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PROTECTIVE ROLE OF CARNITINE SYNERGIZED WITH VITAMIN E AGAINST ISOPROTERENOL INDUCED CARDIAC INFARCTION IN RATS.

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Abstract

Background: The current study aimed to evaluate the role of carnitine in combination with vitamin E in protection against myocardial infarction induced by isoproterenol (ISO) in rats.
Materials and Methods: Rats were grouped into 5 (each 10 rats): Group I. Control fed a standard diet. Group III: Rats were injected with vitamin E (100 IU/kg bw, *i.p*) daily. Group IV: Rats were given carnitine (20 mg/kg bw, *i.p*) daily .Group V: Rats were injected with both vitamin E (100 IU/kg bw, *i.p*) and carnitine (20 mg/kg bw, *i.p*) daily. On 7th, 8th, and 9th day, rats in groups (II-V) were injection *i.p* with ISO (55mg/kg b.w for successive three days)**.** The treatment with carnitine and vitamin E were continuous for 21 days**.**
Results: Canirine combined with vitamin E significantly increased coronary flow (CF) ($P<0.001$) in rats injected with ISO. The recovery of rate pressure product (RPP) and left ventricular developed pressure (LVDP) were significantly improved in treated rats in comparison to untreated. The rats administrated with ISO resulted in a significant elevation of serum enzymes (CK-MB and LDH) compared with control group ($p<0.001$). However, it returned to about normal. ISO administration resulted in a significant elevation in the levels of malondialdehyde (MDA) and nitric oxide (NO) as compared with control ($p<0.001$) and a significant reduction in the activities of GSPxase and GSRase ($p<0.001$) compared with control group. The levels of cardiac inflammatory markers interleukine-6 (IL-6) and tumor necrosis factor (TNF- α) were markedly elevated in rats injected with ISO compared with control group. Vitamin E combined with carnitine reversed these effects. However, pretreatment with vitamin E or carnitine or combined together showed a significant reduction in MDA and NO ($p<0.001$) and a significant elevation in the activities of GSPxase and GSRase ($p<0.001$) as compared to ISO injected group. The combined effect was more significant than individual ones.
Conclusion: Vitamin E combined with carnitine exerts potential protective effect against MI through suppression of inflammatory mediators and enhancement of antioxidant activity.

Key words: Myocardial infarction- ISO- Vitamin E-carnitine.

List of abbreviations: Aspartate transaminase (AST), lacatae dehydrogenase (LDH), and creatine kinase (CKMB)**,** isoproterenol (ISO)**,** interleukine-6 (IL-6) , tumor necrosis factor (TNF- α), malondialdehyde (MDA) and nitric oxide (NO).

Introduction

Myocardial infarction (MI) is one of the most cardiovascular disorders that caused by sudden blockage of blood supply to myocyte and should be reperfused within hour to complete recovery [Kendler,1983]. The etiology of MI including hypertension, diabetes mellitus, lifestyle, environmental pollution and stress [De Viovo and Tein,1990]. Experimentally, MI can be induced by injection of animals with isoproterenol (ISO) that act as sympathomimetic agonist [Rebouche and Chenard,1991].

Isoproterenol (ISO) is a synthetic catecholamine was known to have a toxic effect on the heart. The mechanisms that ISO -induced cardiac damage through generation of reactive oxygen species and depletion of antioxidant capacity. The therapeutic strategy for treatment of MI have many side effects. Replacement therapy by natural products play an important role in the management of many diseases, including cardiovascular diseases.
L-Carnitine is an important fatty acyl carrier from cytosol to mitochondrial matrix for oxidation of long chain fatty acids [Cave etal.,2008]. It obtained from diet or can be synthesized from lysine and methionine [Rebouche, 1992]. Vitamin C, iron, vitamin B6 and niacin are required as coenzymes. Deficiency of carnitine resulted decreased energy supplied by heart, nervous system and skeletal muscles [Lombard etal.,1989]. Vitamin E (γ-tocopherol) one of fat soluble vitamin possesses important biological functions as cell proliferation [9], improvement of endothelial cell [Vaz etal.,2002].

Many trails have been done to explore the rational of carnitine and vitamin E in protection against different diseases. It was found that, methotrexate given in rats showed body weight reduction, elevation in MDA levels, and

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reduction of SOD activity. However, vitamin E and L-carnitine treatments suppressed lipid peroxidation and enhance SOD activity [Rebouche, 2006].

