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Original Research Article

Hepatoprotective Effect of L-Carnitine is Achieved via Activating Nrf2 and Targeting TLR4 Signaling Pathways in Thioacetamide – Induced Liver Fibrosis in Rats

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Abstract: ***Background:*** Liver fibrosis is a critical health problem that can result in serious illness and death. L-carnitine (LC) is a naturally occurring compound which transports fatty acids through the inner mitochondrial membrane for consequent beta-oxidation. It acts as an antioxidant to lessen cellular oxidative stress. Carnitine is essential for the transfer of long-chain fatty acids across the inner mitochondrial membrane for subsequent β-oxidation. This study was carried out to investigate the hepatoprotective effects of LC via modulation of Nrf2 signaling and TLR4 targeting pathways in rats with liver fibrosis induced by Thioacetamide (TAA). ***Methods*****:** Twenty-four adult male Wister rats were assigned into four groups as follows: Group 1 served as a normal non- treated control. Rats in group 2 were injected intraperitoneally (IP) with Thioacetamide (TAA) twice a week at a dose of 200 mg/kg B.wt for 6 weeks to produce liver fibrosis. Two weeks following TAA injections, 50 and 100 mg/kg of LC were administered to the rats in groups 3 and 4, respectively concurrently with TAA injections until end of the experiment (6 weeks). ***Results*****:** Intraperitoneal injection of LC decreased the levels of liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats with liver fibrosis induced by TAA. Malondialdehyde (MDA), tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and toll-like receptor 4 (TLR4) levels were significantly decreased in LC treated groups. LC injection increased albumin, superoxide dismutase (SOD), heme oxygenase-1 (HO-1), nuclear factor erythroid 2-related factor 2 (Nrf2), and glutathione (GSH) levels. Additionally, expression of Phosphoinositide 3-kinase (PI3K) was increased and expression of TLR4 was decreased in LC treated groups according to PCR data. The biochemical findings were supported by histopathological findings. Regarding immunohistopathological examination, the LC treated groups reduced hepatic expression of caspase-3 and α-smooth muscle actin (α-SMA). ***Conclusion***: LC mitigates liver fibrosis in rats induced by Thioacetamide via modifying Nrf2 and TLR4 targeting pathways.

Keywords: Liver fibrosis; L-carnitine; Thioacetamide; Nrf2; TLR4.

INTRODUCTION

Liver fibrosis is characterized by accumulation of extracellular matrix proteins (ECM) especially collagen types I and III, as well as an increase in other extracellular matrix constituents such as proteoglycans, fibronectin and laminin in response to liver injury. It serves as a transitory stage for a number of chronic liver disorders [1]. It may result due to autoimmune disorders, drug-related conditions, viral hepatitis, or other chronic liver diseases, including alcoholic and drug intoxication. Although the pathophysiology of hepatic fibrosis has been extensively studied throughout the last decades, there is currently no existing treatment [2, 3]. The primary liver cells produce matrix are known as hepatic stellate cells (HSCs) which undergo a transformation from latent, cells that store vitamin A, to active cells that resemble myofibroblasts after a fibrogenic stimulation, depositing excessive ECM, particularly type I collagen. Moreover, myofibroblasts also secrete several profibrotic and pro inflammatory cytokines [4].

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Oxidative stress, which results in deterioration of lipids, proteins, and DNA, is essential to the progression of hepatic fibrosis. It results from an imbalance between cells' ability to remove free radicals and their production [5]. As a component of the intricate defense mechanisms of the liver against oxidative damage, Nrf2 is essential for the liver's protection during inflammation and fibrogenesis [6]. Kelch-like ECH-associated protein 1 (Keap1) in a normal state and cytoplasmic Nrf2 form a complex. Upon oxidative stress, Keap1 releases Nrf2, which is then transported to the nucleus and triggers the transcription of genes that contain antioxidant response elements (AREs), including catalase (CAT), HO-1, and SOD [7]. Nrf2 is a fundamental therapy in prevention and treatment of hepatic fibrogenesis due to the activation of its target genes [8].

An innate immune response that involves toll-like receptors (TLRs) and the signaling pathway that underlies it has been linked to inflammation [9]. TLR4, a typical TLRs representative, is essential for generating inflammatory activation and promotes immune responses that are both innate and adaptive [10]. Cytokines that promote inflammation like TNF-α and IL-1β are upregulated in transcription when the main adaptor protein, MyD88 (MyD88)-dependent pathway, activated by TLR4 [11]. High TLR4 levels in the liver have been connected to the development of inflammation and consequently hepatic fibrogenesis [12]. Suppression of TLR4 signaling pathway could lessen liver damage in chronic liver injury animal models [13]. Therefore, preventing TLR4 expression may prevent the hepatic fibrogenesis.

The body produces the nutritional molecule L-carnitine (LC) from the necessary amino acids lysine and methionine [14]. Endogenous carnitine production occurs primarily in the liver [15]. LC is required for the carriage of long chain fatty acids into the matrix of the mitochondria through the activities of specialized acyl transferase [14]. According to numerous research, LC guards against CCl4-induced liver damage and reduces oxidative stress [16]. In addition, LC protects the liver from acute Paracetamol toxicity [17]. According to earlier research, the Nrf2/ARE signaling pathway is implicated in the control of LC on oxidative stress brought on by H2O2 [18]. Additionally, prior research demonstrated that LC reduced neuroinflammation by targeting the TLR4/NF-B pathway [19]. For this reason, we intended to investigate whether L-carnitine could prevent TAA-induced liver fibrosis by activating Nrf2 and blocking TLR4 signaling pathways.

MATERIALS AND METHODS

Experimental animals:

Twenty-four adult male Wister rats weighing 180–220 g at 6–8 weeks of age were obtained from the "Animal House Colony at the National Research Centre (NRC, Egypt)". The environment the rats were kept in with a 25°C temperature and a 12-hour light/dark cycle.

