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Cardioprotective impact of L-carnitine against isoprenaline-induced myocardial infarction by focusing on Nrf2 / Parkin- mediated mitophagy

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Keywords

- Myocardial infarction
- L-carnitine, Oxidative stress
- Nuclear factor erythroid 2-related factor-2
- Parkin

Abstract

Background: Myocardial infarction (MI), a critical condition of ischemic heart disease, significantly contributes to global morbidity and mortality rates. L-carnitine, an essential metabolic cofactor, holds therapeutic promise in management of MI, although its precise mechanisms remain unclear. **Objective:** This research aimed to explore the cardioprotective potential of L-carnitine in male rats subjected to MI, with a particular focus on potential role of Nrf2/Parkin signaling pathway. **Materials and Methods:** Rats were divided into four groups, each comprising eight animals (n=8): a control group, an L-carnitine group (receiving 100 mg/kg/day orally for 14 days), an ISO group (MI induced by subcutaneous administration of 85 mg/kg isoprenaline on days 13 and 14), and an L-carnitine + ISO group (treated with L-carnitine 100 mg/kg/day orally for 2 weeks, followed by ISO administration). **Results:** Isoprenaline induced MI, as indicated by significant ECG changes, elevated cardiac biomarkers, histopathological damage, and reduced desmin expression in immunohistochemistry. Moreover, there was an increase in malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-κB), along with enhanced caspase-3 immunohistochemical staining. These alterations were paralleled by a reduction in total antioxidant capacity (TAC), Parkin levels, and Nrf2 immunohistochemical staining. L-carnitine treatment attenuated these adverse effects. **Conclusion:** L-carnitine exerts cardioprotective effects, which may be attributed to its anti-inflammatory, antioxidant, and anti-apoptotic properties, in addition to promoting mitophagy through upregulation of Parkin via Nrf2 signaling pathway.

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Introduction

Cardiovascular diseases (CVDs), a broad category of disorders impacting both the heart and vascular system, continue to represent the foremost cause of mortality on a global scale [**1**]. Myocardial infarction (MI), a result of an imbalance between coronary blood supply and myocardial oxygen demand, leads to ischemic necrosis of heart muscle tissue. MI is further exacerbated by oxidative stress, inflammation, and apoptosis driven by reactive oxygen species (ROS), lipid peroxides (LPO), defective antioxidant defenses, and release of inflammatory mediators. [**2,3**].

Moreover, evidence has shown that the initiation of mitogen-activated protein kinases (MAPKs) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling cascades plays a pivotal role in promoting inflammatory responses through the upregulation and secretion of pro-inflammatory mediators [**4**]. This suggests that targeting inflammation could serve as a therapeutic approach.

Isoprenaline (ISO), a β-adrenergic agonist, is frequently employed to induce MI in experimental rat models due to its non-invasive nature, ease of use, and low mortality rate **[5]**. ISO induces significant morphological and pathophysiological damage to myocardial tissue, mimicking human MI [**6**]. Its primary effects include hypertension, calcium (Ca2+) overload, altered oxygen utilization, disrupted myocardial metabolism, compromised membrane permeability, electrolyte imbalances, hypoxia, energy depletion, and increased oxidative stress, all of which contribute to myocardial necrosis [**7].**

Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a pivotal role in combating oxidative stress [**8**] by regulating antioxidant response element (ARE), which in turn activates genes encoding key antioxidant enzymes such as heme oxygenase-1 (HO-1) and superoxide dismutase 2 (SOD2) [**9**].

Mitochondrial dysfunction is a major contributor to structural and functional cardiac abnormalities, given its central role in energy metabolism and adenosine triphosphate (ATP) production [**10**]. Mitophagy, a selective form of autophagy, facilitates removal of damaged mitochondria and other cellular debris, thereby maintaining mitochondrial homeostasis [**11,12**]. By selectively targeting and eliminating compromised or malfunctioning mitochondria, the process of mitophagy plays a pivotal role in maintaining mitochondrial balance, thus safeguarding the stability of cellular homeostasis**[13]**. Enhancing mitophagy while simultaneously reducing ROS can significantly improve mitochondrial function **[14]**. A key mechanism driving mitophagy is PTEN-induced putative kinase 1 (PINK1)/Parkin pathway. PINK1 accumulates on outer membrane of impaired mitochondria, facilitating recruitment of Parkin from cytosol. Once activated, Parkin promotes ubiquitination of mitochondrial membrane proteins, initiating mitophagy process to clear out damaged components **[15]**.

Given early involvement of oxidative stress, inflammation, and mitophagy in pathogenesis of cardiac injury, targeting these pathways could be an effective strategy in mitigating ISO-induced MI.

L-carnitine, a quaternary ammonium compound, is synthesized in liver and kidneys and can be found in various meat and vegetable sources **[16]**. Its

primary physiological role lies in facilitating transport of long-chain fatty acids into mitochondria. This process involves moving acyl groups from cytoplasm into mitochondria for β-oxidation **[17]**. In context of MI,heart's energy metabolism is altered due to its reduced capacity to utilize fatty acids for oxidative metabolism under ischemic conditions caused by limited oxygen supply. As a result, fatty acids accumulate, leading to a disruption in fatty acid metabolism. Moreover, inflammation can further interfere with regulation of fatty acid absorption and oxidation, exacerbating impairment of cardiac energy metabolism **[18]**. L-carnitine has been shown to enhance myocardial fat metabolism and improve cardiac function under these conditions **[17, 19]**.

Although extensive research has highlighted L-carnitine's benefits as a metabolic cofactor in MI, its precise efficacy and the fundamental mechanisms driving its action remain inadequately elucidated and continue to be the subject of ongoing investigation. This study aimed to delve deeper into mechanism of action, identifying potential targets for MI protection. To best of our knowledge, no previous studies have demonstrated how L-carnitine can influence mitophagy and mitigate MI in male rats by simultaneously upregulating Nrf2 and downregulating NF-kB, thereby preventing progression of this condition.

