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Toxopathological and biochemical changes in internal organs of albino mice infected with Listeria monocytogenes protective by L *carnitine*

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Abstract— *Listeria Monocytogenes* (LM) can defined as pathogenic bacterium that caused listeriosis, a disease affected both humans and animals, this organism characterized by its ability to live inside host cell and adapt to a wide range environmental condition and lead to sever health problems. Aim of this study to evaluate the biochemical and histopathological impacts of L-carnitine as a protective agent against *Listeria monocytogenes* (LM). A total of Sixty (60) white albino female mice aged 7- 9 weeks and average weight 25-30grms, were randomly divided into three groups (n=20 each), Group1(G1) considered as Negative control, Group2 (G2) was oral administrated with L-carnitine for 21 days followed by intraperitoneal (IP) injection of LM (0.1 ml of 1.5×108 CFU/ml); and Group3 (G3) was injected with LM alone. Two weeks' post-infection, when clinical signs of the listeriosis appeared, all animals were anesthetized for blood collection to assess oxidative stress biomarkers: Malondialdehyde (MDA) and Glutathione (GSH). Subsequently, animals were Euthanized, and internal organs (liver and brain) were harvested and preserved in 10% formalin for histopathological examination using hematoxylin and eosin staining. Results of Biochemical analysis revealed a significant different ($P<0.05$) and elevation in MDA levels in G3 (554.00 ± 13.43) compared to G2 (247.33 ± 8.67) and G1 (229.96 ± 4.89) Conversely, GSH levels were significantly different ($P<0.05$) that revealed higher in G1 (33.37 ± 2.84), and markedly reduced in G2 (11.81 ± 0.6) and G3 (25.66 ± 1.56) 8). Histopathological findings in G2 showed Milder changes, indicating partial protection by L-carnitine while in G3 revealed severe liver damage, including granulomatous lesions, vacuolar degeneration, inflammatory infiltration, and vascular congestion. Similarly, in brain tissue of G2 showed reduced neuronal injury, minimal inflammation, and better tissue preservation, demonstrating the neuroprotective role of L-carnitine while in G3 exhibited perivascular cuffing, neuronal degeneration, microglial activation, and mild meningitis. In conclusion, the results showed that L carnitine act as anti-oxidant by reduce oxidative damage by (LM) infection, this results appeared by measure the levels of MDA and GSH in the serum of female albino mice and result of histological sections.

Keywords — *Listeria monocytogenes*, Listeriosis, L-carnitine, Histopathology, Oxidative stress.

INTRODUCTION

Listeria monocytogenes (LM) is a Gram-positive, rod-shaped bacterium that causes a disease known as listeriosis. It is commonly transmitted through the consumption of contaminated foods such as undercooked meats or unpasteurized dairy products and caused fatal infectious disease in animals especially in cattle, birds, fish and crustaceans (1) This pathogen is capable of surviving in a wide range of environmental conditions, including refrigeration temperatures and high salt concentrations, making it a significant concern in food safety (2). Listeriosis can lead to septicemia, meningitis fetal loss in pregnant women and immunocompromised individuals, and is the third leading cause of foodborne mortality (3). The disease caused by listeria is defined by a variety of disorders classified into two types: severe invasive listeriosis and non-invasive febrile gastroenteritis (4). Due to its ability to cross the intestinal, blood-brain, and placental barriers, *Listeria monocytogenes* is considered a major public health threat worldwide (5,6). L-carnitine defined as important amino acid that the body produced from lysine and methionine, by specific biosynthetic pathway (7). L-carnitine synthesized from the liver and had the ability transport through blood stream that reach to skeletal and cardiac muscle (8). L-carnitine plays a crucial role in transporting long-chain fatty acids into mitochondria for β-oxidation, supporting ATP production and thereby contributing to heart, muscle, and neural health, it's widely recognized as a potent antioxidant that plays a critical role in protecting cells against oxidative damage induced by various pathogenic factors. According to (9), supplementation with L-carnitine significantly reduces oxidative stress markers such as malondialdehyde (MDA) and increases the activity of antioxidant enzymes including superoxide dismutase (SOD). These findings indicate that L-carnitine enhances the cellular antioxidant defense system, thereby limiting lipid peroxidation