Chemicals:

TAA was purchased from Sigma-Aldrich, USA". The supplier of L-carnitine was MEPACO in Egypt. The highest available analytical grade of other chemicals was used in the study.

Experiment protocol:

Twenty-four rats were assigned into four groups, each of six rats. Group 1 was kept as a normal control. Rats in group 2 were injected intraperitoneally (IP) twice a week with TAA for six weeks at a dose of 200 mg/kg B.wt r to induce liver fibrosis [20]. Rats in groups 3 and 4 received oral LC as doses of 50 and 100 mg/kg B.wt) every day, respectively [21], beginning 2 weeks following the TAA injection and continued four weeks later, simultaneously with TAA.

Blood and tissue sample preparation:

After the experiment, under mild ketamine anesthesia, from each rat's retro-orbital venous plexus, blood samples were taken for obtaining serum. Serum samples were kept in a -20°C freezer for biochemical testing. Following blood sampling, cervical dislocation of rats was done. Rinsing in ice-cold saline and drying of the quickly removed livers were done. For molecular and biochemical analyses, a piece of the liver from each rat that was weighted was frozen at -80°C. A different portion was taken out and retained in 10% buffered neutral formalin for immunohistochemistry and histology.

Assessment of liver injury indicators:

Using "Bio diagnostic® commercial kits", Egypt, (Catalog No. AS 10 61 and AL 10 31), "alanine aminotransferase (ALT), aspartate aminotransferase (AST)" activities and albumin levels in serum were all assessed using colorimetric methods.

Assessment of redox imbalance signs:

Colorimetric measurements of the liver tissue homogenate were made to determine the levels of GSH content, MDA, and SOD activity, purchased from Egypt's "Bio-diagnostic® kits" (Refs. GR 25 11, SD 25 21, MD 25 29).

Hepatic pro-inflammatory indicators in the liver (ELISA):

TLR4, TNF-α and IL-1β in hepatic homogenate were measured in accordance with Sunlong Biotec Com. LTD's manufacturing procedures in Zhejiang, China (Catalog No. SL1761Hu, SL0402Ra, and LS-F4846).

Hepatic Nrf2 and HO-1 levels via (ELISA):

In accordance with the directions supplied by Wuhan Fine Biotech Co., Ltd, China and S. Milpitas Blvd., Milpitas, USA (Catalog No. EH3417 and E4525-

100), the liver homogenate Nrf2 and HO-1 levels were evaluated.

Hepatic TLR4 and PI3K expressions in the homogenate (qRT-PCR):

RNA extraction:

All of the groups' homogenized tissues were used to isolate total RNA using Direct-zol RNA Miniprep Plus "USA; California (Cat# R2072, ZYMO RESEARCH CORP)". The extracted RNA was reverse-transcribed using "Superscript IV One-Step RT-PCR kit" (Cat# 12594100) from "Thermo Fisher Scientific", which is sold in Waltham, Massachusetts. The 96-well plate In a thermal profile, the following Step One equipment (Applied Biosystem, USA) was used: For the amplification step, 40 cycles of "10 s at 98 °C, 10 s at 55 °C, and 30 s at 72 °C" make up the initial denaturation phase. Reverse transcription takes place at 45 °C and takes 10 minutes. RT inactivation occurs at 98 °C and takes 2 minutes. After the RT-PCR assay, the information for the target genes and housekeeping gene was communicated using the cycle threshold (Ct). The forward and reverse primer oligonucleotide sequences are listed in Table **1.** Target genes PI3K and TLR4 were normalized for variance in expression using the mean critical threshold (CT) expression. Table 1 presents the oligonucleotide sequences for the forward and reverse primers.

Table 1: Oligonucleotide primers used in qPCR

Gene		Sequence (5′-3′)	Ref./accession No.
PI3K	Forward	"CAGGAGCGGTACAGCAAAGA"	XM_017590649.2
	Reverse	"GCTGTCGATGATCTCGCTGA"	
TLR4	Forward	"ACAGGGCACAAGGAAGTAGC"	NM_019178.2
	Reverse	"GTTCTCACTGGGCCTTAGCC"	

Examination of the liver histopathology:

The paraffinization of rat liver tissue samples from various groups was consistently performed following a twenty-four-hour fixing in 10% neutral buffered formalin. After being cleaned with distilled water, the samples were dried with diluted ethanol and then clarified in xylene. Lastly, paraffin blocks measuring four to five µm thick were cut into pieces. Following deparaffinization and H&E staining, the slices of tissues were put on glass slides [22]. Examination of slides were performed by a qualified investigator who, in order to prevent bias, was blinded during the sample identification procedure.

Immunohistochemistry to evaluate α-SMA and **caspase-3 expression levels:**

Paraffin-embedded liver slices from every treatment group and the control rats were exposed to the application of avidin-biotin peroxidase (Sigma Chemical Company, DAB) to identify caspase-3 and α-SMA expressions immunohistochemical [11]. Vector Laboratories' Vactastain ABC peroxidase kit was used to treat tissue slices with a monoclonal antibody at dilutions of 1:100 and 1:200, respectively, for caspase-3 and α-SMA as well as the components essential for the identification of the antigen-antibody complex using the avidin-biotin peroxidase method. An analysis of each marker's expression was conducted using the "chromagen 3, 3-diaminobenzidine tetra hydrochloride" (DAB, Sigma Chemical Co.). Seven high power microscopic fields were used to measure the optical density of each marker's positive brown area using image analysis software (Image J, 1.46a, NIH, USA).

Statistical Analysis

Using Graph Pad Prism version 5, the results were examined and presented as means with SEM. "The one-way repeated measures analysis of variance" was used, followed by the Tukey test. *p* value of <0.05 was used to determine the significance of differences.

