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RESEARCH ARTICLE

Potential Beneficial Effects of L-Carnitine and Allicin on Doxorubicin Induced Nephrotoxicity in Rats

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A B S T R A C T

The current study was carried out to find out the potential beneficial effects of L-carnitine (LC) and allicin (ALC) to ameliorate the potential toxic effects of doxorubicin on kidneys. For this purpose, a total of 49 rats were divided in 7 groups (7/each) including a control group, a group treated with ALC (20mg/kg, orally), a group treated with DOX (20mg/kg. intraperitoneal injection), a group receiving ALC and DOX combination, a group receiving LC (100mg/kg/day), a group receiving ALC and DOX combination, and a group receiving ALC, DOX and LC in combination. The DOX induced an increase in creatinine and urea, however a significant decrease in total protein and albumin level was observed along with notable renal tissue damage. In terms of stress and oxidative parameters, DOX led to elevated TNF-α expression, increase in MDA (malondialdehyde), decrease in reduced GSH (glutathione), SOD (superoxide dismutase) and CAT (catalase) levels. However, both ALC and LC, separately or in combination, effectively reduced kidney damage and apoptosis induced by DOX. Hence it is concluded that ALC and/or LC may be beneficial supplements for cancer patients undergoing DOX treatment.

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INTRODUCTION

Chemotherapy remains the primary pharmaceutical strategy for treating various cancer types (Yesilkent and Ceylan, 2022). Unfortunately, the medications employed in chemotherapy can result in adverse effects, including cardiac irregularities, cognitive impairments, and dysfunction of the liver and kidneys, leading to death (Demir *et al.*, 2020; Chavalle *et al.*, 2022). Doxorubicin (DOX) belongs to the anthracycline group of compounds, is commonly used to treat the malignant tumors also known as Adriamycin, but it poses serious risks and side effects to multiple organs (Shao *et al.*, 2019; Walters *et al.*, 2022). One of the major concerns is the renal toxicity (Ilkewchi *et al.*, 2021). The DOX induced nephrotoxicity is mainly due to oxidative damage caused by reactive oxygen species (ROS), and inflammatory responses induced by these ROS species and apoptosis that is induced due to these mechanisms (Soliman *et al*., 2024a). ROS stress targets different organs including liver, kidneys and heart and alters their physiological activities due to destructive changes in their histological structures (Afsar *et al.*, 2020). As the kidneys are also responsible for the filtration of excessive materials in body, so DOX accumulates in the renal tubules and induces degenerative changes (Yalcin *et al.*, 2024).

As many of the drugs like doxorubicin (DOX) are the only option available to treat the malignant tumors sometime, so to explore the potential compounds is deemed necessary those can ameliorate or prevent the potential

renal tubular destruction by interfering the ROS production mechanisms and anti-inflammatory activities (Erdogan *et al.,* 2020; Walters *et al.,* 2022). Such auxiliary drugs/agents and their combinations proves to be a practical approach to reduce the tissue destruction associated with the medication. On the other hand, to enhance the treatment efficacy, it is recommended to administer such drugs alongwith the treatment substances to diminish the harmful effects of drug to non-target tissue and body areas. Recently it has been documented that the natural antioxidants can be best option due to their organic origins, decreased side effects and cost-effectivity (Zhang *et al.,* 2013).

Allicin is found in garlic and onion, thiosulfate in nature, has been recognized as a key bioactive compound (Elsafty *et al.,* 2024; Sallam *et al.,* 2024). It has the antioxidant potential through scavenging the oxygen and hydroxyl free radicles alongwith prevention of hepatic lipid peroxidation homogenates- that is the process mainly triggered by the oxidative stress resulting in formation of lipid hydroperoxide (Aboubakr *et al.,* 2023; Sallam *et al.,* 2024). This organic compound has the ability to neutralize the free radicles. Various studies in published literature have highlighted the potential beneficial effects of whole garlic both in humans and animals. Many of them have reported that mainly allicin and its derivatives such as allyl polysulfides or their metabolites like allyl methyl sulfide have the antioxidant, anti-atherosclerotic and lipid-lowering potential (Sallam *et al.,* 2024; Talib *et al.,* 2024). Another very important compound known as L-carnitine, derivative of essential amino acids (including lysine and methionine) also has the antioxidant potential. It is synthesized by the body including liver, kidneys, brain, testes and epididymis (El-Sherbiny *et al.,* 2022; Farag *et al.,* 2024). LC plays an important role for the transportation of fatty acids to mitochondria for the energy production, it is being synthesized by the body and also comes from dietary sources (Belsky *et al.,* 2018; El-Sherbiny *et al.,* 2022; Mesgaran *et al.,* 2022). It has robust antioxidant, anti-inflammatory and anti-apoptotic properties (Elkomy *et al.,* 2020; Mesgaran *et al.,* 2022). It has a very similar mechanism to that of the allicin in terms of tackling the free radicle production and reducing the antioxidant enzyme activity, which is the prime focus for their usages (Aboubakr *et al.,* 2020).