2. Materials and Methods

2.1. Experimental animals:

A cohort of thirty-two adult male albino rodents, each weighing between 180 and 210 grams, were obtained from the Animal House at the Faculty of Veterinary Medicine, Benha University, Egypt. The animals were housed in stainless steel cages under rigorously controlled environmental parameters, with free access to water and food. Ambient temperature was precisely regulated at 23 ± 1°C, with a consistent 12-hour light/dark cycle. All experimental procedures were approved and conducted in strict adherence to Ethical Committee guidelines of Faculty of Medicine, Benha University (approval no. RC 5-3-2024). The protocols adhered to align with the standards outlined in the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996) as well as the ARRIVE guidelines for reporting on animal research.

2.2. Experimental Design:

Following a one-week acclimatization period, the rats were randomly assigned into four equal groups (n=8). The randomization ensured that there were no statistically significant differences in body weight among the groups prior to the initiation of the designated treatments. The groups were then subjected to the following treatment protocols:

Group I (Control group) was injected subcutaneously with normal saline (0.5 ml S.C.) for 2 days on13^{th} and 14^{th} days at a 24-hour interval of2-weeks experimental period.

Group II (L-carnitine group) was given L-carnitine at a dose of 100 mg/kg/day by oral gavage for 2 weeks **[17].**L-carnitine was purchased inform of **syrup** from Global Napi Pharmaceuticals (Egypt).

Group III (ISO group): MI was induced by using 85 mg/kg of subcutaneous ISO once daily on13^{th} and 14^{th} days at a 24-hour interval **[20].** Isoprenaline hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Group IV (L-carnitine + ISO group): rats were received L-carnitine 100 mg/kg/day by oral gavage for 2 weeks then isoprenaline85 mg/kg/sc. on13th and 14th days of experimental period.

Twelve hours after treatment and following an overnight fast,rats were anesthetized using urethane at a dose of 1.5 g/kg to assess cardiac function via ECG monitoring. Urethane was sourced from Sigma-Aldrich (St. Louis, MO, USA). At end of experiment, blood samples were drawn from retro-orbital plexus using microhematocrit tubes. Samples were left at room temperature for 30 minutes to clot and were then centrifuged at 3000 rpm for 15 minutes. Resulting serum was separated using automated pipettes and stored in Eppendorf tubes at -20°C for subsequent biochemical analysis of cardiac enzymes, including Troponin C and CK-MB. Animals were then sacrificed by decapitation, and their thoracic cavities were opened to extract hearts. Heart tissue was quickly excised, rinsed with saline, and blotted dry using filter paper. A portion of tissue was immediately frozen in liquid nitrogen and stored at -80°C for analysis of Parkin levels, oxidative stress markers such as MDA and TAC, as well as inflammatory markers like NF-kB and TNF-α.Remaining cardiac tissue was fixed in 10% buffered neutral formalin for histopathological and immunohistochemical analysis, including assessment of Nrf2, desmin, and caspase-3. Figure 1 provides an illustration of experimental protocol.

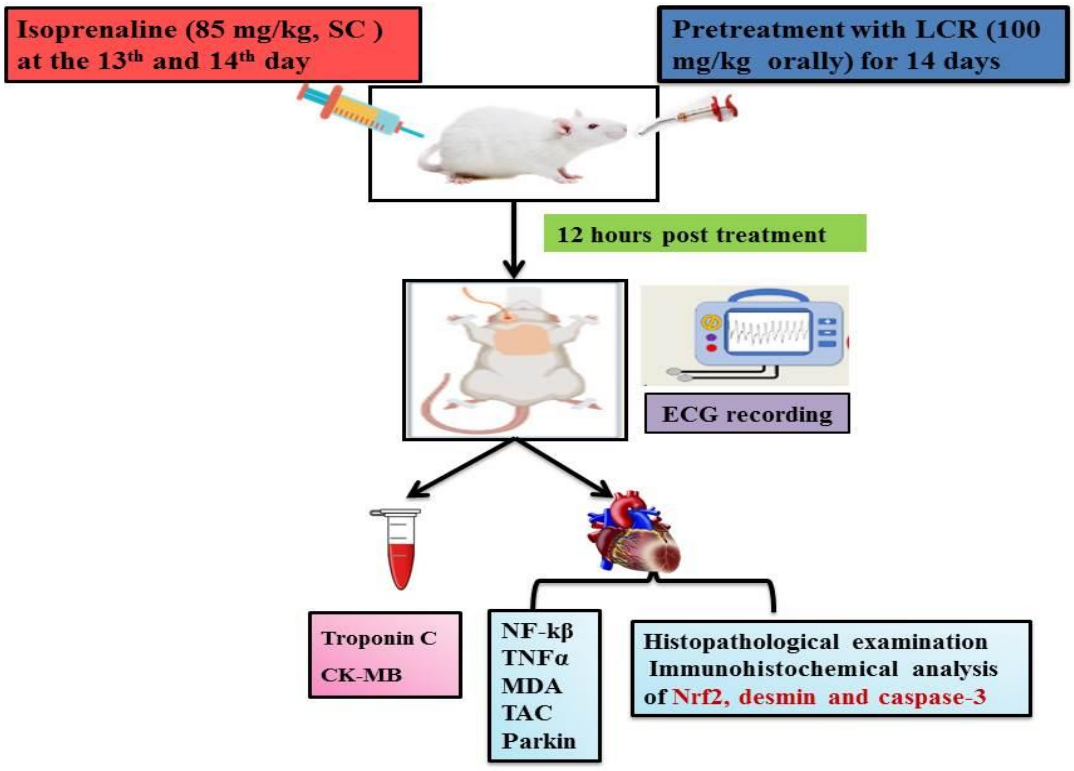

Fig. 1: Illustration of experimental procedure

CK-MB; Creatine kinase-MB.LCR; L-carnitine, MDA; Malondialdehyde, NF-kβ; Nuclear Factor Kappa β, Nrf2; Nuclear factor erythroid-derived 2-like 2, TAC; Total antioxidant capacity, TNF-α; Tumor necrosis factor-α.Illustration was created using software: PowerPoint.

2.3. ECG monitoring

To evaluate cardiac function, anesthetized rats were placed in a supine position. Continuous ECG monitoring was performed using a standard artifact-free lead II configuration. Subcutaneous needle electrodes were positioned on right forelimb, left forelimb, and left thigh to record ECG signals. The electrodes were interfaced with a Power Lab 4/20 data acquisition system (AD Instruments Pty Ltd, Australia) for precise data collection and analysis.ECG data were automatically computed, utilizing voltage calibration (in millivolts). Parameters such as heart rate (beats per minute), R-R interval (seconds), R and T wave amplitudes (millivolts), and ST segment depression were recorded from ECG output.