In addition, skeletal anomalies incidence including limbs, vertebrae, and sternum defects were decreased by L-carnitine. The mean of weight and length of animals' fetuses received L-carnitine were significantly greater than those received only Cyclophosphamide. In conclusion, L-carnitine significantly decreased teratogenicity induced by Cyclophosphamide.

For this reason, the rational of the present study is to assessing the useful of carnitine in combination with vitamin E in protection against MI induced by ISO in rats. We hope to deduce a new regime for protection against CHD.

Materials and Methods

Materials

Isoproterenol (ISO): Isoproterenol hydrochloride was purchased from Sigma Chemical Co., USA. Vitamin E and carnitine were obtained from GNC Store at Jeddah, Saudi Arabia.

Animals

This study was carried out on a total of 50 adult male rats weighing 150–200 g. The animals were housed in steel cages and left for one week- before start the experiment. The handling of animals according to ethical committee of the university.

Design of the experiment

Rats were grouped into 5 groups (each 10 rats): **Group I.** Control fed a standard diet. Rats in groups (II-V) were *i.p* injection with ISO (55mg/kg b.w for successive three days) for induction of myocardial infarction**. Group:** Kept untreated**. Group III:** Rats were injected with vitamin E (100 IU/kg bw, *i.p*) daily. **Group IV**: Rats were given carnitine (20 mg/kg bw, *i.p*) daily .**Group V**: Rats were injected with vitamin E (100 IU/kg bw, *i.p*) and carnitine (20 mg/kg bw, *i.p*) daily. On 7^{th}, 8^{th}, and 9^{th} day of the treatment with carnitine and vitamin E were continuous for 21 days.

Assessment of Cardiac function and coronary flow

Blood sample was collected after anesthesia with thiopental. Sera were separated by centrifugation at 4000 *g* at 4°C for 15 minutes and stored at -80°C. Heart was removed, rinsed from blood in buffer saline pH 7 and stored at -80°C. Heart from different groups treated with either Vitamin E or carnitine or both were excised and transferred to in ice-cold Krebs solution. The heart was cannulated and retrograde perfused through the aorta in Krebs solution (mmol/l: NaCl 118, NaHCO3 25, KCl 4.8, KH2PO4 1.2, MgSO4 1.2, Glucose 11 and CaCl2 1.2) at pH 7.4. Following removal of the left atrial appendage, a deflated water filled latex balloon was inserted through the mitral valve in to the left ventricle. Continuous monitoring of cardiac performance. The index of myocardial function includes heart rate (HR; cardiac spontaneous rhythm was counted per min), and the rate pressure product (RPP=LVDP×HR). (LVDP) left ventricular developed pressure. Coronary flow (CF) was measured by timed collections of the coronary effluent.

Biochemical Assays

Serum aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatine kinase (CKMB) were estimated by colorimetric method using commercial KIT from Biomedical [Thygesen etal., 2010].

Assay of inflammatory cytokines levels of IL-6, TNF-α, malondialdehyde (MDA) and antioxidant enzymes in cardiac tissue.

One gram cardiac tissue was homogenized in 9 volumes of ice-cold saline and centrifuged at 8000 g at 4°C for 20 min. The amount of protein in supernatant was measured according to [16] using bovine serum albumin as standard. Malondialdehyde (MDA) was measured according to [Maron,1979]. Nitric oxide was determined as nitrate according to [Afrah etal.,2013]. SOD activity was determined by the nitro blue tetrazolium reduction method [Habig etal.,1974]. glutathione peroxidase [Pagila and Valentine, 1969], glutathione reductase [Deore etal.,2011]. Levels of cytokines were measured by ELISA kits from BIORAD (England).

Histopathological examination

Heart tissue was fixed in buffer formalin and embedded in paraffin, then serial sections (10 μm thick) were cut using microtome. Each section was stained with hematoxylin and eosin (H&E). The sections were examined under the light microscope. The degree of necrosis was graded according to inflammation and necrosis:

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[−] absence of inflammation edema.
[+] spotted area of inflammation edema.
[++] many area of inflammation, edema and necrosis.
[+++] massive area of inflammation, edema and necrosis.

