Bull. Egypt. Soc. Physiol. Sci. Vol. (42). Issue (3), 213 - 230

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Protective Effect of the Combined use of L-carnitine and L-arginine against Hepatic Ischemia-Reperfusion Injury in Rats

Magdi A. El-Damarawi MD[1], Noha Abdel-Aziz Nassef MD [2]

Department of Medical Physiology, Faculty of Medicine, Tanta University, Tanta, Egypt[1*]

Department of Medical Physiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt[2]

Submit Date: 29 Nov. 2021
Revise Date: 13 March 2022
Accept Date : 13 March 2022

Keywords

- hepatic
- ischemia-reperfusion
- L-carnitine
- L-arginine

Abstract

Background: Hepatic ischemia-reperfusion injury (IRI) can be mainly caused by oxidative stress, decreased nitric oxide (NO) level, activation of hepatic Kupffer cells, neutrophils adhesions, increased level of intercellular adhesion molecules, and inflammatory process. **Aim:** Studying the ability of L-carnitine (LC) and L-arginine (LG) to protect the liver cells against the damage caused by IRI. **Methods:** This study was carried out on 40 male Wistar albino rats which were divided into 5 groups; sham-operated, hepatic IRI, IRI rats pretreated with L-carnitine, IRI rats pretreated with L-arginine, IRI rats pretreated with both substances. **Results:** The pretreatment with LC and LG separately protected the liver cells against IRI damage with preservation of liver functions by significantly improving the oxidative stress and inflammatory states, increasing NO level, decreasing the expression of vascular adhesion molecules in liver tissue, and protecting the liver cells from the damage. It was obvious that LC effects were more significant on all the tested parameters except on the NO level where the LG effect was more significant. Moreover, the combined use of both LC and LG produced a highly significant improvement of all the tested parameters approaching near the control level than that observed by their separate use. **Conclusion:** The combined use of both LC and LG could effectively protect the liver cells from the harmful effects of IRI.

Corresponding author: **Eldamarawi, Magdi** Assistant Professor of Medical Physiology, Faculty of Medicine, Tanta University, Tanta, E mail : magdidamarawi@med.tanta.edu.eg, Tel. 0020403411966.

INTRODUCTION

Hepatic ischemia-reperfusion injury (IRI) is a common and main clinical complication that occurs in many medical conditions as hepatic surgery, trauma, resection, and implantation (1). It also occurs in conditions of hypoxia as shock and cardiac surgery with cardiopulmonary bypass (2). The liver damage due to IRI is the result of many factors as hypoxia, anaerobic metabolism, oxidative stress with a generation of reactive oxygen species (ROS), intracellular calcium overload, decreased nitric oxide (NO), activation of hepatic Kupffer cells (KCs), neutrophils adhesions, and increased level of intercellular adhesion molecules, cytokines, and chemokines (3).

The cellular damage due to ischemia-reperfusion injury is partly caused by ROS generation in the affected tissue (4). These ROS produce damage to cell membranes by lipid peroxidation (5).

The decrease in nitric oxide (NO) level is very prominent in cases of IRI in different organs like the kidneys, liver, and intestine. It is considered a very important factor in the pathophysiological changes that occur in the affected organs due to IR (6).

L-carnitine (β-hydroxy-γ-N-trimethyl ammonium butyrate) is a natural substance that is present in certain types of foods as meat, fish, chicken, and milk. It is needed for the transport of long-chain fatty acids from the cytosol to mitochondria for the release of energy from the β-oxidation of these fatty acids. It was used for the protection of liver cells from IRI (7). Also, L-carnitine plays a very valuable role in the stabilization of the membrane and inhibition of permeability of the mitochondria which contributes to its anti-apoptotic effect (8,9). Moreover, it has an antioxidant, anti-ischemic effect, and protects against cleavage of deoxyribonucleic acid (DNA) (10). Supplementation with L-carnitine might help to prevent hepatic tissue damage caused by oxidative and inflammatory stresses which occur during warm IR (11)

L-arginine (LG) is a semi-essential α-amino acid found in nuts, whole grains, meat, fish, and many other types of foods. It operates as an intermediate in the urea cycle, and as a substrate for the synthesis of NO. In the kidneys, LG is synthesized from l-citrulline, a semi-essential amino acid that is formed from glutamine in the alimentary tract. L-arginine is converted to nitric oxide by the action of the NO synthase isoforms (12). It was proved that supplementation with LG minimizes hepatic injury in cases of hepatic ischemia-reperfusion by improving the microcirculation (13). Many studies reported that the intravenous administration of L-arginine in cases of hepatic IRI significantly increased the NO level and protected the liver against damage (13,14,15).