2.4. Biochemical analysis

2.4.1 Evaluation of cardiac enzymes

Serum analysis of both CK-MB and Troponin C levels were analyzed using Rat CKMB ELISA Kit (catalog #E4608-100; BioVision, Milpitas, CA, USA) and Rat Troponin C ELISA Kit (Code: E-EL-R1253; Elabscience, Texas, USA), respectively.

2.4.2 Assessment of oxidative stress and inflammatory markers

Specimens of cardiac tissue were homogenized in phosphate buffer with a pH of 6 – 7.Homogenized tissue was centrifuged at 10,000 g, 4^{o}C for a total of 15 minutes. supernatant used for quantitative detection of MDA that was performed utilizing lipid peroxide (MDA) kit (Catalog # K739-100; Biovision, Milpitas, CA, USA) and TAC that was performed utilizing TAC kit (Catalog # AMS.E02T0028; Amsbio, Cambridge, USA). Whereas, inflammatory mediators NF-kβ, was measured using ELISA Kit (Catalog # E-EL-R0674; Elabscience, Texas, USA), and TNF-α was measured using Rat TNF-α ELISA kit (CODE: ELR-TNFα-1; Ray Biotech, USA).

2.4.3. Evaluation of Parkin-mediated mitophagy

The Parkin-mediated mitophagy was analyzed using Parkin ELISA Kit (Catalog # ABIN772286; antibodies-online Inc., Limerick, USA).

2.5. Histopathological examination

Cardiac tissue samples were immersed in 10% formalin for a fixation period of 48 hours. Thereafter, 4 μm thick sections were meticulously prepared, processed, and stained using hematoxylin and eosin to examine the histological architecture of the myocardial fibers in detail [**21**]. All sectioned were visualized using high-power option of light microscope and photomicrographs were taken. Myocardial damage was scored based on severity into either; No change = 0, mild change = 1 (damage to focal myocytes or presence of small multifocal degeneration lesions with a minimal degree of inflammation), moderate change = 2 (extensive degeneration of cardiac myofibres), and finally marked change = 3 (the presence of necrosis and diffuse inflammation) [**22**].

2.6. Assessment of Immunohistochemical expression of cardiac Nrf2, caspase-3(apoptotic marker), and desmin(intermediate filaments in cardiac muscle)

For immunohistochemical (IHC) staining, sections were immunostained for primary antibodies. Nrf2 rabbit monooclonal antibody (1:100, Abcam, Cambridge, UK, clone EP1808), caspase-3 rabbit monoclonal antibody (1:40, Cell Signalling Technology, Boston, MA, USA). Desmin rabbit

monoclonal antibody (DE-R-11), (Ventana Medical Systems, Tucson, Arizona, USA). DAB was utilized as a chromogen .IHC staining was performed, using detection kit (Thermoscientific USA) according to manufacturers data. Finally, sections were counterstained with hematoxylin. Pancreatic adenocarcinoma was used as a positive control for Nrf2 and Leiomyoma was used as positive control for desmin. Positive control for caspase-3 was a section from pancreas. Negative control was achieved by omitting primary antibody [**23**]**.** Immunostaining assessments were performed through digital morphometric analysis, utilizing Image-Pro Plus software version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA) for quantification. The mean area percentages of Nrf2, caspase-3, and desmin immunostaining were calculated by examining eight images taken from eight different fields within each group.

3. Statistical Analysis

SPSS software (Version 20; SPSS Inc., Chicago, USA) was employed to conduct statistical analyses of the data. One-way ANOVA was employed to assess group differences, and the Least Significant Difference (LSD) test was employed for post-hoc analysis. Data were expressed as mean ± SD, with statistical significance set at a p-value of less than 0.05. Pearson's 2-tailed correlation coefficient (r) was employed to examine relationships between Nrf2 and Troponin C, NF-kB, MDA, TAC, caspase-3, and Parkin levels. A threshold of statistical significance was set at a p-value ≤ 0.05 for all analyses.

4. Results

4.1. L-carnitine alleviates myocardial injury and ECG changes associated with ISO-induced acute MI in rats

According to our findings, ISO injection has a detrimental effect on heart, which indicated by a discernible rise in serum biomarker levels of CK-MB and troponin C, in ISO group compared to controls($P < 0.05$).Rise in these parameters was dramatically inhibited by L-carnitine in L-carnitine + ISO group in comparison to ISO group ($P < 0.05$) (Table 1). In addition, our results revealed a notable rise in heart rate, a reduction in R-wave amplitude, depression of ST segment, and considerable T-wave inversion in ISO group in comparison to controls ($P < 0.05$). In contrast, L-carnitine ameliorated ISO induced myocardial injury as indicated by remarkable reversal of ST segment depression and T wave inversion, increase in R wave amplitude, increase R-R interval as well as significant decrease in HR in contrast to ISO group ($P < 0.05$) (Table 1, Fig. 2)

Table (1): Serum CK-MB, Troponin C levels and changes of electrocardiogram (ECG) parameters in the experimental groups.

	Control group	L-carnitine group	ISO group	L- carnitine + ISO group
CK-MB (pg/ml)	48.13 ± 2.89	48.20 ± 1.00	167.80 ± 7.85[a, b]	76.24 ± 7.34[a, b, c]
Troponin C (pg/ml)	38.84 ± 1.19	39.19 ± 2.60	152.57 ± 16.49[a, b]	63.63 ± 9.60 [a, b, c]
Heart rate (beat/min)	331.29 ± 8.38	329.29 ± 7.87	486.86 ± 8.71 [a, b]	393.43 ± 15.04 [a, b, c]
R- wave amplitude (mV)	1.150±0.009	1.157 ± 0.020	0.501 ± 0.021 [a, b]	0.801 ± 0.030 [a, b, c]
T- wave amplitude (mV)	0.110 ± .006	0.112 ± 0.004	-0.156 ± 0.017 [a, b]	0.049 ± 0.006 [a, b, c]
R-R interval (S)	0.18 ± 0.005	0.18 ± 0.006	0.12 ± 0.006 [a, b]	0.15 ± .005 [a, b, c]
ST- segment depression (mV)	0.002± 0.003	0.001 ± 0.001	0.168 ± 0.006 [a, b]	0.021 ± 0.004 [a, b, c]

Data are represented as Mean ± SD., n=8. $P < 0.05$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: $P < 0.05$ *vs.* Control group; b: $P < 0.05$*vs.* L-carnitin group. c: $p < 0.05$ *vs.* ISO group. ISO; Isoprenaline, CK-MB; Creatine kinase-MB.