RESULTS

Effect of L-carnitine on serum liver enzymes activities and albumin level in rats receiving TAA:

LC prevented liver fibrosis induced by TAA in rat and its potential benefits, liver function indicators like AST, ALT, and albumin were primarily assessed. The serum activities of ALT and AST in the model group were increased markedly ($P < 0.05$), as shown in Figures 1(a) and 1(b), whereas the serum albumin level was markedly decreased ($P < 0.05$) Figure 1(c), showing that rats' liver damage from TAA was successfully induced. The LC administration could markedly ($P < 0.05$) decreased ALT and AST activities and markedly ($P < 0.05$) elevated serum albumin according to the dose. The findings show that LC improved liver function and protected liver cells.

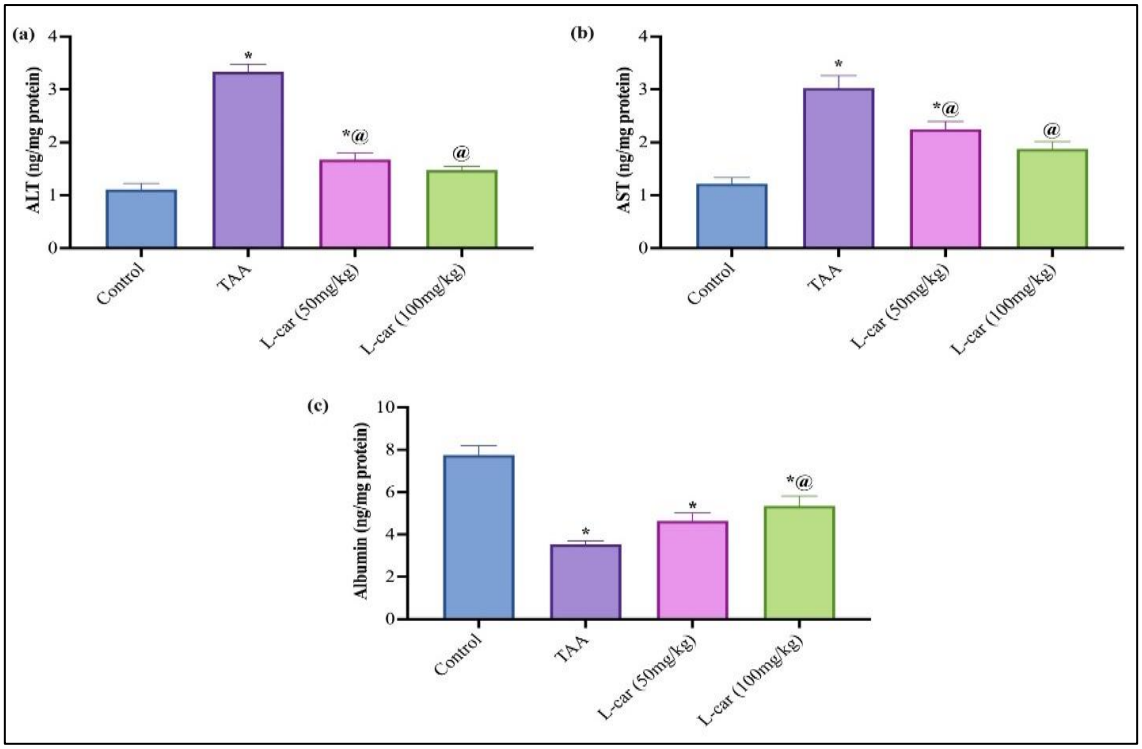


Fig 1: Effect of l-carnitine on serum liver enzymes activities and albumin levels: (Fig 1.a) ALT (U/L), (Fig 1.b) AST (U/L), and (Fig 1.c) albumin (g/dL). The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. "TAA, Thioacetamide; LC, L-carnitine; ALT, alanine transaminase; AST, aspartate transaminase"

Effect of L-carnitine on the hepatic redox imbalance indicators in rats received TAA:

It was determined how LC affects the oxidative damage induced via TAA injections by measuring the MDA level and the antioxidant parameters, GSH content and SOD activity. When compared to control rats, TAA caused a significant increase hepatic MDA and decreased hepatic GSH content and SOD activity ($P < 0.05$). Treatment with LC recovered the decline in GSH content and SOD activity, and considerably reduced the elevated MDA level in the liver when compared to the TAA group ($P < 0.05$) (Fig. 2 a, b, c).

