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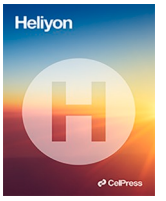

Research article

Protective effects of L-carnitine on isoprenaline -induced heart and kidney dysfunctions: Modulation of inflammation and oxidative stress-related gene expression in rats

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ABSTRACT

The aim of this study was to evaluate the effect of L-carnitine (L-CAR) treatment on isoprenaline (ISO) administered kidney and heart impairment in male Long Evans rats. Four groups of rats were engaged in this study such as control, ISO, control + L-CAR, and ISO + L-CAR, where n = 6 in each group. The rats were also provided with chow food and water ad libitum. At the end of the study, all rats were sacrificed, and blood and tissue samples were collected for bio-chemical analysis. Oxidative stress parameters and antioxidant enzyme activities were determined in plasma and tissues. Antioxidant and inflammatory genes expression were analyzed in the kidney cortex, and histopathological studies of kidney tissues were performed. This study showed that creatinine and uric acid in plasma were significantly increased in ISO-administered rats. L-carnitine treatment lowered the uric acid and creatinine level. ISO-administered rats showed increased lipid peroxidation and declined levels of antioxidant enzymes activities in kidneys and heart. L-carnitine treatment restored antioxidant enzymes activities and protect against oxidative stress in kidney and heart. This effect is correlated with the restoration of Nrf-2-HO-1 genes expression followed by increased SOD and catalase genes expression in the kidney. L-carnitine treatment also prevented the TNF-α, IL-6, and NF-κB expression in kidneys of ISO administered rats. Histopathology staining showed that L-carnitine treatment prevented kidney damage and collagen deposition in ISO administered rats. The result of this study exhibited that L-carnitine treatment reduced oxidative stress and increased antioxidant enzyme activities by enhancing antioxidant genes expression in ISO administered rats.

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1. Introduction

Chronic kidney disease is responsible for many deaths and disabilities among the world population, especially in Asia [1]. Almost 1.4 million deaths were recorded globally by The Global Burden of Disease study from 2010 to 2019 [2]. The prevalence of developing CKD is increasing day by day in developing countries as well as developed countries [3]. CKD is involved in the development of several other diseases like hypertension, cardiovascular diseases, and end-stage renal dysfunction which may require costly management processes like dialysis and kidney transplantation [4]. Obesity and diabetes are two major risk factors for the development of CKD and cardiovascular diseases [5]. Other risk factors include pesticides, heavy metals, smoking, and underground water with high fluoride and arsenic levels, might be associated with the development of CKDs in South Asian populations [4,6].

Systemic inflammation and oxidative stress are closely linked to the pathogenesis of CKD. C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor- (TNF-α), adipokines, and adhesion molecules are thought to be the main inflammatory markers in the development of CKD [7]. Increased expression of cytokines such as TNF-α may increase the risk of heart failure in chronic kidney disease [8]. In CKD patients, NF-κB activation positively controls the expression of inducible nitric oxide synthase (iNOS) and the production of cytokines [7]. Reactive oxygen species (ROS) can be overproduced due to inflammation and CKD progression. Elevated ROS levels have been observed in a number of investigations in CKD patients and animal models of renal damage [9]. Early CKD stages have been linked to increased oxidative stress [10]. It is conceivable that the kidneys' two main ROS producers are the mitochondrial respiratory chain and enzymes like NADPH oxidase (NOX). Via the induction of pro-inflammatory mediators in renal failure, it may potentially further amplify the inflammatory response [11,12]. Impairment in nuclear factor erythroid 2-related factor 2 (Nrf2) responses is also associated with renal failure. Superoxide dismutase (SOD) and catalase are two examples of enzymes and proteins involved in detoxification and antioxidant defense. Nrf2 is a transcription factor that controls their genes [12,13]. Decreased SOD levels have been observed in CKD, suggesting that increased superoxide is associated with oxidative stress in renal failure [11].

A synthetic adrenergic receptor agonist is called isoprenaline (ISO). Oxidative damage in the heart and kidneys may be exacerbated by high dosages of ISO [14,15]. ISO administration in rats causes infarcts, including cardiac lesions that eventually develop inflammation and fibrosis [15]. A previous report also showed that ISO administration in rats declined antioxidant enzyme activities and increased lipid peroxidation [16]. A potential way of mitigating oxidative stress-related complications and kidney and cardiovascular disorders would be antioxidant therapy. Our previous investigation showed that antioxidants may prevent lipid peroxidation and restore the antioxidant enzymes function in the heart and kidney of ISO-administered rats [15,16].

L-carnitine (L-CAR) is a natural antioxidant, which is produced from essential amino acids like lysine and methionine, and can be obtained from the brain, kidney, and liver [17,18]. About 40–50 μM/L of L-CAR is available in the muscle which is 70-fold higher than plasma [19]. L-CAR depletion may develop pathogenesis such as skeletal muscle myopathy and cardiomyopathy [20]. Carnitine acetyltransferase uses carnitine as a substrate to transfer fatty acids into the inner matrix of mitochondria [21]. In the case of myocardial function, L-CAR may play a key role and enhance myocardial fat metabolism by utilizing free fatty acid [22]. Previous reports suggest that L-CAR treatment is beneficial in various experimental disease models such as oxygen-induced retinopathy [23], imatinib-induced cardiotoxicity [24], tilmicosin-induced cardiac toxicity [25], and monosodium glutamate-induced nephrotoxicity [26]. L-CAR prevented the rise of caspase −9 expression and increased the Bcl-2 expression in kidneys of rats received monosodium glutamate [26]. Another report suggests that L-CAR may prevent renal apoptosis probably by enhancing adenosine mono phosphate kinase (AMPK) mediated peroxisome proliferator-activated receptor-γ (PPAR-γ) activation in carboplatin induced renal impairment in mice [27]. L-CAR also prevented the methotrexate induced renal oxidative stress probably by upregulates the silent information regulator 1 (SIRT1) and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) [28]. The nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) level were also increased in kidneys of methotrexate induced male Sprague-Dawley rats [28]. However, increased adrenergic stimulation is one of the major causes of chronic kidney impairment [29], the beneficial effect of L-CAR administration in such condition was not well studied and the mechanisms are little explained to date. Thus, in this investigation, the beneficial effect of L-CAR treatment was assessed on isoprenaline (ISO) induced oxidative stress, heart and kidney dysfunction in Long Evans male rats. Moreover, the genes related to antioxidants and inflammation pathways in kidney were also evaluated.

2. Methods and materials

2.1. Experimental animals

Long Evans rats were used in this investigation, which were collected from the animal house at North South University, Department of Pharmaceutical Sciences, Dhaka, Bangladesh. For this study, 24 male rats, each about 8–10 weeks old and weighing about 180–190 g were used. Environmental conditions were provided according to standard animal house condition, consisted with 12 h/12 h light and dark cycle, relative humidity of about 55 %, and temperature of about 25 ± 2 °C. Guidelines were followed according to The Council for International Organization of Medical Sciences and The International Council for Laboratory Animal Science. Furthermore, Ethical clearance and experimental protocols were approved by the Institutional Animal Care and Use Committee, North South University (AEC 012–2018).

2.2. Experimental design

The rats were divided into four groups, each group consisting of 6 rats. The groups were as such.

1. Control group.
2. ISO (Isoprenaline) group.
3. Control + L-CAR (L-carnitine) group.
4. ISO + L-CAR group.

The control group was fed a diet consisting of chow food and drinking water. The ISO group was administered with 50 mg/kg of isoprenaline subcutaneously every 3 days for 2 weeks. Water and chow food were given to the ISO group along with the isoprenaline. The Control + L-CAR group was also given chow food and water and additionally, L-CAR was also administered orally at a dose of 100 mg/kg daily for 2 weeks. The group ISO + L-CAR was given both ISO and L-CAR. The ISO + L-CAR group received isoprenaline subcutaneously at a dose of 50 mg/kg every 3 days for 2 weeks and L-CAR was administered orally, using a stainless steel gavage needle, at a dose of 100 mg/kg daily for 2 weeks. The ISO + L-CAR group was additionally fed chow food and water. After completion of 14 days of the procedure, the rats were weighed and then sacrificed on the 15th day. Blood was collected and centrifuged at 1600 g for 15 min, with a temperature of 4 °C in order to collect the plasma. Following that, the required organs (kidney and heart) were collected and weighed. The harvested organs, kidneys and heart tissues were stored for bioassay and one part of the kidney was stored in neutral buffer formalin for the use of histology staining. The plasma and tissues collected for bioassay were stored at −18 °C.