Statistical Analysis

Data obtained were analyzed using the program SPSS 15.0 for Windows. Data were presented as mean ± SD. For comparison between multiple groups, data were analyzed by ANOVA. Differences were considered to be statistically significant when $P<0.05$.

Results

Cardiac function and coronary flow

The heart efficacy function parameters showed that, the mean of CF in ISO rats treated with combined carnitine and vitamin E significantly increased in compared with ISO untreated (17.15 ± 0.6 Vs14.3 ± 0.52 and 13.13 ± 1.00 ml/min). Also, rats received carnitine or vitamin E alone showed CF significantly increased but less than combined (15.2 ± 1.49). However, in the treated groups, the RPP and LVDP indices were significantly better in than untreated ($p<0.001$). The data presented in Table (1) showed that, rats administrated with ISO resulted in a significant elevation of serum enzymes (LDH, AST and CK-MB) activity compared with normal rats ($p<0.001$). Pretreatment with vitamin E or carnitine or combined together showed a significant reduction in the activity of these enzymes compared with untreated. The reduction doesn't reach to normal value but higher than normal. The combined effect is more significant in effect on CK-MB better than LDH and AST ($p<0.01$).

Table (2**)** showed the marker oxidative stress (MDA and NO) in the cardiac tissue homogenate**.** The rats administrated ISO resulted in a significant elevation in the levels of MDA and NO as compared with control ($p<0.001$). However, pretreatment with vitamin E (100 IU/kg b.w, ip) or carnitine (20 mg/kg b.w, *ip*) or combined together showed a significant reduction. The combined treatment tends to normalize MDA but in NO still higher than normal. The activities of cardiac antioxidant enzymes (SOD, GSPxase and GSRase) in the cardiac tissue revealed that, a significant elevation in the activity of SOD ($p<0.001$) whereas a significant reduction in the activities of GSPxase and GSRase ($p<0.001$ and $p<0.01$) in rats injected with ISO compared to control group. Pretreatment with with vitamin E (100 IU/kg b.w, ip) or carnitine (20 mg/kg b.w, *ip*) or combined together showed a significant decrease in the activity of SOD ($p<0.001$) and a significant elevation ($p<0.001$) in the activities of GSPxase and GSRase as compared to ISO injected group. The combined effect was more significant on GSRase better than other enzymes. But in all treatment not reach to normal values.

Results in table (3) revealed that the levels of IL-6 and TNF-α in tissue extract were significantly elevated ($p<0.001$) in ISO injected rats. Administration of vitamin E with carnitine reduced it ($p<0.001$) compared with ISO untreated. The combined treatment exerted a significant potent action than individual treatment.

Table 1: The activities of serum Lactate dehydrogenase (LDH), Aspartae transaminase (AST) and creatine kinase (CK-MB) inn all studied groups (mean±SD).

Groups	*Control*	*Iso*	*Iso+Vit E*	*Iso+carnitin*	*Iso+VitE+carnitn*
LDH (IU/L)	83.4±6.5	260±23 [a]	220±16.5 [b]	199.9±12.3 [b]	134.8±19 [c]
AST (IU/L)	45.7±3.5	122.4±8.5 [a]	93.4±7.5 [b]	90.4±11 [b]	63.9±4.5 [c]
CK-MB (U/L)	113±11	273.4±16[a]	184±14 [b]	165±9[b]	123±11[c]

Results were expressed as mean ± SD ($p<0.05$), analysis of variance for multiple comparison by Dennett's test.
a: ISO vs. control.
b: ISO vs. individual treatment.
c: combined treatment vs. individual treatment.

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Table 2: Cardiac malondialdehyde (MDA), nitric oxide (NO) levels and the activities of superoxide dismutase(SOD), glutathione peroxidase (GSPaxe) and glutathione reductase (GSRase) in all studied groups (mean± SD)

Groups	***Control*** N=10	***ISO*** N=9	***Iso+Vit E*** N=9	***Iso+*carnitine N=8**	***Iso+VitE+****carnitine N=9*
MDA (nmol/g)	23±2.5	77±13 [a]	53±11 [b]	41±7.5 [b]	31.8±1.9 [c]
NO (ug/g)	103±12	270±33 [a]	201±19.5 [b]	220±16.5 [b]	131.8±15 [c]
SOD(U/g)	90.1±8.5	190±33 [a]	159±19 [b]	140±13 [b]	154.8±12
GSPXae(U/g)	490±63.	240±43 [a]	320±43 [b]	370±16.5 [b]	380±19
GSRase	1290±193	880±76 [a]	998±98 [b]	1110±176 [b]	1106±200

Results were expressed as mean ± SD ($p<0.05$), analysis of variance for multiple comparison by Dunnett's test.
a: ISO vs. control.
b: ISO vs. individual treatment. c: combined treatment vs. individual treatment.

