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THE MODULATORY ROLE OF L-CARNITINE ON INFLAMMATION IN EXPERIMENTALLY-INDUCED TESTICULAR TORSION IN WISTAR RATS

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ABSTRACT

Testicular torsion (TT) is a urological emergency that leads to testicular tissue loss due to ischaemic damage. Quick diagnosis and urgent treatment is essential in the management of testicular injury. Reperfusion following torsion leads to generation of reactive oxygen species, inflammatory cytokines and adhesion molecule which are harmful to the testicular environment. 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, blood samples were collected from each of the animals through cardiac puncture and automated haematological analyzer was used to determine the white blood cells. 1.6ml of blood was used to determine Erythrocyte sedimentation rate and serum was harvested to assay TNF-α, Interleukin-1α and Interleukin-1β. The results obtained showed significant decrease (0.90±0.45 3mm/hr) in ESR in the IRI for 22 followed by significant increase (4.00±0.70 3mm/hr) in IRI+L for the same duration when compared with the Sham (2.80±1.11 3mm/hr) treated group. There was also significant decrease (9.50±0.30 $\times 10^3$ µL) in WBCs in TT+L for 62 days when compared with sham for the same period (18:75±75 $\times 10^3$ µL). There was no significant difference in the results obtained for both TNF-α and IL-1α across all the groups but there were significant differences in the TT and TT+L for 42 days, and TT for 62 days when compared with their respective sham treated groups. From our findings, we can conclude that L-carnitine was able to ameliorate the level of inflammation occasioned by TT by lowering the levels of ESR, WBCs and IL-1α which are markers of inflammation.

Keywords: Testicular Torsion, L-carnitine, Inflammation, Cytokines

INTRODUCTION

Ischaemic-reperfusion injury (IRI) is an injury that takes place when blood flow is restored to a tissue or organ after a period of ischaemia (Zhao *et al.*, 2024). Ischaemia refers to a decreased delivery of oxygen and nutrients to a tissue or an organ of the body. Reperfusion to the ischaemic organ is the only way to rescue the organ from ischaemic injury. Ironically, reperfusion itself induces the injury, which is more severe than the previous injury caused by ischaemia resulting into ischaemic-reperfusion injury (Kuldeep *et al.*; 2021). Ischaemic-reperfusion injury (IRI) is a complex phenomenon that causes cell damage through a dual process-ischaemia and reperfusion. This results in oxidative damage via the reactive oxygen species, inflammatory cytokines and adhesion molecule generated during reperfusion (Bebekah *et al*., 2023).

Testicular torsion is a condition in which a testis rotates around its longitudinal axis and twists the spermatic cord. This consequently leads to a significant reduction in blood flow and perfusion of testicular tissue. Testicular torsion leads ischaemia, heat stress, hypoxia, and oxidative and nitrosative stress. Testicular torsion should be seen as an emergency condition and surgical intervention as the sole treatment option-orchipexy (detorsion) and orchidectomy (surgical removal of a completely necrotic testis). Possible testicular detorsion side-effects occur due to reperfusion and endothelial cells injury, microcirculation disturbances, and intense germ cells loss (Minas *et al*., 2023).

L-Carnitine is an amino acid derivative that plays an important role in the metabolism of fatty acids. It acts as a carrier that shuttles long-chain fatty acyl CoA across the mitochondrial membrane for fuel mitochondrial β-oxidation. In addition, L-carnitine reduces oxidative damage and plays an essential role in the maintenance of cellular energy homeostasis (Carillo *et al*., 2020). L-Carnitine is produced in the body of mammals from two essential amino acids, methionine and lysine, when it is not received from diet. The synthesis can occur in the brain, liver, and kidneys (Cave *et al*., 2008).

MATERIALS AND METHODS

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, red blood cell pipette, chromic suture, Petri dish, electronic balance, Enzyme-linked immunosorbent assay (ELISA) kits for inflammatory cytokines 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 torsion/detorsion
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).

Erythrocyte sedimentation rate
1.6 ml of blood taken through cardiac punction was thoroughly mixed, the cap of the container was removed and sample put in ESR stand. A Westergren pipette was inserted vertically. Using a safe suction, blood was drawn to the 0 mark. The ESR stand and pipette were not exposed to direct sunlight. After about one hour the level at which the plasma met the red cells in mm was recorded.