2.3. Drugs and chemicals

Isoprenaline hydrochloride was purchased from Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). Standards and all other reagents for MDA, NO, APOP assays, and Picrosirius red staining reagents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). SOD standard and other assay components were purchased from SR Group (Delhi, India). Assay kits for creatinine kinase-muscle brain (CK-MB), uric acid and creatinine were purchased from DCI diagnostics (Budapest, Hungary). GeneJET RNA Purification Kit, RevertAid First Strand cDNA Synthesis Kit (Catalog number: K1621) and SYBR™ Green PCR Master Mix were purchased from Thermo Fisher Scientific Inc. (Waltham, Massachusetts, United State of America). L-carnitine was received from General Pharmaceuticals Pvt. Ltd, Dhaka, Bangladesh as a gift sample.

2.4. Assessment of creatinine kinase-muscle brain (CK-MB) activities in plasma and kidney-specific marker such as creatinine, and uric acid level in plasma test

CK-MB activities were also assessed using assay Kit followed by the manufacturer's protocol. Kidney specific marker tests such as creatinine, and uric acid were also performed using plasma following the manufacturer's protocol.

2.5. Tissue homogenization for oxidative stress and antioxidant enzyme activity

The kidney and heart tissues were homogenized in phosphate buffer solution (pH 7.4) to assess oxidative stress and antioxidant enzyme activity parameters. The tissues were also centrifuged for 15 min at 4 °C, at 5000 g. After centrifugation, the supernatant was collected for analysis of oxidative stress and antioxidant enzyme activity parameters.

2.6. Determination of malondialdehyde (MDA)

To determine MDA, a colorimetric test was performed using thiobarbituric acid reactive substances [30]. A combination of TBA-TCA-HCl was taken with a ratio of (1:1:1), where the reagent was composed of equal amounts of TBA (0.37 %), TCA (15 %), and HCl (0.25 N). This homogenate mixture was then added with 0.1 mL of supernatant tissue as mentioned earlier and kept in water bath for about 15 min. After 15 min, the mixture was allowed to cool to room temperature before measuring the clear supernatant at 535 nm against a reference blank.

2.7. Determination of nitric oxide (NO)

In order to determine nitric oxide (NO), both nitrate and nitrite were determined by following a previous study [30]. The Griess-Illosvay reagent was used and was adjusted by naphthyl-ethylenediamine-dihydrochloride (0.1 % w/v). 0.5 mL of phosphate buffer saline and 2 mL of supernatant or plasma were added and then incubated for more than 2 h. The unit expression was signified as nmol/mL and nmol/g tissue. The absorbance taken was about 540 nm against a blank solution.

2.8. Determination of advanced oxidation protein product (AOPP)

AOPP concentration was calculated following a previously established protocol [30]. Compounds such as acetic acid (0.2 mL) and potassium iodide (1.16 M) in 0.1 mL amount were used with plasma and phosphate buffer solution in the ratio of 1:5. This sample was

kept still for 2 min while a blank was measured at the absorbance of 340 nm. The absorbance of the sample was immediately measured after measuring the blank.

2.9. Estimation of myeloperoxidase (MPO)

The method for MPO activity assay was described in a previous study [31]. To use this method, di-anisidine-H_2O_2 was used. Potassium phosphate buffer (pH 6.0, 50 mM) and 10 μl plasma sample or supernatant samples were mixed together. To this solution, H_2O_2 (0.15 mM) and *O*-dianisidinedihydrochloride (0.53 mM) were then added and mixed homogeneously. Absorbance was taken at 460 nm.

2.10. Determination of catalase

The catalase activity was measured by following a method described previously (29). Three compounds were mixed for this test, sample (0.1 mL), phosphate buffer solution (Concentration of 50 mmol at pH 5.0 and amount 2.5 mL), and H_2O_2 (5.9 mmol of 0.4 mL). The time interval for absorbance was about 1 min and absorbance was 240 nm.

2.11. Determination of superoxide dismutase (SOD)

Following a previous study, SOD was measured [31]. Phosphate buffer (90 μl) was mixed with sample (10 μl) and adrenaline (100 μl). The absorbance was recorded at 490 nm.

2.12. Determination of reduced glutathione (GSH)

As described in a previous study, glutathione was estimated [31]. Sulphosalicylic acid (about 1 mL) was mixed with the sample (1 mL) and the sample mixture was stored in ice. Centrifugation was done to the mixture at 4 °C for 20 min at 80 g. After centrifugation, 0.2 mL DTNB (100 mM) was mixed with 3 mL of sample and 2.7 mL of phosphate buffer solution (0.1 M). Absorbance was taken immediately at the absorbance of 412 nm.

2.13. RT-PCR for anti-oxidant and inflammation gene expression

Kidney cortex tissue was used for RT-PCR and mRNA extracted using an RNA purification kit purchased from Thermo-Fisher Scientific (MA, USA). The amount of RNA samples was then measured with a NanoDrop 2000 (Bio-Rad, California, USA). From these samples, about 1 μm samples were taken for the next step generation known as cDNA according to the manufacturer's protocol of RevertAid First Strand cDNA Synthesis Kit (Thermo-Fisher Scientific, USA). Target genes were designed using the Primer 3 online

Table-1
The forward and reverse sequence of the primer applied in this experiment.

Name of gene	Type	Sequence
Nrf-2	Forward	5′-CCC AGCACA TCC AGACAGAC-3′
	Reverse	5′-TATCCAGGGCAAGCGACT C-3′
Heme oxigenase-1 (HO-1)	Forward	5′-TGCTCGCATGAACACTCTG-3′
	Reverse	5′-TCCTCTGTCAGCAGTGCCT-3′
Heme oxigenase-2 (HO-2)	Forward	5′-CACCACTGCACTTTACTTCA-3′
	Reverse	5′-AGTGCTGGGGAGTTTTAGTG-3′
MnSOD	Forward	5′-GCTCTAATCACGACCCACT-3′
	Reverse	5′-CATTCTCCCAGTTGATTACATTC-3
Catalase	Forward	5′-ATTGCCGTCCGATTCTCC-3′
	Reverse	5′-CCAGTTACCATCTTCAGTGTAG-3′
Glutathione peroxidase (GPx)	Forward	5′-GGGCAAAGAAGATTCCAGGTT-3′
	Reverse	5′-GGACGGCTTCATCTTCAGTGA-3′
IL-1	Forward	5′-ATGCCTCGTGCTGTCTGACC-3′
	Reverse	5′-CCATCTTTAGGAAGACACGGGTT-3′
IL-6	Forward	5′-AGCGATGATGCACTGTCAGA-3′
	Reverse	5′-GGTTTGCCGAGTAGACCTCA-3′
TNF-α	Forward	5′-ATGTGGAACTGGCAGAGGAG-3′
	Reverse	5′-CCACGAGCAGGAATGAGAAGAG-3′
TGF-β	Forward	5′-AAGAAGTCACCCGCGTGCTA-3′
	Reverse	5′-TGTGTGATGTCTTTGGTTTTGTC-3′
iNOS	Forward	5′-TGGTCCAACCTGCAGGTCTTC-3′
	Reverse	5′-CAGTAATGGCCGACCTGATGTTG-3′
NF-κB	Forward	5′-TGTGAAGAAGCGAGACCTGGAG-3′
	Reverse	5′-GGCACGGTTATCAAAAATCGGATG-3′
β-Actin	Forward	5′-GCGAGAAGATGACCCAGATC-3′
	Reverse	5′-GGATAGCACAGCCTGGATAG-3′

software (Table 1). Quantitative RT–PCR was then analyzed using Maxima SYBR Green qPCR master mix (Thermo-Scientific, USA). Finally, PCR was performed according to the previously described method (29) and the machine used was the CFX96 C1000 Touch real-time PCR detection system "Bio-Rad, California, USA". Data were collected and analyzed using CFX Manager™ software (Bio-Rad, CA, USA), and mRNA levels of various genes at the transcript level were normalized using standard β-actin.

2.14. Histopathological studies

Kidney tissue was kept in neutral buffered formalin for several days, a process known as fixation. Kidney tissue was fixed, treated with ethanol and xylene, and embedded in paraffin blocks. After that, it was sliced at a thickness of 5 μm to prepare the section slides. All the tissue sections were then stained with Hematoxylin and eosin. Sirius staining was also performed. Finally, images were taken using a microscope at 40× magnification.

Histological scoring system on kidney damage in lab animals were also used, based on the EGTI scoring system, in which 4 separate components such as *endothelial, glomerular, tubular*, and *interstitial* damage in kidney sections were examined [32,33]. The percentages of fibrosis were also determined in kidney and heart sections using Image J free software (Version 4.0) from National Institute of Health of United State of America [15,34].

2.15. Statistical analysis

All the values were expressed as mean ± SEM (Standard error of mean). For statistical significance, One Way ANOVA followed by Newman Keuls multiple comparison post hoc test was performed using GraphPad Prism 9 software. Overall statistical significance was assessed as $p < 0.05$ in all cases.