According to the mechanisms involved in the occurrence of IRI, the protection from the harmful effects of hepatic IRI should be directed to the use of natural substances that possess antioxidant activity, can increase NO levels, help to decrease the elevated levels of pro-inflammatory cytokines and cell adhesions molecules.

The present work aimed to study the protective effect of the combined use of L-carnitine and L-arginine against hepatic IRI in rats. To our knowledge, this is the first research to investigate the effect of this combination for the protection against hepatic ischemia-reperfusion injury. We chose these two substances because of their antioxidant and anti-inflammatory effects. Also, they are available, not expensive,

present in various types of food, and easy to be used by the patients.

MATERIAL AND METHODS:

After approval from the Research ethics committee of the Faculty of Medicine, Tanta University (Approval code: 34350/12/20), this study was carried out on forty male Wistar albino rats weighing 180–200 g. The rats were obtained from the animal house in Medical Ain Shams Research Institute (MASRI), Faculty of Medicine, Ain Shams University. All animals were kept under the same environmental conditions, fed standard commercial rat chow, and had free access to tap water throughout the study. The rats were housed in isolated animal cages (5 rats per cage) and kept under a 12-hour light-dark cycle at controlled temperature (23±1°C) and humidity 70–75%. They were divided into five groups of 8 rats each and allowed to acclimatize to their environment for one week before the start of the experiment. The handling of the animals was carried out following the ethical guidelines for investigations.

Research design

The present study was performed on 40 male Wistar albino rats which were divided into 5 groups (8 rats in each group):

- **Group 1 (Sham-operated group):** No IR operation was done. The rats were given saline (1ml/kg/day) by orogastric tube (oral gavage) for one week, then a midline laparotomy incision was performed, and the liver was isolated.
- **Group 2 (hepatic IR+ saline):** The rats were given saline (1ml/kg/day) by orogastric tube (oral gavage) for one week before the time of IR operation. Then, the operation was performed.
- **Group 3 (hepatic IR+ L-carnitine):** The animals were pretreated with oral L-carnitine in a dose of 200 mg/kg/day (16) dissolved in 1 ml normal saline by orogastric tube (oral gavage) for one week before the time of the IR operation. After this period, the operation was done.
- **Group 4 (hepatic IR + L-arginine):** The rats were pretreated with L-arginine orally in a dose of 100 mg/kg/day (17) dissolved in 1 ml saline by orogastric tube (oral gavage) for one week before the time of the operation. Then, the operation was performed.
- **Group 5 (hepatic IR+ L-carnitine+ L-arginine):** The rats were pretreated with both substances in the same doses and in the same way for one week before the time of the IR operation. Then, the operation was done.

Surgical procedures:

After overnight fasting, the rats were anesthetized with intraperitoneal administration of ketamine 50 mg/kg and xylazine 5 mg/kg. The abdomen was shaved and disinfected with 75% ethanol. A midline incision was performed, and the hilum of the liver was exposed. All structures in the portal triad (hepatic artery, portal vein, and bile duct) were moved to the left and median liver lobes vessels were occluded by a clamp to produce 70 % hepatic ischemia. Sixty minutes later, reperfusion of the liver was done for 90 minutes by opening the clamp (18). At the end of the experimental procedure, the animals were sacrificed, and blood and liver tissue samples were obtained for further

biochemical and histopathological investigations. The blood was centrifuged at 3000 rpm for 15 min to separate serum for measurement of liver enzymes. For histopathological examination, liver tissue samples were preserved in formalin 10% solution and processed in the usual way until paraffin blocks were obtained.

Chemicals:

L-carnitine and L-arginine were purchased from Sigma-Aldrich (St. Louis, Mo., USA).