Leucocyte blood count
The blood sample was collected into a test tube containing an anticoagulant (EDTA). A haematology analyzer machine was used to carry out a complete blood count. Seventeen (17) µL was aspirated through narrow tubing followed by an aperture and a laser flow cell. It conducted quantitative and qualitative analyses of complete blood count and results of the leucocyte count analyzed.

Cytokines Assays
Rat specific enzyme linked immune-sorbent assay (ELIZA) of TNF-α, IL-1α, IL-1β were purchased from Wuhan Fine Biotech Co., Ltd, China and assays were done according to manufacturer's protocol. Below is the summary for each assay.

Tumour Necrosis Factor-Alpha
Standard, test samples and control were set on wells on the pre-coated plate and then their positions were recorded. 0.1ml of standard, blank or sample were added per well. The plate was sealed and incubated for 90 minutes at 37°C. Each well was aspirated and washed, repeating the process twice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper. 0.1 ml Biotin-labelled antibody working solution was added to each well at the bottom without touching the side walls of the plates. The plate was sealed and incubated at 37°C for minutes. After the incubation, the sealer was removed and the plates washed three times using wash solution. 0.1 ml of SABC working solution was added to each well covered with a new sealer and incubated for 30 minutes at 37°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 25 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.

Interleukin-1 alpha
The HRP-Streptavidin Conjugate (SABC) working 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. 0.1ml of standard, blank or sample were added per well. The plate was sealed and incubated for 90 minutes at 37°C. Each well was aspirated and washed, repeating the process twice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper. 0.1 ml Biotin-labelled antibody working solution was added to each well at the bottom without touching the side walls of the plates. The plate was sealed and incubated at 37°C for minutes. After the incubation, the sealer was removed and the plates washed three times using wash solution. 0.1 ml of SABC working solution was added to each well, covered with a new sealer and incubated for 30 minutes at 37°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 25 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.

Interleukin-1 beta
Standard, test samples and control were set on wells on the pre-coated plate and then their positions were recorded. 0.1ml of standard, blank or sample were added per well. The plate was sealed and incubated for 90 minutes at 37°C. Each well was aspirated and washed, repeating the process twice using wash buffer. The plate was then inverted and patted against thick clean absorbent paper. 0.1 ml Biotin-labelled antibody working solution was added to each well at the bottom without touching the side walls of the plates. The plate was sealed and incubated at 37°C for minutes. After the incubation, the sealer was removed and the plates washed three times using wash solution. 0.1 ml of SABC working solution was added to each well, covered with a new sealer and incubated for 30 minutes at 37°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 25 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.

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.

RESULTS AND DISCUSSION

Table 1: Effect of L-carnitine on Erythrocyte sedimentation rate in testicular torsion in Wistar rats

Duration (days)	Sham (3mm/hr)	IRI	IRI+L
22	2.80 ± 1.11^{a,x}	0.90 ± 0.45^{b,x}	4.00± 0.70^{c}
42	6.32 ± 0.97^{y}	3.50 ± 0.50^{y}	4.00 ± 0.84
62	1.75 ± 0.80^{z}	2.50 ± 0.81^{y}	2.00 ± 0.32

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

^{x,y,z} = Means on the same row with different superscript letters differ significantly (P < 0.05)

Table 2: Effect of L-carnitine on Leucocyte count in testicular torsion in Wistar rats

Duration (days)	Sham ($\times10^3/\mu L$)	TT	TT+L
22	4.20 ± 2.20	13.34 ± 2.61	15.72 ± 1.99^{x}
42	14.87 ± 1.22	21.02 ± 9.96	9.13 ± 2.30^{y}
62	18.75 ± 3.17^{a}	8.94 ± 0.63^{b}	9.50 ± 0.30^{b,y}

^{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)