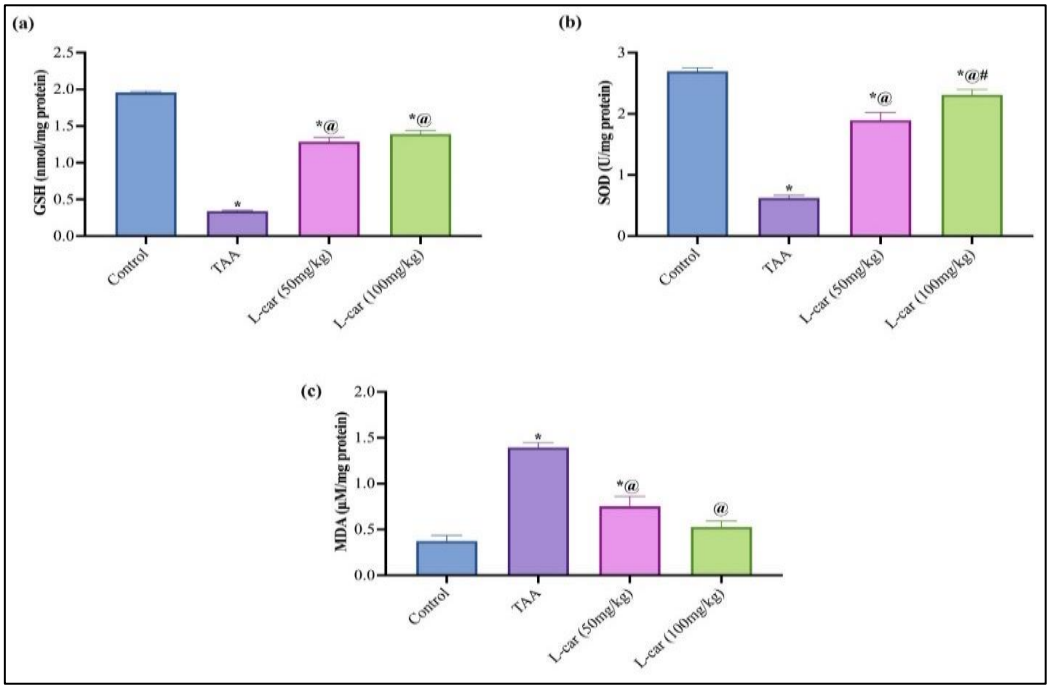


Fig 2: Effect of L-carnitine's on the hepatic redox imbalance indicators in rats receiving TAA: (Fig 2.a) GSH (nmol/mg protein), (Fig 2.b) SOD (U/mg protein) activity, (Fig 2.c) MDA (µM/mg protein). The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. "TAA, Thioacetamide; LC, L-carnitine; MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase"

Effect of l-carnitine on pro inflammatory markers in the liver of rats given TAA:

Compared to the negative control group, TAA significantly ($P < 0.05$) increased the levels of hepatic TLR4, TNF-α, and IL-1β. As evidenced by (Fig 3 a, b, c), treatment with LC significantly ($P < 0.05$) decreased TLR4 level and its downstream cytokines, TNF-α and IL-1β levels in rat livers in a manner dependent on dosage, compared to TAA group.

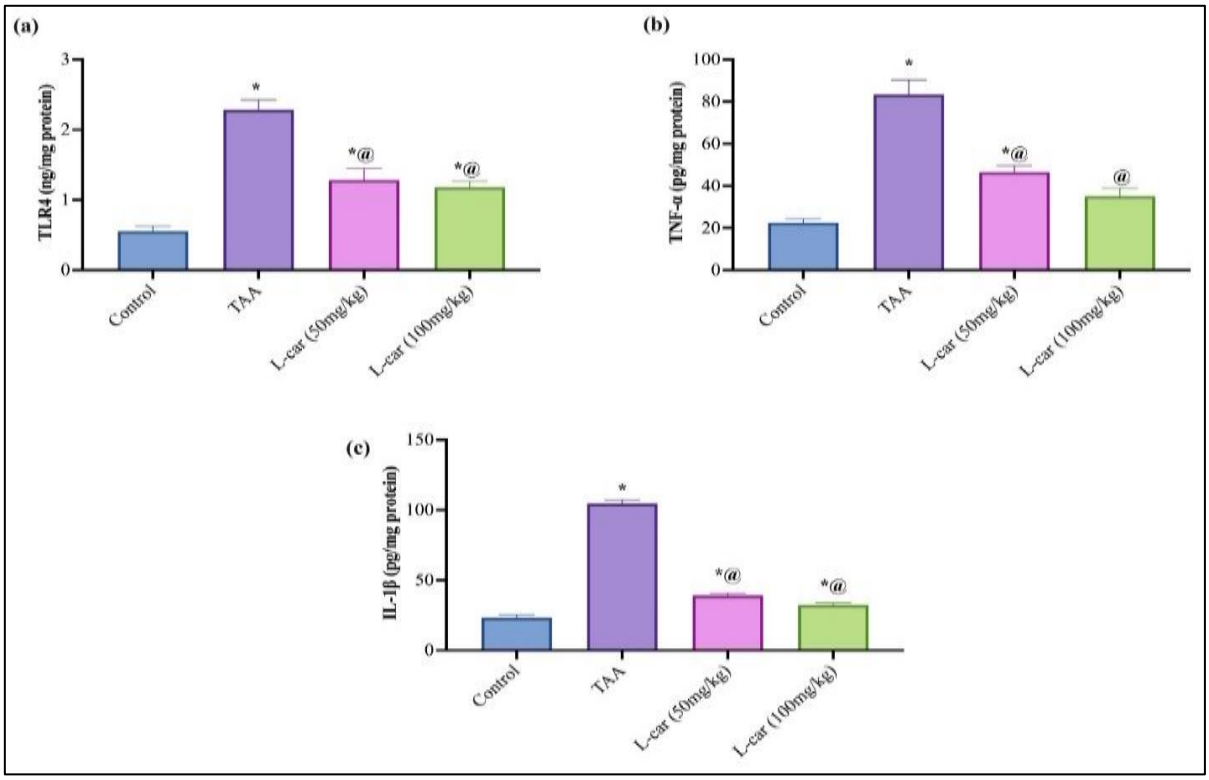


Fig 3: Effect of l-carnitine on pro inflammatory markers in the liver of rats given TAA: (Fig 3.a) TLR4 (ng/mg protein), (Fig 3.b) TNF-α (pg/mg protein), (Fig 3.c) IL-1β (pg/mg protein). The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. "TAA, Thioacetamide; LC, L-carnitine; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin 1 beta"

Effect of l-carnitine on the hepatic antioxidant pathway, Nrf2 and HO-1, levels in rats received TAA:

Rats with hepatic fibrosis induced by TAA-induced significantly lowered levels of Nrf2 and its target gene, HO-1, ($P < 0.05$) than the negative control group (Fig 4 A, B). Contrasted with TAA rats, the antioxidant Nrf2 and HO-1 pathways were markedly ($P < 0.05$) elevated in the rats administered both dosages of LC.