Biochemical assays:

The liver enzymes were measured by the available commercial kits according to the manufacturer's instructions. The oxidative stress markers were estimated in the liver tissue as follows: malonaldehyde (MDA) by the method of Ohkawa et al. (19), superoxide dismutase (SOD) activity by the method of Marklund and Marklund (20), catalase (CAT) activity by the method of Aebi (21) and activity of glutathione peroxidase (GSH-Px) by the method of Lawrence and Burk (22).

Vascular adhesion molecule-1 (VCAM-1) was assayed by using Rat Vascular Cell Adhesion Molecule 1 ELISA Kit (Elabscience, USA), and intercellular adhesion molecule-1 (ICAM-1) in liver tissue was estimated by using Rat Intercellular Adhesion Molecule 1 (ICAM-1) ELISA Kit (MyBioSource, USA).

Tumor necrosis factor-alpha in liver tissue was measured by using Rat TNF-α ELISA Kit (CUSABIO Technology LLC, Houston, USA), and IL-1β in hepatic tissue was measured by using Rat IL-1β ELIZA Kit **(**Sigma-Aldrich, St. Louis, Mo., USA).

Nitric oxide (NO) in liver tissue was assayed using BioVision Nitric Oxide Colorimetric Assay Kit (BioVision Incorporated, Milpitas Boulevard, Milpitas, USA). The biochemical assays were performed blinded to any data.

Histopathological examination:

Fresh biopsy specimens were fixed in 10% formalin for 24–48 hours. Paraffin blocks were prepared then sectioned at 4–5μm. All sections were stained with routine hematoxylin & eosin using standard protocols. The slides were observed by light microscopy, using the 40× objective power. Histopathological examination was evaluated blinded to any data.

Assessment of histopathological changes of IRI:

The histopathological changes caused by IR were assessed and graded according to the severity of injury based on the degree of portal inflammation, sinusoidal and central vein (CV) congestion, cytoplasmic hydropic degeneration, and hepatocyte necrosis, and they were categorized into 4 grades: grade 0, minimal or no evidence of injury; grade 1; mild injury in the form of cytoplasmic hydropic degeneration with mild sinusoidal and CV congestion and mild portal inflammation; grade 2; moderate injury consisting of marked ballooning of hepatocyte, focal hepatocyte necrosis with moderate sinusoidal and CV congestion and moderate inflammation, and grade 3; severe injury in the form of extensive hepatocyte necrosis and drop out with marked sinusoidal and CV congestion and marked inflammation (23).

The techniques:

The different techniques of the study were carried out in the Physiology department of Faculty of Medicine, Ain Shams University, Pathology department of Faculty of Medicine, Tanta University, and Biochemistry department of Faculty of Medicine, Cairo University.

Statistical analysis:

Data were processed using SPSS program V. 20. They were expressed as mean (M) and standard deviation (SD). Comparisons between groups were done using a one-way analysis of variance (ANOVA) test followed by inter-group comparisons using a Post hoc test. The results were considered statistically significant if the p-value was <0.05.

RESULTS

- **Effect of hepatic IRI and pre-treatment with L-carnitine and L-arginine separately and in combination on serum liver enzymes (Table 1):**

  The results of our study showed that in group 2, the induction of hepatic IRI produced a significant elevation in the serum ALT and AST levels as compared to group 1 (sham-operated). While the pre-treatment of the rats with LC in group 3 caused a significant reduction in the serum level of both enzymes as compared to group 2 ($p<0.001$). Similarly, the administration of LG one week before the IRI in group 4 produced the same significant effect as compared to group 2 ($p<0.001$). Interestingly, the combined use of both LC and LG in group 5 resulted in a similarly significant effect as the use of each of them separately compared to group 2 ($p<0.001$) and approaching nearly the control level ($p>0.05$ compared to group 1).

- **Effect of hepatic IRI and pre-treatment with L-carnitine and L-arginine separately and in combination on oxidative stress markers levels in hepatic tissue (Table 2):**

  Hepatic IRI in group 2 resulted in a significant increase in the level of MDA with a significant reduction in the level of SOD, CAT, and GSH-Px antioxidant enzyme activities in the liver tissue when compared to their results in group 1. Comparing the results of group 3 to that of group 2 showed that the pre-treatment with LC significantly reversed the effect of IRI in group 2 with a significant decrease in MDA level and significant elevation of the activity of the antioxidant enzymes in liver tissue indicating the improvement of the oxidative stress state caused by IRI. While the pre-treatment with LG in group 4 caused similar less significant effects (compared to the IRI group) than that produced by LC. Finally, it was obvious that the use of both substances in group 5, resulted in similar more significant improvement than that produced by using each of them separately when compared to group 2 ($p<0.05$ for SOD and $p<0.001$ for the other parameters) and approaching nearly the control levels when compared to group 1 ($p>0.05$).