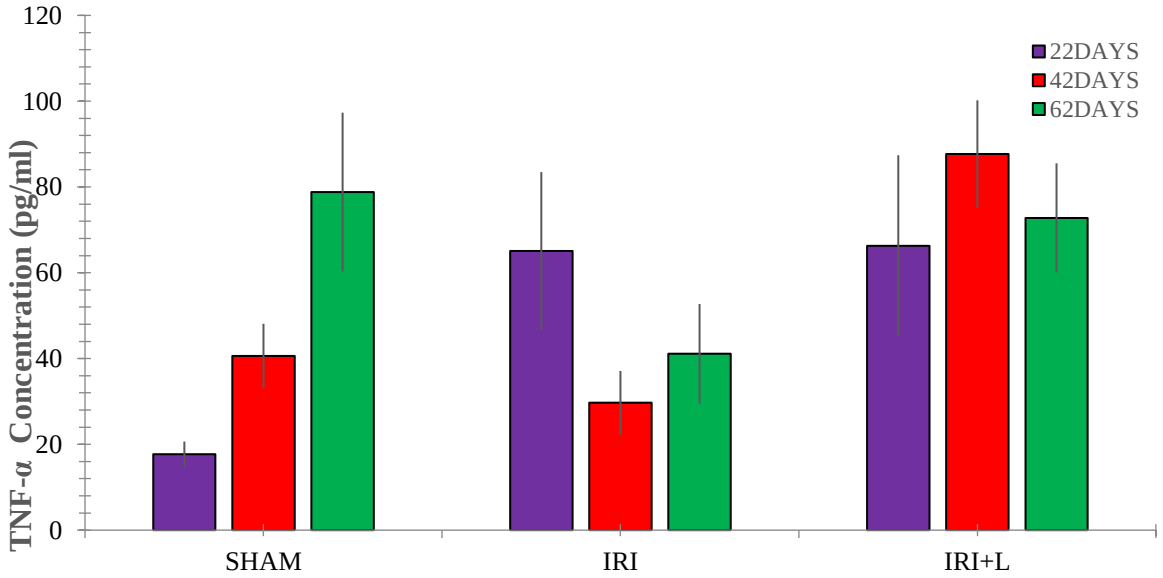

Figure 1: Effect of L-carnitine on Tumour necrosis factor-alpha in testicular IRI in Wistar rats.
Each bar represents mean of five animals

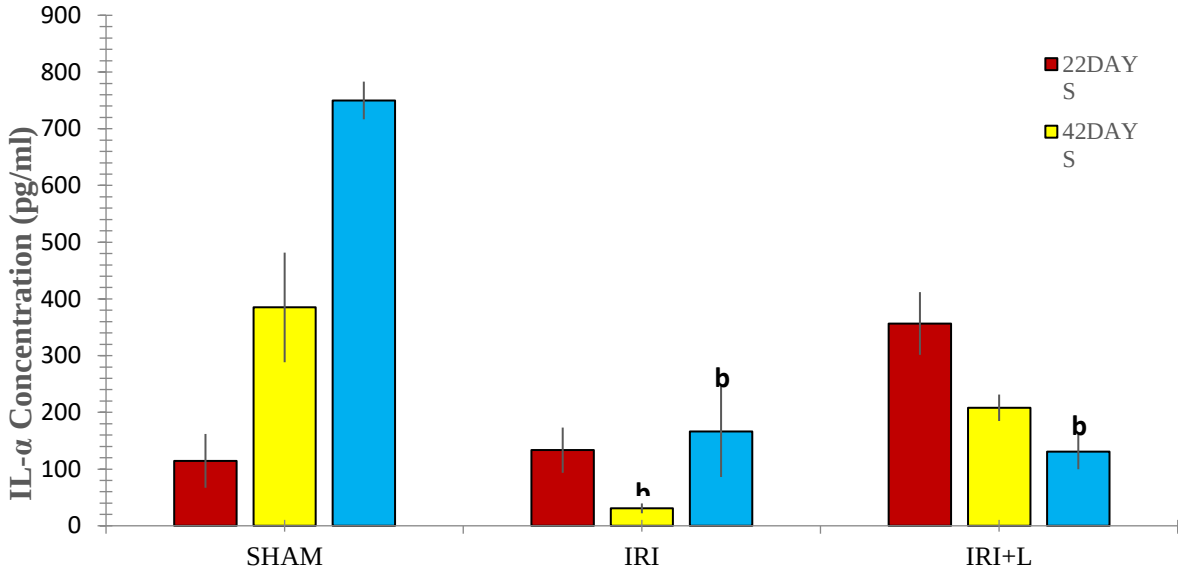

Figure 2: Effect of L-carnitine on IL-1α in testicular torsion in Wistar rats
b = significant difference (P<0.05) compared to the sham group
Each bar represent mean of five animals
Bars with superscripts letter differ significantly (P < 0.05) compared with sham groups