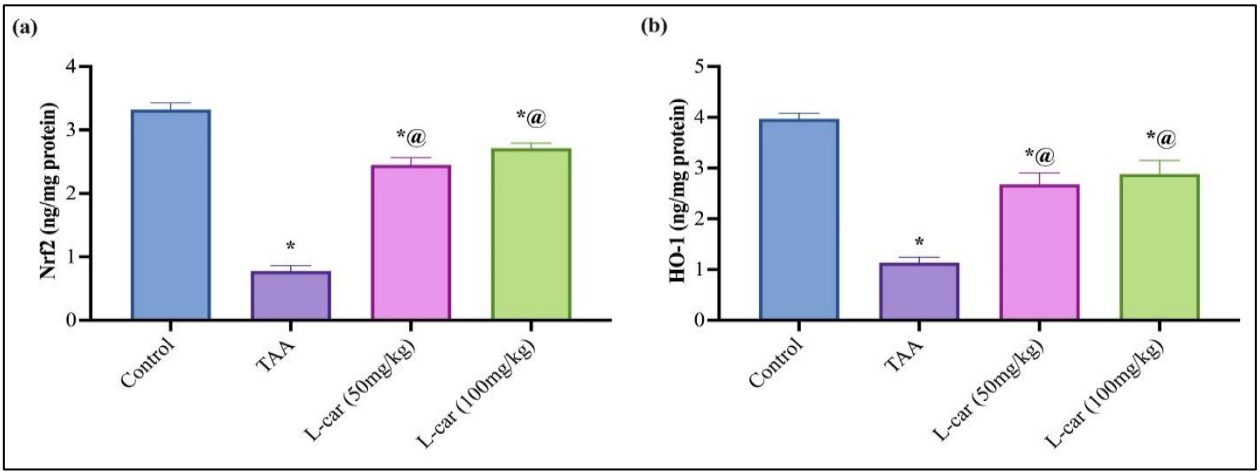


Fig 4: Effect of l-carnitine on the hepatic Nrf2 and HO-1 levels in rats receiving TAA. (Fig 4.a) Nrf2 (ng/mg protein), (Fig 4.b) HO-1 (ng/mg protein). The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. "TAA, Thioacetamide; LC, L-carnitine; Nrf2, nuclear factor erythroid 2-related factor; HO-1, heme-oxygenase"

Effect of l-carnitine on PI3K and TLR4 gene expression in rats receiving TAA:

Fig 5a and Fig. 5b show a significant ($P < 0.05$) increase in TLR4 gene expression and a significant ($P < 0.05$) decrease in PI3K gene expression in TAA group when compared to the control group. The LC treatment markedly ($P < 0.05$) boosted the mRNA expression of PI3K in comparison to the TAA group while markedly ($P < 0.05$) decreased the expression of TLR4.

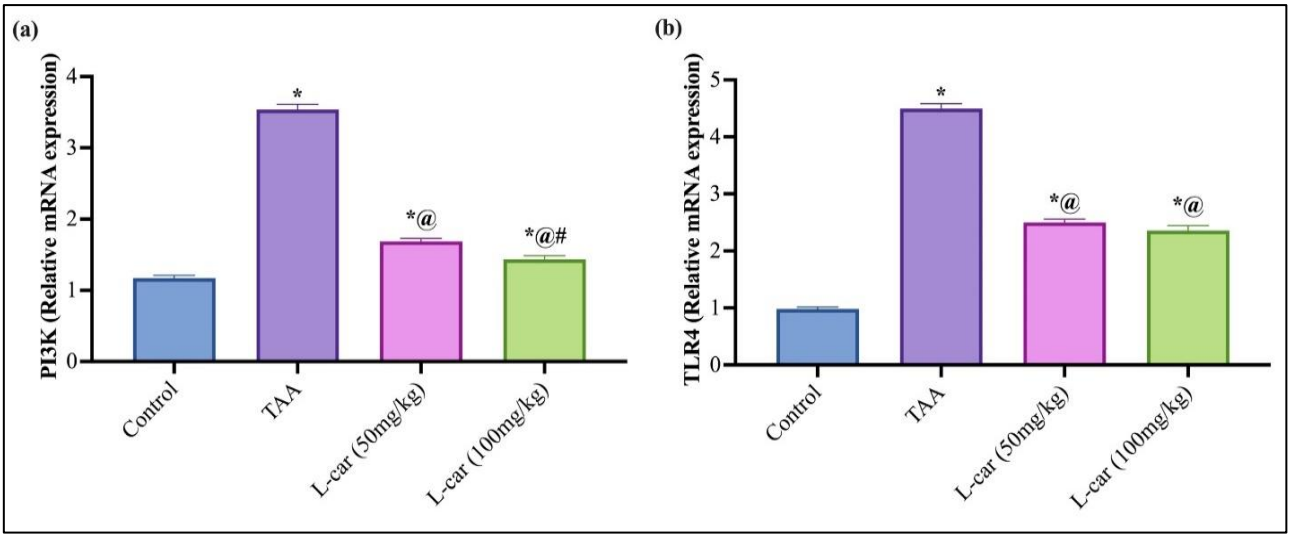


Fig 5: Effect of l-carnitine on the hepatic PI3K and TLR4 gene expression in rats receiving TAA: (Fig 5.a) PI3K, (Fig 5.b) TLR4. The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. "TAA, Thioacetamide; LC, L-carnitine; PI3K, phosphoinositide 3-kinase; TLR4, Toll-like receptor 4"

Effect of l-carnitine on the liver histological examination results in rats receiving TAA:

Livers of control rats showed normal portal triads, central veins, and hepatic parenchymal cells, all of which were consistent with normal histological structure (Fig 6a). While considerable fibroplasia was visible in the livers of TAA-administered rats, appeared as enhanced fibrous tissue proliferation in portal locations, followed by portal-to-portal bridging fibrosis and variable-sized nodular regeneration (Fig 6b). In addition to oval cell hyperplasia, proliferation of the bile duct epithelium, blockage of the blood vessels, and infiltration of mononuclear inflammatory cells were also visible in the later portal areas (Fig 6c). There was a noticeable parenchymal pseudo-lobulation caused by the outward expansion of variable lengths fibrous bands toward the parenchyma (Fig 6d). These pseudo-lobules contained liver cells that had vacuolar degeneration, necrosis, and dispersed apoptosis. Along the fibrous septa, it was possible to see lymphoplasmacytes and some histocyte infiltrates, proliferating bile ductules, and congested blood vessels.