3. Results

3.1. Effect of L-carnitine on total heart wet weight, LV heart wet weight, and RV heart wet weight and kidneys wet weight in isoprenaline (ISO) administered rats

Fig. 1A shows the total wet weights of the hearts. The LV heart wet weight rose considerably in the ISO group **($p < 0.001$)** compared to the control group (Fig. 1A). On the other hand, when compared to the ISO group, the weight of the ISO + L-CAR group was considerably lower **($p < 0.01$)** (Fig. 1A). Moreover, when compared to the ISO group, the Control + L-CAR group's LV heart wet weight decreased significantly **($p < 0.0001$)** (Fig. 1A). RV heart-wet weight did not differ significantly **($p > 0.05$)** across any of the groups (Fig. 1A).

In terms of kidney wet weight, there was no discernible difference between the ISO group and the control group **($p > 0.05$)** (Fig. 1B). Rats in the ISO + L-CAR group showed no variation in wet kidney weight compared to the ISO group **($p > 0.05$)** (Fig. 1B). Moreover, there were no differences in kidney wet weight between the control and Control + L-CAR group (Fig. 1B).

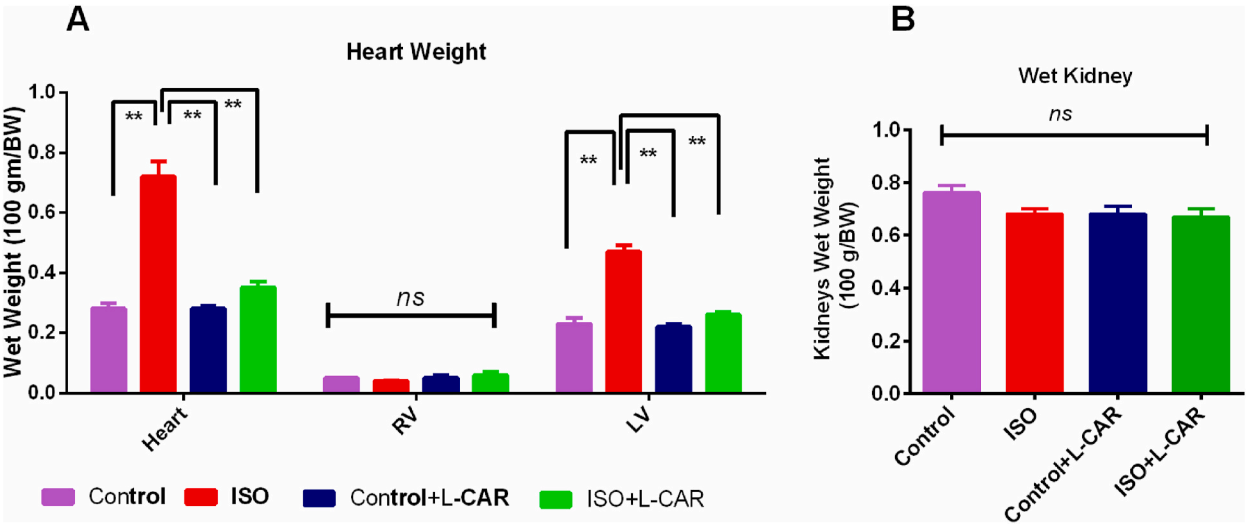

Fig. 1. Effect of L-carnitine on heart, right ventricle (RV), left ventricle (LV) weight and kidney wet weight. A. Heart wet weights; B. Kidney wet weight, C. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

3.2. Effect of L-carnitine on plasma uric acid and creatinine level in isoprenaline (ISO) administered rats

For each group, the plasma uric acid concentration was assessed. When compared to the control group, the ISO group had a higher concentration of uric acid **($p < 0.01$)** (Fig. 2A). The uric acid plasma concentration was reduced significantly **($p \leq 0.01$)** for the ISO + L-CAR group compared to the ISO group (Fig. 2A). No abnormalities in plasma uric acid concentration was observed in the Control + L-CAR group (Fig. 2A).

Rats that received subcutaneous administration of ISO, increased the levels of creatinine plasma concentration **($p < 0.01$)** compared to the control group (Fig. 2B). Comparing the ISO + L-CAR group to the ISO group, the ISO + L-CAR group significantly normalized creatinine plasma levels **($p < 0.01$)** (Fig. 2B). Creatinine plasma concentrations were normal **($p < 0.01$)** in the Control + L-CAR group compared to the ISO group (Fig. 2B).

3.3. Effect of L-carnitine on CK-MB activities in plasma and myeloperoxidase (MPO) activities in kidney cortex of isoprenaline (ISO) administered rats

ISO-administered rats showed an increased CK-MB and MPO activity in plasma and kidneys **($p < 0.01$)** respectively compared to the control group (Fig. 3A and B). Rats administered with ISO raised CK-MB activity in the heart significantly **($p < 0.001$)** compared to the Control group (Fig. 3A and B). ISO administered rats treated with L-CAR showed decreased CK-MB activity in plasma significantly **($p < 0.01$)** compared to rats that only received ISO (Fig. 3A).

ISO administered rats treated with L-CAR showed decreased MPO activity in kidney significantly **($p < 0.01$)** compared to the ISO administered rats (Fig. 3B). L-CAR treatment in control rats did not alter the CK-MB or MPO activities compared to the control rats (Fig. 3).

3.4. Effect of L-carnitine on MDA in plasma, kidneys and heart of isoprenaline (ISO) administered rats

The first parameter, known as MDA, a by-product of lipid peroxidation, was significantly increased in plasma and kidney of ISO-treated rats compared to controls ($p < 0.01$) (Fig. 4A and C). The ISO + L-CAR group showed a declined concentration of MDA in plasma and MDA in the kidneys significantly **($p < 0.01$)** compared to the ISO group (Fig. 4A and C). The Control + L-CAR group did not show any alteration in the MDA levels in the plasma or the kidneys (Fig. 4A and C). In the heart, rats that received ISO showed increased levels **($p < 0.001$)** of MDA concentration compared to the control group (Fig. 4B). The ISO + L-CAR group significantly normalized MDA concentrations in the heart compared to the ISO group ($p < 0.01$) (Fig. 4B). The control + L-CAR group also showed normal levels of MDA concentrations in the heart **($p < 0.001$)** compared to the control group (Fig. 4B).

3.5. Effect of L-carnitine on NO and AOPP level in plasma, kidney, and heart in isoprenaline (ISO) administered rats

The next parameter for oxidative stress is NO. In the ISO group, NO concentration in plasma, kidneys, and heart were **increased** significantly **($p < 0.01$)** compared to the control group (Fig. 5A, B, 5C). Rats belonging to the ISO + L-CAR group showed lower NO concentration in plasma, kidneys, and heart **($p < 0.01$)** compared to the rats that were administered with ISO. This showed that L-CAR can normalize the NO concentrations in rats even after administration with ISO (Fig. 5A, B, 5C). The Control + L-CAR group showed a normal level **($p < 0.001$)** of NO concentration in plasma, kidney, and heart compared to the control group (Fig. 5A, B, 5C) (see Fig. 6).

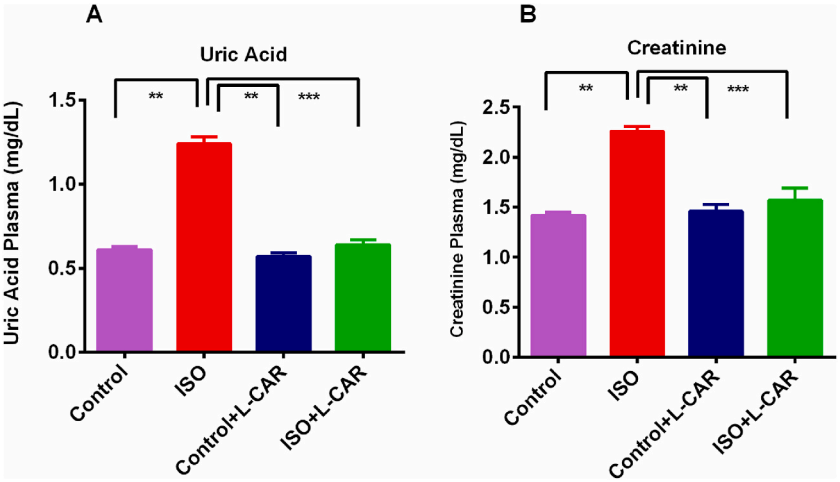

Fig. 2. Effect of L-carnitine on uric acid and creatinine level in ISO administered rats. A. Uric acid Plasma, B. Creatinine Plasma. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

