EFFECT OF L-CARNITINE ON GONADOSOMATIC INDEX AND SEX HORMONES IN EXPERIMENTALLY-INDUCED TESTICULAR TORSION IN WISTAR RATS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

***R. J. Bebekah; M. I. A. Saleh., A. Mohammed., Y. Tanko and A. Jimoh**
Department of Human Physiology, Faculty of Basic Medical Science, Ahmadu Bello University, Zaria, Nigeria.
Address for correspondence: **drbebekah@gmail.com**

Received: December 14, 2023 Accepted: March 28, 2024

Abstract: Testicular torsion (TT) is the ischaemic-reperfusion injury that occurs in the tesis. It is the twisting of the testis along the axis of the spermatic cord which is commonly seen in neonates, children and adolescents. Timely intervention is key to the salvage of the testicular tissue against irreversible damage. The aim of the study was to investigate the modulatory effect of L-carnitine on inflammatory mediators during TT. Forty-five adult male Wistar rats were used for to conduct the experimental. The rats were weighed and randomly divided into three groups comprising of fifteen each group. Five animals from each group i.e. Sham, TT and TT treated with 500mg/kg of L-carnitine were sacrificed on day twenty two (22), forty two (42) and sixty two (62) of the study respectively. At the end of the experiment, epididymis and testes were removed and Gonadosomatic indices were evaluated. Blood samples were collected from each of the animals through cardiac puncture and serum was harvested to assay for follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T_4). The results obtained showed significant decrease in the epididymal body ratio for TT +L for 62 days, lack of significance for testicular body ratio and increase in T_4 and FSH concentrations in the L-carnitine treated groups. In conclusion, L-carnitine was found to reduce epididymal body ratio, protect the testicular germ cells against reactive oxygen species mediated damage and caused increase in the secretion of FSH.

Keywords: Testicular torsion, epididymal, L-carnitine, testis, spermatic

Introduction

Testicular torsion occurs due to the rotation of the spermatic cord around its longitudinal axis, leading to strangulation of the vessels that irrigate the scrotum. It is one of the main acute urological emergencies that affect boys which can be extravaginal or intravaginal, that is more common in the perinatal period and, this in the other ages. The main risk factor for the occurrence of testicular torsion is the presence of clump anomaly, allowing movement of the testicle within the tunic. The spermatic cord usually twists 180° to 720°, disrupting blood flow to the testis, causing ischemia and possible necrosis of this organ. The condition consists of sudden onset, unilateral severe scrotal pain that is less than 24 hours in duration and may be associated with nausea, vomiting, scrotal edema, testicular tenderness, and erythema. Time is an extremely important issue when it comes to the diagnosis and treatment of torsion, since the longer it takes to do them, the lower the testicular salvage rate (Da Conceicao *et al*., 2024)

L-carnitine is a vital molecule that is found in all living cells. It is a quaternary amine (3-hydroxy-4-*N*-trimethylaminobutyrate) whose main function in mammalian cells is the transfer of long chain fatty acids across the inner mitochondrial membrane for β- oxidation and generation of ATP energy. This process requires specific enzymes and transporters and any defects in these can cause disorders of the carnitine cycle. Once fatty acids are inside mitochondria, they undergo the process of β-oxidation through a series of acyl-CoA dehydrogenase enzymes that mediate the shortening of long-chain fats used to produce ATP. Deficiency of any of these enzymes can cause symptoms similar to those encountered in disorders of the carnitine cycle (Virmani and Cirulli, 2022).

The aim of this study was to determine the effect of treatment with supplemental L-carnitine on gonadosomatic indices and sex hormones following TT.

Materials and Methodology

Materials

Well-aerated ages, clean water, feed, normal saline, scissors, buffered distilled water, containers, 1 ml, 2 ml and 5ml of syringes, soap, masking tape, oral intragastric tube, watch, cotton wool, dissection kit, filter paper, chromic suture, Petri dish, electronic balance, Enzyme-linked immunosorbent assay (ELISA) kits for hormonal assay were purchased from Wuhan Fine Biotech Co., Ltd., China and L-Carnitine was purchased from GNC Well, USA.