and membrane damage caused by reactive oxygen species (ROS). Furthermore, (10) reported that increased serum levels of L-carnitine are positively correlated with reduced cellular alterations under oxidative stress conditions, such as hypoxia or infection. This suggests that L-carnitine not only scavenges free radicals but also stabilizes mitochondrial function, which is often impaired during infection or inflammation. And this substance proved in some studies that its used to treat some cases as Alzheimer's and hepatic encephalitis (11). The aim of this study was to evaluate the effect of infection with *Listeria monocytogenes* on oxidative stress markers in animal tissues by measuring malondialdehyde (MDA) and glutathione (GSH) levels, as well as to investigate the protective effect of L-carnitine in reducing oxidative damage and improving antioxidant status compared with the Control Group.

Experimental design

In this study, sixty (60) white female mice are used; aged 7-9 weeks and average weight 25-30gms, housed and maintained in the animal house of Veterinary Medicine college with optimal conditions and feed on special formula (food pellets) with clean water, the all experimental animals were housed in a clean plastic cages, which were contain sawdust as bedding changed twice a week to ensure a clean environment.Sixty (60) albino female mice were divided randomly into four groups as follows: G1: (n=20) Negative control group fed on special formula (food pellets) with clean water. G2:(n=20) firstly oral administration of L carnitine daily for 21 days then infected with *Listeria monocytogenes* 0.1ml ($1.5X10^8$ CFU/ml) intraperitoneal on day 22 and sacrificed after 2 weeks (35days). G3: (n=20) infected with *Listeria monocytogenes* ($1.5X10^8$ CFU/ml) intraperitoneal on day 22 and sacrificed after 2 weeks (35days). Evaluate levels of Biochemical test by measure MDA and GSH and study the histopathological findings of liver and brain by light microscope for all groups.

Determination of MDA with GSH by ELISA technique:

ELISA Kit obtained from Elabscience U.S.A Used for detected concentration of Malondialdehyde and glutathione from serum of female mice as (nmol/ml) and this test performed according to the protocol of the company.

MATERIALS AND MTHODS

Preparation of culture media

All media in this study were preparation according to instructions of manufacturing companies keep in refrigerator at 4°C, finally it used for growth of *Listeria monocytogenes* (12).

Histopathological examination

Ten percent formalin was prepared by taking 10 standard solution concentration 37-40% formalin and completed with 90 ml of tap water according to (13).

Preparation of *Listeria monocytogenes*

According to McFarland tube number 0.5 LM prepared for injection of female mice (14).

Preparation of L carnitine.

The calculated dose was 0.1 mL per 10 g of mouse body weight, which corresponds to a dose of 0.5 mg/kg of L carnitine according to (15).

Statical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant. (16).

RESULT AND DISCUSSION

Results of biochemical assay;

Malondialdehyde

The results in Table 1 reveal significant differences in Malondialdehyde (MDA) levels among the groups ($P<0.05$). Group G3 (mice injected with *Listeria monocytogenes* only) exhibited the highest MDA concentration (554.00 ± 13.43), indicating a substantial increase in lipid peroxidation. In contrast, groups G2 and G1 showed markedly lower levels, with G2 at (247.33 ± 8.67) and G1 at (229.96 ± 4.89). These findings suggest that Listeria infection alone (G3) significantly elevated oxidative stress, while treatments or interventions in G1 and G2 were associated with reduced MDA levels.

Table 1. refers to statical value of MDA level in albino female mice

Groups	MDA nmol/ml
G1	229.96±4.89bc
G2	247.33±8.67b
G3	554.00±13.43a
LSD = 30.52 Means with different small letters in the same rows are significantly different. * (P<0.05)	

Glutathione

The results presented in Table 2 indicate significant differences in Glutathione (GSH) levels among the groups ($P<0.05$). Group G1 showed the highest GSH concentration (33.37 ± 2.84a), whereas a marked decrease was observed in group G2 (11.81 ± 0.68c). Group G3, on the other hand, displayed values similar to those of G1 (33.37 ± 2.84a), suggesting that the change in GSH levels was mainly associated with the treatment effect in G2, while no substantial differences were observed between G1 and G3.