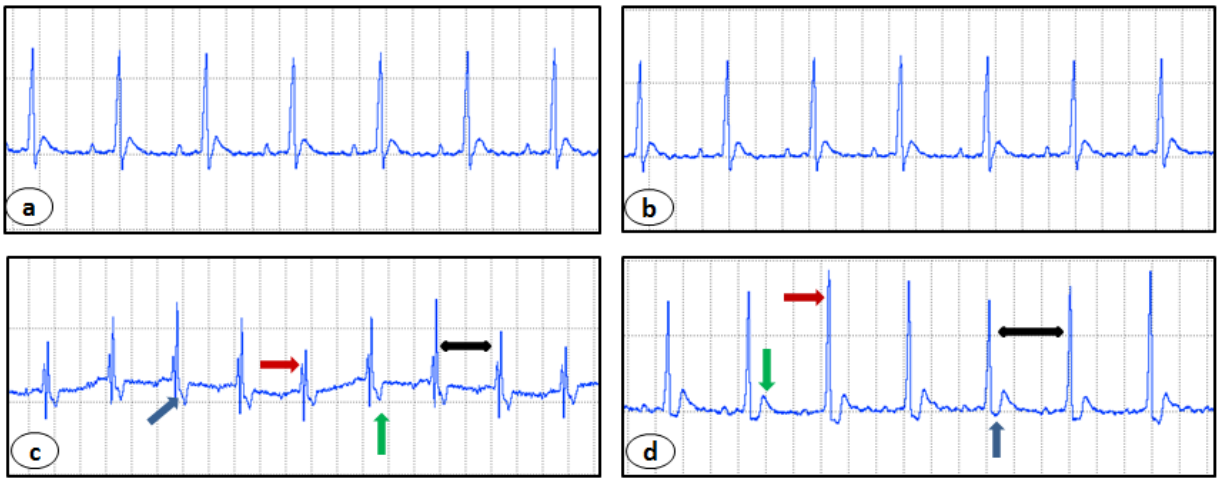

Fig. 2: ECG trace pattern (lead II) of experimental groups
(a): control group and **(b)**: L-carnitine group showing regular ECG pattern; **(c):** ISO group showing ST segment depression (blue arrow), inverted T wave (green arrow), R wave amplitude decrease (red arrow), decrease inR-R interval (black arrow) and increase in heart rate; **(d):** L-carnitine + ISO group showing obvious reduction in ST segment depression(blue arrow), reversal of T wave inversion(blue arrow), increase in R wave amplitude(red arrow), increase in R-R interval (black arrow) as well as significant decrease in heart rate.

4.2. L-carnitine ameliorates ISO-induced cardiac oxidative stress and inflammation in rats

As compared to controls, ISO injection resulted in oxidative stress which was established by a substantial rise in cardiac MDA with a significant reduction in cardiac TAC ($P < 0.05$). Regarding inflammatory markers, there was a notable increase in NF-kβ and TNF-α in ISO group when compared to controls ($P < 0.05$). Nevertheless, L-carnitine administration resulted in a remarkable reduction in cardiac MDA in parallel with significant enhancement of cardiac TAC when compared to ISO group ($P < 0.05$). Furthermore, L-carnitine attenuates ISO induced inflammation which indicated by a valuable decrease in NF-kβ and TNF-α when compared to ISO group ($P < 0.05$)indicating antioxidant and anti-inflammatory protective effects of L- carnitine (Table 2).

4.3. L-carnitine improves Parkin mediated mitophagy

Findings of the current study demonstrated a significant suppression of mitophagy in ISO-induced MI group indicated by a discernible drop in cardiac Parkin level when compared with controls ($P < 0.05$). On contrary, L-carnitine treatment considerably enhanced cardiac Parkin level with respect to ISO group($P < 0.05$)(Table 2).

Table (2): Cardiac TAC, MDA, NF-kB, TNF-α and Parkin levels in the experimental groups.

	Control group	L-carnitine group	ISO group	L- carnitine + ISO group
TAC (ng/mg)	1.73 ± 0.09	2.35 ± 0.59	0.37 ± 0.12[a, b]	1.05 ± 0.20[a, b, c]
MDA (nmol/mg)	0.45 ± 0 .06	0.42 ± 0.07	1.56 ± 0.12[a, b]	0.73 ± 0.09[a, b, c]
NF-κβ (pg/mg)	58.52± 4.72	57.54 ± 5.80	170.37 ± 4.77[a, b]	83.67 ± 16.66[a, b, c]
TNF-α (pg/ mg)	57.14 ± 2.79	54.43 ± 3.64	109.00 ± 3.92[a, b]	79.00 ± 1.83[a, b, c]
Parkin (ng/mg)	2.002 ± 0.16	2.061 ± 0.21	0.51 ± 0.01[a, b]	1.084 ± 0.16[a, b, c]

Data are represented as Mean ± SD., n=8. $P < 0.05$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: $P < 0.05$ *vs*. Control group; b: $P < 0.05$*vs*. L-carnitine group. c: $p < 0.05$ *vs.*

ISO group .ISO;Isoprenaline, MDA; Malondialdehyde, NF-kB; Nuclear Factor Kappa β ,TAC; Total antioxidant capacity, TNF-α; Tumor necrosis factor-α.

4.4. Effect of L-carnitine on histopathological evaluation of cardiac tissue.

The histopathological evaluation of cardiac tissue in control and L-carnitine groups revealed regular branching cardiomyocytes with central rounded nuclei (Fig. 3a and 3b respectively). On other hand, ISO group showed areas of coagulative necrosis with loss of structure, widening of interstitial spaces, and inflammatory cell infiltrate (Fig. 3c) with a significant increase in histopathological scoring when compared to control and L-carnitine groups ($P < 0.05$) (Fig. 3e). In contrast, L-carnitine dramatically improved myocardial injury denoted by preserved cardiac cells by decreasing cellular infiltrate and congested blood vessels (Fig. 3d), in parallel with remarkable reduction in histopathological scoring when compared to ISO group ($P < 0.05$) (Fig. 3e).