Animals

Forty five (45) Male Wistar rats weighing 104g-230g were used for the study. The animals were purchased from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were housed in well aerated laboratory cages and allowed access to growers mash from Labar Feeds and Grains Merchant, Zaria, Nigeria and water *ad libitum* throughout the duration of the experiment.

Methodology

Group 1 (Sham): In this group, the testes were brought through the scrotal incision and then returned backto the scrotal sac and sutured. The rats were given distilled water 1 ml/kg each day orally after reperfusion and five animals each were sacrificed on day 22 day 42 and day 62 respectively.

Group 2 (TT): Rats in this group were induced with torsion for 6 hours followed by detorsion. Five animals each were sacrificed on day 22, day 42 and 62 respectively.

Group 3 (TT+L-Carnitine): Rats in this group were induced with torsion for 6 hours followed by detorsion and then treated with 500mg/kg of L-Carnitine (Dokmeci *et al*., 2007) orally. Five animals each were sacrificed on day 22, day 42 and 62 respectively.

Induction induction of testicular torsion

The rats were anaesthetised through chloroform inhalation in a closed chamber and consequently sacrificed. The testes were exposed through identically-opened and closed right-sided ilio-inguinal incision. The testes were exposed by opening the *tunica vaginalis*. The spermatic cords were exposed and torsions were created by rotating the testes 720^0 clockwise. The rotated testes were maintained for six hours by fixing them medially and laterally to the scrotum using a surgical silk suture. The detorsions were carried and the animals kept until the time of sacrifice. The testes were surgically removed through a lower abdominal incision according to the method described by Akusu *et al.* (1985) and Oyeyemi and Ubiogoro (2005).

Gonado-somatic index

The testes and epididymis were dissected and immediately separated out from the adherent tissue and weighed to the nearest mg on an electronic balance according to the method described by Franca *et al*. (2006). The GSI calculated using the formula:

$$\text{GSI} = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Where gonad weight= (weight of right testis + weight of left testis)/2

Hormonal assays

Follicle stimulating hormone

Standard, test samples and control were set on wells on the pre-coated plate and then their positions were recorded. 50µL of standard, blank or sample were added per well. The blank well is added with sample/standard dilution buffer. Immediately 50µL of Biotin-detection antibody working solution was added to each well. The plate was sealed and incubated for 74 minutes at 27°C. Each well was aspirated and washed, repeating the process thrice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper.100µL of SABC working solution was added to each well, covered with a new sealer and incubated for 58 minutes at 27°C. After the incubation, aspiration of the plate and 5 times washing was carried out. 90µL of TMB substrate was added to each well, covered with a new plate sealer and incubated for 15 minutes at 37°C. 50µL of stop solution was added at the end of incubation period and the coloured changed to yellow. The optical density was determined using microplate reader at 450 nm.

Luteinising hormone

The HRP-Streptavidin Conjugate (SABC) working solution solution was prepared within 30 minutes before the experiment. The total volume of the working solution was estimated by multiplying 0.1ml/well by quantity of wells and 0.1-0.2 ml in excess of the total volume is allowed. The SABC was diluted with SABC dilution buffer at 1:100 and mixed thoroughly

Standard, test samples and control were set on wells on the pre-coated plate and then their positions were recorded. 50µL of standard, blank or sample were added per well. The blank well is added with sample/standard dilution buffer. Immediately 50µL of Biotin-detection antibody working solution was added to each well. The plate was sealed and incubated for 88 minutes at 27°C. Each well was aspirated and washed, repeating the process thrice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper.100µL of SABC working solution was added to each well, covered with a new sealer and incubated for 62 minutes at 27°C. After the incubation, aspiration of the plate and 5 times washing was carried out. 90µL of TMB substrate was added to each well, covered with a new plate sealer and incubated for 45 minutes at 37°C. 50µL of stop solution was added at the end of incubation period and the coloured changed to yellow. The optical density was determined using microplate reader at 450 nm.