Additionally, L-carnitine has anticancer properties by inhibiting the angiogenesis (Baci *et al.,* 2019). So, keeping in view all these potential benefits of these two compounds (ALC and LC), the current study was designed to evaluate their potential ameliorative effects on DOX-induced nephrotoxicity in rats as animal model.

MATERIALS AND METHODS

Chemicals: All the chemicals were procured from locally from different sources in Egypt like DOX (doxorubicin) was procured from EIMC United pharmaceuticals (Egypt) in injectable form (2mg/ml), Allicin (35% powder) (Delta Vet Center, Cario, Egypt) and L-carnitine (MEPACO company, Inshas Elraml, Egypt).

Animals: A total of forty-nine (49) male wistar albino rats (175-200g body weight) were procured from Egyptian Organization for Biological Products and Vaccines. Animals were housed under standard environmental conditions at 25±2ºC, with a 12-hours light/dark cycle. Standard pellet diet was provided to them and drinking water *ad libitum.* All the rats were acclimatized for one week and then divided into seven (7) groups having seven rats in each group. Group 1 served as control and only normal saline was given. Group 2 was treated with ALC (20 mg/kg/day, orally) for 30 days (Aboubakr *et al.,* 2023), group 3 treated with LC (100 mg/kg/day, orally) for 30 days (Elkomy *et al.,* 2020), group 4 was administered normal saline for 30 days and then after the 24 hours of the final saline dose was injected with DOX (20 mg/kg intraperitoneal injection) following AlAsmari *et al.* (2022). Group 5 was treated with ALC and DOX combination, and group 6 with DOX and LC in combination, and group 7 was treated with ALC, DOX and LC combination. This study was duly approved by the Research Ethical committee of the Faculty of Veterinary Medicine, Cairo, Egypt vide letter number Vet CU 25122023820.

Blood sampling and serum biochemical markers: 48hrs following the DOX injection, all rats were anesthetized using isoflurane, and blood samples were collected from the retro-orbital plexus. The blood samples were allowed to coagulate at room temperature before being centrifuged at 1200g for 15 minutes. The serum samples were stored at -20°C till further analysis. Renal function parameters, including levels of creatinine, urea, total protein, and albumin, were evaluated in the serum.

Tissue oxidative stress markers: Commercial Kits provided by Bio-diagnostics company based in Giza, Egypt were used to analyze the parameters including MDA (malondialdehyde), GSH (glutathione), SOD (superoxide dismutase) and CAT (catalase) in renal tissue as per following the instruction provided by the kit manufacturer.

Histopathological examination: Renal tissues were prepared for histopathological examination following the standardized protocol described by Bancroft and Gamble (2013). Histological sections were stained with hematoxylin and eosin (H&E) to assess structural alterations under a light microscope. Histological changes were evaluated semi-quantitatively based on four primary parameters: tubular degeneration, tubular dilatation, inflammatory infiltration, and overall tissue integrity. Each parameter was graded on a scale from 0 to 3, where 0 indicated no change, 1 represented mild change, 2 corresponded to moderate change, and 3 reflected severe change.

Tubular degeneration was characterized by the loss of epithelial integrity, cytoplasmic vacuolation, and nuclear alterations. Tubular dilatation referred to the widening of the tubular lumen with flattened epithelial cells. Inflammatory infiltration was assessed based on the presence and extent of inflammatory cells within the interstitial space. Overall tissue integrity encompassed general architectural disruptions, including the presence of necrosis or fibrosis. The total histopathological score for each treatment group was determined by summing the scores of all parameters, with higher scores signifying greater severity of tissue damage. To ensure consistency and reliability, observations were independently performed by two pathologists who were blinded to the treatment

groups. This approach minimized bias and enhanced the robustness of the histopathological evaluation.

Immunohistochemical analyses of apoptotic markers in renal tissues: Immunohistochemical staining for TNF-α was performed according to the method of Hsu *et al*. (1981), with modifications to optimize staining quality. Renal tissue sections, cut to a thickness of 4µm, were deparaffinized in xylene and rehydrated through a graded series of ethanol concentrations. Antigen retrieval was achieved by heating the sections in 10mM citrate buffer (pH 6.0) using a microwave oven for 10 minutes. To quench endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide in methanol for 10 minutes at room temperature.

Subsequently, the sections were incubated overnight at 4°C with a primary TNF-α antibody (diluted 1:100; Santa Cruz Biotechnology, USA). This step was followed by incubation with a biotinylated secondary antibody and application of the avidin-biotin-peroxidase complex (Vectastain ABC Peroxidase Kit, USA). Visualization of immunoreactivity was accomplished using 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Chemicals, Perth, Australia) as the chromogen. After color development, the sections were counterstained with hematoxylin, dehydrated, and mounted for microscopic examination.

Positive TNF-α expression was identified by brown cytoplasmic staining in renal tissue cells. Negative control slides, prepared by omitting the primary antibody, confirmed the specificity of the staining. Staining intensity was evaluated semi-quantitatively by two independent, blinded observers. Tubular and glomerular TNF-α expression was scored separately on a scale of 0 to 3, where 0 indicated no staining, 1 mild staining, 2 moderate staining, and 3 intense staining. The overall score for each section was calculated as the sum of the tubular and glomerular scores, providing a comprehensive assessment of TNF-α expression across experimental groups.