Table 3: cardiac IL6 and TNF- α levels in different studied groups (Mean ±SD).

Animal groups	Control N=10	ISO N=9	***Iso+Vit E*** N=8	***Iso+carnitin*** N=8	***Iso+VitE+carniti n*** N=9
IL-6 (ng/g) Mean± SD	178± 28	973±66 [a]	810±75 [a,b]	604±33 [a,c]	401±59 [a,,b,c]
TNF- α (ng/g) Mean ± SD	10.1±0.9	22± 3.2 [a]	17±2.6 [a,b]	15±3.9 [a,c]	13±1.9 [a,,b,c]

Results were expressed as mean ± SD ($p<0.05$), analysis of variance for multiple comparison by Dunnett's test.
a: ISO vs. control.
b: ISO vs. individual treatment.
c: combined treatment vs. individual treatment.

Histopathological Examination

It was found that, control rats showed normal histological architecture manifested by normal coronary artery wall thickness, normal size and appearance of cardiac muscles with oval central euchromatic nuclei and normal blood capillaries and no necrosis as illustrated in **(Figure A).** In rats injected with isoproterenol, irregular thickening of coronary wall with perivascular mast cell infiltrate and narrow of lumen due to buildup of atheromatous plaque, disorganization of cardiac muscle structure with acidophilic dark regions or bands indicating mild necrosis and marked congestion of blood capillaries as demonstrated in **(Figure B).** Rats treated with carnitine showed highly improvement of pathological structure and showed normal thickness of coronary artery with wide lumen without any narrowing, preservation of normal structure of cardiac muscles with absence of degenerated dark bands and the nuclei of cells looked active and euchromatic **(figure C).** Treatment with vitamin E showed also normal heart section cardiac muscles with no congestion or degeneration with oval central euchromatic nuclei, normal thickness of coronary artery with wide lumen without any narrowing (**figure D).** Mixture of carnitine and vitamin E revealed a marked improvement in histological architecture of the heart tissue and marked congestion blood capillaries and perfective thickness of coronary artery with moderate lumen without cholesterol deposition **(figure E).**

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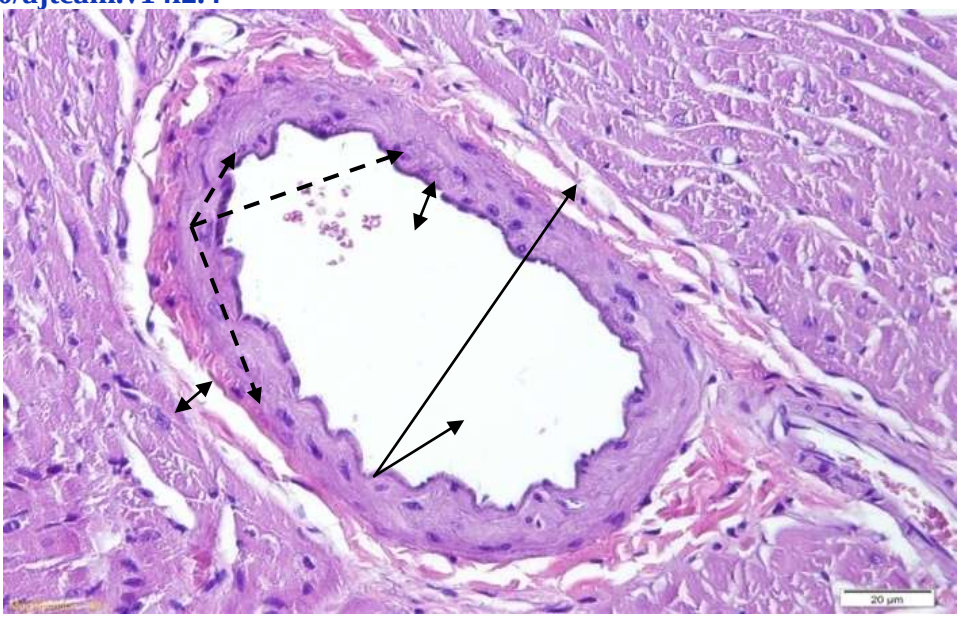

Figure 1: Represent myocardium of control rats showing normal architecture of the muscle fibers with abundant wavy cytoplasm and small nuclei.