Testosterone

Standard, test samples and control were set on wells on the pre-coated plate and then their positions were recorded. 50µL of standard, blank or sample were added per well. The blank well is added with sample/standard dilution buffer. Immediately 50µL of Biotin-detection antibody working solution was added to each well. The plate was sealed and incubated for 45 minutes at 37°C. Each well was aspirated and washed, repeating the process thrice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper.100µL of SABC working solution was added to each well, covered with a new sealer and incubated for 50 minutes at 27°C. After the incubation, aspiration of the plate and 5 times washing was carried out. 90µL of TMB substrate was added to each well, covered with a new plate sealer and incubated for 30 minutes at 27°C. 50µL of stop solution was added at the end of incubation period and the coloured changed to yellow. The optical density was determined using microplate reader at 450 nm.

Statistical Analysis

All values were expressed as mean± standard error of the mean (mean±SEM). The significance of the data obtained was evaluated by using one-way analysis of variance (ANOVA). Differences between means were analysed using Turkey's post hoc test. Values of $P < 0.05$ were considered significant.

Epididymal body ratio

There was no significant difference ($P > 0.05$) in the L-carnitine groups that received treatment for 22 and 42 days when compared with the sham groups. However, there was significant decrease in the L-carnitine group that received

treatment for 62 days (0.77±0.00 mg/Kg) when compared to the sham treated group (0.80±0.04 mg/Kg).

Table 4. 1: Effects of L-carnitine on Epididymal body ratio in TT in Wistar rats

Duration (days)	Sham (mg/Kg)	TT (mg/Kg)	TT+L (mg/Kg)
22	0.80 ± 0.20	0.94 ± 0.13	0.84 ± 0.22
42	0.99 ± 0.09	0.79 ± 0.09	0.77 ± 0.12
62	0.80 ± 0.04 [a]	0.72 ± 0.04 [a]	0.77 ± 0.00[b]

[a,b,]= Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the sham groups

Testicular body ratio

There was no significant (P>0.05) difference across all the treated groups when compared with the sham treated groups. Length of time was not able to improve the testicular body weight despite treatment for maximum 62 days.

Table 4. 2: Effects of L-carnitine on Testicular body ratio in testicular TT in Wistar rats

Duration (days)	Sham (mg/Kg)	TT (mg/Kg)	TT+L (mg/Kg)
22	6.69 ± 0.53	4.87 ± 0.96	3.77 ± 0.66
42	5.45 ± 1.01	3.29 ± 0.36	3.92 ± 0.46
62	4.15 ± 0.45	4.02 ± 0.36	3.04 ± 0.09

Follicle stimulating hormone

There was significant increase (P<0.05) in the FSH levels in the group that were induced with TT and kept for 62 days compared with the sham groups that were kept for 22 and 62 days. There was also significant increase (P<0.05) in the group that were induced with TT and treated with 500 mg/Kg of L-carnitine for 22 days compared with the sham groups for 22 days and L-carnitine for 62 days.

Table 4. 3: Effects of L-carnitine on Follicle stimulating hormone concentration in testicular TT in Wistar rats

Duration (days)	Sham (mlU/ml)	TT	TT+L
22	4.50 ± 0.39[a]	25.80 ± 12.13[b]	93.80 ±1.36[c,x]
42	21.40 ± 15.94	27.40± 17.63	10.00 ±1.76[d,y]
62	5.50 ± 0.50[b]	67.00± 16.11[c]	10.00 ±1.76[d,y]

[a,b,c,d]= Means on the same column with different superscript letters differ significantly (P < 0.05)

[x,y]= Means on the same row with different superscript letters differ significantly (P < 0.05)

Leuteinizing hormone

There was significant increase in TT group for 62 (34.75±7.24 MIU/ml) days when compared with TT groups for 22 (23.30±10.20 MIU/ml) and 42 (8.70±2.80 MIU/ml) days respectively. There was also significant increase (34.75±7.24 MIU/ml) when compared with the sham treated group (5.65±1.28 MIU/ml) for the same duration. There was significant decrease in TT treated for 62 days (4.33±0.24 MIU/ml) when compared with sham treated group for 62 days (5.65±1.28 MIU/ml).

