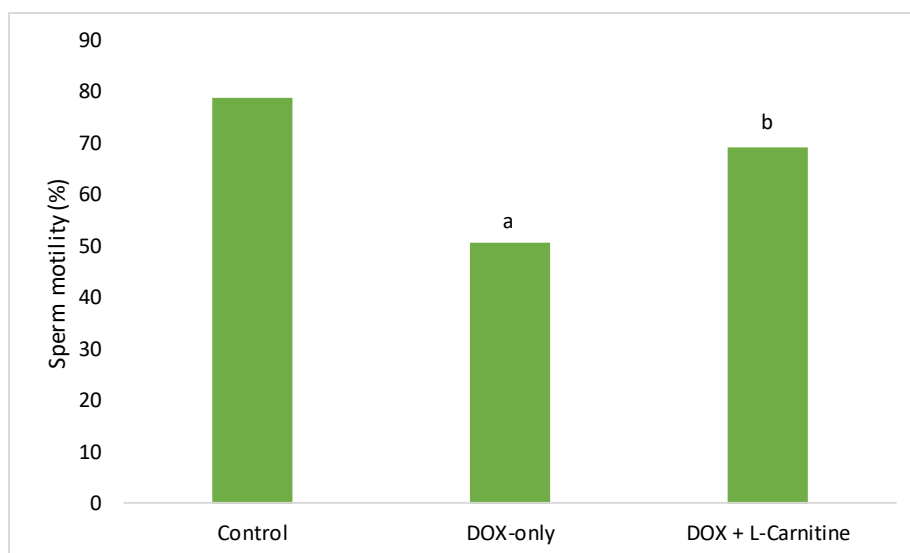


Fig 2: Comparison of sperm motility in the different experimental groups. Values are expressed as Mean  $\pm$  SEM, n = 5.  
a =  $p < 0.05$  vs Control  
b =  $p < 0.05$  vs DOX-only

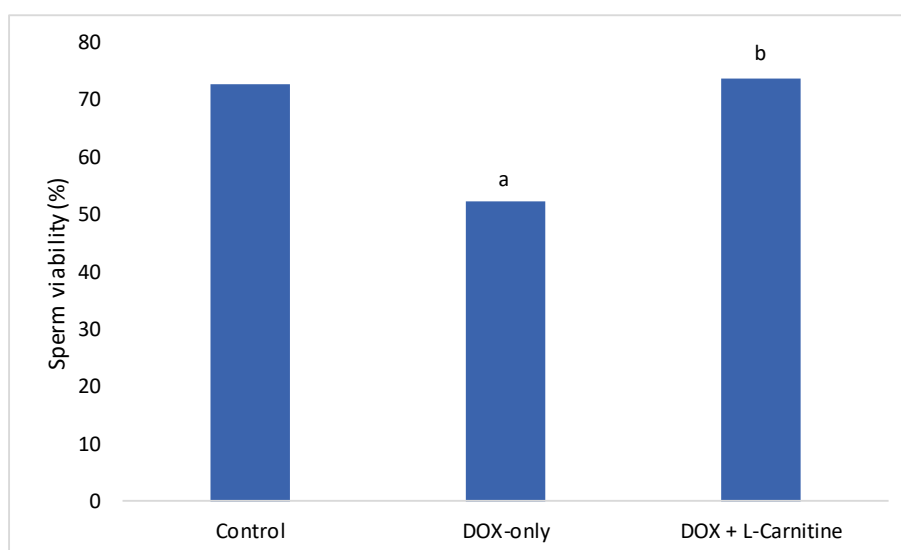


Fig 3: Comparison of sperm viability in the different experimental groups. Values are expressed as Mean  $\pm$  SEM, n = 5.  
a =  $p < 0.05$  vs Control  
b =  $p < 0.05$  vs DOX-only

### Comparison of serum reproductive hormones in different groups.

#### Testosterone

The mean testosterone (ng/mL) concentrations were  $5.11 \pm 0.85$ ,  $2.03 \pm 0.52$  and  $4.84 \pm 0.54$  for control, doxorubicin-only, and doxorubicin + L-carnitine groups respectively. It was significantly ( $p < 0.05$ ) lower in the doxorubicin-only compared with control group but significantly ( $p < 0.05$ ) higher in the doxorubicin + L-carnitine compared with the doxorubicin-only group. Table 2.

#### Luteinizing hormone

The mean serum concentrations (ng/mL) of LH were  $5.31 \pm 0.73$ ,  $1.68 \pm 0.21$  and  $4.74 \pm 0.07$  for control, doxorubicin-only, and doxorubicin+L-carnitine groups respectively. It was significantly ( $p < 0.05$ ) lower in doxorubicin-only compared with control and doxorubicin + L-carnitine groups, Table 2.

