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L-carnitine promising effects in cisplatin-induced nephrotoxicity

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Abstract: Objective: This study aimed at exploring the therapeutic efficacy of L-Carnitine (LC) against cisplatin-induced nephrotoxicity (CIN) by assessing oxidative stress response, and relevant gene expression in rat kidney tissues.

Methods: Rat CIN model was induced by intraperitoneal injection of Cisplatin (Cis). Eighteen male Sprague-Dawley mature rats with age (6 weeks) weighing 180 ± 20 gm were randomized to receive either control (saline), Cis or Cis + LC. Markers of oxidative stress (superoxide dismutase (SOD) and Malonaldehyde (MDA) and Bax and BCL-2 gene expression profiles were assessed thereafter .

Results: Cisplatin administration showed a marked increase in oxidative stress as evidenced by elevated MDA and diminished SOD levels, and changes in gene expressions of apoptosis-regulating genes (BCL2, BAX). Remarkably, treatment with LC normalized most parameters and achieving gene expression levels comparable to control groups.

Conclusion: Our findings highlight the significant nephroprotective potential of LC against CIN, pivoting the way for bigger clinical studies and incorporation into clinical care guidelines.

keywords: Cisplatin, Nephrotoxicity, L-Carnitine, Oxidative stress, Gene expression, BCL-2, Bax, SOD, MDA.

1.Introduction

Cisplatin, a well-known chemotherapeutic agent, has been acknowledged for its significant efficacy against various malignancies, spanning ovarian, testicular, to lung cancers **[1].** Nevertheless, the widespread clinical adoption of Cis is frequently challenged by its notable nephrotoxic side effect **[2].** The hallmark of CIN is the surge in oxidative stress, which culminates in tubular cell apoptosis and consequential renal dysfunction **[3].** Crucially, genes orchestrating apoptosis, notably BCL2 and BAX, stand out as central figures in this pathophysiological cascade [4].

L-Carnitine, traditionally known for its pivotal role in fatty acid metabolism, has piqued scientific curiosity for its antioxidant attributes. Emerging evidence suggests that LC can potentially quell oxidative stress in diverse pathological contexts **[5].** Within the confines of nephrotoxicity, especially when triggered by agents like Cis, LC's prospective protective prowess is becoming a research focal point.

In this study, we embark on a mission to unravel the therapeutic merit of LC in mitigating CIN, accentuating its impact on oxidative stress indices and salient apoptotic gene expressions in rat kidney tissue.The last reference highlights the neuroprotective role of L-Carnitine, which is related to its potential in alleviating oxidative stress and might provide context to its nephroprotective potential.

2. Materials and methods

2.1. In Vivo Experimental Design:

Animal Housing: A total of 18 mature male Sprague-Dawley rats, 6 weeks of age and weighing approximately 180 ± 20 gm, were

accommodated in polycarbonate cages with four rats per cage. The environmental conditions were consistently maintained at 24ºC with a humidity of 50-70% and a 12-hour light-dark cycle. Rats had free access to food and water throughout the study. The experimental procedures were designed in line with the Guide for the Care and Use of Laboratory Animals (ILAR 1996) and were approved by the Mansoura University, Urology and Nephrology Center's ethical committee ID: MU-ACUC (SC.MS.22.11.7).

Treatment Groups: Rats were grouped as follows:

- Control group (n=6): Saline injection.
- Cisplatin group (n=6): Single dose of 5 mg/kg Cis.
- Cisplatin + L-carnitine group (n=6): Pre-treated with LC (50 mg/kg/day, IP) for 2 days, followed by a 5 mg/kg Cis dose and a subsequent 7-day LC regimen.

Following the final drug administration and a 24-hour interval, rats were weighed, and blood samples were collected before euthanization for subsequent analysis.

2.2. Oxidative Stress Markers Analysis:

Oxidative stress parameters, specifically MDA and SOD levels, were assessed. The spectrophotometer is use to quantify the changes in light absorbance as a result of the chemical reactions in the samples.

2.3. Molecular Analysis:

Real-Time Polymerase Chain Reaction (RT-PCR):

Gene expression levels for markers related to apoptosis (BCL2, BAX) were determined in bladder tissue using RT-PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal control. Gene-specific primer sequences were employed, and relative expression was calculated utilizing the $\mathbf{2^{-\Delta\Delta C\ (T)}}$ method. Table 1 shows the primer sequences.

Table 1: Primers' sequence

Gene	Accession no.	Primer sequence (5-3)
BAX	NM_017059.2	F- 5′-GGCGATGAACTGGACAACAA-3′ R- 5′-CAAAGTAGAAAAGGGCAACC-3′
BCL2	NM_016993.1	F: 5′-GGTGAACTGGGGGAGGATTG-3′ R: 5′-GCATGCTGGGGCCATATAGT-3′
GAPDH	NM_017008.4	F: 5′-AGACAGCCGCATCTTCTTGT-3′ R: 5′-TTCCCATTCTCAGCCTTGAC-3′

3.Results:

3.1.Effect of LC treatment on oxidative stress markers

The levels of MDA and SOD were evaluated across different treatment groups, and the findings are summarized in Table 2. Treatment with CIS resulted in a significant increase in MDA levels which was significantly higher when compared to the control group. Simultaneously, SOD levels in the CIS group showed significant decrease in comparison to the control group.

