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Modulatory Effect of L-carnitine on Red Blood Cell and Indices in Testicular Ischaemic-Reperfusion in Wistar Rats

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Abstract: *Ischaemic-reperfusion injury (IRI) is a complex phenomenon that induces cell damage through a biphasic process and reperfusion. This results in oxidative damage via the reactive oxygen species, inflammatory cytokines and adhesion molecule generated during reperfusion. This study aimed to investigate the modulatory effect of L-carnitine on Red blood cells and their indices following testicular torsion. Forty-five adult male Wistar rats were used for this study. The animals were divided into three groups comprising fifteen (n=5) in each group. Five (n=5) animals from each group i.e. Sham, IRI and IRI 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 study, blood samples were collected from each of the animals through cardiac puncture and a full blood count was done using an automated haematological analyzer to determine the Red blood cells and their indices. Our findings revealed a significant increase in RBCs count (6.30±0.44 x10^6 µL) for the group the IRI+L treated 62 groups when compared with sham treated group for the same duration and also when compared with the 1RI+L for 22 days. The Haemoglobin concentration (13.49±0.35 g/dL) increased significantly in the IRI+L for 42 days when compared with the Sham group for the same duration. A similar trend also was recorded in the Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC). Treatment of Wistar rats induced with testicular torsion with L-carnitine improved significantly the RBC and their indices.*

Keywords: *Ischaemic-Reperfusion Injury, Testicular Torsion, Sham, L-carnitine, Modulatory, Blood Count*

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1.0 Introduction

Ischemia-reperfusion (I/R) injury is a deleterious clinical entity in the organism that occurs when blood circulation is restored after an episode of acute ischemia. In this type of injury the blood supply of the tissue is interrupted initially which leads to damage of metabolically active tissues, but the restoration of blood flow to the tissues, which initiates

paradoxic cascade of events, leads to further cellular and tissue damage eventually (Akbas *et al*.,2005).

Successful salvage of the torsioned testis is directly related to the time elapsed from the onset of ischemia (Ramachandra *et al*., 2015). If exploration is performed within 4-6 hours of symptom onset, salvage rates may approach 90%; with delayed intervention, however, these rates drop dramatically to 50% at 12 hours after symptom onset and to almost 10% after 24 hours. In contrast, perinatal testicular torsion almost always results in loss of the involved testis (salvage rate < 5%) (Davenport, 1996).

Blood act as a pathological reflector of the status of the exposed animals to toxicants and other conditions. The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutritional and pathological status of an animal. Blood constituents change the physiological status of an animal. These changes are important in assessing the response of farm animals to various physiological situations (Etim, 2014).

L-carnitine (L-3-hydroxytrimethylaminobutan -oate) is a naturally occurring substance that can be synthesized in mammals from the essential amino acids lysine and methionine or ingested in diet (Kraemer *et al*.; 2008). It acts as a carrier for fatty acids across the inner mitochondrial membrane for subsequent β-oxidation (Rebouche, 2004). It is also an antioxidant that reduces metabolic cells (Gulcin, 2005). Therefore, LC has an important function by transporting the medium-, short- and long-chain fatty acids through the mitochondria membrane in the oxidation process that strengthens animal immunity, improving antioxidant status and increasing reproductive performance (Pirestani *et al*., 2009)

Many investigations have been carried out on the protective effects of exogenous substances on testicular torsion concerning oxidative stress biomarkers. They include the protective effect of famotidine onischemia–reperfusion injury following testicular torsion in rats (Tanriverdi *et al.,* 2021), Antioxidant property of *Plantago major* leaf extracts reduces testicular torsion/detorsion-induced ischemia/reperfusion injury in rats (Moradi-Ozarlou *et al.,* 2020), Protective effects of crocin on testicular torsion/detorsion in rats (Ganjiani *et al.,* 2021) amongst others. In the studies cited above, the duration of ischaemia was short, as well as the reperfusion cycle. In our study, we try to mimic what is obtainable in real life. From the onset of scrotal pain to arrival at differential diagnosis of testicular torsion, there is usually a time-lapse and in most cases, the time interval may span into several hours and even days. Six hours or more may be about the minimum time needed before surgical intervention and treatment. The treatment in this study is longer than what is reported in most studies. Longer treatment is also required to ascertain the effect of the antioxidant on the blood cells and testicular tissue. L- carnitine has been found to contain anti-oxidative and anti-inflammatory properties. In general, it seems to be well tolerated by the body; harmful effects related to high-dose have not been established (Hendler *et al*.; 2011).