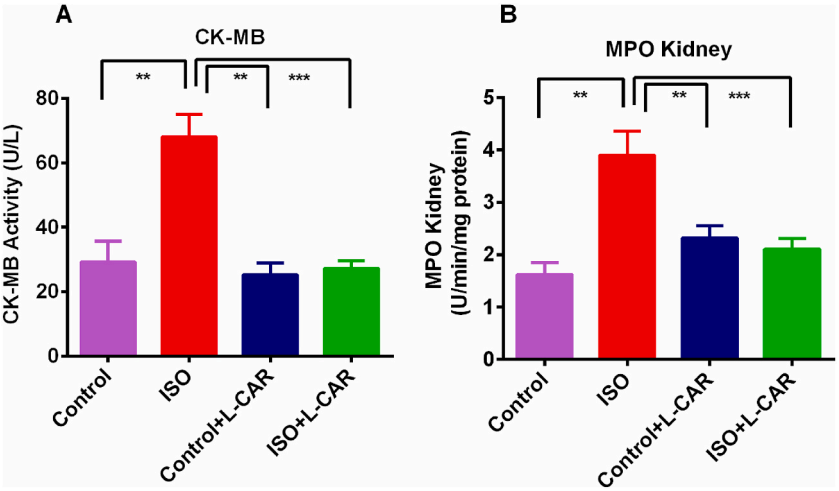

Fig. 3. Effect of L-carnitine on CK-MB activity in plasma and MPO activity in kidney tissues of ISO administered rats. A. CK-MB Plasma and B. MPO in Kidney. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

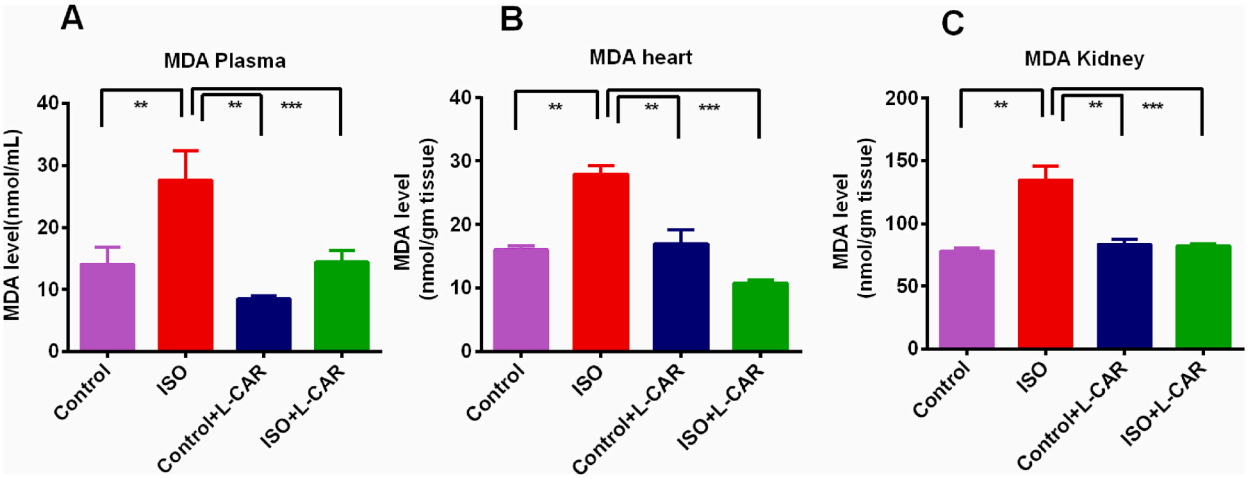

Fig. 4. Effect of L-carnitine on MDA level in plasma and tissues homogenates of ISO administered rats. A. MDA Plasma, B. MDA Kidney, C. MDA Heart. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

AOPP is another parameter of oxidative stress. The concentration of AOPP was increased **($p \leq 0.01$)** in plasma, kidneys, and heart by the administration of ISO compared to the control group **(6A, 6B, 6C)**. Rats that were treated with L-CAR and received ISO as well, showed declined levels of APOP in plasma, kidneys, and heart **($p < 0.01$)** compared to the rats which were only administered with ISO **(6A, 6B, 6C)**. Control + L-CAR group did not show any changes in APOP level in plasma, kidney, and heart compared to the control rats **(6A, 6B, 6C)**.

3.6. Effect of L-carnitine on SOD activity in plasma, kidney, and heart in isoprenaline (ISO) administered rats

SOD enzymatic activity was significantly decreased in plasma, kidney, and heart in ISO-administered rats compared to controls ($p \leq 0.01$) (Fig. 7A, B, 7C). Whereas, the SOD enzyme activity for the ISO + L-CAR group was restored in plasma, kidneys, and the heart significantly **($p < 0.01$)** compared to ISO administered rats. This indicates that L-CAR can improve the SOD effect even when administered with ISO **(7A, 7B, 7C)**. The Control + L-CAR group showed normal levels **($p < 0.001$)** of SOD enzyme activity in plasma, kidneys, and the heart compared to the control group **(7A, 7B, 7C)**.

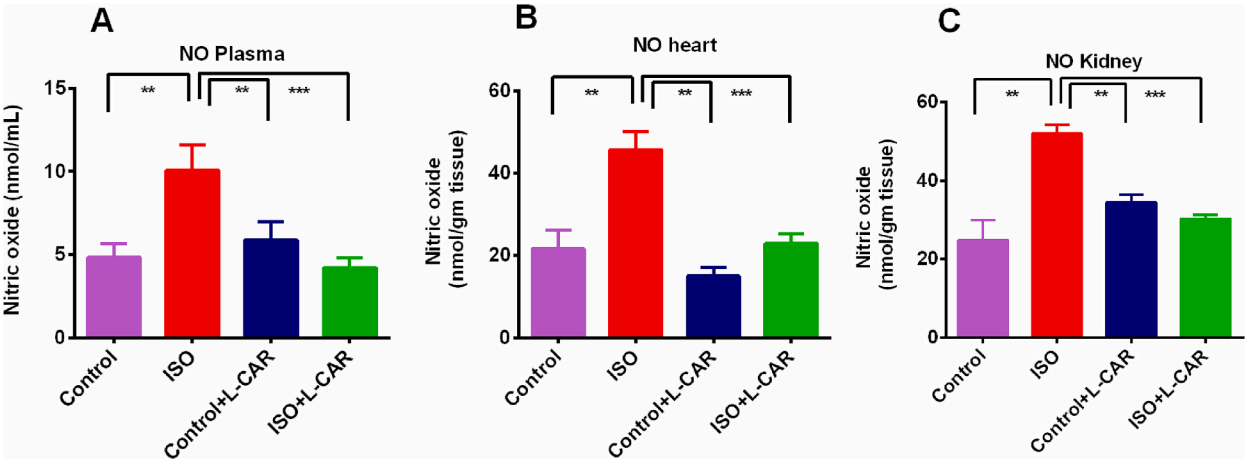

Fig. 5. Effect of L-carnitine on NO level in plasma and tissues homogenates of ISO administered rats. A. NO Plasma, B. NO Kidney, C. NO Heart,. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

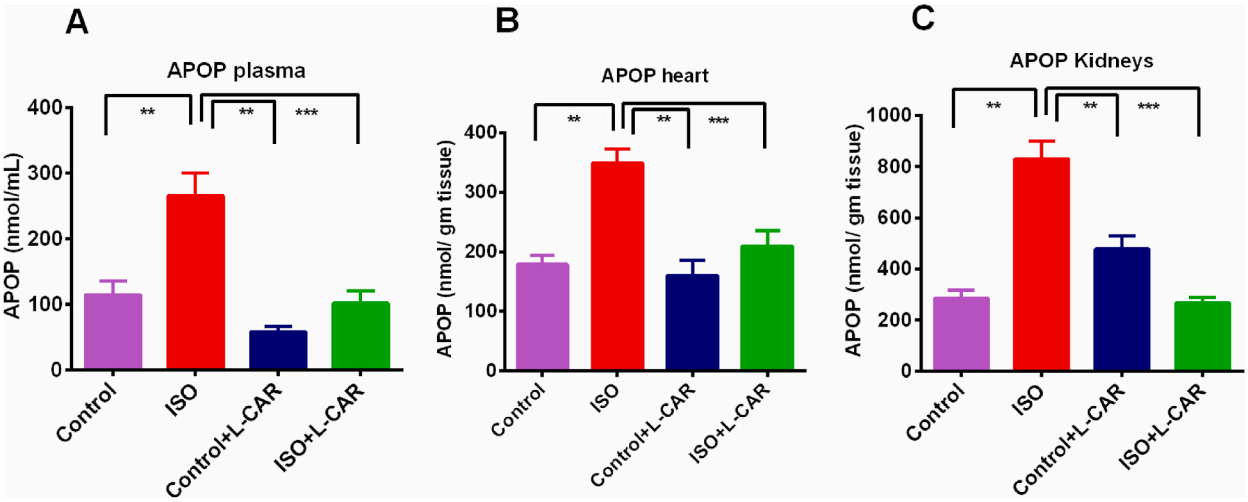

Fig. 6. Effect of L-carnitine on APOP level in plasma and tissues homogenates of ISO administered rats. A. APOP Plasma, B. APOP Heart, F. APOP Kidney. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