Microscopic analysis of liver sections of the LC treated groups, different degrees of fibrous proliferative regression were visible, particularly in the group of rats given a high dose of LC (100 mg/kg). The livers of the low dose LC treated group (50 mg/kg) showed minor fibroplasia in the portal areas, a few infiltration of inflammatory cells, and mild bile duct epithelial proliferation (Figs 6e and f). Some portal triads displayed incomplete thin fibrous strands that were extending out without bridging. Hepatocellular degeneration, sporadic necrosis, and apoptosis were all evident to a modest extent. The group treated with a high dose of LC displayed sparse fibrous tissue growth in their hepatic portal areas along with modest alterations, mild hepatocellular degenerative and necrotic changes cells (Figs 6g and h).

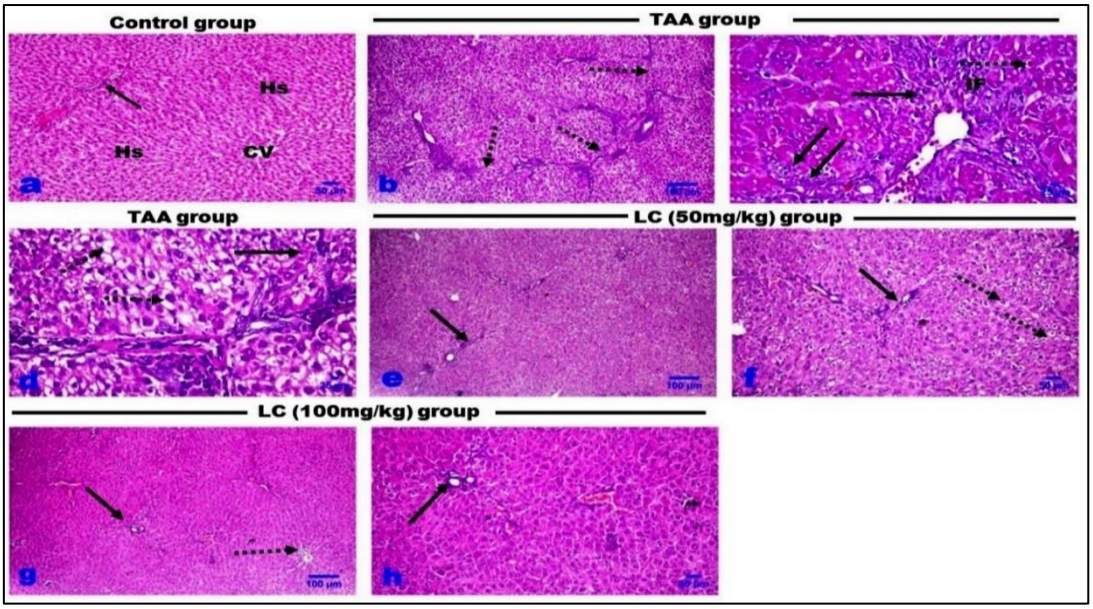
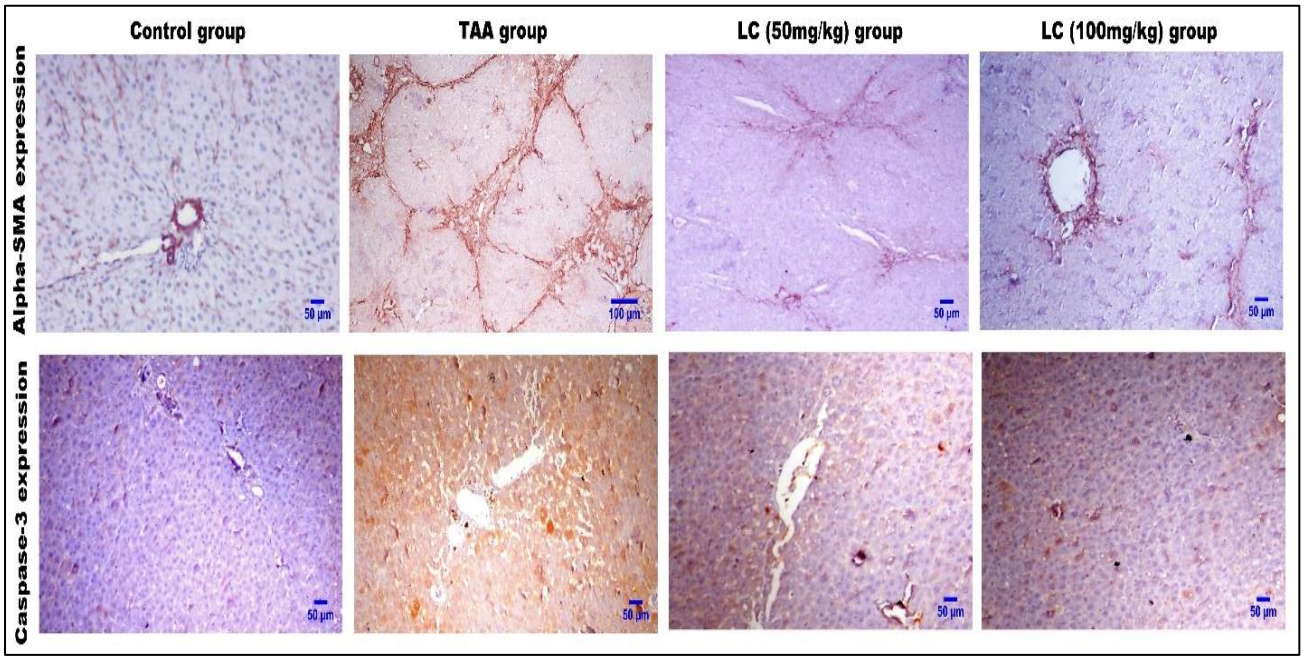