2.0 Materials and Methods

2.1 Materials

Cages, normal saline, scissors, buffered distilled water, EDTA bottles, syringes (1 ml, 2 ml and 5 ml), soap, masking tape, oral intragastric tube, watch, cotton wool, chloroform, dissection kit, filter paper, , chromic suture, Haematology analyzer

2.2 Animals

Forty-five (45) Male Wistar rats weighing 104g-230g were used for the study. The animals were purchased in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were kept in well-aerated laboratory cages and

given access to growers' mash from Labar Feeds and Grains Merchant, Zaria, Nigeria and water *ad libitum*.

2.3 Methods

The animals were weighed and divided randomly into three (3) groups made up of fifteen (15) animals each.

GROUP 1 (SHAM): In this group, the testes were brought through the scrotal incision and then replaced with the scrotal sac and sutured. The rats were given distilled water 1 ml/kg each day orally after detorsion and five Animals each were sacrificed on day 22 day 42 and day 62 respectively.

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

GROUP 3 (IRI+L-CARNITINE): Rats in this group were experimentally 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.

2.3 Surgical induction of torsion and detorsion

The rats were anaesthetised by chloroform inhalation in a closed chamber and thereafter sacrificed. The testes were exposed through identically-opened and closed right-sided ilio-inguiral incision. The testicles were exposed by incising 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 remained in that state until sacrifice were carried on. The testicles were surgically removed through a lower abdominal incision according to the method described by Akusu *et al.* (1985) and Oyeyemi and Ubiogoro (2005).

2.4 Full blood count

The blood sample was drawn into the test tube containing an anticoagulant (EDTA). A haematology analyzer was used to perform a complete blood count. 17µL was aspirated through narrow tubing followed by an aperture and a laser flow cell. It carried out quantitative and qualitative analyses of red and white blood cells. Detection by electrical impedance involved determining cell number by conductivity variations and volume by changes in voltage. Optical systems emitted a beam of light whose path was altered by the blood cells which allowed their size and content to be determined. Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Haemoglobin (Hb) in the RBCs were determined.

3.0 Results and Discussion

Table 1 Shows the Effects of L-carnitine on Red blood cell count in testicular IRI in Wistar rats. There was a significant decrease in the IRI+L for 42 days when compared with the sham-treated group for the same time and when compared with IRI+L for 22 days. There was also a significant increase in the IRI+L for 62 days when compared with sham treated group for the same duration and when compared with IRI+L for 22 days.

Table 1: Effects of L-carnitine on Red blood cells count in testicular IRI in Wistar rats

Duration (days)	Sham ($\times10^6$/µL)	IRI	IRI+L
22	7.25 ± 0.24	7.04 ± 0.19	6.57 ± 0.21
42	7.32 ± 0.23	8.31 ± 0.14^x	6.30 ± 0.44^x
62	6.72 ± 0.47	6.30 ± 0.44^y	7.96 ± 0.16^y

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

Table 2 Shows the Effects of L-carnitine on Haemoglobin concentration in testicular IRI in Wistar rats. There was a significant increase in the IRI+L for 42 days when compared with sham treated group for the same duration and when compared with IRI+L for 22 days.

Table 2: Effects of L-carnitine on haemoglobin concentration in testicular IRI in Wistar rats

Duration (days)	Sham (g/dL)	IRI	IRI+L
22	13.00 ± 0.41	12.70 ± 0.30	12.30 ± 0.44
42	12.96 ± 0.11	14.80 ± 0.75^{a,x}	13.49 ± 0.35^{b,z}
62	13.20 ± 1.53	15.22 ± 0.82^{y}	14.25 ± 0.29

^{a,b}= Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the sham groups
^{x,y,z}= Means on the same row with different superscript letters differ significantly (P < 0.05) compared with the groups treated for 22 days

Table 3 shows the Effects of L-carnitine on Mean cell volume in testicular IRI in Wistar rats.