3.7. Effect of L-carnitine on catalase activity in plasma, kidney, and heart in isoprenaline (ISO) administered rats

Rats receiving ISO showed decreased catalase enzymatic activity in plasma and kidney compared to the controls ($p < 0.01$) (Fig. 8A, B, 8C). Rats belonging to the ISO + L-CAR group retained catalase enzyme activity in plasma and kidneys significantly **($p < 0.01$)** compared to the ISO group (Fig. 8A, B, 8C). The Control + L-CAR group also showed normal catalase enzyme activity in both plasma and kidneys (Fig. 8A, B, 8C). The catalase enzyme activity in the heart was reduced **($p < 0.001$)** for ISO-administered rats compared to the control group **(8A, 8B, 8C)**. The ISO + L-CAR group normalized the catalase enzyme activity in the heart significantly **($p < 0.01$)** compared to the ISO group (Fig. 8A, B, 8C). The Control + L-CAR group also showed a normal catalase enzyme activity in the heart compared to the control group (Fig. 8A, B, 8C).

3.8. Effect of L-carnitine on GSH level in plasma, kidney, and heart in isoprenaline (ISO) administered rats

Another parameter of antioxidant enzyme activity is GSH. The concentration of GSH is lowered **($p < 0.01$)** in plasma, kidneys, and the heart when administered with ISO compared to the control group (Fig. 9A, B, 9C). ISO administered rats that received L-CAR as treatment showed increased GSH concentration in plasma, kidneys, and the heart significantly **($p < 0.01$)** compared to ISO group (Fig. 9A, B, 9C). The Control + L-CAR group did not show any change in GSH level in plasma, kidneys, and the heart compared to the

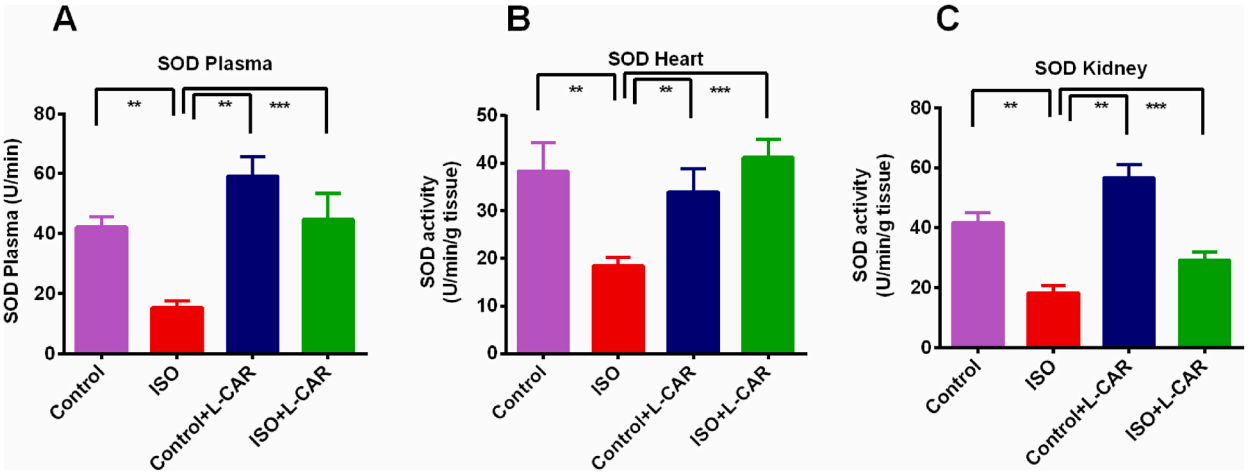

Fig. 7. Effect of L-carnitine on SOD activity in plasma and tissues homogenates of ISO administered rats. A. SOD Plasma, B. SOD Heart, C. SOD Kidney. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

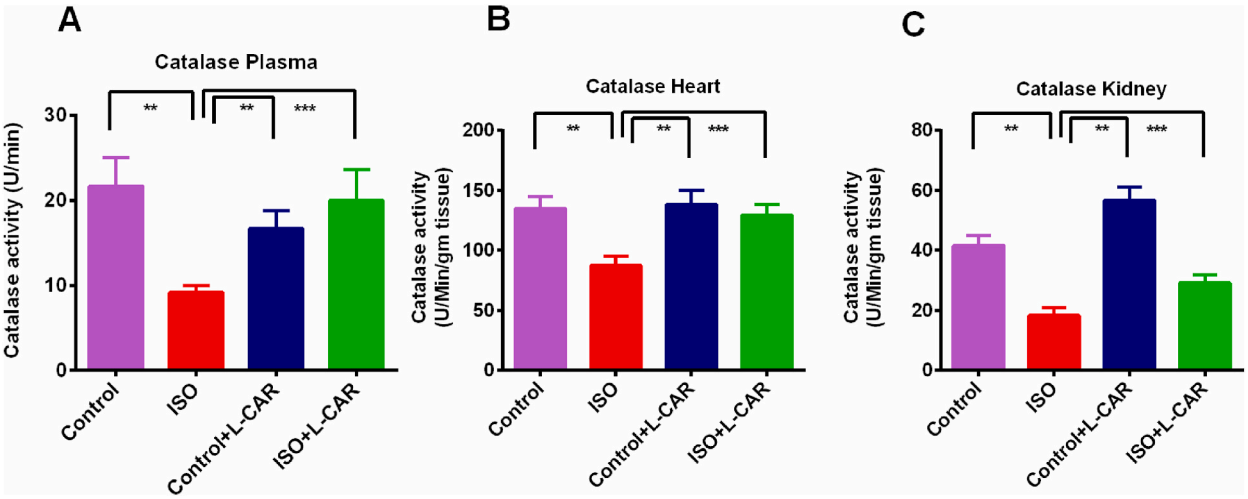

Fig. 8. Effect of L-carnitine on catalase activity in plasma and tissues homogenates of ISO administered rats. A. Catalase Plasma, B. Catalase Heart, C. Catalase Kidney. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

control rats (Fig. 9A, B, 9C).

3.9. Effect of L-carnitine on antioxidant genes expression of kidney cortex in isoprenaline (ISO) administered rats

ISO-administered rats showed reduced renal Nrf-2 transcript levels compared to controls (Fig. 10). Treatment with L-carnitine restored her Nrf-2 expression in the kidneys of ISO-administered rats (Fig. 9). A significant **($p < 0.01$)** up-regulation of HO-1 and HO-2 transcript levels was also detected in ISO-administered rats treated with L-carnitine (Fig. 10). In addition, gene expression of endogenous antioxidant enzymes such as SOD, catalase, and GPx was also decreased in the kidneys of ISO-administered rats. Furthermore, L-carnitine treatment significantly enhanced gene expression of these antioxidant enzymes in the kidneys of ISO-treated rats (Fig. 10).

3.10. Effect of L-carnitine on inflammation genes expression in kidney cortex of isoprenaline (ISO) administered rats

In this experiment, evaluation of gene expression that induces inflammation and fibrosis in the kidneys of ISO-administered rats was done of six genes such as interleukin-1 (IL-1), interleukin-6 (IL-6), transforming growth factor beta-1 (TGF-β1), tumor necrosis factor alpha (TNF-α), nuclear factor kappa B (NF-κB) and inducible nitric oxide synthase (iNOS) (Fig. 11). The study results showed