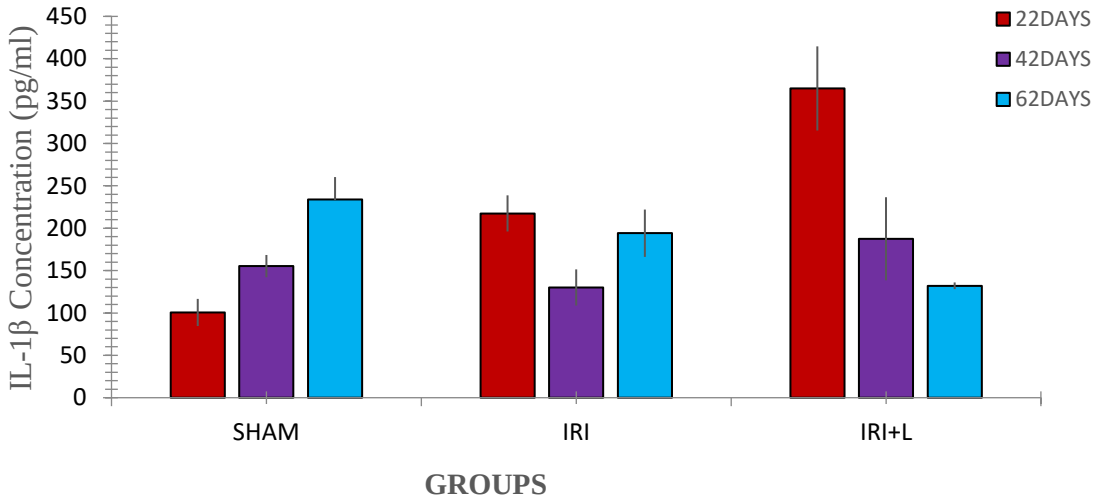

Figure 3: Effect of L-carnitine on IL-1β in testicular torsion in Wistar rats
Each bar represents mean of five animals

Discussion

Oxidative stress, inflammation and germ cell death are the main features of testicular ischaemic-reperfusion injury, which is seen as the underlying mechanism for testicular torsion and detorsion (Almarzouq and Al-Maghrebi, 2023). In a living organisms, systemic inflammation can be evaluated by several markers and these include leukocyte, neutrophil and lymphocyte counts (Girgin *et al*., 2018). A significant increase in leukocyte count in patients with TT has been established in different studies (Cicek *et al*., 2015; Yang *et al*., 2011). White blood cells are markers of infection and inflammation. In the TT for 42 days, there was significant increase in WBCs when compared with the Sham treated group for the same period. Treatment with L-carnitine for 42 days significantly reduced the WBCs value. The TT+L for 62 days recorded significant decrease in WBCs when compared with TT+L for 42 and 62 days respectively. The decrease in WBCs in groups treated with L-carnitine may be due to its ability to enhance tissue repair and recovery (Kononov *et al*., 2022)

Erythrocyte sedimentation rate (ESR) is a commonly performed hematology test that may signify and monitor an increase in inflammation within a biologic system occasioned by one or more conditions such as autoimmune disease, infections, or tumors. The ESR is not a differential for any single disease but is used alongside with other tests to evaluate the presence of increased inflammatory activity. The ESR has long been used as a "sickness indicator" due to the fact it can be reproduced and is inexpensive (Tishkwoski and Gupta, 2024). There was a significant decrease in TT for 22 days when compared with the Sham treated group for the same duration. The ESR test records the rate at which the erythrocytes, in a whole blood sample fall to the bottom of the Westergren tube. This process of "falling" is called sedimentation (Plebani and Piva, 2002). The erythrocytes typically fall at a faster rate in individuals with inflammatory conditions such as infections, cancer, or