Statistical analysis: The data are presented as mean±standard deviation (SD). Statistical analyses were performed using GraphPad Prism 9 software (San Diego, CA, USA). A one-way ANOVA was conducted, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was determined by asterisks, * indicating $P<0.05$, ** indicating $P<0.01$, *** indicating $P<0.001$, and **** indicating $P<0.0001$, compared to the DOX-treated groups.

RESULTS

Effect of ALC and LC on serum biochemical parameters in DOX-intoxicated rats: The administration of DOX resulted in renal damage, as indicated by elevated serum levels of renal biomarkers (Fig. 1). In comparison to the control rats, those treated with DOX exhibited significant increases in creatinine and urea concentrations, along with a reduction in serum levels of total protein and albumin. However, treatment with ALC, LC, or their combination (ALC and LC) significantly attenuated these parameters (creatinine and urea) in the DOX-treated rats compared to the DOX group. Notably, the group of DOX-intoxicated rats that received both ALC and LC demonstrated a more pronounced decrease in these parameters than those receiving either ALC or LC alone. Consequently, the combination treatment of ALC+LC+DOX conferred enhanced protection against DOX-induced renal damage compared to either therapy administered independently. Moreover, the concentrations of total protein and albumin gradually returned toward control values in the groups treated with ALC+DOX, LC+DOX, and ALC+LC+DOX.

Effect of ALC and LC on renal oxidative stress indices in DOX- DOX-intoxicated rats: The findings presented in Fig. 2 illustrate the impact of DOX exposure and treatment with ALC, LC, or a combination of both on MDA, GSH, and antioxidant enzyme levels in renal tissues. In rats subjected to DOX, MDA levels exhibited a significant increase, while CAT, SOD, and GSH levels showed a marked decrease in comparison to the control group. Treatments with either ALC or LC were effective in alleviating the adverse effects of DOX on MDA, CAT, SOD, and GSH levels in the renal tissues; however, these values remained significantly different from those of the control group. Notably, co-treatment with ALC and LC resulted in a significant enhancement of renal tissue protection against the oxidative damage induced by DOX, surpassing the effects of ALC or LC administered individually.

Histological analyses of kidney sections: Histological analysis of kidney sections revealed distinct architectural variations among the treatment groups. In Group 1 (Control), the typical renal architecture was preserved, featuring well-defined glomeruli and intact tubular structures (Fig. 3A). Groups 2 (ALC) and 3 (LC) maintained the standard kidney architecture, showing no histopathological changes or signs of cellular damage or abnormalities (Fig. 3B and 3C). In stark contrast, Group 4 (DOX) displayed significant pathological alterations, including tubular dilatation, hydropic degeneration of tubules, vacuolization, and indications of nephritis characterized by extensive inflammatory cell infiltration in the renal interstitium (Fig. 3D and 3E). Treatments in Group 5 (ALC+DOX) and Group 6 (LC+DOX) mitigated the severity of pathological damage to some extent, evidenced by a reduction in inflammatory cell infiltration and varying degrees of tissue preservation: Group 5 demonstrated slight restoration of renal architecture, while Group 6 exhibited less tubular degeneration and diminished glomerular damage (Fig. 3F and 3G). The combination treatment in Group 7 (ALC+LC+DOX) showed the most well-preserved architecture among the treated groups, with minimal cellular degeneration and well-maintained tubular integrity (Fig. 3H), suggesting potentially synergistic protective effects of ALC and LC when administered alongside DOX.

A semi-quantitative evaluation of histological changes in kidney tissues is illustrated in Fig. 3I. Group 1 (Control) exhibited no changes, receiving a score of 0 across all categories. The ALC group showed no tubular degeneration but recorded a score of 1 for tubular dilatation, indicating minimal changes. The LC group presented mild tubular degeneration, with a score of 1, and no alterations in other parameters. The DOX group experienced significant tissue damage, achieving the