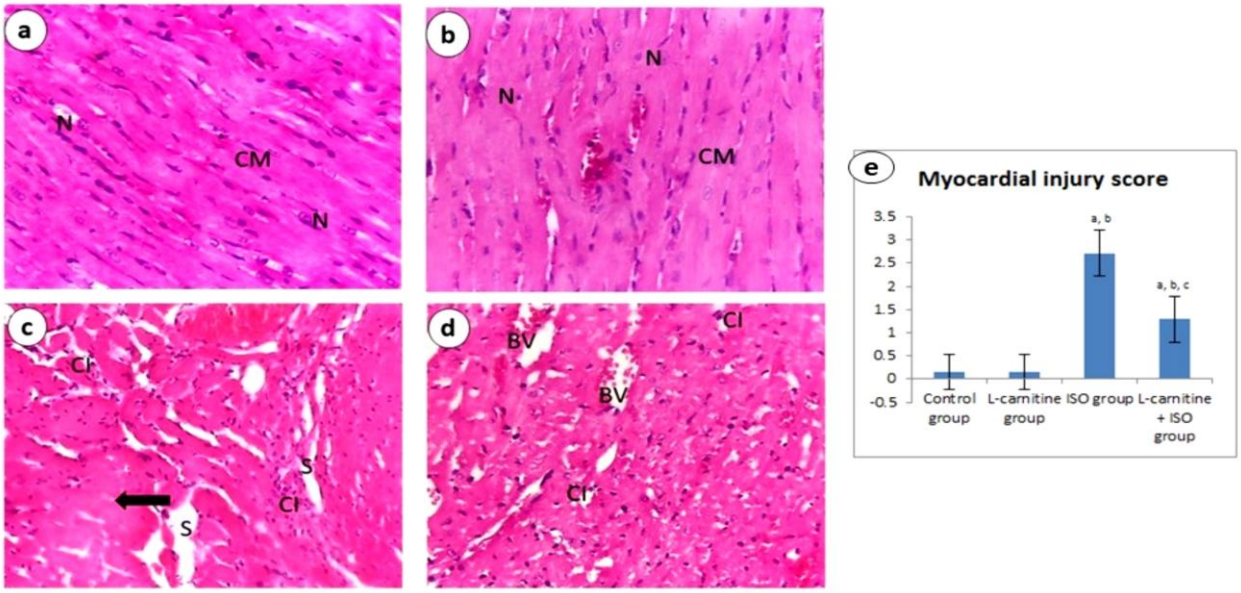

Fig. 3: Photomicrographs of sections in cardiac muscle cells of rats stained with Hematoxyline and Eosin. **(a) and (b)** control and L-carnitine group: showing regular branching cardiomyocytes (CM) with central rounded nuclei (N) **(c)** ISO group: showing area of coagulative necrosis (black arrow) with loss of structure, Widening of interstitial spaces (S) and inflammatory cell infiltrate (CI) **(d)** L-carnitine + ISO group: showing largely preserved cardiac cells with few cellular infiltrate (CI) and congested blood vessels (BV)**(Magnification power,400x).(e)** Cardiac injury score in experimental groups, data are represented as Mean ± SD., n=8. $P < 0.05$ is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: $P < 0.05$ *vs*. Control group; b: $P < 0.05$*vs*. L-carnitine group. c: $p < 0.05$ *vs.* ISO group. ISO; Isoprenaline.

4.5. Effects of L-carnitineon immunohistochemistry expression of Nrf2, caspase-3 (apoptotic marker), and desmin (intermediate filaments in cardiac muscle) in cardiac tissue

Immunohistochemical analysis of cardiac expression of Nrf2 protein, antioxidant marker, showed a strong Nrf2 nuclear and cytoplasmic expression in cardiac muscle cells in both control and L-carnitine group (Fig. 4a, 4b respectively). While, ISO- induced MI distinctly down-regulated Nrf2 expression (Fig. 4c) when compared to controls. Moreover, there was moderate Nrf2 nuclear expression in cardiac muscle cells when

rats were given L-carnitine prior to MI (Fig 4d). Also in this regard, there was a significant decline in digital morphometric analysis in ISO group *vs.* controls($P < 0.05$), while there was a notable rise in L-carnitine + ISO group when compared to ISO group ($P < 0.05$) (Fig. 4e).

Regarding Immunohistochemical expression of caspase-3 protein, apoptotic marker, there was a negative nuclear expression of caspase-3 in cardiac muscle cells in control and L-carnitine groups (Fig. 4f, 4g). On other hand, there was an intense nuclear expression of caspase-3 in cardiac muscle cells in ISO group (Fig. 4h), while L-carnitine + ISO group showed a mild caspase-3 nuclear expression in cardiac muscle cells (Fig. 4i). Concerning digital morphometric scoring of caspase-3 expression, there was a considerable increase in ISO group when compared to controls ($P < 0.05$), while there was a notable decline in L-carnitine + ISO group *vs*. ISO group ($P < 0.05$) (Fig. 4j).

Desmin, a vital intermediate filament protein, plays a crucial role in maintaining the structural stability and integrity of muscle fibers. It is primarily located around Z-disk, where it helps connect myocardial cells **[24]**. Its immunohistochemical staining revealed a moderate expression in cardiac muscle cells in control and L-carnitine groups (Fig. 4k, 4l). Furthermore,I SO group showed a negative desmin expression in cardiac muscle cells (Fig. 4m), while L-carnitine +ISO group: showed mild desmin expression in cardiac muscle cells (Fig. 4n). In keeping with these findings, there was a considerable decline in digital morphometric scoring of desmin immunohistochemical expression in ISO group *vs.* controls ($P < 0.05$), while there was a significant rise observed in L-carnitine + ISO group *vs*. ISO group ($P < 0.05$) (Fig. 4o).

4.6 Correlation between Nrf2 level and serum troponin C, cardiac MDA, TAC, NF-kβ, Parkin and caspase-3 levels:

There is an observable negative correlation between Nrf2 with troponin C ($r = - 0.96$; $p < 0.001$), MDA ($r = - 0.97$; $p < 0.001$), NF-kβ ($r = - 0.98$; $p < 0.001$) and caspase-3 ($r = - 0.95$; $p < 0.001$). In contrary, a positive correlation with TAC ($r = 0.87$; $p < 0.001$) and Parkin ($r = 0.92$; $p < 0.001$) was observed(Table 3).