Table 2: refers to statical value of GSH level in albino female mice

Groups	GSH nmol/ml
G1	33.37±2.84a
G2	25.66±1.56b
G3	11.81±0.68c
LSD = 5.47 Means with different small letters in the same rows are significantly different. * (P<0.05)	

Histopathological examination

The results of histopathological study in Control negative showed no clear pathological changes in liver and brain while in G3 in liver section shows multiple granulomatous lesion in liver parenchyma Figure (1) addition to vacuolar degeneration

and congested blood vessels Figure(2) and the histopathological section of the brain show sever edema Figure (3) with congested blood vessel, edema and vacuolar degeneration Figure(4) and in results of G2 the liver section show infiltrations of inflammatory cell, congested of blood vessels and vacuolar degeneration of hepatocyte Figure (5) in other sections appeared moderate inflammatory cell aggregation and edema Figure(6). While brain tissue shows clear edema Figure (7,8).

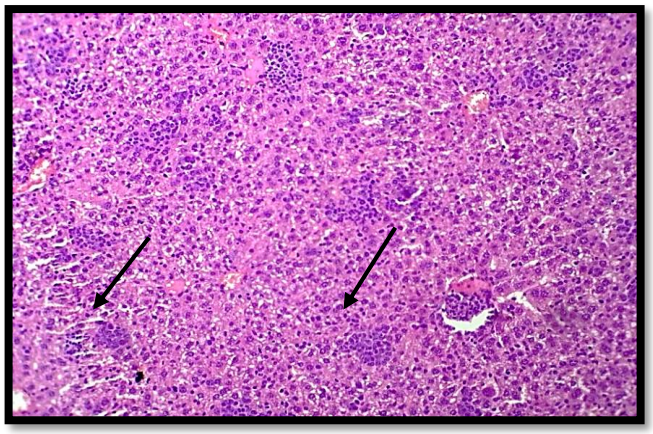

Figure 1. section in liver of female mice at two weeks' post-infection with *Listeria monocytogenes* shows multiple granulomatous lesions in the liver parenchyma (black arrows) (H& E stain 10X).

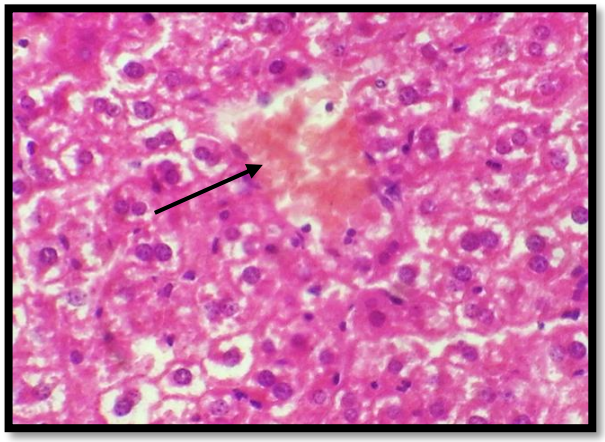

Figure 2. section in liver of female mice at two weeks' post-infection with Listeria monocytogenes shows congestion of central vein (black arrow) and vacuolar degeneration of hepatocyte (green arrows) (H& E stain 40X).

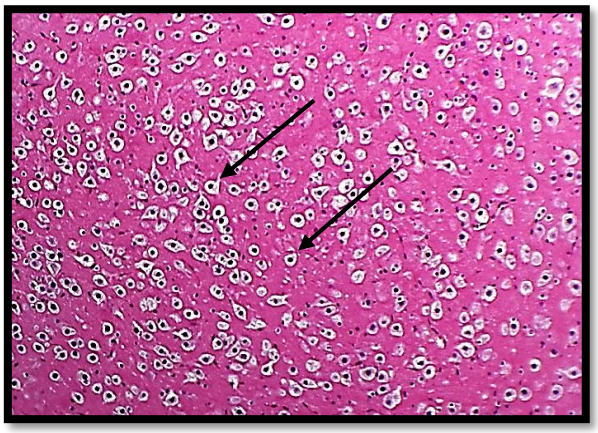

Figure 3.section in brain of female mice at two weeks' post-infection with Listeria monocytogenes shows sever edema (black arrows) (H& E stain 40X).