Fig 6: Effect of l-carnitine on the liver histological examination results in rats receiving TAA: H&E-stained liver sections: (a) Control rat's liver showing normal hepatocytes (Hs), central vein (CV), and portal area (arrow). (b-d) liver of TAA-administrated rat showing; (b) portal-to-portal bridging fibrosis (dotted arrow), nodular regeneration (NR), (c) portal area fibroplasia with inflammatory cells infiltration (IF) and proliferation of bile duct epithelial cells (arrow), and apoptotic bodies (dotted arrow), (d) dispersed apoptosis (arrow) and hepatocellular vacuolar degeneration (dotted arrow). (e and f) rats treated with LC at a dose of 50 mg/kg had a liver that displayed mild portal tract fibrosis and incomplete thin fibrous strands extending peripherally from certain areas (arrow), and considerable hepatocellular vacuolation (dotted arrow). (g and h) Liver of LC (100mg/kg) treated rat showing scarce portal fibrosis (dotted arrow), some triads are near to normal (arrow) and very mild degenerative changes in the hepatic cells

Effect of l-carnitine on the immunohistochemistry expressions of caspase-3 and α-SMA in rats received TAA:

TAA injection in rats (I/P) significantly elevated the immune-expressions of α-SMA and caspase-3 (Figures 7) in their livers in comparison to the treated and control sets. LC (50 and 100 mg/kg) administration markedly reduced α-SMA and caspase-3 expression, especially in livers of the high dose treated group (Figure 7). Comparing the TAA-treated group to the other therapy groups, the quantitative analysis of the positive brown color of both α-SMA and caspase-3, expressed as staining score, revealed a significant ($P < 0.05$) expression of both proteins (Figure 7).



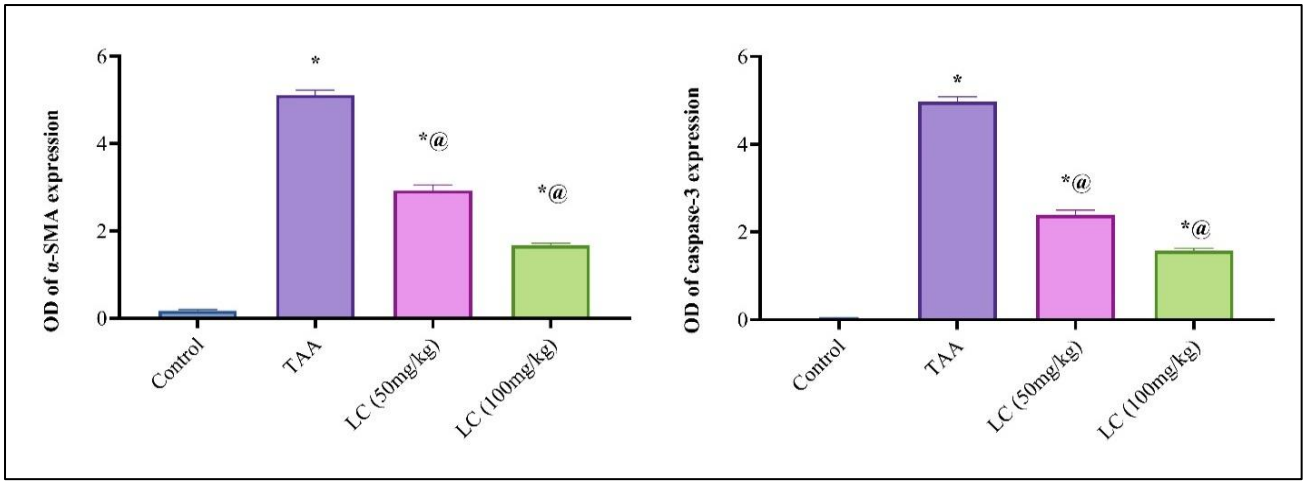


Fig 7: Effect of l-carnitine on the immunohistochemistry expression of α-SMA and caspase-3. Photomicrographs of liver of various groups presenting the immune-expression of α-SMA and caspase-3, notice the marked expression of α-SMA in TAA group in portal areas, in the bridging fibrous septa and among the hepatic cells as well as, the marked expression of caspase-3 in TAA group compared with control and LC treated groups. The six rats' mean± SE is represented by each bar. *control group, @vs. TAA group. TAA, Thioacetamide; LC, L-carnitine; α-SMA, α-smooth muscle actin

DISCUSSION

In order to closely mimic human liver fibrosis and cirrhosis, the organosulfur chemical Thioacetamide (C2H5NS) is widely used to induce efficient animal models for acute and long-term liver damage [23]. Via the CYP450, TAA is changed into the extremely hazardous reactive metabolite TAA-sulfur dioxide (TAA-S-dioxide), which attaches to liver parenchyma and macromolecules, triggering necrosis and activating HSCs [24]. TAA S-dioxide compromises the integrity and stability of cellular membranes, triggering them to become more permeable and allowing the escape of liver enzymes, AST and ALT [25]. Our study results demonstrated that TAA significantly damaged the liver, showed by a significant rise in serum ALT and AST activities and a marked drop in albumin level. These results agreed with those of earlier investigations [26, 27]. The lowered enzyme activity and restored serum albumin level show that treatment with LC significantly preserved hepatocyte integrity in a dose-dependent manner. Our data proved that LC offers protection against liver damage caused by TAA. Our findings are consistent with earlier research [15, 28].