Table 3: Effects of L-carnitine on mean cell volume in testicular IRI in Wistar rats

Duration (days)	Sham (fL)	IRI	IRI+L
22	52.62 ± 1.34	54.10 ± 1.05	61.80 ± 1.40
42	53.67 ± 1.11	52.00 ± 0.95^{b}	64.58 ± 0.90^{a,y}
62	56.80 ± 3.44	56.22 ± 3.78	52.56 ± 0.68^{x}

^{a,b,}= Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the sham groups
^{x,y}= Means on the same row with different superscript letters differ significantly (P < 0.05) compared with the groups treated for 22 days

There was a significant difference in IRI+L for 42 days when compared with sham treated group for the same duration and IRI+L for 22 days. There was a significant decrease in IRI+L for 62 days when compared with sham treated group for the same duration and when compared with IRI+L for 22 days.
Table 4 shows the effects of L-carnitine on Mean cell haemoglobin in testicular IRI in Wistar rats. There was a significant increase in the IRI+L for 42 days when compared with IRI+L for 22 days and a significant decrease when compared with sham and IRI for the same duration.

Table 4: Effects of L-carnitine on Mean cell haemoglobin in testicular IRI in Wistar rats

Duration (days)	Sham (pg)	IRI	IRI+L
22	17.94 ± 0.42	18.04 ± 0.25	18.70 ± 0.31
42	50.30 ± 9.23^{x}	71.40 ± 2.84^{b,x}	24.60 ± 2.80^{a,x}
62	19.78 ± 2.36^{y}	19.68 ± 1.72^{y}	17.93 ± 0.22^{y}

^{a,b}= Means on the same column with different superscript letters differ significantly (P < 0.05) compared with the sham groups
^{x,y}= Means on the same row with different superscript letters differ significantly (P < 0.05) compared with the groups treated for 22 days

Table 5 shows the effects of L-carnitine on Mean cell haemoglobin concentration in testicular IRI in Wistar rats. There was a significant increase in the IRI+L for 42 days when compared with sham and IRI treated group for the same duration and when compared with IRI+L for 22 days.
Antioxidants have been shown to have a number of beneficial effects, protecting against RBC lipid peroxidation and increasing levels of reduced glutathione (GSH) while reducing levels of ROS (Balushi *et al*., 2019). The main function of red blood cells is the

transport of respiratory gases along the vascular tree. To fulfil their task, RBCs can elastically deform in response to mechanical forces and pass through the narrow vessels of the microcirculation.

Table 5: Effects of L-carnitine on mean cell haemoglobin concentration in testicular IRI in Wistar rats

Duration (days)	Sham (g/dL)	IRI	IRI+L
22	34.12 ± 0.10	33.44 ± 0.19^{a}	30.30 ± 0.32^{b}
42	19.52 ± 3.86^{x}	13.44 ± 0.39^{a,y}	37.73 ± 3.73^{b}
62	34.44 ± 1.83^{y}	34.82 ± 0.63^{z}	34.07 ± 0.02

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

^{x,y,z}= Means on the same row with different superscript letters differ significantly (P < 0.05) compared with the groups treated for 22 days

Decreased RBC deformability is observed in pathological conditions linked to increase in oxidative stress as seen in TT (Diederich *et al*., 2018). Oxidative stress decreases the level of antioxidant capacity, irreversibly damages erythrocytes, resulting in their eventual damage by haemolysis and their removal from circulation because mature RBCs are cells without nucleus and other cell organelles, they have no capacity to repair the damaged components (Waggiallah and Alzohairy, 2011).

In our study, there was a significant increase in the number of RBCs in the IRI+L for 62 days when compared with both the sham-treated group for the same duration and with IRI+L for 22 days. From the result obtained, the length of time during treatment with L-carnitine significantly increased the number of RBCs. This could have been achieved the ability of the L-carnitine to reduce the permeability of the transport pathways mediating hydration (Balushi *et al*.; 2019). The result agrees with the findings of Smercioz *et al*., (2017) in which they recorded an increased in the number of RBCs in the groups treated with antioxidants following TT as against control and sham-treated groups. The erythrocyte cytoskeleton consists of several proteins that form a filamentous network under the lipid bilayer. The network is composed of spectrin, ankyrin, actin, and protein 4.1. Cytoskeletal proteins interact with integral proteins and lipids of the bilayer to maintain membrane integrity. The cytoskeleton has an important role in erythrocyte shape, flexibility, and lipid organization. According to literature, L-carnitine stabilizes the membrane of red blood cells via interaction with cytoskeletal membranes proteins (Konov *et al*.; 2022). The stability confer on the cell membrane of the animals treated with L-carnitine for 62 days could be responsible for the increase seen when compared with the sham treated group for the same duration and also with the group that was just treated with L-carnitine for 22 days. This implies that length of time also improved the number of RBCs significantly.