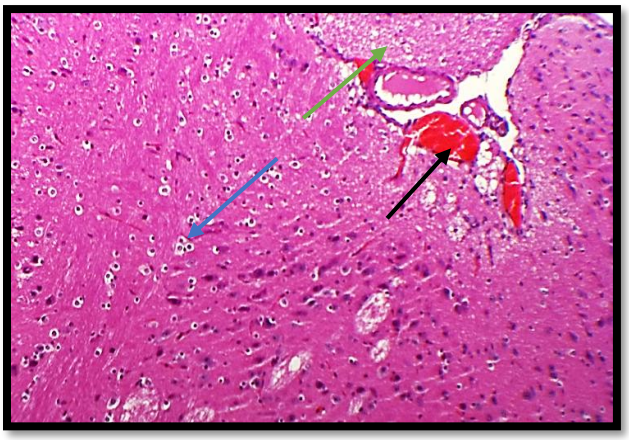

Figure 4. section in brain of female mice at two weeks' post-infection with *Listeria monocytogenes* shows congested blood vessels (black arrows), vacuolar degeneration (blue arrow) and edema (green arrow) (H& E stain 10X).

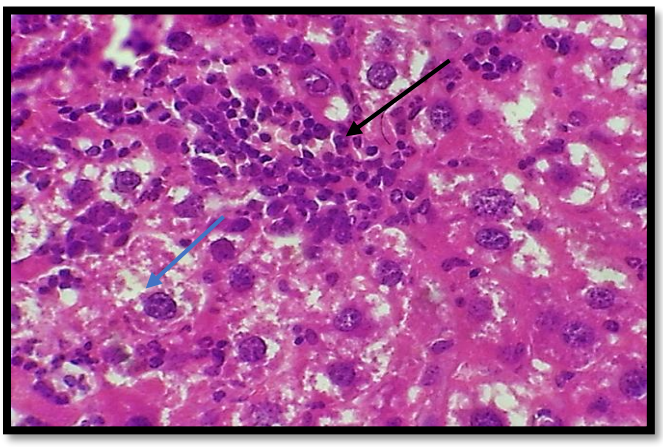

Figure 5. Histopathological section in the liver of female mice at 21 days administrated with L carnitine then infected with *Listeria monocytogenes*, shows inflammatory cells aggregation in the liver parenchyma (black arrow) with vacuolar degeneration of hepatocyte (blue arrow) (H& E stain 40 X).

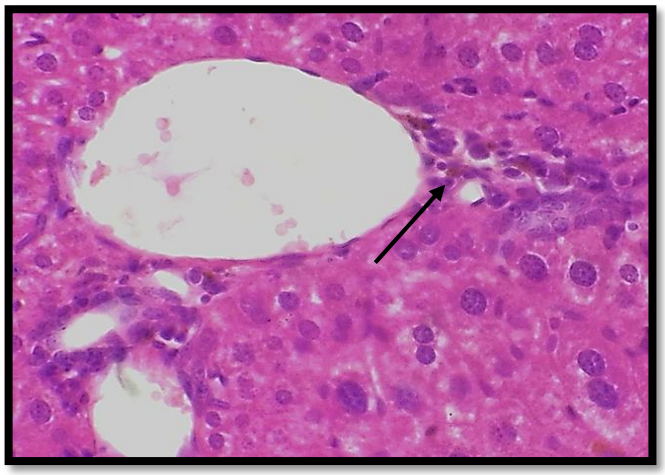

Figure 6. Histopathological section in the liver of female mice at 21 days administrated with L carnitine then infected with *Listeria monocytogenes*, shows inflammatory cells infiltration in the portal area (black arrow) (H& E stain 40X).

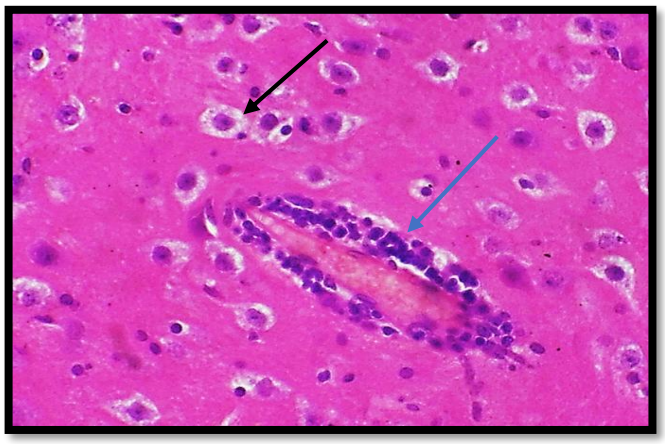

Figure 7. Histopathological section in the brain of female mice at 21 days administrated with L carnitine then infected with *Listeria monocytogenes*, shows edema (black arrow) with moderate inflammatory cells aggregation around congested blood vessels (blue arrow) (H& E stain 40X).