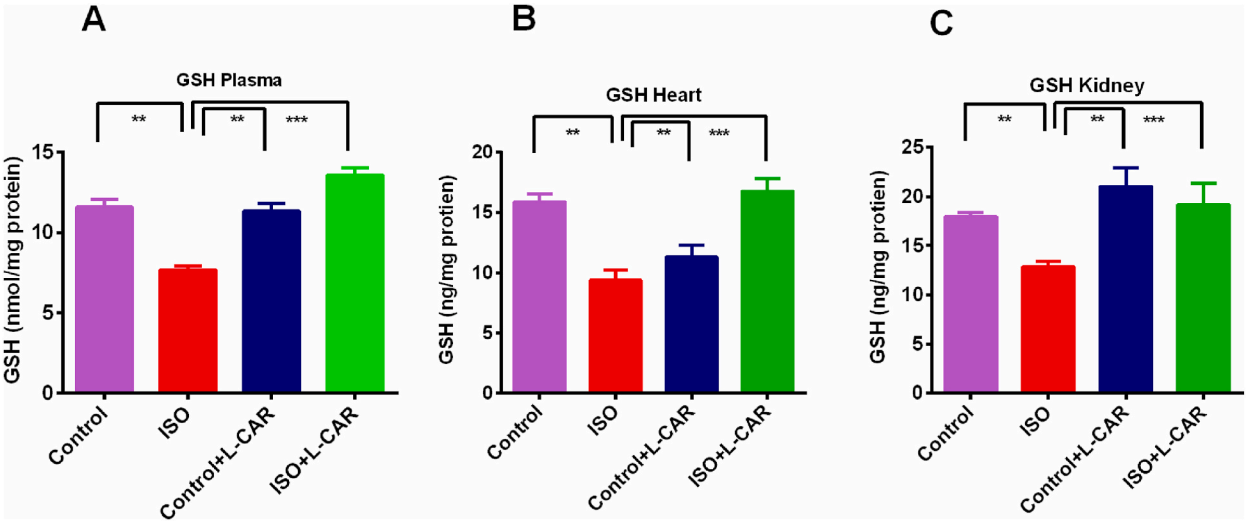

Fig. 9. Effect of L-carnitine on GSH level in plasma and tissues homogenates of ISO administered rats. A. GSH Plasma, B. GSH Heart and C. GSH Kidney. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

that IL-1, IL-6, and TNF-α gene expression were significantly **($P < 0.001$)** increased in the kidneys of ISO-administered rats (Fig. 11). Expression of TGF-β1, iNOS, and NF-κB were also significantly increased in ISO-administered rats compared to controls (Fig. 11). In this study, we also found that L-carnitine treatment successfully suppressed the expression of all these pro-inflammatory and inflammatory genes in the kidneys of ISO-administered rats. It should be noted that gene expression of key fibrosis-associated proteins such as TGF-β1 and IL-1 was significantly decreased **($P < 0.001$)** by L-carnitine treatment in ISO-administered rats (Fig. 11).

3.11. Effect of L-carnitine on histology of kidney in isoprenaline (ISO) administered rats

On the top part of the figure, Hematoxylin and Eosin staining is shown (Fig. 12). The control group showed no inflammatory cells infiltration or disorientation of kidney glomerular structure (Fig. 12A). The proximal tubules and distal tibular internal structures were not changed in control rats. However, the ISO group, showed focal necrosis of epithelial lining renal tubules associated with mononuclear cells infiltration (Fig. 12B). The glomerular basement membrane was thickened and the mesangial matrix was increased in ISO group (Fig. 12B). Conversely, the ISO + L-CAR group showed a healing process from inflammatory cells infiltration and the pathological alterations notably decreased compared to ISO administered rats (Fig. 12D). The Control + L-CAR group showed normal histoarchitecture and no inflammatory cells infiltration (Fig. 12C).

On the lower part of the figure, Sirius red staining is shown (Fig. 12). The control rats showed no collagen deposition (Fig. 12E), but in the ISO group, collagen deposition is observed clearly (Fig. 12F). The Control + L-CAR group also did not show any collagen deposition (Fig. 12G), whereas the ISO + L-CAR group showed less collagen deposition compared to the ISO administered rats. This indicates that L-CAR treatment decreases collagen deposition and fibrosis in the kidneys (Fig. 12H). The histological changes in kidneys are semi-quantitatively analyzed and presented in Fig. 12 I, J, K, L. The % of fibrosis were significantly increased in ISO administered rats compared to control rats; which were significantly normalized by L-CAR treatment (Fig. 12 L).

4. Discussion

In this investigation, the administration of isoprenaline (ISO) developed renal and cardiac pathogenesis. The ISO administration in rats showed significant increase in wet weight in the left ventricle (LV) of the heart as well as inflammation and fibrosis. The ISO administration in rats also increased kidney fibrosis and structural abnormalities followed by increased creatinine and uric acid level in plasma. These pathologies were mitigated and reduced by the treatment with L-CAR.

ISO administration increased the CK-MB activity in plasma which is a marker of myocardial damage in the heart. ISO administration may lead to the oxidative burst in the heart by auto-oxidation and causes tissue damage. Antioxidant supplementation may prevent the myocyte loss and preserved the cardiac tissues in ISO administered rats [35]. In this investigation, L-CAR prevented the myocardial damage in the heart of ISO administered rats. Previous report suggests that L-CAR treatment may decrease the CK-MB in tilmicosin-induced rats [36]. Apart from this CK-MB activity, creatinine and uric acid level were also analyzed as a diagnostic test for the kidney function in ISO administered rats. Previous investigation confirmed that kidney function may get affected secondarily by myocardial infarction [37]. ISO administration increased the levels of creatinine and uric acid. These findings are supported by a previously reported investigation showed that ISO administration increased the uric acid level [38]. L-CAR treatment reduced plasma levels of both creatinine and uric acid. Previous reports also support these findings that L-carnitine may prevent the rise of both

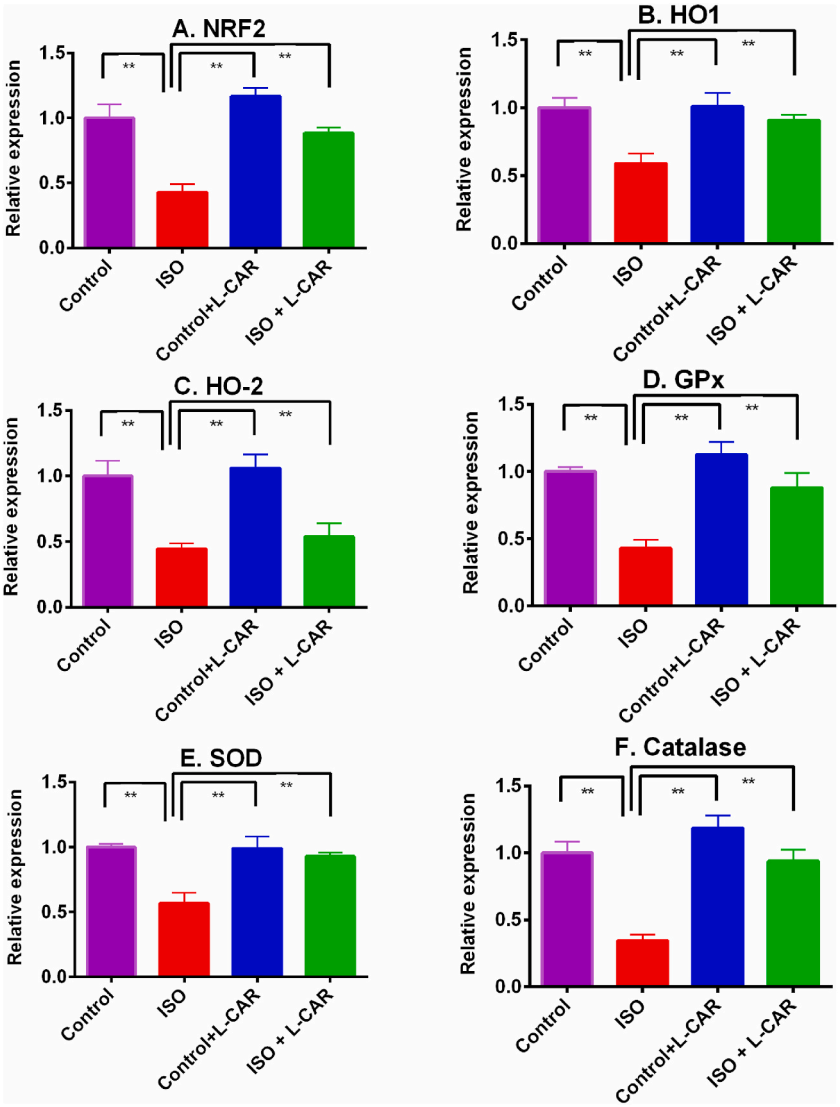

Fig. 10. Effect of L-carnitine on antioxidant genes expression in kidney cortex of ISO administered rats. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

creatinine and uric acid and ameliorates the nephrotoxicity in rats [39].