- **Effect of hepatic IRI and pre-treatment with L-carnitine and L-arginine separately and in combination on pro-inflammatory cytokines (IL- 1β & TNF-α) and nitric oxide (NO) levels in hepatic tissue (Table 3):**

  The level of the pro-inflammatory cytokines (IL-1β and TNF-α) showed significant elevation associated with a significant reduction of NO level in hepatic tissue in group 2 when compared to the sham-operated group. While the use of LC in group 3 significantly decreased the level of these proinflammatory cytokines and increased the level of NO as compared to the IRI group ($p<0.05$). Also, by comparing the results of group 4 to that

of group 2 we found that the administration of LG significantly reduced TNF-α level ($p<0.05$) and increased NO level ($p=0.001$) but it caused an insignificant reduction of IL-1β tissue level ($p>0.05$). Interestingly, the LG effect on TNF-α was less significant than that produced by LC while its effect on NO level was more significant than that of LC. Lastly, in group 5, the concomitant use of both LC and LG produced more significant effects on these parameters than their separate use as compared to the results of group 2 ($p<0.001$ for TNF-α & NO and $p<0.05$ for IL-1 β) and approaching nearly the control levels when compared to group 1 ($p>0.05$).

- **Effect of hepatic IRI and pre-treatment with L-carnitine and L-arginine separately and in combination on cell adhesion molecules levels in hepatic tissue (Table 4):**

Comparing the results of the hepatic IRI group to that of the sham-operated group showed that liver IRI caused a significant elevation in the level of vascular cell adhesion molecule-1 (VCAM-1) and intercellular Adhesion Molecule 1 (ICAM-1) in the liver tissue ($p<0.001$). In group 3, the pre-treatment with LC produced a significant decrease in the level of VCAM-1 ($p<0.001$) and ICAM-1 ($p=0.003$) when compared to group 2. Interestingly, the use of LG in group 4 caused a similar but less significant effect ($p=0.007$ for VCAM-1 and $p=0.017$ for ICAM-1) than that produced by LC. Ultimately, the use of both substances in group 5 resulted in similar more significant effects ($p<0.001$) compared to that of group 2 than separate use of each of them and these results are near the control results as compared to group 1 ($p>0.05$).

Table 1: Effect of hepatic IRI and pre-treatment with L-carnitine (LC) and L-arginine (LG) separately and in combination on serum liver enzymes.

Parameter	Group 1 (Sham-operated)	Group 2 (IRI)	Group 3 (IRI+LC)	Group 4 (IRI+LG)	Group 5 (IRI+LC+LG)
Serum ALT (U/L)	19±8.7	69.57±29.3$^{\#}$	30.15±8.4**	32±14.2**	26.38±5.7** †
Serum AST (U/L)	21±9.1	57.71±19.4$^{\#}$	22.3±8.4 **	28.57±9.2**	21.5±3.5**†

Data are expressed as mean±S. IRI= Ischemia-reperfusion injury,
ALT= Alanine aminotransferase, AST= Aspartate aminotransferase
# $p < 0.05$ compared to group 1. * $p < 0.05$ compared to group 2.
** $p < 0.001$ compared to group 2. † $p > 0.05$ compared to group 1.

Table 2: Effect of hepatic IRI and pre-treatment with L-carnitine (LC) and L-arginine (LG) separately and in combination on oxidative stress markers levels in hepatic tissue.