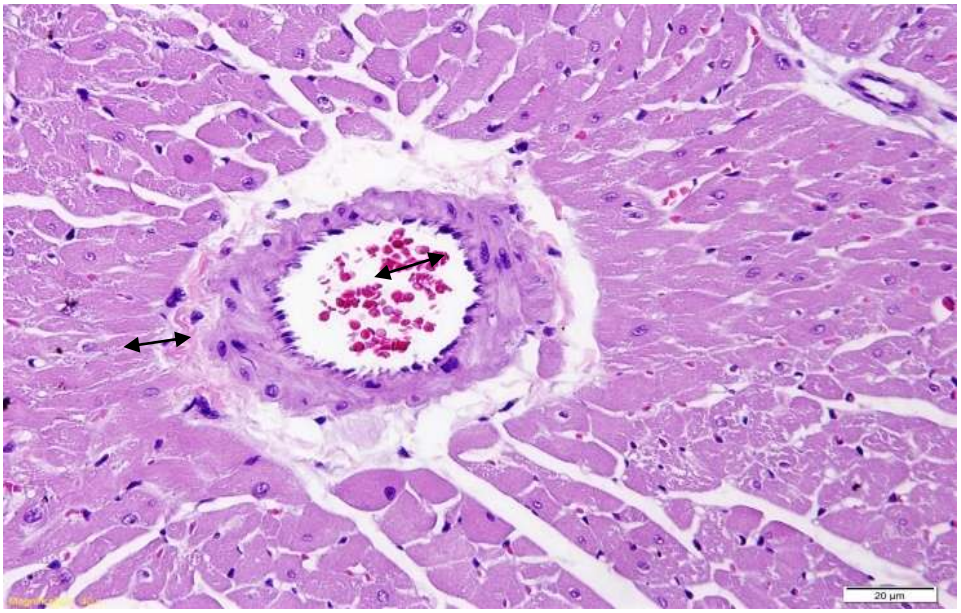

Figure 2: Section of myocardium of rats injected with ISO and that most of the myocytes showed increased eosinophilia infiltration inside cardiomyocyte.

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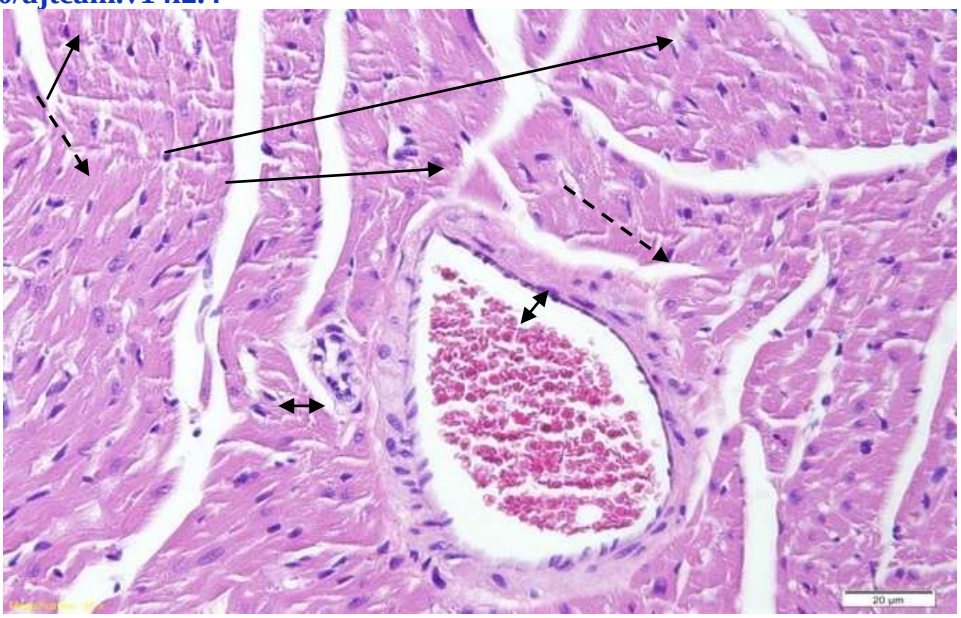

Figure 3: Section of myocardium of rats pretreated with carnitine showed most of myocytes have normal pale granular central nuclei.