Table 4. 4: Effects of L-carnitine on Leuteinizing hormone concentration in testicular IRI in Wistar rats

Duration (days)	Sham (MlU/ml)	TT	TT+L
22	6.80 ± 0.44	23.30 ± 10.20[x]	11.16 ± 4.42
42	11.70 ± 5.63	8.70± 2.18[x]	0.00 ± 0.00
62	5.65 ± 1.28[a]	34.75± 7.24[b,y]	4.33 ± 0.24[c]

[a,b,c]= Means on the same column with different superscript letters differ significantly (P < 0.05)

[x,y]= Means on the same row with different superscript letters differ significantly (P < 0.05)

Testosterone

There was significant increase in the T_4 concentration in TT group for 42 days (6.62±2.14 ng/ml) when compared with the sham treated group for 42 days (5.82±1.46 ng/ml). The TT group that was treated for 62 days with L-carnitine was significantly higher (8.02±1.15 ng/ml) in T_4 and significantly lower (8.02±1.15 ng/ml) when compared with the TT group for 62 days (12.17±0.09 ng/ml) and sham treated group for 62 days (7.25±1.68 ng/ml).

Table 4. 5: Effects of L-carnitine on Testosterone concentration in testicular IRI in Wistar rats

Duration (days)	Sham ng/ml	TT	TT+L
22	5.02 ± 1.72	7.34 ± 1.99	8.60 ± 1.08
42	5.82 ± 1.46[a]	6.62± 2.14[b]	0.00 ± 0.00
62	7.25 ± 1.68[a]	12.17± 0.09[a]	8.02 ± 1.15[b]

[a,b]= Means on the same column with different superscript letters differ significantly ($P < 0.05$)

[x,y]= Means on the same row with different superscript letters differ significantly ($P < 0.05$)

Discussion

The gonadosomatic index which is either the testicular volume per body weight or testicular weight per body weight is species-specific and varies with age and breeding season, even within the same species (Pochon *et al*., 2002). It was reported that this index predicts the rate of sperm function in a given species (Adebayo, 2009). It is also use to measure sexual maturity in relation to the sexual development of testes or ovaries, hence an index of fertility. For the epipidymal body ratio, there was significant decrease in the TT+L for 22 days when compared with the sham treated group for the same duration. The epididymis contains high concentration of fat which serve as a substrate for the exogenous L-carnitine given to the animals during treatment. Coenzyme A present in the blood binds with the fatty acid, activates and then move it into the cytosol. A carnitine molecule then attaches, which allows the transporting receptor, carnitine-palmitoyl-transferase 1 (CPT1), which recognizes and transport the fatty acid across the outer mitochondrial into the inter membrane space. Then, a carnine-specific transfer enzyme in the inner mitochondrial membrane brings the fatty acid into the mitochondrion for breakdown by carnitine-palmitoyl-transferase 2 (CPT2) and the carnitine molecule returns back to the cytosol to shuttle more fatty acids (Mehta, 2014). Continuous mobilization of fatty acids for β-oxidation could be responsible for the epididymal body ratio established in this study. There was no significant difference in the testicular body ratio of the TT and TT+L of all the durations when compared with the sham treated groups despite treatment with L-carnitine. The two major events in TT are ischaemia and reperfusion. These result into elevated production of ROS, activation of nitrogen-activated protein kinases, induction of transcription and growth factors, including vascular endothelial growth factors, triggering apoptotic machinery and induction of several inflammatory cytokines, including TNF-α and IL-1β. This pathological cascade is responsible for testicular atrophy, decreased blood flow and impaired spermatogenesis (Atavilla, 2012). The results obtained disagreed with the findings of Abdel-Emam, and Ahmed, (2021). They reported decrease in testicular weight in rats that were subjected to trauma and treated with 100 mg/Kg of L-carnitine.