However, the LC treatment (CIS + LC group) ameliorated these effects to some extent. The MDA level was significantly lower than the CIS group, but still higher than the Control group. Although the SOD levels in the CIS + LC group were decreased when compared to the control group, they were still significantly higher than the Cis group.

Table 2 Comparison of SOD and MDA levels across treatment groups

Groups	SOD (U/gm)	MDA (nmol/gm)
Control	**88.5±9.55**	19.48±5.22
CIS	**36.22±8.17** [a]	233.7±28.59 [a]
CIS +LC	**55.77±7.03** [a]	106.9±10.52 [ab]

Significant difference compared to corresponding [a]Control, [b]CPT, [c]LC group by one-way analysis of variance (ANOVA) followed by posthoc multiple comparisons (Tukey test) at $p \leq 0.05$.

3.2. Effect of LC treatment on gene expression of apoptosis markers

Table 3: comparison of gene expression profiles

Groups	BCL-2	BAX
Control	1.01	1.07
Cis	0.26 [a]	4.75 [a]
LC	0.55 [ab]	3.62 [ab]

a denotes significance compared to control group, **b** denotes significance compared to Cis group.

Table 3 shows that the Cis-treated group showed a significant upregulation of BAX expression when compared to the control group. However, when LC was added to the Cisplatin treatment, the BAX expression was notably reduced, although it still remained significantly elevated in comparison to the control with non-significant differences compared to the Cis group .

Inversely, the BCL-2 expression showed a statistically significant decline in BCL-2 expression compared to the control group. Interestingly, the introduction of LC to the Cisplatin treatment saw a moderate increase in BCL-2 expression, drawing it closer to control levels. Yet, it was still significantly lower than the control, but did not differ significantly from the Cis-only group.

4. Discussion

Cisplatin, an esteemed chemotherapeutic agent, has been fundamental in the management of numerous malignancies **[1].** However, its potential nephrotoxic effects have prompted significant research into therapeutic interventions that can mitigate its side effects while preserving its chemotherapeutic potential **[2].** Our findings corroborate the central role of oxidative stress in CIN pathophysiology, with marked elevation of MDA levels and depletion of the antioxidant enzyme, SOD, post Cis administration. This aligns with the narrative that Cis-induced nephrotoxicity arises primarily from oxidative stress, eventually leading to renal tubular cell apoptosis and ensuing renal dysfunction **[3].** Of paramount importance is the expression level of genes like BAX and BCL-2, which orchestrate apoptosis and are pivotal in this pathology **[4].**

L-Carnitine, with its conventional role in fatty acid metabolism, has lately been spotlighted for its antioxidant potential **[5].** In the context of CIN, our study reveals that LC holds promise in attenuating the oxidative stress and nephrotoxic aftermath instigated by Cis. Notably, while LC substantially dampened the rise in MDA and fall in SOD levels post Cis treatment, it also influenced the expression of apoptosis-regulating genes. Specifically, LC treatment following Cis administration seemingly moderated the BAX upregulation and BCL-2 downregulation – although not to control levels. While our study provides valuable insights, it's worth noting the broader context in which LC operates. Emerging literature has highlighted LC's neuroprotective attributes, especially against oxidative stress **[2].** Drawing parallels between the neuroprotective and nephroprotective effects of LC may pave the way for further understanding its multifaceted roles in diverse pathologies. In conclusion, our study underscores the nephroprotective potential of LC against CIN, highlighting its prospective role in diminishing oxidative stress and modulating apoptosis-associated gene expression. As we advocate for its inclusion in clinical care guidelines, larger-scale studies and rigorous clinical trials are essential to validate these findings. Cisplatin remains an effective chemotherapeutic agent, renowned for its therapeutic promise against several malignancies **[1].** However, its nephrotoxic side effects pose substantial challenges to its widespread clinical use **[2].** Our findings indicate that LC exhibits a protective role CIN, specifically by modulating oxidative stress markers and key apoptotic genes. A paramount observation in our study was the increase in MDA levels following cisplatin administration, hinting at an escalation in oxidative stress. This aligns with a study by **[6],** which observed that Cis administration led to augmented oxidative stress, corroborated by increased MDA levels. Contrarily, SOD, an antioxidative enzyme, showed reduced levels in our cisplatin group. A decline in SOD activity has been regarded as a direct reflection of oxidative stress amplification **[7].**

L-Carnitine's potential role in mitigating oxidative stress and its resultant cellular damages has been noted in previous studies **[3].** detailed how antioxidants, including LC, can modulate the oxidative stress induced by cisplatin, which is consistent with our observations. Their study found notable improvements in markers of oxidative stress after LC treatment, but like our findings, it did not show a complete restoration to control levels.