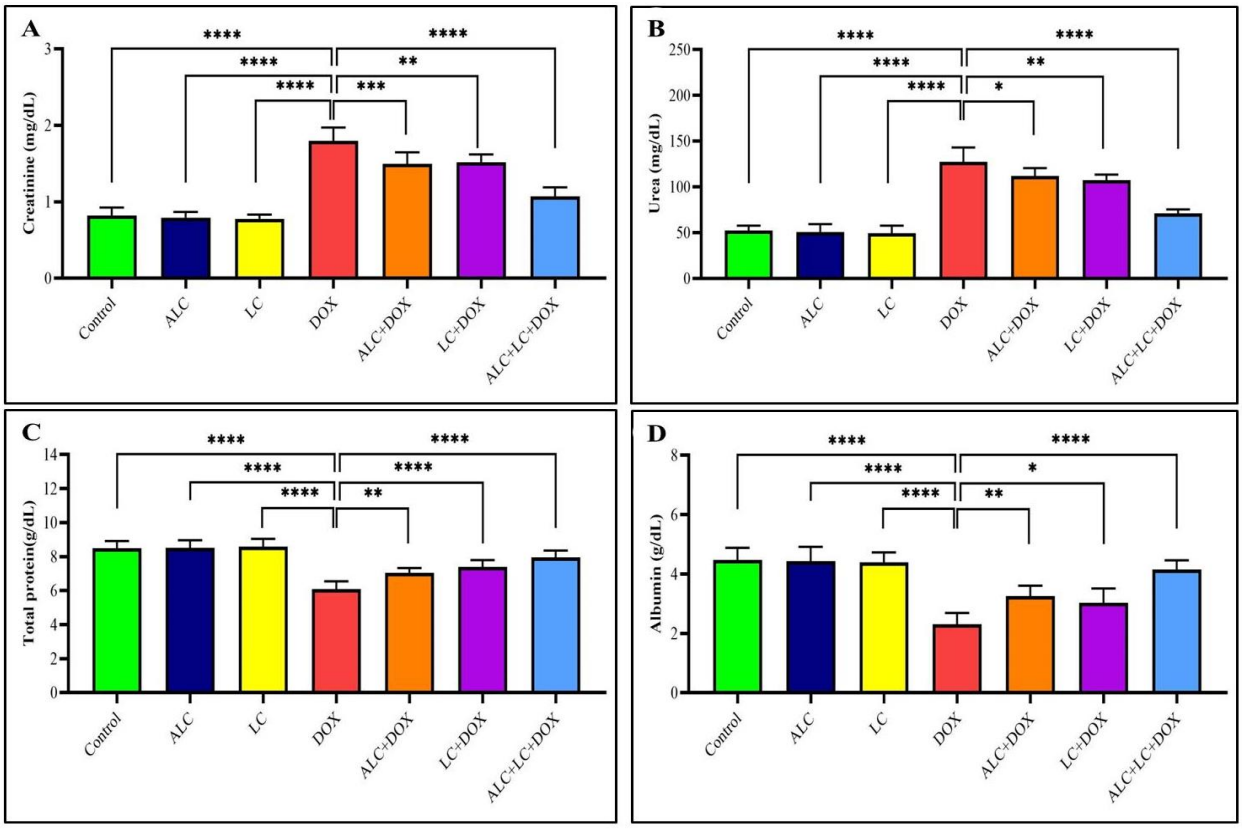

Fig. 1: Effect of allicin (ALC), L-carnitine (LC) and doxorubicin (DOX) on renal biochemical parameters in rats, (A) Creatinine, (B) Urea, (C) Total Protein and (D) Albumin. Statistical significance was determined by asterisks, indicating $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, and $^{****}P<0.0001$, compared to the DOX-treated groups.