Oxidative stress-mediated pathogenesis of kidney damage and myocardial infarction is mediated by reactive oxygen species such as hydrogen peroxide and superoxide radicles, as well as other critical indicators such as MDA, NO, AOPP, and MPO [40,41]. Several complications such as irreversible damage and intracellular calcium overload are all associated with free radicals mediated lipid peroxidation [42]. In this investigation, ISO administration increased the concentration of MDA, a byproduct of lipid peroxidation in experimental rats. Previous studies also showed that ISO administration also increased lipid peroxidation and MDA level in kidneys and heart [40,41,43]. The concentration of MDA was found to be lower in the L-CAR treated rats. Hence, in order to reduce the lipid peroxidation, L-CAR may play a vital role. This finding is supported by previous study showed that L-CAR reduces the concentration of lipid peroxidation product MDA in the heart of ISO administered rats [44]. For kidney damage and cardiac problems such as myocardial infarction, nitrosative stress is the most important parameter. In this study, ISO administration resulted in higher NO levels in both heart and kidneys. The high NO concentration led to myocardial infarction [45]. In this experiment, it is seen that L-CAR reduces the concentration of NO. In another study, it is proved that L-CAR reduces NO production [46]. Peroxynitrite radicals resulted due to nitrosative stress which is associated with NO and inducible nitric oxide protein up-regulation due to the stimulation of β-adrenergic system [47]. Another oxidative stress parameter is known as AOPP, was assayed. Higher levels of AOPP were observed in ISO-administered rats, whereas declined levels were found in ISO administered rats treated with L-CAR. In a previous study, it was confirmed that L-CAR treatment may reduce both the AOPP and MDA levels [48].

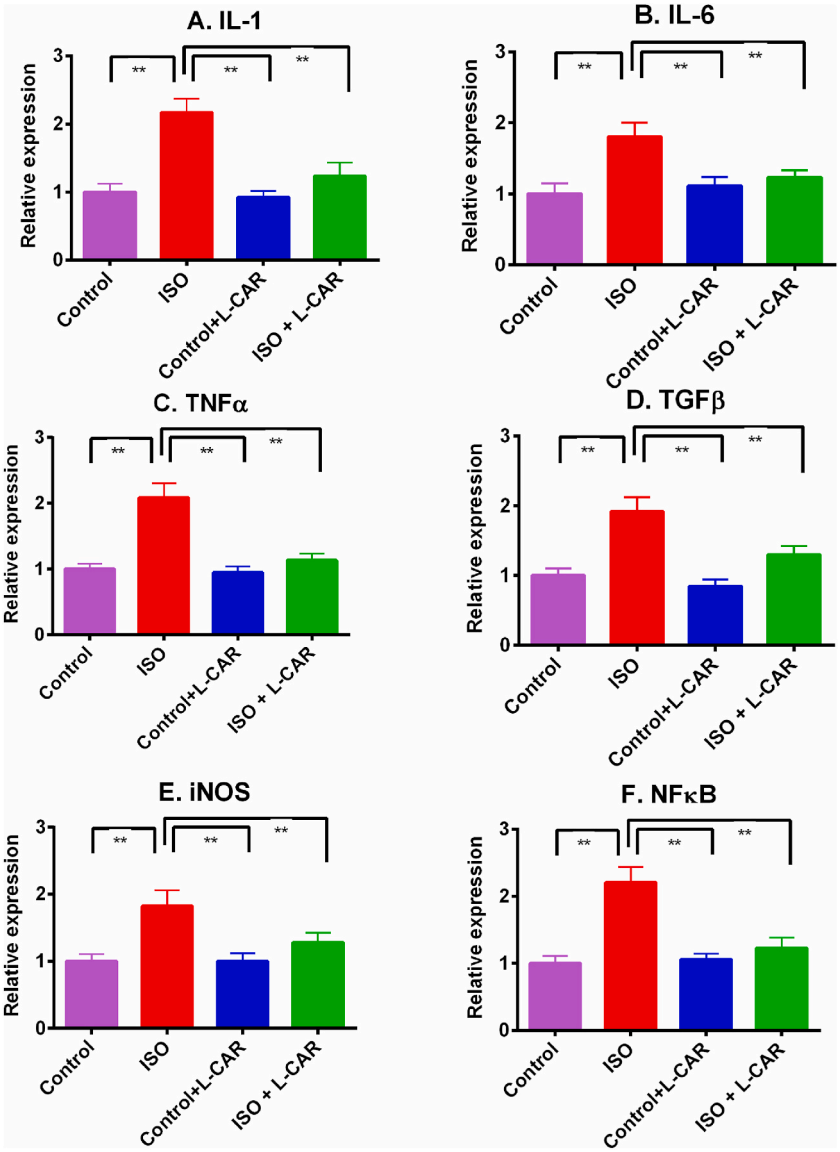

Fig. 11. Effect of L-carnitine on anti-inflammatory genes expression in kidney cortex of ISO administered rats. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by Newman-Keuls multiple comparisons test were done where significance was indicated as **ns** means $p > 0.05$; * means $p \leq 0.05$; ** means $p \leq 0.01$.

MPO is also considered as another crucial oxidative stress parameter mediated through neutrophil infiltration [49]. MPO-hydrogen peroxide-chloride system is a pro-oxidant system to generate a family of tyrosyl radicals [49]. MPO may also use nitrite as a precursor to initiate lipid peroxidation and nitrogen dioxide radical formation [50]. It is well established that MPO activity is significant in many kidney diseases [51,52]. ISO-administered rats showed increased MPO activities in kidney cortex. Treatment with L-CAR normalized the MPO activity in kidneys of ISO administered rats; which is supported by previous report showed that L-CAR was beneficial in normalizing the MPO activity in aspartame induced nephrotoxicity in rats [53].

There are few antioxidants enzymes available such as GSH, catalase, and SOD for the prevention of oxidative stress in tissues [54]. In this experiment, tissue and plasma oxidative stress parameters such as MDA, NO and AOPP levels were increased and caused kidney and cardiac abnormalities, probably due to the lack of antioxidant enzyme activities. It was observed that catalase, SOD activities and GSH level were decreased in kidney and heart of ISO administered rats [55,56]. The L-CAR treatment restored the normal levels of these antioxidant enzymes in ISO administered rats. The L-CAR as an antioxidant may scavenge the free radicle mediated damage to the kidneys and heart as well as enhance the antioxidant enzymes activities in case of oxidative stress. Previous study confirmed that a single dose of L-CAR increased all antioxidant capacities including SOD, catalase and GSH in plasma of healthy human [57]. The similar findings were also reported by a previous study showed that L-CAR may increase SOD, catalase and GPx in monosodium glutamate administered rats [58].