autoimmune disorders. These health conditions lead to an increase in the number of proteins in the blood. This increase causes erythrocytes to stick together (clump) and settle faster. A group of RBCs clumped together will form a stack (similar to a stack of coins) called a rouleau (pleural, rouleaux) (Abramson, 2006). The elevation seen in the TT for 22 days could be due to the increase in proteins in the blood occasioned by TT. The TT+L for 22 days recorded decrease in ESR. This is due to the ability of L-carnitine to mop up the free radicals and inflammatory cytokines produced during TT. Reduction in ROS and cytokines due to L-carnitine suppress the leukocytosis that was triggered by TT.

Tumour necrosis factor alpha (TNF-α) is a multifunctional cytokine that has well-established immunological roles in innate and adaptive immunity and in the normal physiological functions of immune cells, where its production and actions tend to be both temporally and spatially limited (Sethi and Hotamisligil, 2021). There was no significant difference amongst all the treated groups. Our findings disagrees with those of Olugbodi *et al*., 2021 in which they treated animals with L-carnitine after torsion and recorded significant decrease in TNF-α. The lack of significance could be due to the inability of L-carnitine to significantly improve the antioxidant status of the body after elevated production of ROS as a result of TT.

Interleukin-alpha (IL-1α) is a unique member of the IL-1 family in that it is produced in a normal state and rarely linked with disease conditions. IL-1α activity is regulated by two types of IL-1 receptors (IL-1R1 and IL-1R2) although the cytokine binds with greater affinity to IL-1R1 (Dinarello, 2018; Khazim *et al*., 2018). During cellular necrosis, IL-1α is shedded into the extracellular environment, where it stimulates sterile inflammatory signaling (Khazim *et al*., 2018). There was significant increase in IL-1α in TT+L for 42 days when compared with the sham for 42 days. There was a possibility of inflammation and apoptosis still remaining in the tissue of the animal even after reperfusion and treatment had taken place. However, there was significant decrease in the TT+62 for 62 when compared with the sham and IRI treated groups for the same duration. This could be due to the ability of the L-carnitine to stimulate the expression of soluble IL-1α receptor antagonist which in turn reduced the production of IL-1α. This result again corroborate with that of Olugbodi *et al*., 2021.

IL-1β is largely secreted by mononuclear cells and is active only after binding by caspase-1 and release from producing cells. It is active at a much longer duration than IL-1α, and this is the reason why IL-1 inhibitors are more active for IL-1β than for IL-1α. Both IL-1α and IL-1β have haematopoietic capacity (one of the original names of IL-1 is hemopoietin-1), in particular in response to damage ("emergency" hematopoiesis), and seem to be associated with the age-dependent increased inflammatory status (Mai *et al*., 2018; Calvani *et al*., 2005). There was no significant difference in IL-1β levels across groups after treatment.

CONCLUSION

In conclusion, the study demonstrated that TT resulted into elevated levels of ESR, WBCs and IL-1α. Treatment with 500 mg/kg of L-carnitine was able to mitigate the effect of inflammation.

REFERENCES

Abramson N. (2006). Rouleaux formation. Blood. *Case Reports*, Jun 01;107(11):4205.

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.

Almarzouq, D. and Al-Maghrebi, M. (2023). NADPH Oxidase-Mediated Testicular Oxidative Imbalance Regulates the TXNIP/NLRP3 Inflammasome Axis Activation after Ischemia Reperfusion Injury. *Antioxidants*, 12(1):145. https://doi.org/10.3390/antiox12010145

Bebekah, R. J., Saleh, M. I. A., Mohammed, A. and Tanko, Y. (2023). Modulatory effect of L-carnitine on red blood cells and indices in testicular ischaemic-reperfusion in wistar rats. *Communication in Physical Sciences*, 10(2): 09-12

Calvani, N., Tucci, M., Richards, H. B., Tartagila, P. and. Silvestris, F. (2005). Th 1 cytokines in the pathogenesis of lupus nephritis: The role of IL-18. *Autoimmunity Reviews*, 4(8): 542-548