Table (3) Correlation between Nrf2 level and serum troponin C, cardiac MDA, TAC, NF-kB, Parkin and caspase-3 levels in experimental groups

Parameter	Nrf2	
	RS	P value
Troponin C	- 0.96	P < 0.001
MDA	-0.97	P < 0.001
TAC	0.87	P < 0.001
NF-kB	-0.98	P < 0.001
Parkin	0.92	P < 0.001
Caspase-3	-0.95	P < 0.001

Data were analyzed using Pearson's correlation coefficient (r) 2-tailed test. $P < 0.05$ was considered statistically significant. MDA; Malondialdehyde, NF-kB; Nuclear Factor Kappa β , TAC; Total antioxidant capacity, TNF-α; Tumor necrosis factor-α.

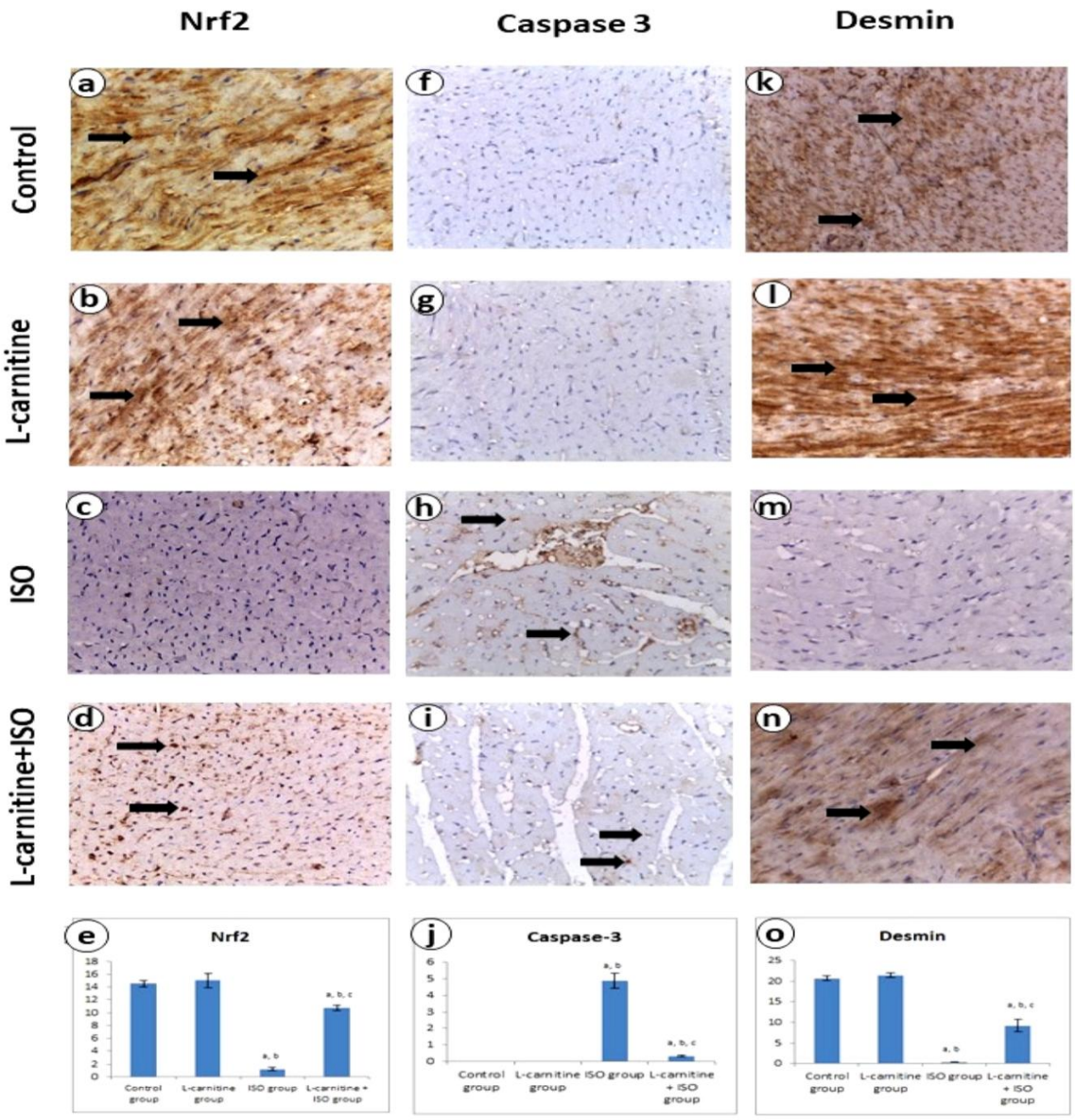

Fig. 4: Photomicrographs of sections in cardiac muscle cells of rats stained with anti-Nrf2, anti-caspase-3 and anti-desmin antibodies. **(a) and (b)** control and L-carnitine group: showing strong cytoplamic Nrf2 expression in cardiac muscle cells in form of brown color (black arrows). **(c)** ISO group: showing negative Nrf2 expression in cardiac muscle cells. (**d**) L-carnitine + ISO group: showing moderate nuclear Nrf2 expression in cardiac muscle cells (black arrows)**(Magnification power,400x)**. **(e)** Digital morphometric scoring of Nrf2 in experimental groups, data are represented as Mean ± SD., n=8. P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: P < 0.05 *vs*. Control group; b: P < 0.05*vs*. L-carnitine group. c: p < 0.05 *vs.* ISO group. ISO;Isoprenaline. **(f) and (g)** control and L-carnitine group: negative caspase -3 expression in cardiac muscle cells. **(h)** ISO group: showing moderate nuclear caspase3 expression in cardiac muscle cells in form of brown color (black arrows) **(i)** L-carnitine + ISO group: showing mild caspase-3 expression in cardiac muscle cells (black arrows). **(j)** Digital morphometric scoring of caspase-3 in experimental groups, data are represented as Mean ± SD., n=8. P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: P < 0.05 *vs*. Control group; b: P < 0.05*vs*. L-carnitine group.c: p < 0.05 *vs.* ISO group. ISO;Isoprenaline.**(k) and (l)** control and L-carnitine groups: showing intense cytoplasmic desmin expression in cardiac muscle cells in form of brown color (black arrows). **(m)** ISO group: showing negative desmin expression in cardiac muscle cells (**n**) L-carnitine + ISO group: showing mild desmin expression in cardiac muscle cells (black arrows)**(Magnification power,400x)**. **(o)**Digital morphometric scoring of desmin in experimental groups, data are represented as Mean ± SD., n=8. P < 0.05 is significant tested by one-way analysis of variance (ANOVA) and post hoc multiple comparison LSD method. a: P < 0.05 *vs*. Control group; b: P < 0.05*vs*. L-carnitine group.c: p < 0.05 *vs.* ISO group. **(Magnification power, 400x)**