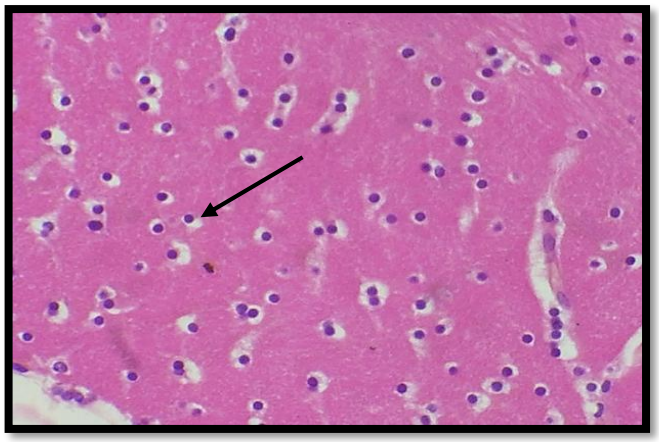

Figure 8. Histopathological section in the brain of female mice at 21 days administrated with L carnitine then infected with *Listeria monocytogenes*, shows moderate edema (black arrow) (H& E stain 40X).

The result of this study revealed significant chief effect in(table 1), this table showed significant increase in levels of MDA in G3 compared with G1 and G2, this increased attributed to oxidative stress induced by listeriosis, elevation of MDA used as indicator of damaged tissue by listeriosis, this result agreed with (17), as stated in their study that bacterial infection by *listeria monocytogens* can trigger an acute inflammatory response leading to excessive production of reactive oxygen species (ROS) and reactive nitrogen species. While in G2 the levels of MDA showed significant decrease when compared with result in G4 and marked elevated when compared with G1, indicating the antioxidant and cytoprotective effect of l-carnitine does not caused obvious tissue damage and the increase in levels occurred due to l-carnitine enhance the cellular metabolic activity and mitochondrial activity therefore MDA may remain slightly higher than levels in G1 but still within normal or non-pathological limits. These results agreed with (18) reported that L- carnitine used as antioxidant that reduce the oxidative stress and reactive oxygen species that induced due to bacterial infection. The result that showed in table 2 reveals significant increase due to GSH that serves as a major intracellular antioxidant that protecting cells against oxidative stress that induced by *listeria monocytogens* infection. This increased in G2 due to l-carnitine serve as a major antioxidant that reduce the production of ROS and improvement the mitochondrial activity with reduce production of ROS all that caused increased in GSH levels while in G3 the levels of GSH decreased due to L.M infection caused production ROS inside the cells and tissue that caused rapid conception to GSH because its acts as an antioxidant addition to it used in utilizing ROS these results agreed with (19).

The histopathological findings in group G3 (group of animal that infected with *Listeria monocytogenes* only), showed severe liver and brain lesions including granulomatous lesions, central vein congestion, vacuolar degeneration. these result agreed with (20) who reported that presence of multiple granulomatous lesions appear in the liver after infection with *Listeria monocytogenes*, indicating an active immune response to contain the bacteria. The histopathological lesion of this study shows also vacuolar degeneration and cytoplasmic swelling these result agreed with (21) who reported that these lesions caused cellular injury resulting from oxidative stress and acute inflammation. The result of Histopathological examination of liver sections of group G2 (group of animals that administrated l carnitine then infected with *Listeria monocytogenes*) agreed with (22) who reported that protective effect of L-carnitine against Listeria-induced hepatic injury, as vacuolar changes and immune cell infiltration indicate cellular stress and immune activation. L-carnitine is known to exhibit antioxidant and anti-inflammatory properties, which may have mitigated the severity of hepatic damage by reducing oxidative stress and modulating immune responses. L-Carnitine in this study used to decreased the harmful effects of *listeria monocytogenes*.

In contrast, the liver tissues of group G2, which received an L-carnitine supplement prior to infection, exhibited less severe histological alterations. Mild vacuolar degeneration, vascular congestion, and a limited number of immune cells

were observed. These findings suggest that L-carnitine exerted a protective effect, due to its antioxidant properties and its role in maintaining cellular homeostasis, thereby minimizing the extent of cellular injury. Overall, the histological findings demonstrate that *Listeria monocytogenes* causes notable and potentially harmful effects on liver tissue.