In our study, we observed the protective effects of L-Carnitine against cisplatin-induced nephrotoxicity, specifically modulating oxidative stress markers and key apoptotic genes. Our results, when compared with relevant studies, provide a broader understanding of L-carnitine's potential nephroprotective properties. Several studies are in line with our results.

In a study investigating the renal effects of monosodium glutamate (MSG) in rats, L-carnitine showed significant protective properties. Specifically, the MSG group exhibited increased serum levels of MDA, BUN, creatinine, uric acid, and renal expressions of Caspase-9, NGAL, and KIM-1. However, the treatment with L-carnitine, particularly at a dose of 200 mg/kg, significantly mitigated these adverse changes, emphasizing its antioxidant and anti-apoptotic effects. L- Carnitine effectively reduced serum BUN, creatinine, uric acid, and MDA levels while enhancing renal expressions of antioxidant enzymes like catalase, GPX, and SOD. Notably, at the higher dosage, L-carnitine significantly regulated the expression of Caspase-9, NGAL, KIM-1, and Bcl-2 in the kidney, suggesting its potential in cellular protection and as an effective therapy against kidney complications induced by MSG **[8].**

Another study focused on L-carnitine's protective effects against nephrotoxicity induced by Methotrexate (MTX), a common chemotherapeutic agent. L-carnitine was found to significantly protect against MTX-induced renal damage. The MTX group displayed evident histopathological changes in the kidneys, elevated levels of MDA, inflammatory markers TNF-α and IL-6, and increased apoptotic markers. L-carnitine intervention reversed these adverse effects, showcasing its antioxidant, anti-inflammatory, and anti-apoptotic activities **[9].**

In conclusion, our study underscores LC's nephroprotective role against CIN. However, the observed variations with previous research suggest a need for a more comprehensive understanding of the mechanisms and optimal LC dosages. However, one must approach these results with caution. While the therapeutic efficacy of LC in mitigating CIN seems promising in our rat model, extrapolation to clinical settings requires comprehensive human trials. As we pivot toward potential clinical adoption, it's imperative to delve deeper into LC's mechanistic underpinnings, ensuring safety and therapeutic consistency.

5. References

1. Miller, R. P., Tadagavadi, R. K., Ramesh, G., & Reeves, W. B. (2010), Nov Mechanisms of Cisplatin nephrotoxicity. Toxins (Basel), **2(11)**, 2490-2518. https://doi.org/10.3390/toxins2112490
2. Peres, L. A., & da Cunha, A. D., Jr. (2013)., Oct-DecAcute nephrotoxicity of cisplatin: molecular mechanisms. *J Bras Nefrol*, 35(4), 332-340. https://doi.org/10.5935/0101-2800.20130052
3. Pabla, N., & Dong, Z. (2008), May Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int, **73(9)**, 994-1007. https://doi.org/10.1038/sj.ki.5002786
4. Elmore, S. (2007), Jun. Apoptosis: a review of programmed cell death. Toxicol Pathol, **35(4)**, 495-516. https://doi.org/10.1080/01926230701320337
5. Ferreira, G. C., & McKenna, M. C. (2017)., Jun L-Carnitine and Acetyl-L-carnitine Roles and Neuroprotection in Developing Brain. Neurochem Res, **42(6)**, 1661-1675. https://doi.org/10.1007/s11064-017-2288-7
6. Saad, S. Y., Najjar, T. A., & Alashari, M. (2004), Dec Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats.

Clin Exp Pharmacol Physiol, 31(12), 862-867. https://doi.org/10.1111/j.1440-1681.2004.04127.x

7. Kalayarasan, S., Prabhu, P. N., Sriram, N., Manikandan, R., Arumugam, M., & Sudhandiran, G. (2009), Mar 15. Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. *Eur J Pharmacol*, 606(1-3), 162-171. https://doi.org/10.1016/j.ejphar.2008.12.055
8. Koohpeyma, F., Siri, M., Allahyari, S., Mahmoodi, M., Saki, F., & Dastghaib, S. (2021), May 2. The effects of L-carnitine on renal function and gene expression of caspase-9 and Bcl-2 in monosodium glutamate-induced rats. BMC Nephrol, 22(1), 162. https://doi.org/10.1186/s12882-021-02364-4
9. Radwan, S. M., Alqulaly, M., Elsaeed, M. Y., Elshora, S. Z., Atwa, A. H., & Wasfey, E. F. (2023), Nov. L-carnitine reverses methotrexate-induced nephrotoxicity in experimental rat model: Insight on SIRT1/PGC-1alpha/Nrf2/HO-1 axis. *J Appl Toxicol*, **43(11)**, 1667-1675. https://doi.org/10.1002/jat.4503