#### Follicle stimulating hormone

The serum FSH (ng/mL) concentrations were  $6.59 \pm 0.91$ ,  $1.66 \pm 1.10$ , and  $5.63 \pm 0.63$  for control,

doxorubicin-only, and doxorubicine + L-carnitine groups respectively. The FSH was significantly ( $p<0.05$ ) lower in the doxorubicin-only compared with control group but increased significantly ( $p<0.05$ ) in the doxorubicin + L-carnitine compared with the doxorubicin-only groups. Table 2.

### Comparison of testicular concentration and activities of oxidative stress biomarkers in different experimental groups

#### Malondialdehyde (MDA)

The mean testicular MDA concentrations (mmol/mL) were  $8.72 \pm 0.53$  for control,  $16.99 \pm 1.05$  for doxorubicin-only and  $10.07 \pm 1.11$  for doxorubicin + L-Carnitine groups. Malondialdehyde concentration was significantly ( $p<0.05$ ) higher in the doxorubicin-only compared with the control group. It was however significantly ( $p<0.05$ ) lower in Doxorubicin + L-carnitine compared with doxorubicin-only group, Table 3.

#### Superoxide dismutase (SOD)

The values of mean SOD activity (IU/mg protein) were  $33.09 \pm 1.09$ ,  $19.37 \pm 1.08$  and  $30.54 \pm 1.40$  for

control, doxorubicin-only and doxorubicin+L-Carnitine groups respectively. It was significantly ( $p<0.05$ ) lower in the doxorubicin-only compared with the control group but significantly ( $p<0.05$ ) higher in the doxorubicin + L-Carnitine compared with the doxorubicin-only group, Table 3

#### Glutathione peroxidase (GPx)

The values of mean GPx activity (IU/mg protein) were  $22.43 \pm 0.84$  for control,  $12.21 \pm 0.89$  for doxorubicin-only and  $20.60 \pm 1.64$  for DOX+L-Carnitine groups. The activity was significantly ( $p<0.05$ ) lower in doxorubicin-only compared with control groups but increased in the doxorubicin + L-Carnitine compared with the control groups as shown in Table 3.

#### Catalase (CAT)

The values of mean CAT activity (IU/mg protein) were  $12.99 \pm 1.46$  for control,  $5.15 \pm 1.15$  for doxorubicin-only and  $10.16 \pm 1.15$  for doxorubicin + L-Carnitine group. It was significantly ( $p<0.05$ ) lower in the doxorubicin-only compared with the control group but significantly ( $p<0.05$ ) elevated in the doxorubicin + L-Carnitine compared with doxorubicin-only group, Table 2.

**TABLE 2: Comparison of lipid [reoxidation product (MDA) and anti-oxidant enzymes (SOD, GPx and CAT) in the different experimental groups**

Group	Testosterone (ng/mL)	LH (ng/mL)	FSH (ng/mL)
Control	$5.11 \pm 0.85$	$5.31 \pm 0.73$	$6.59 \pm 0.91$
Doxorubicin-only	$2.03 \pm 0.52^a$	$1.68 \pm 0.21^a$	$1.66 \pm 1.10^a$
Doxorubicin + L-carnitine	$4.84 \pm 0.54^b$	$4.74 \pm 0.07^b$	$5.63 \pm 0.63^b$

Values are expressed as mean  $\pm$ SEM, n = 5.

a=  $p<0.05$  vs Control

b =  $p<0.05$  vs DOX-only

**TABLE 3: Comparison of lipid [reoxidation product (MDA) and anti-oxidant enzymes (SOD, GPx and CAT) in the different experimental groups**

Group	MDA (mmol/mL)	SOD (IU/mg protein)	GPx (IU/mg protein)	CAT (IU/mg protein)
Control	$8.72 \pm 0.53$	$33.09 \pm 1.09$	$22.43 \pm 0.84$	$12.99 \pm 1.46$
Doxorubicin-only	$16.99 \pm 1.05^a$	$19.37 \pm 1.08^a$	$12.21 \pm 0.89^a$	$5.15 \pm 1.15^a$
Doxorubicin + L-carnitine	$10.07 \pm 1.11^b$	$30.54 \pm 1.40^b$	$20.60 \pm 1.64^b$	$10.16 \pm 1.15^b$

Values are expressed as mean  $\pm$ SEM, n = 5.

## DISCUSSION

The possible ameliorative effects of L-carnitine on doxorubicin-induced male reproductive toxicity in male Wistar rats were evaluated. Our findings are discussed.

The significant disease in relative body weight noted in the doxorubicin-only compared with the control group might be due to doxorubicin-induced cachexia. This is similar to the findings by de Lima Junior *et al.*, (de Lima Junior *et al.*, 2016). Doxorubicin is said to cause cytotoxicity via oxidative stress which may result in cell death and eventual weight loss (Takar *et al.*, 2013). The weight loss was mitigated following supplementation with L-carnitine which might have been due to the antioxidant effect of L-carnitine (Sawicka *et al.* 2020).

The relative testicular weights being significantly reduced in the doxorubicin-only compared with the control could have been due to the toxic effect of doxorubicin on the testes as also reported by Brilhante *et al.*, (Brilhante *et al.*, 2011). The antioxidant effect of L-carnitine might have at least in part been responsible for the increase in testicular weight following its co-treatment with doxorubicin (Sawicka *et al.* 2020).