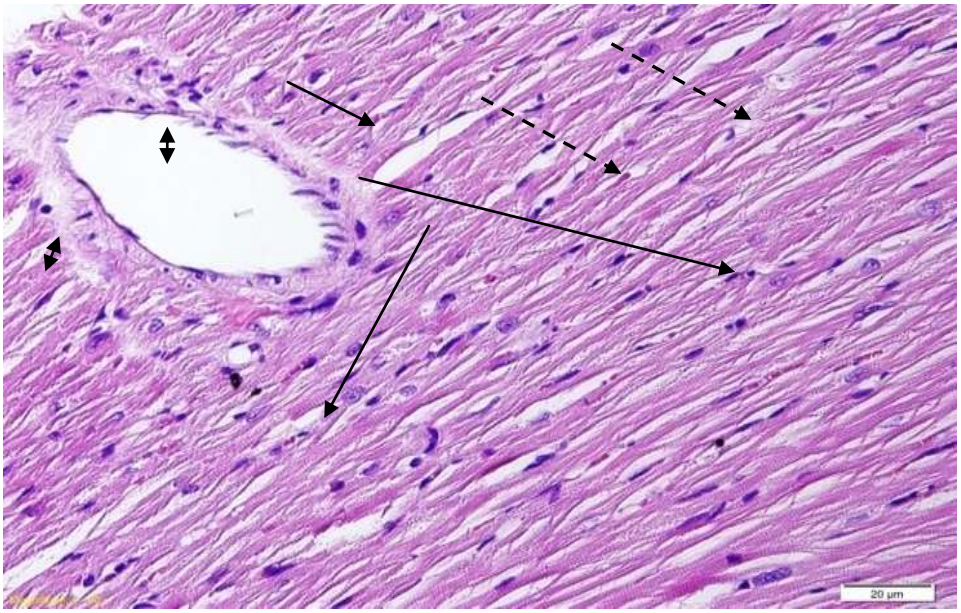

Figure 4: Section of myocardium of rats pretreated with vitamin E showed congestion of blood capillaries and absence of cross striations.

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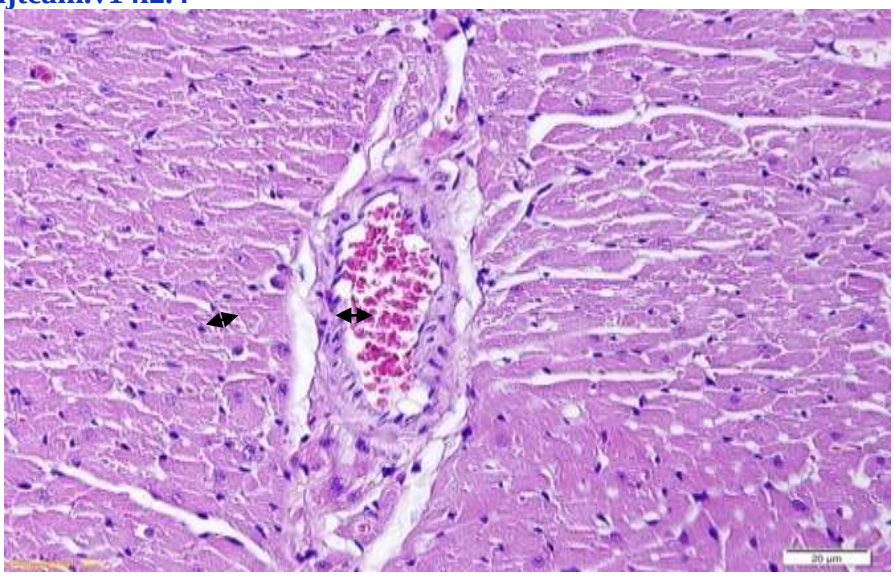

Figure 5: Section of myocardium section of myocardium of rats pretreated with carnitine and vitamin E showed no apparent changes in cardiac myocyte staining intensity.