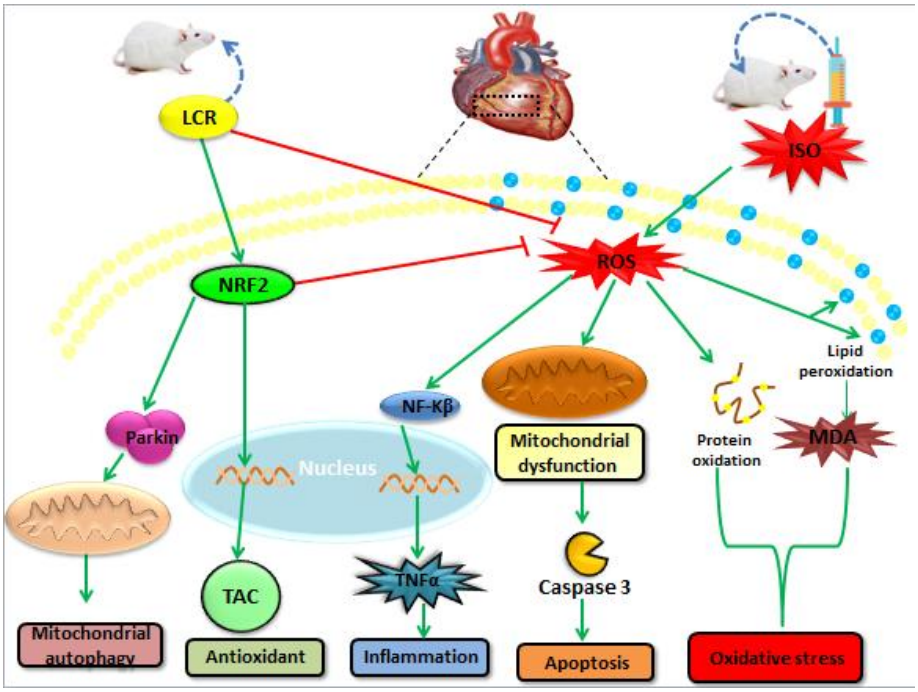

Fig. 5: A schematic diagram summarizes proposed mechanisms of protective effect of L-carnitine against ISO- induced MI

In heart, ISO induce myocardial damage trough induction of oxidative stress and inflammation. In addition, it led to mitochondrial dysfunction which promotes apoptosis. LCR exerts its protective affects via quenching of ROS, that mitigates MDA while enhances TAC. This in turn inhibits inflammatory sequences by down regulation of NF-kB and TNFα. Furthermore, LCR promotes up-regulation of Nrf2 that block oxidative stress. Moreover, Nrf2 can remarkably trigger mitophagy throughNrf2/Parkin signaling pathway. ISO;Isoprenaline, LCR; L-carnitine, MDA; Malondialdehyde, NF-kβ; Nuclear Factor Kappa β, Nrf2 Nuclear factor erythroid-derived 2-like 2, ROS; reactive oxygen species, TAC; Total antioxidant capacity, TNF-α; Tumor necrosis factor-α.illustration was created using software: PowerPoint.

4. Discussion

The incidence of ischemic heart disease (IHD) continues to rise, accounting for 50% of morbidity and mortality linked to cardiovascular diseases, with a higher prevalence in low-income countries. As indicated by Global Burden of Disease study, IHD is primarily driven by AMI, with angina contributing to a lesser degree**[25,26]**. This study concludes that L-carnitine exerts a cardioprotective effect against ISO-induced MI by attenuating oxidative stress, reducing inflammation, inhibiting apoptosis, and preventing myocardial tissue damage. To our knowledge, this is first study to investigate L-carnitine's role in MI, emphasizing Nrf2/Parkin-mediated mitophagy.

The myocardial damage induced by ISO was confirmed through ECG monitoring, biochemical analysis, and histological evaluation. Administration of ISO, a β-adrenergic receptor agonist, resulted in a shortened RR interval, elevated heart rate, ST segment depression, and significant T wave inversion on ECG, all indicative of myocardial ischemia due to reduced myocardial perfusion caused by tachycardia. Additionally, ISO prolonged QRS complex and increased R wave amplitude, potentially due to tachycardia-induced bundle branch block or ischemia, aligning with findings by **Diab et al. [27] and Boarescu et al. [28].** On biochemical level, ISO treatment caused a significant rise in

serum levels of troponin-C and CK-MB. Troponin-C is a specific diagnostic marker for MI, while CK-MB reflects release of enzymes from cardiomyocytes following cell membrane damage, indicating myocardial injury **[29]**. Histological analysis further supported these observations, revealing significant loss of cardiac muscle fibers, leukocyte infiltration, and vascular congestion in ISO-treated rats. There was also a marked decrease in desmin protein immunohistochemical staining, which is essential for maintaining structural and mechanical integrity of cardiac contractile proteins. These findings are consistent with prior experimental research on MI **[29, 30,and 31]**. Our results affirm validity of ISO-induced MI model in studying cardioprotective effects of L-carnitine.

Importantly, pretreatment with L-carnitine notably, restored ECG near normal, and this was in agreement with a previous study **[32].** Also, L-carnitine pretreatment successfully stopped alterations in cardiac biomarkers and slightly enhanced architecture of cardiac tissue and increased desmin expression with respect to ISO group. This is consistent with earlier researches by **Aboubakr et al. [33] and Khedr et al. [34]** and presents L-carnitine as a promising cardioprotective nutrient.