Mammalian reproduction is mainly driven and regulated by the hypothalamic-pituitary-gonadal (HPG) axis. Follicle-stimulating hormone (FSH), which is synthesized and secreted by the anterior pituitary gland, is a key regulator that ultimately affects animal fertility. As a dimeric glycoprotein hormone, the biological specificity of FSH is mainly determined by the β subunit. Since FSH is a key regulator in the hypothalamic-pituitary-gonadal (HPG) axis, it plays an indispensable role in mammalian reproductive activities (Wang *et al*., 2021). In our study, there was significant increase in both FSH in IRI and IRI+L for 22 days when compared with the sham treated group for the same duration. According to Ahmed et al., (2016), the increase in FSH in TT could be due to testicular damage caused by ischaemia. Trauma to the testis affects the testicular cells, namely, Leydig cells, Sertoli cells and germ cells. The assault on these cells stimulate the release of GnRH which in turn stimulate the anterior pituitary gland to release FSH. There was also significant increase in the FSH level in the L-carnitine group that was treated for 22 days compared to all the sham treated groups. The result obtained agrees with the findings of Rezaei *et al*., (2018) that treatment of L-carnitine elevated FSH levels and could improve sexual potency. The increase in FSH in TT+L for 22 days could be due to the ability of L-carnitine to mop up free radicals, adhesion molecules and inflammatory cytokines occasioned by TT.

Luteinizing hormone (LH) is a glycoprotein hormone that is co-secreted along with follicle-stimulating hormone by the gonadotrophin cells in the adenohypophysis (anterior pituitary). Luteinizing hormone is a part of a neurological pathway comprised of the hypothalamus, the pituitary gland, and gonads. In this pathway, LH release is stimulated by gonadotropin-releasing hormone (GnRH) and inhibited by estrogen in females and testosterone in males (Nedresky, and Singh, 2023). In the result of this study, there was significant increase and significant decrease in the LH concentration in TT and TT+Lfor 62 days respectively when compared with the sham treated group for the same duration. The increase in LH concentration in TT, just like we established in the FSH concentration could due to the response to the damage of the testicular tissue as a result of ischaemia (Ahmed *et al*., 2016). The decrease could be due to the ability of L-carnitine to mop up free radicals generated, repair the damaged cells and restore the tissue to normal state.

Testosterone is the primary male hormone responsible for regulating sex differentiation, producing male sex characteristics, spermatogenesis, and fertility (Nassar and Leslie, 2023). There was significant increase in the Testosterone concentration in the TT+L for 62 days when compared with the sham treated group for the same duration. L-carnitine due to its antioxidant properties, reduced testicular oxidation stress, led to prevention of the destruction of Leydig cells, and thereby increases testosterone levels without changing gonadotropin levels. L-carnitine could also directly affected the testosterone secretion by decomposition of long-chain fatty acid, which reduced the aromatase enzyme in the adipose tissue, since this enzyme converts testosterone to estrogen, thereby

inhibiting the activity of this enzyme and increasing testosterone levels in the blood (Ishii *et al*., 2015).

Conclusion

The result of this study established that TT created IRI and treatment with L-carnitine resulted into decrease epipidymal body ratio and increase in FSH and T4 concentrations. This could be due to the ability of L-carnitine to confer stability to the cells of the body exposed to oxidative stress, repair damaged cells and also mop up free radicals and inflammatory cytokines.

Competing interests

The authors declare no conflict of interests

Recommendation

Consumption of foods rich in dietary carnitine should be encouraged before and after surgical intervention (ochiopexy) to correct testicular torsion.

Acknowledgement

The authors sincerely appreciate the technical assistance of the staff of Department of Human Physiology and Faculty of Veterinary Medicine, Ahmadu Bello University during the period of the research.

References

Abdel-Emam, R. A., and Ahmed, E. A. (2021). Ameliorative effect of L-carnitine on chronic lead-induced reproductive toxicity in male rats. *Veterinary Medicine and Science*, *7*(4), 1426-1435.

Adebayo, A. O., Oke, B., and Akinloye, A. K. (2009). Characterizing the Gonado-somatic index and its relationship with age in greater cane Rat (*Thryonomys swinderianus*, *Temminck).Veterinary Anatomy*, 2 (2): 53-59.

Ahmed, A. I., Lasheen, N. N., and El-Zawahry, K. M. (2016). Ginkgo biloba ameliorates subfertility induced by testicular ischemia/reperfusion injury in adult Wistar rats: a possible new mitochondrial mechanism. Oxidative Medicine and Cellular Longevity, 2016. Vol. 2016, article ID 6959274, 19 pages, 2016. https://doi.org/10.1155/2016/6959274

Akusu, M. O., Akpokodje, J. U., Ogwnegbu, S. O., and Oke, B. O. (1985).Differences in morphology of bull spermatozoa from normal and pathological testis duringepididymaltransit. *Nigerian Veterinary Journal*, 14(2), 30-33.