Carillo, M. R.., Bertapelle, C., Scialo, F., Siervo, M., Spangnuolo, G., Simeone, M., Peluso, G. and Digilio, F. A. (2020). L-carnitine in Drosophila: A review. *Antioxidants*, 9(12): DOI. 10.3390/antiox9121310

Cave, M. C., Hurt, R. T., Frazier, T. H., Matheson, P. J., Garrison, R. N., McClain, C. J. and McClave, S. A. (2008). Obesity, inflammation and the potential application of pharmaconutrition. *Nutrition in Clinical Practice*, 23(1): 16-34

Cicek, T., Togan, T., Akbaba, K., Narcı, H. and Aygun, C. (2015). The value of serum mean platelet volume in testicular torsion. *Journal of Internal Medical Research*, 43:452-459

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunology Reviews*, 281:8-27

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 Biochemistry and Function*, 25:611-618.

Girgin, R., Cinar, O., Bulut, E., Akduman, B and Mungan, N. A. (2018). The Role of the Platelet Mass Index (PMI) as a New Prognostic Factor in Fournier's Gangrene. *African Journal of Urology*, 24:226-232

Khazim K, Azulay EE, Kristal B, Cohen I. Interleukin 1 gene polymorphism and susceptibility to disease. Immunology Reviews, 281:40-56

Kononov, S. U., Meyer, J., Frahn, J., Kersten, S., Kluess, J., Meyer, U., Huber, K. & Danicke, S. (2021). Effects of Dietary L-carnitine supplementation on placenta and Erythrogram of Dairy cows with special with special emphasis on parturition. *Dairy*, 2, 1, pp. 1-13.

Kuldeep, K., Nirmal, S., Amteshwar, J. S. and Leonid, M. (2021). Clinical applicability of conditioning techniques in ischaemia-reperfusion: A review of literature. *Current Cardiology*, 17 (3): 306-318

Mai, S., Li, C., Xie, X., Xiong, H., Xu, M., Zeng, F., Guo, Q. and Han, Y. (2018). Increased serum IL-36α and IL-36ϒ

levels in patients with systemic lupus erythematosus: Association with disease activity and arthritis. *International Immunopharmacology*, Pp 58:103-108

Minas, A., Gamchi, M., Mahmoudabadi, S., Gamchi, N. S., Antoniaass, M. P., Alizadeh, A. and Bertolla, R. P. (2023). Testicular torsion in *vivo* models: Mechanisms and treatments. *Andrology*, 11(7): 1267-1285

Olugbodi, J. O., Samaila., K., Lawal, B., Anunobi, O. O., Baty, R. S., Ilesanmi, B. O. and Batiha, G. E. (2021). Computational and Preclinical evidence of anti-ischaemic properties of L-carnitine-rich supplement via stimulation of anti-inflammatory and antioxidant events in testicular torsed rats. Oxidative Medicine and Cellular Longevity, Volumre 2021/Article ID 5543340/ https://doi.org/10.1155/2021/5543340

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.

Plebani M, Piva E. (2002). Erythrocyte sedimentation rate: use of fresh blood for quality control. American Journal of Clinical Pathology, 117 (4): 621-626

Sethi, J. K. and Hotamisligil, G. S. Metabolic Messengers: tumour necrosis factor. *Nature Metabolism*, 3:1302-1312. https://doi.org/10.1038/s42255-021-00470-z

Tishkowski K, and Gupta V. (2024). Erythrocyte Sedimentation Rate. [Updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https: //www.ncbi.nlm.nih.gov/books/NBK557485

Yang, C. Jr, Song, B., Liu X, Wei, G. H., Lin, T. and He, D. W. (2011). Acute scrotum in children: an 18-year retrospective study. *Paediatric Emergency Care*, 27:270-274

Zhao, L., Yanghong, L., Chen, Y., Yu, Z. and Luo, H. (2024). A novel postconditioning approach attenuates myocardial ischaemia-reperfusion injury in rats. *Reviews in Cardiovascular Medicine*, 25 (2): DOI: 10.31083/j.rcm2502067