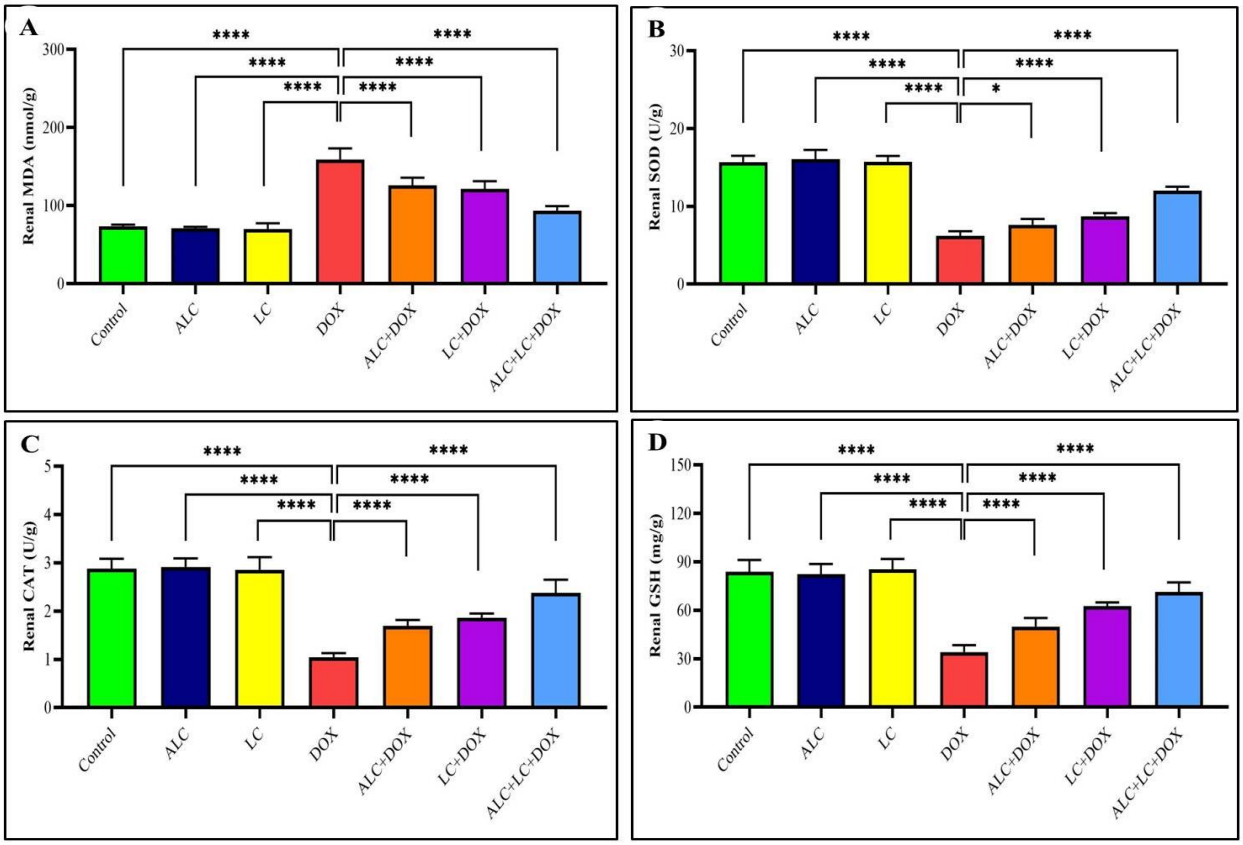

Fig. 2: Effect of allicin (ALC), L-carnitine (LC) and doxorubicin (DOX) on antioxidant parameters in renal tissues in rats (A) MDA, (B) SOD, (C) CAT and (D) GSH. Statistical significance was determined by asterisks, with $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, and $^{****}P<0.0001$, compared to the DOX-treated groups.

highest scores for tubular degeneration and inflammatory infiltration, culminating in an overall score of 8. In comparison, the ALC+DOX group demonstrated less tissue damage than the DOX-only group, with a total score of 4, suggesting potential protective effects of ALC. Similarly, the LC+DOX group also received an overall score of 4, likely reflecting reduced damage attributable to the protective properties of LC. Lastly, the ALC+LC+DOX group exhibited no tubular degeneration and obtained a total score of 2, which may indicate a synergistic protective effect arising from the combination of ALC and LC with DOX.

Immunohistochemical analysis of TNF-α expression in kidney tissues: In the Control group, kidney tissues exhibited no detectable TNF-α expression in either the tubules or glomeruli, with an average tubular and glomerular score of 0, indicating a clear absence of inflammatory response in the renal cortex (Fig. 4A). The ALC and LC groups similarly showed no significant changes in TNF-α expression, perfectly mirroring the normal control findings, as both groups maintained a consistent score of 0 (Fig. 4B and 4C). In sharp contrast, the DOX-treated group displayed a significant increase in TNF-α expression, characterized by strong staining in renal tubular cells and faint staining in the glomeruli, leading to an impressive average tubular score of 2.6 and a glomerular score of 1. This resulted in an overall score of 3.6, clearly indicating a strong inflammatory reaction likely due to the pharmacological effects of Doxorubicin (Fig. 4D and 4E).