Altavilla, D., Romeo, C., Squadrito, F., Marini, H., Morgia, G., Antonuccio, P., & Minutoli, L. (2012). Molecular pathways involved in the early and late damage induced by testis ischemia: Evidence for a rational pharmacological modulation. *Current Medicinal Chemistry*, 19(8), 1219–1224

Da Conceição, H. N., Diogo, M. P. S., Vitorino, T. C. T., Juntolli, A. C. V., Castanheira, E. P., Huff, C. R., Siquara Neto, E. De J. and Gonçalves, J. S. (2024). Clinical and Pathophysiological Aspects of Testicular Torsion: *Brazilian Journal of Health Review*, Vol. 7, No. 1, Pp. 4021–4030, 2024. Doi: 10.34119/Bjhrv7n1-327

Dokmeci, D., Inan, M., Basaran, U. N., Yalcin, O., Aydogdu, N., Turan, F. N. and Uz, Y. H. (2007). Protective effect of L-carnitine on testicular Ischaemia-reperfusion injury in rats. *Cell and Biochemistry Function*, 25:611-618.

Ishii, T., Matsuo, N., Sato, S., Ogata, T., Tamai, S., Anzo, M., ... and Hasegawa, T. (2015). Human chorionic gonadotropin stimulation test in prepubertal children with micropenis can accurately predict Leydig cell function in pubertal or postpubertal adolescents. *Hormone Research in Paediatrics*, 84(5), 305-310

Franca, L. R., Suescun, M. O., Miranda, J. R., Giovambattista, A., Perello, M., Spinedi E., and Calandra, R. S. (2006). Testis structure and function in anongenetichyperadipose rat model at prepubertal and adultages. *Endocrinology, 147*(3), 1556-1563.

Ishii, T., Matsuo, N., Sato, S., Ogata, T., Tamai, S., Anzo, M., ... and Hasegawa, T. (2015). Human chorionic gonadotropin stimulation test in prepubertal children with micropenis can accurately predict Leydig cell function in pubertal or postpubertal adolescents. *Hormone Research in Paediatrics*, 84(5), 305-310

Nassar, G. N and Leslie, S. W. (2023). Physiology, Testosterone. In: StatPearls. StatPearls Publishing, Treasure Island (FL); PMID: 30252384

Nedresky, D. and Singh, G. (2023). Physiology, Luteinizing Hormone. In: StatPearls. StatPearls Publishing, Treasure Island (FL); PMID: 30969514

Oyeyemi, M. O. and Ubiogoro, O. (2005).Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of Large White boar in Nigeria. *International Journal of Morphology 23*(3), 235-239.

Pochon, S. T., Wright, P. C., Schaentzter, E., Ippolito, M., Rakonirina, G., and Rakotosoa, R. (2002). Effect of season and age on Gonado-somatic index of Milne Ewards' silfakas (*Propithecus diadema Edwards*) in Ranomafana National Park, Madagascar. *International Journal of Primatology*, 53(2), 355-364.

Rezaei, N. , Mardanshahi, T. , Shafaroudi, M. M. , Abedian, S. , Mohammadi, H. , and Zare, Z. (2018). Effects of L-carnitine on the follicle-stimulating hormone, luteinizing hormone, testosterone, and testicular tissue oxidative stress levels in streptozotocin-induced diabetic rats. *Journal of Evidence-Based Integrative Medicine*, 23, 1-10. 10.1177/2515690X18796053

Virmani, M. A., and Cirulli, M. (2022). The role of l-carnitine in mitochondria, prevention of metabolic inflexibility and disease initiation. *International journal of molecular sciences*, *23*(5), 2717.

Wang, H. Q., Zhang, W. D., Yuan, B., and Zhang, J. B. (2021). Advances in the regulation of mammalian follicle-stimulating hormone secretion. *Animals*, 11(4), 1134