Hemoglobin in the red cell continuously undergoes redox reactions. Redox reactions with oxygen produce superoxide and hydrogen peroxide. Hydrogen peroxide can then react with hemoglobin producing ferryl hemoglobin and eventually degrading the heme (Rifkind *et al.,* 2003). There is a strong significant effect of oxidative stress (reduced glutathione) on glutathione peroxidase, glutathione reductase level, these may reduce haemoglobin concentration. Glutathione reductase plays an important role in protecting haemoglobin, red cell enzymes and biological cell membranes against oxidative damage by increasing the level of reduced glutathione. Reduction or deficiency in the enzyme can lead to haemolysis of the cells and degradation of the haemoglobin (Waggiallah and Alzohairy, 2011). In the present study, there was an increase in the Hb concentration of the IRI+L for 42 days when compared with the sham-

treated group for the same duration. There was also an increase in the Hb concentration of the IRI+L for 42 days when compared with IRI+L for 22 days.

The red cell is of subject of interest in any research that involves oxidative stress due to ischaemia. It undergoes a high endogenous rate of production of H_2O_2 from haemoglobin autoxidation which can markedly increase in cells with unstable haemoglobin (Waggiallah and Alzohairy, 2011). RBC indices such as MCV, MCHC, and red cell distribution width are laboratory parameters that are frequently overlooked in clinical practice but they can assist in establishing a diagnosis in anaemic patients (Cavaliere, 2004). MCV, MCH and MCHC are the three leading RBCs that help measure RBCs' average size and haemoglobin composition (Moshtagh *et al.*, 2023). MCH denotes the haemoglobin average mass per RBC in the blood sample, MCHC denotes the haemoglobin level in packed red blood cell volume (Adeyemo-Salami and Ewuola, 2015) while MCV denotes the average volume of RBC and gives an idea of the size of each RBC in an animal (Somade *et al*.; 2022). Our study revealed significant increase in the MCV, MCH and MCHC in all the groups with IRI+L for 42 when compared with the groups that received treatments for just 22 days. The increased in Hb content in the red cells as seen in the significant increase in MCHC and MCH values may be as a result of the compensatory mechanism to improve the oxygen-carrying capacity of the blood already compromised by haemolysis of the RBCs occasioned TT. MCH and MCHC define the concentration of Hb and suggest the restoration of oxygen-carrying capacity of the blood (Afolabi *et al*.; 2019).

4.0 Conclusion

Treatment of Wistar rats that were experimentally induced with TT with 500mg/kg of L-Carnitine resulted in improvement in the values of RBC count and its indices. The results obtained could play a very vital role in using inexpensive test like the evaluation of haematological parameters to improve differential diagnosis of acute scrotum cases and eventually anticipate the fate of the testis in TT.

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7.0 References

Adeyemo-Salami, O. & Ewuola, E. O. (2015). Haematological effects of graded doses of the methanol extract of Paullinia pinnate (Linn) leaves in Wistar rats. *Pharmacognosy Research,* doi:10.4103/ -0974-8490-8490.150522

Afolabi, O. K., Oyewo, E. B., Adeleke, G. E., Badmusi, J. A. & Wusu, A. D. (2019). Mitigation of aluminium phosphide-induced haematotoxicity and ovarian oxidative damage in Wistar Rats. *American Journal of Biochemistry*, 9, 1, pp. 7-16

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

Akbas, H., Ozden, M., Kanko, M., Marat, H., Bulbul, S. & Yavuz, S. (2005). Protective antioxidant effects of carvediol in a rat model of Ischaemic-reperfusion injury. *Journal of international medical research,* 33, pp. 528-538.

Balushi, H. A.; Hannemann, A., Rees, D., Brewin, J. & Gibson, J. S. (2019). Effects of antioxidants on the properties of Red blood cells from patients with sickle cell anemia. *Frontiers in Physiology*, 10:976, doi: 10.3389/fphys.2019.00976

Cavaliere, T. A. (2004). Red blood cell indices: Implications for Practice. *Newborn and Infant Nursing Reviews*, 4, 4, pp. 231-239

Davenport, M. (1996). ABC of generalsurgery in children. Acute problems of the srotum. *British Medical Journal, 312, pp.* 435-437.