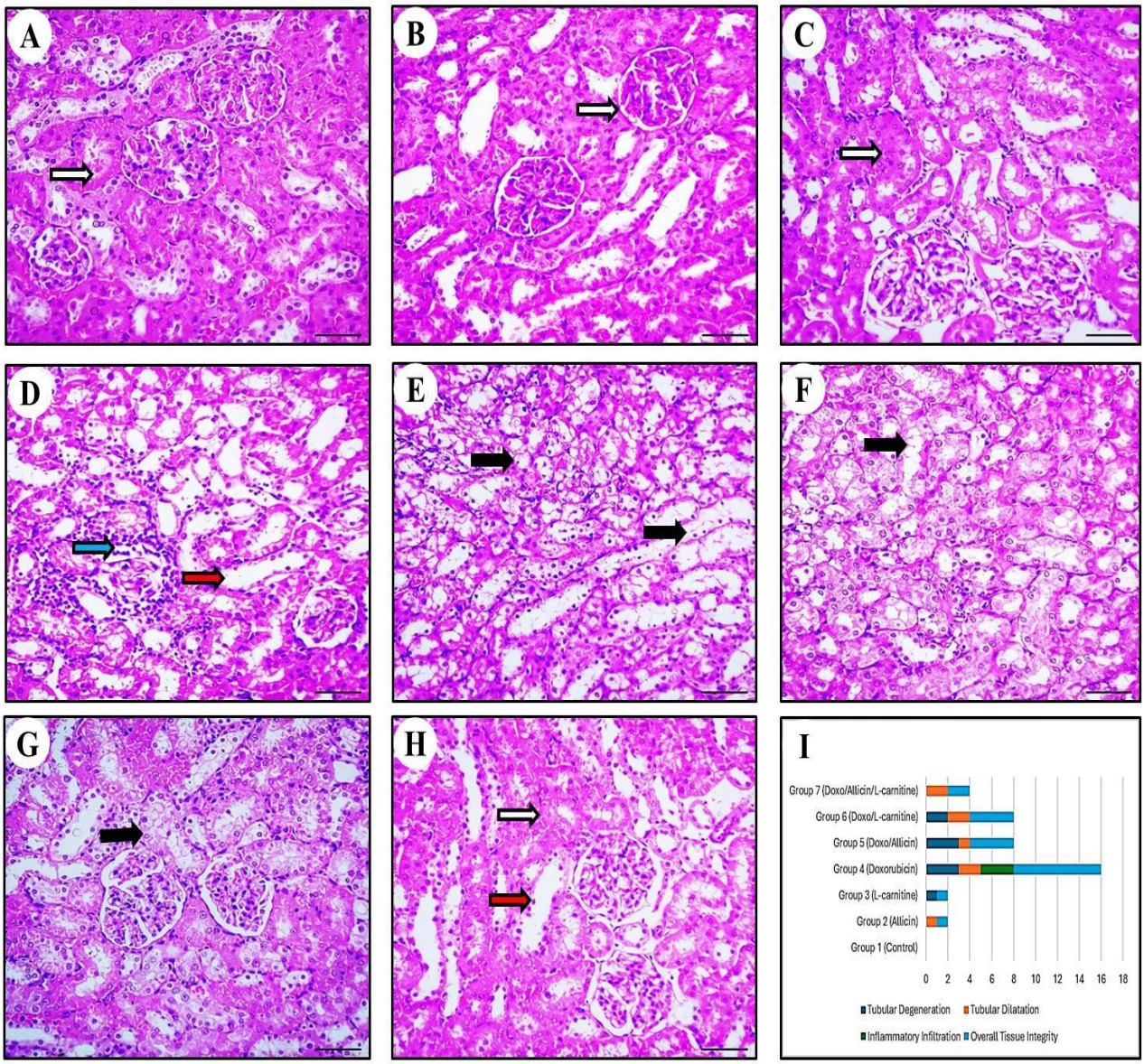

Fig. 3: Histological analysis of kidney sections illustrating structural differences across treatment groups. (A) Group 1 (Control) shows preserved renal architecture with intact tubules and glomeruli. White arrows indicate normal tubular structures. (B, C) Groups 2 (ALC) and 3 (LC) display normal kidney architecture with no significant alterations. (D, E) Group 4 (DOX) reveals significant pathological changes, including tubular degeneration (black arrows), tubular dilatation (red arrows), and inflammatory cell infiltrations (blue arrows). (F, G) Groups 5 (ALC+DOX) and 6 (LC+DOX) demonstrate varying degrees of tissue preservation with some areas showing a return towards normal architecture. (H) Group 7 (ALC+LC+DOX) shows the most preserved architecture with minimal structural changes. (I) Semi-quantitative scoring of histopathological changes with parameters for Tubular Degeneration, Tubular Dilatation, Inflammatory Infiltration, and Overall, Tissue Integrity.