Sperm motility was significantly decreased in the doxorubicin-only group but this was ameliorated when doxorubicin was co-administered with L-carnitine. The doxorubicin-associated decline in sperm kinematics is similar to the reports by Ijaz *et al.* (2004) and Oyovwie *et al.* (2022). Co-treatment with L-carnitine mitigated this effect likely due to its antioxidant nature.

Supplementation with L-carnitine has been shown to improve sperm motility associated its role in energy metabolism (Samiei *et al.*, 2025; Kooshesh *et al.* 2023). This might have contributed to the observed improvement in sperm motility in the L-carnitine-only group compared with control. The doxorubicin-induced decrease in sperm count seen in this study is similar to findings by Silva *et al.*, (2013) and Ijaz *et al.*, (2004) but this was prevented by co-administration with L-carnitine. This mitigative effect of L-carnitine could be linked with its antioxidant effect which prevented the damaging effects on tissues and cells by free radicals (Sawicka *et al.*, 2020).

An increase in teratozoospermia by doxorubicin was observed in this study. This observation is similar that made by Ijaz *et al.*, (2004), Sakai *et al.*, (2018) and Ceribasi *et al.*, (2012). The increase in the percentage of abnormal sperm cells caused by doxorubicin was prevented following supplementation with L-carnitine. Oxidative stress can lead to defects in cellular antioxidant action. The increase in teratozoospermia induced by doxorubicin might have contributed to the decrease in sperm motility.

The significantly reduced sperm viability in the doxorubicin-only when compared with the control groups is in line with the reports by Al-Garaghile *et al.* (2023) and Ijaz *et al.* (2004). One of the mechanisms of cytotoxicity by doxorubicin is oxidative stress (Rossi, 2013). Supplementation with L-carnitine as an antioxidant could therefore prevent impairment in the viability of the sperm cells as noted in this study.

Testosterone is an essential hormone in spermatogenesis. The doxorubicin-induced dysregulation of testosterone noted in our study is in tandem with the observation by Ijaz *et al.*, (2004) and Oyovwi *et al.* (2022). This decline in serum testosterone caused by doxorubicin is likely a contributing factor to the reduction in sperm count earlier reported in this study as testosterone is essential for spermatogenesis. The dysregulation was largely prevented by co-administration of L-carnitine due to its antioxidative protective effect against doxorubicin toxicity and its natural ability to boost testosterone biosynthesis (Al-Daraji and Tahir, 2024). The significantly increased concentration of testosterone in the group treated with only L-carnitine compared with the control confirms the positive role L-carnitine plays in the biosynthesis of testosterone as also reported by Tanis *et al.* (2010) and Al-Daraji and Tahir (2024).

Our observed significant reduction in serum concentration of LH induced by doxorubicin is consistent with findings reported by Agan *et al.*, (2024) which suggests a primary effect on anterior pituitary function to secrete the hormone (Hall & Hall, 2021). The doxorubicin-induced decrease in LH might have contributed to the low testosterone concentration in the doxorubicin-only treated rats reported earlier. Luteinizing hormone stimulates Leydig cells in the testes to produce testosterone (Hall and Hall, 2021). The ability of L-carnitine co-treatment with doxorubicin to prevent this decrease suggests that at least in part, the mechanism by which doxorubicin induces the LH derangement was oxidation stress-mediated.

Follicle-stimulating hormone is an important primary hormone that stimulates spermatogenesis and supports Sertoli cell function (Caroppo and Niederberger (2023) and secreted by the anterior pituitary gland. The decrease in serum concentration of FSH induced by doxorubicin is similar to the findings by Agan *et al.* (2024) and which may be due to a primary pituitary toxicity. Co-administration of L-carnitine mitigated this effect likely due to its antioxidant action.

Malondialdehyde is one of the end products of lipid peroxidation (Caroppo and Niederberger (2023),

a process that liberates reactive Oxygen species into tissues and which could damage tissues (Benchekroun and Roberts, 1992).

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The increase in lipid peroxidation observed is similar to the findings by previous researchers (Hrelia *et al.*, 2002). L-carnitine supplementation mitigated this effect most likely due to its antioxidant effects.

The decline in the activities of SOD, GPx and CAT in the DOX-only compared with the control groups is in line with observations by previous studies (Angsutararux *et al.*, 2015). Antioxidant substances have been known to mitigate the negative effects of different toxins on the redox status of the body (Innocent *et al.*, 2021; Aribio *et al.*, 2024; Nwangwa *et al.* (2024).

## CONCLUSION

Doxorubicin induces testicular oxidative stress, impairs sperm functions and dysregulates male reproductive hormone functions which are mitigated by L-carnitine supplementation.

## Disclosure of conflict of interest

The authors declare that there are no competing interests.

## Statement of ethical approval

The protocol for this study was approved by the Animal Ethics and Research Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigerian. The data presented herein are part of our findings in a PhD thesis.

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