Diederich, L., Suvorava, T., Sansone, R., Keller, T. C. S., Barbarino, F., Sutton, R. T., Kramer, C. M., Luckstadt, W., Isakson, B. E., Gohlke, H., Feelisch, M., Kelm, M. & Cortese-Krott, M. M. (2018). On the effects of reactive oxygen species and nitric oxide on Red blood cell deformability. *Frontiers in Physiology*, 11, pp. 9:332, doi:10.3389 /fphys.2018-.00332

Dokmeci, D., Inan, M., Basaran, U. N., Yalcin, O., Aydogdu, N., Turan, F. N. & Uz, Y. H. (2007). Protective effect of L-carnitine on testicular Ischaemia-reperfusion injury in rats. *Cell Biochemistry Function*, 2, pp. :611-618.

Etim, N. (2014). Haematological Parameters and factors affecting their Values. *Agricultural Science*, 2, 1, pp. 37-47

Ganjiani, V., Ahmadi, N., Divar, M. R., Sharifiyazdi, H. & Meimandi-Parizi, A. (2021). Protective effects of crocin testicular torsion/detorsion in rats. Theriogenology, 173, pp. 241-248.

Gulcin I. (2006). Antioxidant and antiradical activities of L-Carnitine. *Life science78*, pp. 803- 811.doi: 10.:1016/J.IFS.2005. 05.103.

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.

Kraemer, W. J., Volek, J. S., & Dunn-Lewis, C. (2008). L-carnitine supplementation: Influence upon physiological function. *Current Sports Medicine Reports*, *7*(4), 218-223.

Moradi-Ozarlou, M., Javanmardi, S. & Tayefi-Nasrabadi, H. (2020). Antioxidant Property of *Plantago major* leaf extracts reduces testicular torsion/detorsion-induced ischaemia/reperfusion injury in rats. *Veterinary Research Forum*, 11, 1, pp. 27-33

Moshtagh, M., Moodi, M., Moezi, S. A., Sharifi, F. & Khazdair, M. R. (2023). Inflammatory and oxidative stress Biomarkers in the elderly, the Birjand Longitudinal Aging. *Biochemistry and Biophysics Reports*, https://doi.org/10. -1155/2023/4683542

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

Pirestani, A., Rkh, S., Tabatabaei, S. N., Ghalamkari, G, R. & Alibabaei, Z. (2009). Effect of L-carnitine supplement in diet transitional cows on reproduction indices and mil parameter. *Veterinary Journal of Islamic Azad University, Tabriz Branch*, 3, pp. 205-208.

Ramachandra, P., Palazzi, K. L., Holmes, N. M. & Marietti, S. (2015). Factors influencing rate of testicular salvage in acute testicular torsion at a tertiary pediatric center. *Western Journal of Emergency Medicine* 16(, 1, pp. 190-194.

Rebouche C. J. (2004). Kinetics, pharmacokinetics and regulation of L-Carnitine and acetyl-L-Carnitine metabolism. *Annals of New York Academic of Science,*1033, pp. 30-41. doi 10.1196/annals. 1320.003.

Rifkind, J. M., Nagababu, E., Ramasamy, S. & Ravi, L. B. (2003). Haemoglobin redox reactions and oxidative stress. *Redox Report*, 8, 5, pp. 234-237

Somade, O. T., Oyinloye, B. E., Ajiboye, A. O., Osukoya, O. A. & Adeyi, O. E. (2022). Effects of syringic acid on steroid and gonadotrophic hormones, haematological indices, sperm characteristics and morphologies and markers of tissue damage in methyle cellosolve-administered rats. *Biochemistry and Biophysics,* 32, https://doi.org/10.1016/j.bbrep.2022.101360

Tanriverdi, H. L., Senel, U., Gevrek, F. & Akbas, A. (2021). Protective effect of famotidine on Ischaemia-reperfusion injury following testicular torsion in rats. *Journal of Paediatric Urology*, Pages 167.e1-167.e7, https://doi.org/10.1016/j.jpurol.2020.09.019

Waggiallah, H. & Alzohairy, M. (2011). The effect of oxidative stress on human cells glutathione peroxidase, glutathione reductase levels and prevalence of anaemia among diabetics. *North American Journal of Medical Science*, 3(, 7, pp. 344-347

Compliance with Ethical Standards
Declarations

The authors declare that they have no conflict of interest

Data Availability

All data used for this study will be readily available to the public

Consent for Publication

Not Applicable

Availability of Data and Materials

The Publisher has the right to make the Data Public

Competing interests

The authors declare no conflict of interests

Authors' Contribution

R. J. Bebekah designed and carried out the experiment while M. I. A. Saleh, A. Mohammed and Y. Tanko supervised.