The antioxidant defense mechanisms, which include antioxidant enzymes (SOD, CAT, etc.) and non-enzymatic (GSH, etc.) reduce oxidative stress formation and clearance in a cellular homeostasis [29]. The development of numerous hepatic disorders is ultimately caused by a pro-oxidant/antioxidant imbalance that results in the depletion or inactivation of antioxidants in hepatocytes [30]. In our investigation, TAA triggered a significant oxidative stress, which was demonstrated by a marked elevation in liver MDA level and a noticeably lower levels of SOD and GSH. These results are consistent with prior investigations [31, 32]. Inhibiting lipid peroxidation and increasing SOD activity and GSH levels in LC treated groups allowed us to assess the antioxidant ability of LC against oxidative stress [33]. Previous studies [34, 35]. were consistent with the present findings

An essential mechanism for controlling TAA-induced oxidative damage and inflammation is the Nrf2/HO-1 pathway. Inhibiting oxidative stress and the inflammatory response is a defense mechanism that is mediated by Nfr2 and its downstream antioxidant genes [36, 37]. Specific genes for antioxidant defense, including SOD, CAT, HO-1, and GSH, are regulated by Nrf2-ARE pathway activation [38]. When Nrf2 is stimulated and translocates to the nucleus, it activates the transcription of anti-oxidant genes through phosphorylation of AKT by activated phosphatidylinositol kinase (PI3K). According to this study, the hepatic Nrf2/HO-1, and PI3K expressions were downregulated in the TAA group but were upregulated in LC treated groups. These findings are consistent with earlier ones [20]. In HL7702 hepatocytes treated with hydrogen peroxide (H_2O_2), LC pretreatment was found to improve Nrf2 nuclear translocation, DNA binding activity, and HO-1 expression [39], which agrees with our findings. Therefore, an increase in nuclear Nrf2 expression that coincides with an increase in endogenous antioxidant defense may constitute LC's protective mechanism in TAA-induced liver fibrosis. These results demonstrated the critical contribution of the Nrf2/HO-1 pathway to the antifibrotic activity of LC.

The major pathway involved in the production and release of inflammatory mediators during inflammation, is the TLR4/NF-κB signaling pathway [40, 41]. TLR4 is crucial for controlling inflammation, HSC activation, and liver fibrosis [42]. TNF-α and IL-1β play a role in the pathogenesis of inflammatory liver diseases [43]. In the TAA group, TLR4 and its downstream target cytokines, IL-1β and TNF-α, were increased. The findings of this study are consistent with

earlier researches [44, 45]. LC treated groups showed a marked reduction in TLR4 level and expression, which was in parallel with the decrease in the levels of its downstream inflammatory cytokines, IL-1β and TNF-α. Previous studies are consistent with our findings [46, 47]. It's possible to explain LC's anti-inflammatory effects by the fact that it suppressed TLR4 pathway [48].

In the fibrogenic liver, HSCs proliferate, migrate, and transformed into myofibroblasts like cells [49]. Smooth muscle actin (α-SMA) is the most common myofibroblasts marker [50]. Immunohistochemical evaluations of liver sections in our investigation revealed increased α-SMA positive cells in the TAA group. Due to liver injury, HSCs become more active, which causes an increase in α-SMA release [51, 52]. As a result, monoclonal α-SMA antibody immunohistochemical staining indicates the presence of activated HSCs. Expression of α-SMA was significantly downregulated by LC treatment. This outcome is consistent with prior research [53, 54].

Additionally, TAA caused an increase in caspase-3, a sign of apoptosis [55]. Caspase-3 is associated with liver fibrosis and is a sensitive indication of liver damage [56]. The increased caspase-3 expression in the TAA group is thought to indicate the death of hepatocytes that resulted from the inflammation. Both dosages of LC considerably reduced the increase of caspase-3, indicating a protective and anti-apoptotic effect of LC. This outcome is consistent with other investigations [57, 58].

Histological analysis in the current investigation verified that LC had a protective effect against harmful effects of TAA. When combined, the findings of this research indicate that LC may activate the hepatic Nrf2/HO-1 signaling pathway, which may help to lessen the negative consequences of oxidative stress induced via TAA. Additionally, The TLR4 signaling pathway may be an important target for LC in the prevention of liver fibrosis.

CONCLUSION

Liver fibrosis induced by TAA is efficiently prevented in the liver by L-carnitine. The increased activity of underlying antioxidants and the decrease of oxidative stress through the Nrf2/HO-1 signaling pathway are two signs that LC has an antioxidant impact. LC reduces the production of pro-inflammatory cytokines by blocking the TLR4 pathway. Moreover, the biochemical data are confirmed by the outcomes of histopathogy and immunohistochemistry. These results show that LC as a potential agent for prevention of liver fibrosis in rats.

Declarations:

All experimental and animal management procedures in this study followed the ARRIVE guidelines. The current study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Cairo, University, Egypt. The approval number is: Vet CU 09092023791, dated at 5/ 11/ 2023.

Author Contributions:

Mostafa A. Shalaby and Amer Ramadan: suggested the subject, planned it and wrote the draft manuscript. Sahar S. Abd El-Rahman: performed histopathology and immunohistochemistry.

Hany M. Fayed: prepared charts and figures. Mostafa A. Shalaby: wrote the final manuscript,

Data availability: The authors declare that all relevant raw data will be freely available.

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Consent for publication: Not applicable

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Competing Interest: The authors declare that there are not competing interests

Acknowledgements: Not Applicable

Data Availability: All relevant data are within the manuscript.

Abbreviations:

TAA: Thioacetamide
Nrf2: Nuclear factor erythroid 2-related factor 2
TLR4: Toll-like receptors
MDA: Malondialdehyde
SOD: Superoxide dismutase
CAT: Catalase
TNF-α: Tumor necrosis factor
PI3K: Phosphoinositide 3-kinase
H_2O_2: Hydrogen peroxide
HO-1: Heme oxygenase-1

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