Oxidative stress plays a crucial role in promoting cardiac cell apoptosis during progression of MI. Consistent with earlier research **[31,30],**our study also demonstrated a significant increase in lipid peroxidation, indicated by elevated MDA levels, along with a marked decrease in TAC levels in ISO group compared to controls. ISO likely produces oxidative radicals that irreversibly damage cardiac proteins, disrupt mitochondrial function, and lead to cell death. Additionally, antioxidant enzymes are employed to neutralize generated free radicals **[30]**.

L-carnitine treatment reduced oxidative stress by increasing TAC and lowering MDA levels compared to ISO group. Antioxidant properties of L-carnitine are attributed to its ability to stabilize free radicals by conjugation. As a key cofactor for carnitine palmitoyltransferase 1 (CPT1), L-carnitine facilitates transport of fatty acids into mitochondria for β-oxidation, thereby reducing production of ROS **[35]**. Additionally, L-carnitine may serve as a buffer for excess acetyl groups within the mitochondria, thereby limiting mitochondrial superoxide production under hypoxic conditions, particularly in ischemic tissue environments **[36]**. These findings are in agreement with those from previous studies conducted by **Li et al. [32] and Khedr et al. [34].**

In order to get further insight into molecular mechanisms behind L-carnitine's antioxidant properties, Nrf2 signaling pathway in rat heart tissue was examined. Nrf2 has been demonstrated to play a pivotal role in exerting potent antioxidant effects, contributing significantly to myocardial protection. Nrf2 is a critical transduction factor controlling oxidative stress and maintaining body's redox homeostasis **[37**]. Our results revealed that rats given L-carnitine had noticeably greater levels of Nrf2 expression in their heart tissue, indicating that L-carnitine had triggeredNrf2 signaling pathway. These results corroborated those of **Zhao et al. [38]** who showed that L-carnitine alleviates oxidative stress in cardiomyocytes to relieve myocardial ischemia-reperfusion injury. Nevertheless, ML385 (an Nrf2 blocker) inhibited Nrf2/HO-1 signaling pathway, which led to elimination of L-carnitine's protective impact. This

indicates that L-carnitine exerts its cardioprotective effects by activating the Nrf2/HO-1 signaling cascade, thereby safeguarding cardiomyocytes**.** In contrary, current study demonstrated impaired Nrf2 signaling, as shown by a decrease in Nrf2 expression in heart tissue of rats that had MI caused by ISO. These results aligned with the outcomes of previous studies, corroborating earlier findings [**39, 40**].

Mitophagy is a mechanism that governs mitochondrial quality and homeostasis by removing damaged and senescent mitochondria selectively [**41**]. There is mounting evidence that conventional mitochondrial autophagic pathway PINK1/Parkin exists [**15]**. Activation and subsequent translocation of Parkin are driven by the accumulation of PINK1 on the outer mitochondrial membrane, triggering the selective degradation of mitochondrial proteins in response to mitochondrial dysfunction [**42**].

Our results demonstrate that L-carnitine therapy induced mitophagy by enhancing Parkin level in cardiac tissue. Similarly, **Li and coauthors [43]** found that treatment with L-carnitine enhanced PINK1-Parkin-dependent mitophagy, which reduced mitochondrial damage and boosting cell survival following hyperglycemia/free fatty acid insult. Furthermore, this effect was linked to L-carnitine's ability to lower expression of protease presenilin-associated rhomboid-like protein (PARL), a mitochondrial rhomboid protease, which is an inhibitor of. Additionally, it prevented PARL from dissociating from prohibitin 2 (PHB2), a PARL inhibitor, thus restricts PARL's protease activity to degrade PINK1, consequently promoting mitophagy through PINK1-Parkin pathway.

Interestingly, our results revealed a positive correlation between Nrf2 and Parkin. This makesNrf2 signaling pathway more likely to be involved, which in turn regulates activity of membrane potential, biogenesis, antioxidant defense, and mitochondrial integrity **[44, 45]**. This came in line with previous researches that revealed Parkin expression and enhanced mitophagy were caused by Nrf2 activation **[46].**

In contrast, Parkin was shown to be downregulated in heart tissue of rats treated with ISO compared to controls, implying that ISO hindered mitophagy and mitochondrial function. These findings were consistent with **Rostamzadeh et al. [47]** found that ISO altered balance between oxidative stress and mitochondrial fission, fusion, and mitophagy.

Furthermore, growing evidence indicates that inflammation plays a critical role in exacerbating cardiac damage following ISO administration. Neutrophils infiltrate infarcted region, releasing ROS, various inflammatory cytokines, proteolytic enzymes, and chemokines. In present study, a significant increase in NF-kB and TNFα was observed in ISO group, consistent with previous findings **[48]**. Ischemia during MI stimulates macrophages and cardiomyocytes to produce elevated levels of NF-kB, which in turn drives production of TNFα and other pro-inflammatory cytokines **[49],** thereby worsening hypoxic conditions [**48]**. Conversely, pretreatment with L-carnitine mitigates increase of TNFα and NF-kB. This is consistent with a prior investigation by **Aziz et al. [50],** which found that increased NF-kB with doxorubicin-induced cardiotoxicity is reduced by L-carnitine.

Inflammation and oxidative stress are important mechanisms in development of apoptosis.

Activation of cysteine proteases called caspases is one of most important steps in process of apoptosis. One of these proteases is caspase-3, a major effector caspase in many cells, which activates DNase and subsequently fragments DNA [**51**]. According to our research, ISO group exhibits a substantial upregulation of caspase-3 immunohistochemistry expression. Similar results have been shown by **Davidson and colleagues [52].** Considerably, its expression dropped in group that received L-carnitine pretreatment. This is consistent with earlier research [**53**] and evidenced anti-apoptotic impact of L-carnitine**.**

Conclusion

This study demonstrates cardioprotective effects of L-carnitine in a rat model of MI. Underlying mechanisms for these protective effects likely involve reduction of inflammation and apoptosis, alongside its antioxidant properties, which may be explained by upregulation of Nrf2 proteins in cardiac tissue. L-carnitine appears to significantly promote mitophagy throughNrf2/Parkin signaling pathway. As a result, L-carnitine holds potential as a therapeutic option for CADs.

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All authors contribute equally in the study design, collection of samples, data analysis, and manuscript writing.

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