Discussion

The patho-physiological and morphologic alterations in the heart of this non-coronary myocardial necrotic rat model are similar to those taking place in human myocardial infarction [Spagnoli etal.,1982]. By studying the biochemical alterations that take place in an animal model, it is possible to gain more insight into the mechanisms leading to the altered process in human MI [Weitz etal.,1991]. The present study has clearly demonstrated that, carnitine and vitamin E possess antioxidant activity which could prevent the occurrence of heart related diseases via free radical scavenger.

It has been suggested that the cardio protective effect of carnitine and vitamin E is due to anti-inflammatory and antioxidant activities on coronary endothelial function and vasoreactivity [Yuncu etal.,2015]. Also, it has been shown that Vitamin E improved endothelial function with CHD [Mahmoud etal.,2015]. In current study carnitine and vitamin E increased the CF in rats injected with ISO compared with untreated. This is may be due to their vasodilator activity on blood vessels.

Serum LDH, AST and CK-MB activities are sensitive markers to evaluate the severity of myocardial infarction [Whellen,2005]. In the present study, the increased activities of these enzymes after administration of ISO in rats as a marker for the occurrence of myocardial dysfunction was in accordance with results as mentioned [Dhalla etal.,1996]. Pretreatment with carnitine and vitamin E reduced the activities of these enzymes compared with untreated. This is an indication of the protective effect of the combined action of carnitine and vitamin E in reversing cardiac damage and recovery of myocyte. Previous study indicated that, L-carnitine administration have a cardio protective role in cardio-myopathy, prevention of myocardial infarction [Lango etal.,2001]. Arsenian *et al* [1996] demonstrated a decrease in mortality and incidence of circulatory failure in patients with acute myocardial infarction who were administered of L-carnitine with glucose, insulin, potassium and magnesium.

The antioxidant enzymes play an important role in free radical scavenger and protection against oxidative stress. Superoxide dismutase convert peroxyradical to superoxide which converted by catalase to water. In the current study, ISO injected to rats resulted in enhancement in initiation and propagation of lipid peroxidion in myocyte that decrease the efficacy of myocyte and lower the blood supply to the heart [Yusuf etal.,2000]. ISO induced myocardial infarction by stimulation of SOD activity, and a significant reduction in GSPaxe and GSrase compared with control. Carnitine and vitamin E treatment reverse the action of ISO by reduction of SOD and elevation of GSPaxe and GSrase. This result may be due to upregulation effect of carnitine and vitamin E on GSPaxe and GSrase and downregulation effect on SOD. Previous study indicated that, the incidence of infarction decreased 5 % among intake of vitamin E compared with the non-recipients. However, the observed effect did not reach statistical significance that is in accordance with the present study.

In the current study, rats injected with ISO caused a significant elevation of inflammatory markers as (IL-6 and TNF-α) that cause damage of liver tissue. Treatment with combination of vitamin E and carnitine caused a reduction in these mediators and recovery of cardiocyte as indicated by a decrease in CK-MB and LDH activities. The histopathological examination support the biochemical study as indicated by improvement of myocyte fibers in treatment with vitamin E and carnitine and disappearance of necrosis. This study explained that administrations of ISO induced production of cardiac lipid peroxidation and inflammatory mediators that caused necrosis and infarction. However, pretreatments of carnitine and vitamin E act as radical scavenger and suppression release of these mediators to reverse this action. This is in accordance to previous study reported, oxidative stress has been implicated in

cardiovascular disease. Many of the deleterious cellular myocardium may be attributed to ROS and oxidative stress [Mann etal.,2004]. Experimental studies with antioxidants support the hypothesis that oxidative stress is pathogenic in myocardial remodeling and failure. The histopathological examinations support the biochemical analysis by improving the caridocyte with reduction in infiltration of monocyte in rats supplemented with both carnitine and vitamin E.

Conclusion

Combination of carnitine with vitamin E pretreatment normalized the levels of malondialdehyde and antioxidant enzymes of cardiac tissue in rats injected with ISO. Further studies are needed to determine signal mediated by which vitamin E and carnitine protective effect against MI.

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