Similarly, the histological examination of the brain tissues from each experimental group demonstrated clear pathological changes induced by *Listeria monocytogenes*, with the severity of these alterations varying depending on the administration of L-carnitine. In group G3, which was infected with Listeria only, the brain sections showed marked inflammatory infiltration, particularly around the meninges and ventricles, accompanied by neuronal degeneration and occasional perivascular edema. This result is consistent with the observations of **(**23**)**, who reported that neuropathological changes in brain tissues exhibited significant meningeal inflammation, perivascular cuffing, and neuronal damage due to the activation of microglial cells and infiltration of peripheral immune cells. While in group G2, which received L-carnitine prior to Listeria infection, exhibited less pronounced histopathological alterations. Mild inflammatory infiltration was observed and reduced neuronal degeneration. The maintenance of more intact brain architecture suggests that L-carnitine exerted a neuroprotective effect through its antioxidant activity and its ability to support mitochondrial function, thereby mitigating oxidative stress and limiting the extent of neuronal injury.

Conclusions

This study results showed that infection with (LM) lead to a marked increase in oxidative stress, as indicated by elevated MDA levels and a significant decrease in GSH levels, reflecting tissue damage. In contrast, in group of animals that administrated L-carnitine reduced MDA levels and improved or increased GSH levels, demonstrating its antioxidant effect and protective role against oxidative damage by (LM).

Acknowledgements
N/A
Conflict of Interest
The authors declare no conflict of interest

REFERENCES

1) FDA. (2024). Listeria (Listeriosis). U.S. Food and DrugAdministration.https://www.fda.gov/food/foodborne-pathogens/listeria-listeriosis

2) Centers for Disease Control and Prevention. (2023). Listeria: Clinical overview for healthcare providers. U.S. Department of Health & Human Services. https://www.cdc.gov/listeria/hcp/clinical-overview/index.html

3) Abdallah, M. S., Al-Khatib, G. M., & Alwan, M. J. (2004). Effect isolation and identification of *Listeria monocytogenes* from aborted women with studying of its pathogenic. The Iraqi Journal of Veterinary Medicine,28(1),95–108. https://doi.org/10.30539/ijvm.v28i1.1070

4) Centers for Disease Control and Prevention. (2025). Clinica loverview of listeriosis. https://www.cdc.gov/listeria/hcp/clinical-overview/index.html

5) Huda Abdal-Hadei Ali Al-Nasrawi. (2018). Study of the inhibitory effects of some medicinal plants extracts on growth of *Listeria monocytogenes* isolated from human, cow and sheep in Al-Qadisiyah governorate. (2018). The Iraqi Journal of Veterinary Medicine, 41(2), 38-47. https://doi.org/10.30539/iraqijvm.v41i2.46

6) Vázquez-Boland, J. A., Krypotou, E., Scortti, M., & Pucciarelli, M. G. (2024). *Listeria monocytogenes*: A multifaceted food-borne pathogen. Microbiology Spectrum, 12(1), e03252-23https://doi.org/10.1128/spectrum.03252-23

7) Rebouche, C. J. (2014). Carnitine. In A. C. Ross, B. Caballero, R. J. Cousins, K. L. Tucker, & T. R. Ziegler (Eds.), Modern nutrition in health and disease (11th ed., pp. 252–253). Lippincott Williams & Wilkins. https://doi.org/10.1146/annurev.nutr.18.1.39

8) Shiblawi, W. M., & Ismael, S. H. (2017). The effect of L-carnitine as an adjuvant supplement on lipid profile in Iraqi diabetic patients. Iraqi Journal of Pharmaceutical Sciences, 24(2), 48-52. https://doi.org/10.31351/vol24iss2pp48-52

9) Fathizadeh, H., Milajerdi, A., Reiner, Ž., Amirani, E., Asemi, Z., Mansournia, M. A., & Hallajzadeh, J. (2020). The effects of L-carnitine supplementation on indicators of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials. Journal of Diabetes & Metabolic Disorders, 19(2), 1879–1894. https://doi.org/10.1007/s40200-020-00627-9