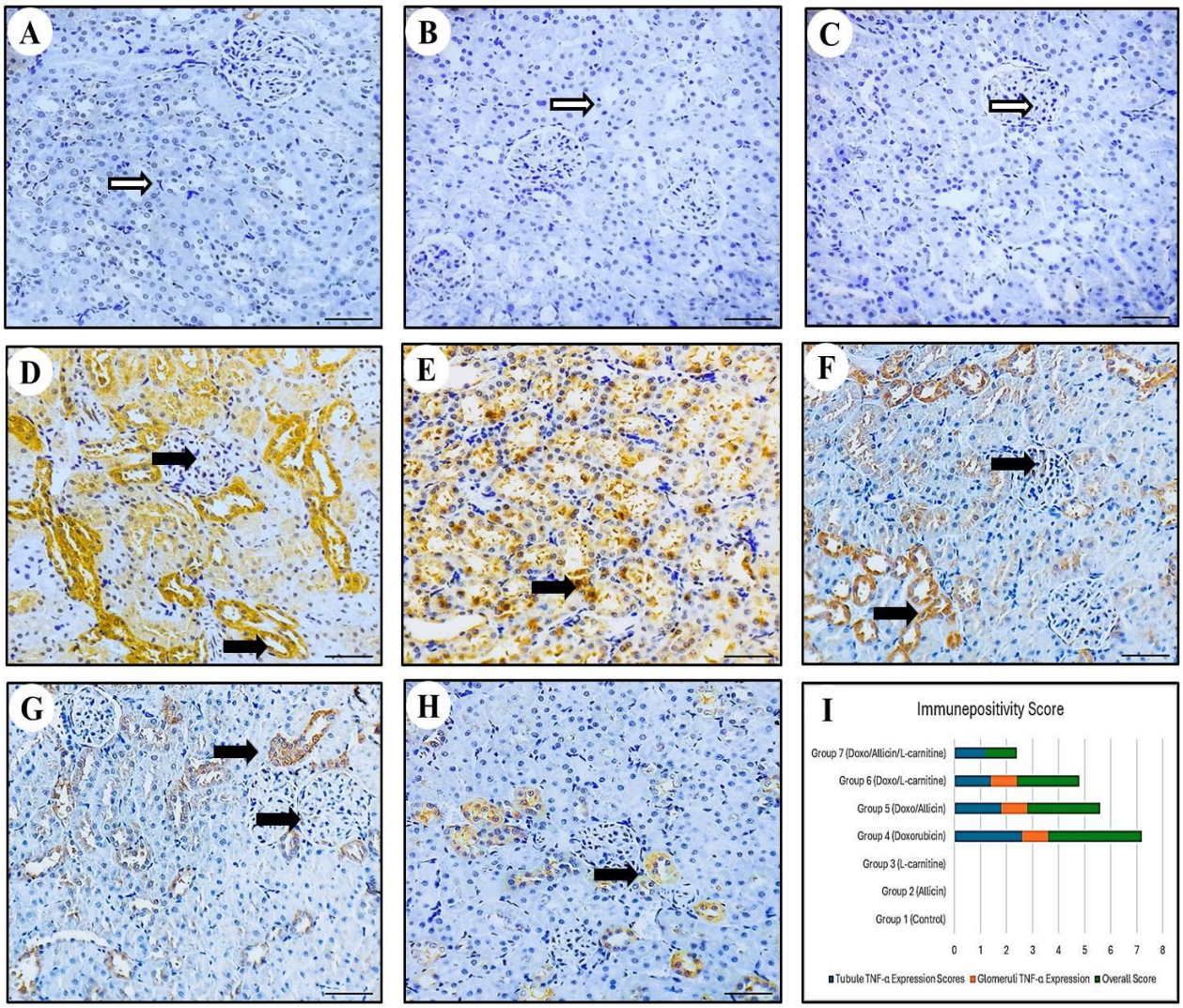

Fig. 4: Immunohistochemical Analysis of TNF-α Expression in Kidney Tissue across treatment groups. White arrows denote areas with no TNF-α expression, while black arrows highlight regions of positive TNF-α expression. (A) Group I (Control) showed a complete absence of TNF-α expression in both tubular and glomerular structures. (B, C) Groups 2 (ALC) and 3 (LC) paralleled the control with no significant TNF-α expression. (D, E) Group 4 (DOX) exhibited intense TNF-α expression, especially in the tubules. (F, G) The ALC+DOX and LC+DOX groups showed mitigated TNF-α expression, primarily in the tubules with glomerular expression. (H) The combination treatment in Group 7 (ALC+LC+DOX) resulted in the most significant reduction of TNF-α expression, and absence of glomerular staining. (I) The immunohistochemical reaction scores highlight the semi-quantitative assessment of TNF-α expression levels in kidney tissues of different treatment groups.

ALC+DOX and LC+DOX exhibited decreased TNF-α expression, particularly in the tubules, with average scores of 1.8 and 1.4, respectively. These groups also maintained a consistent glomerular score of 1. Consequently, the overall scores were 2.8 for Group 5 and 2.4 for Group 6, indicating a protective effect against the inflammatory induction caused by Doxorubicin, as illustrated in Fig. 4F and 4G. Notably, ALC+LC+DOX demonstrated the most significant reduction in TNF-α expression, achieving a tubular score of 1.2 and showing no expression in the glomeruli, resulting in the lowest overall score of 1.2. The lack of expression in the glomeruli and the reduced tubular scores are emphasized in Fig. 4H. The glomerular TNF-α expression and overall scores are depicted in Fig. 4I.

DISCUSSION

While anti-cancer therapies primarily focus on targeting rapidly dividing cancer cells, chemotherapeutic agents can unintentionally harm other quickly proliferating healthy cells throughout the body (Erdogan *et al.,* 2020; Chavelle *et al.,* 2022). This disruption of cellular homeostasis can result in undesirable physiological consequences and negatively affect various organs (Ayla *et al.,* 2011). Therefore, the side effects experienced during or after treatment may not always lead to the most severe outcomes, but they do raise significant concerns regarding cancer management in patients (So *et al.,* 2019). Consequently, there is an urgent need to develop targeted therapies and/or identify adjunctive agents that can alleviate the harmful toxicity associated with these treatments, while maintaining their effectiveness and clarifying their protective mechanisms.

Although the doxorubicin is much effective to prevent the cancer induced mortality, yet it has the potential to induce delayed cardiomyopathy, hepatic impairment, renal toxicities that eventually results in renal failure. This renal failure is a clinical challenge for the physicians in DOX treated patients (Ikewuchi *et al.,* 2021; Lawson *et al.,* 2022). This is the most documented potential hazard of

DOX treatment in cancer patients and has been linked to many other pathological outcomes (Troxell *et al.,* 2016). It directly harms the tubular epithelial cells (Manawy *et al.,* 2024). The same findings in the current study have been recorded, where DOX treatment resulted significant increase in creatinine and urea level as compared to the control group, which is an evidence for the renal damage. This has already reported in literature that DOX resulted in increase in creatinine and urea levels (Kuzo *et al.,* 2019; Altinoz *et al.,* 2022; Wu *et al.,* 2023; Manawy et al., 2024). In contrast, the treatment groups received ALC, LC in addition to DOX have significantly lower values for the same parameters. These findings prove the potential of ALC and LC to ameliorate the toxic potential of DOX on renal tubular cells. which already has been mentioned in literature.