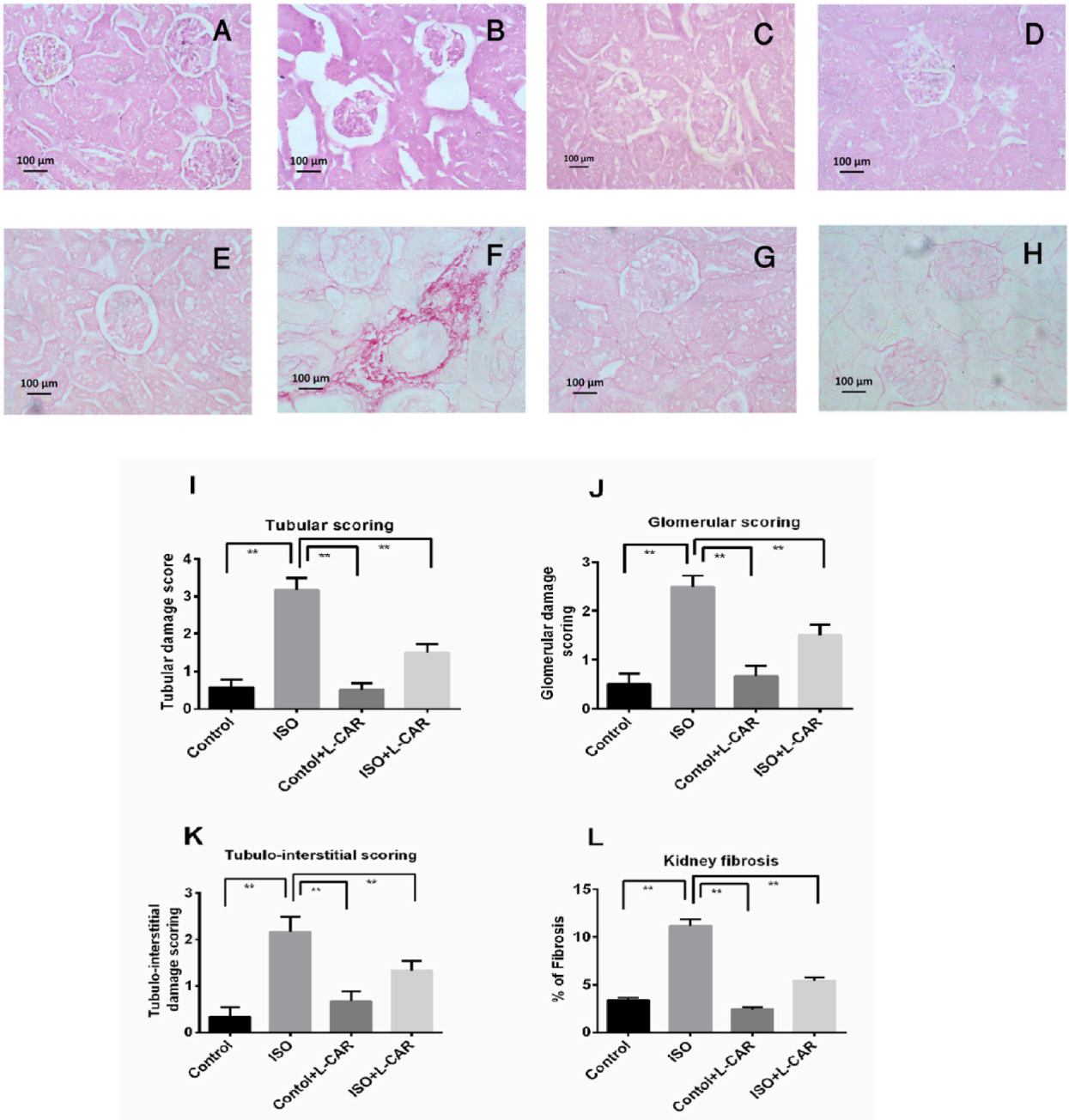

Fig. 12. Effect of L-carnitine on kidney histology in ISO administered rats. Upper portion is Hematoxylin and Eosin staining and Lower portion is Sirius red staining. A, E– Control; B, F– ISO; C, G- Control + L-CAR and D, H– ISO + L-CAR. I- Tubular scoring, J-Glomerular scoring, K- Tubulo-interstitial scoring and L- % of kidney fibrosis. Mean ± SEM were used and for statistical calculation, One way ANOVA followed by Newman-Keuls multiple comparisons test were done, where N = 6. Statistical significance was considered as $P < 0.05$. ** means $P \leq 0.01$.

In this investigation, antioxidant enzymes and inflammation related genes expressions were analyzed in the kidney cortex. Among them, expression of six genes, including Nrf-2, HO-1, HO-2, catalase, SOD, and GPx, were involved in antioxidant enzyme activity. On the other hand, inflammation linked genes expression included IL-6, IL-1, iNOS, TGF-β, TNF-α, and NFκB. In this investigation, ISO-administration in rats decreased the genes expression of antioxidant enzymes such as Nrf-2 followed by HO-1 and HO-2. L-CAR treatment in ISO administered rats improved these genes expression. Nrf-2 is the first respondent against oxidative stress and enhanced the other antioxidant genes such as catalase and SOD [59]. L-CAR augmented gene expression of Nrf-2 and suppressed oxidative stress in liver of bisphenol administered rats [60]. The HO-2 and HO-1 expression was observed in another study and was found that treatment with L-CAR normalized the condition of neurotoxicity [61]. The antioxidant genes, GPx, SOD, and catalase were increased

by L-CAR treatment in monosodium glutamate administered rats [58].

In this study the, ISO administration enhanced the inflammatory cytokines genes expression in kidney cortex of rats. This finding is supported by previous reports showed that oxidative stress may enhance the cytokine gene expression in kidneys [62,63]. In a previous study, L-CAR treatment inhibited the secretion of inflammatory cytokines such as TNF-α [64]. This investigation also suggests that L-CAR treatment may lower the IL-1, IL-6, and TNF-α gene expression in kidney cortex of ISO administered rats. ISO administration also increased the gene expression of TGF-β and NF-κB. L-CAR treatment decreased the expression of these two genes. Previous report also suggests that doxorubicin administration may increase the TGF-β and NF-κB genes expression which may be prevented by the treatment with L-CAR [65]. TGF-β may regulate the extra cellular matrix deposition in tissues and develop fibrosis and inflammation [66]. This investigation also revealed that the iNOS expression in kidneys was also increased due to ISO administration. This iNOS expression may be a causative factor for the increased amount of NO in kidneys and are responsible for nitrosative stress. In a previous study, it was observed that treatment with L-CAR decreased gene expression of iNOS in cultured hepatocytes [67]. This study also suggests that L-CAR treatment may lower the iNOS expression and NO level in kidneys of ISO administered rats.

Finally, a histopathological examination was done on kidney tissues. The kidney sections were stained with Hematoxylin and Eosin. ISO administration in rats showed necrosis in kidneys and develop abnormal glomerular structure which are prevented by L-CAR administration. These histological changes are supported by the biochemical and gene expression studies which are linked to oxidative stress and inflammation in kidneys. Another staining such as Sirius red staining determines the collagen deposition in kidney sections. In the ISO-administered rats, collagen deposition was found to be increased significantly, which was reduced by L-CAR treatment. This reduction of collagen deposition and reduced fibrosis development by L-CAR treatment is correlated with the decreased oxidative stress and inflammation; probably mediated through decreased cytokines and TGF-β expression in kidneys of ISO administered rats.

L-CAR and its derivatives are direct scavenger of free radicles generated in tissues like heart [68–70], and kidney [71]. L-CAR may also be responsible for the generation of anti-inflammatory mediators [72], which serves as protective agents during tissue damage. The anti-inflammatory function of L-CAR could follow the suppression of TNF-α and NF-κB mediated signaling cascade [73]. However, the other possible pathways of L-CAR action may adhere to the function of energy metabolism in mitochondria. A recent review paper discussed the L-CAR mediate metabolic flexibility and stability of cellular membrane because of its amphiphilic nature due to charged tri-methylamino group and the carboxylic group which can interact with the membrane phospholipids and other molecules [74]. L-CAR also preserved the buffer condition of acetyl-CoA and acyl-CoA, which may allow the functioning of relevant reactions in the cells and ATP production [75,76]. Moreover, L-CAR is also responsible for the detoxification of acyl residues as well as abnormal organic acids in cells [74,77].

Dietary L-CAR is degraded by intestinal bacteria to trimethyleamine and gamma-butyrobetaine [78]. Trimethyleamine is secreted mostly in urine and gamma-butyrobetaine is found in the feaces [78]. Further, γ-butyrobetaine is also a precursor molecule, which will be converted into L-CAR in its biosynthetic pathways in the liver and kidneys [79]. γ-Butyrobetaine hydroxylase is the enzyme responsible for the conversion of gamma-butyrobetaine to L-CAR, which is only available in certain tissues like liver and kidneys. However, inhibition of γ-butyrobetaine hydroxylase by a compound known as MET-88, which inhibits γ-butyrobetaine conversion to L-carnitine, showed cardioprotective effect in H_2O_2-induced metabolic derangement and in ischemia/reperfusion induced experimental insult in isolated heart [80,81]. Direct administration of MET-88 in isolated heart did not improve the cardiac function, only the pretreated animal with MET-88 showed potential to improve the cardiac function [80]. This protective effect was attributed to the low accumulation of long-chain acylcarnitine in the heart [81]. Previous report also showed that gamma-butyrobetaine prevented endothelial dysfunction in high glucose administered rats [82]. Lack of literature in systemic experimental work has been found for the beneficial role of gamma-butyrobetaine in cardiac and renal complications.

5. Conclusion

In conclusion, L-CAR treatment prevented oxidative stress in plasma and tissues and restored antioxidant enzymes activities in kidneys and heart of ISO administered rats. Moreover, L-CAR treatment also lowered the inflammation, inflammatory cells infiltration, fibrosis, and collagen deposition in kidneys of ISO administered rats. According to these experimental results, this protective effect is mediated through enhancing the antioxidant genes such as Nrf-2-HO-1 pathway; and lowering the expression of the inflammatory genes in the kidney cortex of ISO administered rats. The role of L-CAR metabolite gamma-butyrobetaine is not assessed in this study. However, considering the dual role of gamma-butyrobetaine in tissue physiology, further investigation is suggested in revealing the L-CAR metabolites effect on tissues protection.

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Data availability

All data included in this article. The raw data will be available from the corresponding author after reasonable request.

CRediT authorship contribution statement

Tammana Tabassum Eysha Chisty: Writing – original draft, Methodology, Investigation, Conceptualization. **Sumaia Sarif:** Writing – original draft, Investigation, Formal analysis. **Ishrat Jahan:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Iffat Nowshin Ismail:** Writing – original draft, Software, Investigation, Formal analysis. **Faizul Islam Chowdhury:** Writing – original draft, Project administration, Investigation, Formal analysis, Conceptualization. **Shahnaz Siddiqua:** Writing – original draft, Methodology, Investigation, Formal analysis. **Tahmina Yasmin:** Writing – original draft, Supervision, Investigation, Data curation. **Md Nurul Islam:** Writing – original draft, Supervision, Data curation, Conceptualization. **Ferdous Khan:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Conceptualization. **Nusrat Subhan:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Data curation, Conceptualization. **Md Ashraful Alam:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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