10) Fathizadeh, H., Milajerdi, A., Reiner, Ž., Amirani, E., Asemi, Z., Mansournia, M. A., & Hallajzadeh, J. (2020). The effects of L-carnitine supplementation on indicators of inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials. Journal of Diabetes & Metabolic Disorders, 19(2), 1879–1894. https://doi.org/10.1007/s40200-020-00627-9

11) Aram, J., Al-Shamma, K. J., & Al-Hassani, A. N. (2017). Effect of L-carnitine, multivitamins and their combination in the treatment of idiopathic male infertility. Iraqi Journal of Pharmaceutical Sciences, 21(1), 14–20. https://bijps.uobaghdad.edu.iq/index.php/bijps/article/view/435

12) Atlas, R. M. (2010). Handbook of microbiological media (4th ed.). CRC Press.

13) Wehrle, E., Günther, D., Mathavan, N., Singh, A., & Müller, R. (2024). Protocol for preparing formalin-fixed paraffin-embedded musculoskeletal tissue samples from mice for spatial transcriptomics. STAR Protocols, 5(2), 102987. https://doi.org/10.1016/j.xpro.2024.102987

14) Baron, E.J., Peterson, L.R., and Finegold, S.M. (1994). Bailey and Scott's Diagnostic Microbiology 9th ed the C.V Mosby company, pp. 333-351.

15) Fakhr al-Din, M. B. M. R., & Fulayyih, N. K. (2012). Effect of L-carnitine administration to pregnant mice on some reproductive hormones and organs. The Iraqi Journal of Veterinary Medicine, 36(1), 68–74. https://doi.org/10.30539/iraqijvm.v36i1.550

16) SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

17) Virmani, M. A., & 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. https://doi.org/10.3390/ijms23052717

18) Ribas, G. S., Vargas, C. R., & Wajner, M. (2014). L-carnitine supplementation as a potential antioxidant therapy for inherited neurometabolic disorders. Gene, 533(2), 469–476. https://doi.org/10.1016/j.gene.2013.10.017

19) Chatterjee, S. S., Portman, T. A., & colleagues. (2021). Detoxification of methylglyoxal by the glyoxalase system is required for glutathione availability and virulence activation in *Listeria monocytogenes*. PLoS Pathogens, 17(8), e1009819. https://doi.org/10.1371/journal.ppat.1009819

20) Hu, Xiaowei; Chen, Min; Li, Li; Wang, Yan; Zhang, Jing. (2021). Persistent *Listeria monocytogenes* infection induces a unique immune transcriptional signature in hepatocytes. Frontiers in Cellular and Infection Microbiology, 11, Article 761945. https://doi.org/10.3389/fcimb.2021.761945

21) Haggag, N. A., Aziz, M. A., Zahra, A. E. A., Belih, S. S., & Khalifa, H. O. (2022). Ameliorative effects of L-carnitine on florfenicol-induced hepatotoxicity in broilers. Journal of the Hellenic Veterinary Medical Society. https://doi.org/10.12681/jhvms.24660

22) Alhusaini, A. M., Alsoghayer, R., Alhushan, L., Alanazi, A. M., & Hasan, I. H. (2023). Acetyl-L-carnitine and liposomal co-enzyme Q10 attenuate hepatic inflammation, apoptosis, and fibrosis induced by propionic acid. International Journal of Molecular Sciences, 24(14), 11519. https://doi.org/10.3390/ijms241411519

23) Koopmans, M. M., Engelen-Lee, J. Y., Brouwer, M. C., Hack, C. E., van de Beek, D., & van der Ende, A. (2018). Characterization of a *Listeria monocytogenes* meningitis mouse model. Journal of Neuroinflammation, 15(1), 257. https://doi.org/10.1186/s12974-018-1293-3

24) Al-Khatib, G. M., Al-Haddad, Z. A. A., Al-Hissen, S. F., & Al-Kutbi, S. H. (2004). Preparation of Listerine antigen as a tool for diagnosis of listeriosis. The Iraqi Journal of Veterinary Medicine, 28(1), 43–54. https://doi.org/10.30539/ijvm.v28i1.1065

25) Isolation and identification of Staphylococcus aureus, *Listeria monocytogenes*, E. coli O157:H7 and Salmonella specious from raw beef and lamb meat in Baghdad by PCR. (2022). Iraqi Journal of Science, 57(3B). https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/6876