Serum total protein and albumin are significantly reduced following the DOX treatments and these findings are supported by the study conducted by Amarasiri *et al.* (2021), who recorded the same observation. Allicin has been found to mitigate the potential renal toxic impacts induced by various agents like glycidamide (Wang *et al.,* 2021), gentamycin (El-Kashef *et al.,* 2015), cyclosporine A (El-Kashef *et al.,* 2015), diclofenac sodium (Orabi *et al.,* 2020) and acetaminophen (Elsafty *et al*., 2024). It has also been documented by the Wang *et al.* (2015) particularly reducing the creatinine and urea levels. Similar effects have been documented for the L-carnitine against the renal damage induced by the glycerol (Kunak *et al.,* 2016), cisplatin (Elkomy *et al.,* 2020) and doxorubicin and cyclophosphamide (Morid *et al.,* 2023). It has an additional effect that LC enhances the total protein and albumin levels in rats with renal damage caused by above mentioned toxicities. One important factor in the process of destroying tissues is oxidative stress. It happens when there is an imbalance between the body's capacity to neutralize or repair the negative effects of reactive oxygen species (ROS) and their production (Aboubakr *et al*., 2023a; Aboubakr *et al*., 2023b; Elsayed *et al*., 2024; Soliman *et al*., 2024a; Soliman *et al*., 2024b).

In current study, the rats in DOX exposed group have a significant decrease in GSH (glutathione) activity, CAT (catalase) and SOD (superoxide dismutase) levels. However, the MDA (malondialdehyde) was significantly higher due to ROS overproduction leading to oxidative damage to renal epithelial cells. In literature, various researchers have reported that the DOX toxicity is associated with oxidative stress, inflammatory responses and apoptotic damage induced (Afsar *et al.,* 2020; Altinoz *et al.,* 2022; Yesilkent and Ceylan, 2022; Chang *et al.,* 2023; Manawy *et al.,* 2024).

Derivative micronutrients formed due to therapeutics and environmental exposure facilitate the redox reactions that ultimately lead to reactive oxygen species production. Various enzymes including CAT, SOD and GSH are responsible for the redox balance inside cells (Yesilkent and Ceylan, 2022). In current study, a significant decrease in these three enzyme activity has been recorded in kidney tissue exposed to DOX, while an increase in lipid peroxidation was recorded in the same group. The same findings have been reported by various researchers including Kuzu *et al.* (2019), Yesilkent and Ceylan (2022) and Wu *et al.* (2023). The positive effects of ALC and LC were recorded on DOX nephrotoxicity. This was due to the protective effects of antioxidant defense system. Hence in this study it was proven that DOX disrupted antioxidant system can be reinstated by ALC and it has already been reported by Zhang *et al.* (2013) and Abdel-Daim *et al.* (2019), and allicin also has the anti-inflammatory and it has been observed in various animals (El-Kashef *et al.,* 2015). Similarly, LC has the antioxidant potential, which has been reported against glycerol induced toxicity (Kunak *et al.,* 2016), cisplatin (Elkomy *et al.,* 2020) and cyclophosphamide (Morid *et al.,* 2023) in rats and El-Sherbiny *et al.* (2022) reported the antioxidant potential of LC administration in rams.

In current study, histological changes were recorded in DOX treated group including hemorrhage, glomerular congestion, infiltration and vacuolization and these potential effects were alleviated ALC and LC, which has also been previously following DOX administration (Kuzu *et al.,* 2019; Abd-Ellatif *et al.,* 2022; Altinoz *et al.,* 2022; Chang *et al.,* 2023; Wu *et al.,* 2023; Manawy *et al.,* 2024). The groups treated with ALC showed the ameliorative effects on renal tubules and reversed the histological changes significantly and it has also been previously reported the positive effects of ALC against gentamicin-induced renal toxicity (El-Kashef *et al.,* 2015), cyclosporine-A (El-Kashef *et al.,* 2017), diclofenac sodium (Orabi *et al.,* 2020) and paracetamol (Elsafty *et al.,* 2024). Similar results have been reported for LC in case of renal histopathology changes induced by glycerol (Kunak *et al.,* 2016), cisplatin (Elkomy *et al.,* 2020), DOX and Cyclophosphamide (Morid *et al.,* 2023) nephrotoxicity in rats.

Apoptosis induced by DOX, also plays a crucial role in the onset of nephrotoxicity and mainly regulated by the collaboration between pro and anti-apoptotic proteins (Amarasiri *et al.,* 2021). This study also revealed the same results, alongwith significant increase in TNF-α due to DOX treatment due to immunoreactivity, which is also endorsed by Entezari Heravi *et al.* (2018). Allicin has been shown to mitigate TNF-α overexpression in cases of renal injury linked to metabolic syndrome (Arellano Buendia *et al.,* 2023), whereas LC administration has been found to reduce TNF-α expression in renal toxicity induced by glycerol (Kunak *et al.,* 2016).

Conclusions: It has been concluded that DOX induced nephrotoxicity toxicity can be mitigated by the supplementation of allicin or L-carnitine, which is evident from the biochemical and histological changes in the tissues. These positive effects of supplemented compounds are attributed to their antioxidant and anti-inflammatory potential. Hence these two compounds including allicin and L-carnitine have the potential to be used as promising agents against renal toxicity induced by doxorubicin, to protect the cancer patients from chemotherapy related challenges. The molecular pathways underlying the synergistic effects of ALC and LC and testing the protective effects in other organs should be investigated.

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Authors contribution: AS, MA; methodology and designed the experimental protocol. MA; histopathology and IHC. AA, MHA, HIG, AF; manuscript draft preparation, review and editing. All the authors have gone through the final draft and approved its final submission.

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