Parameter	Group 1 (Sham-operated)	Group 2 (IRI)	Group 3 (IRI+LC)	Group 4 (IRI+LG)	Group 5 (IRI+LC+LG)
MDA (nmol/mg protein)	42.33±15.1	89.41±38.2$^{\#}$	63.34±14.9*	65.16±19.3*	43.81±17.7**†
SOD (U/mg protein/min)	29.15±9.9	19.10±10.1$^{\#}$	28.34±3.2*	26.71±4.4*	29.04±6.07*†
GSH-Px (U/mg protein/min)	85.10±7.6	53.80±20.9$^{\#}$	70.85±8.9*	67.19±6.68*	81.21±8.11**†
CAT (U/mg protein/min)	117.88±10	81.03±28.4$^{\#}$	111.07±8.38*	99.93±16.03*	115.81±11.03**†

Data are expressed as mean±SD IRI= Ischemia-reperfusion injury,
MDA= Malonaldehyde, SOD= superoxide dismutase, GSH-Px= Glutathione peroxidase, CAT= Catalase
# $p < 0.05$ compared to group 1. * $p < 0.05$ compared to group 2.
** $p < 0.001$ compared to group 2. † $p > 0.05$ compared to group 1.

Table 3: Effect of hepatic IRI and pre-treatment with L-carnitine (LC) and L-arginine (LG) separately and in combination on pro-inflammatory cytokines (IL 1β and TNF-α) and nitric oxide (NO) levels in hepatic tissue.

Parameter	Group 1 (Sham-operated)	Group 2 (IRI)	Group 3 (IRI+LC)	Group 4 (IRI+LG)	Group 5 (IRI+LC+LG)
IL-1β (pg/mg)	107.83±19.6	153.04±56.4$^{\#}$	110.53±11*	143.29±51.9	110.94±11.8*†
TNF-α (pg/mg)	46.83±14.1	95.96±34.6$^{\#}$	59.31±14.3*	72.54±15.3*	54.04±14.5**†
NO (nmol/mg)	36.24±7.71	13.7±4.19$^{\#}$	22.95±8.43*	28.74±11.30*	34.61±5.92**†

Data are expressed as mean±SD IRI= Ischemia-reperfusion injury,
IL-1β= Interleukin-1β, TNF-α= tumor necrosis factor- α, NO= Nitric oxide
# $p < 0.05$ compared to group 1. * $p < 0.05$ compared to group 2.
** $p < 0.001$ compared to group 2. † $p > 0.05$ compared to group 1.

Table 4: Effect of hepatic IRI and pre-treatment with L-carnitine (LC) and L-arginine (LG) separately and in combination on cell adhesion molecules levels in hepatic tissue.

Parameter	Group 1 (Sham-operated)	Group 2 (IRI)	Group 3 (IRI+LC)	Group 4 (IRI+LG)	Group 5 (IRI+LC+LG)
VCAM-1 (pg/mg)	218.12±12.8	286.24±49.7$^{\#}$	221.5±9.6**	252.24±8.7*	213.3±6.7**†
ICAM-1 (pg/mg)	2.38±0.8	6.57±3.2$^{\#}$	3.6±1.2*	4.24±1.9*	2.65±1.1**†

Data are expressed as mean±SD IRI= Ischemia-reperfusion injury,
VCAM-1=Vascular cell adhesion molecule 1, ICAM-1= intercellular Adhesion Molecule 1
# $p < 0.05$ compared to group 1. * $p < 0.05$ compared to group 2.
** $p < 0.001$ compared to group 2. † $p > 0.05$ compared to group 1.

- **Effect of hepatic IRI and pre-treatment with L-carnitine and L-arginine separately and in combination on liver histopathology (Figure 1):**

Histopathological results

Histopathological examination of group I specimens (sham-operated group) showed normal lobular architecture, normal CV and sinusoidal calibers, normal arrangement of hepatocyte cords with an absence of portal inflammation (Figure 1A). While group II (ischemia-reperfusion injury) specimens showed grade 3 liver injury in which there was extensive hepatocyte necrosis, marked sinusoidal and CV congestion with marked portal inflammation (Figure 1B&C). Group III (IRI+LC) showed grade 1 liver injury in which hepatocytes underwent mild hydropic degeneration with mild sinusoidal and CV congestion (Figure 1F&G). Examination of group IV (IRI+LG) revealed grade 2 liver injury evidenced by marked ballooning of hepatocyte, focal hepatocyte necrosis with moderate sinusoidal and CV congestion, and moderate portal inflammation (Figure 1D&E). No evidence of liver injury (nearly normal liver) or minimal injury (grade 0) was observed in group V (IRI+LC+LG) rats (Figure 1H).

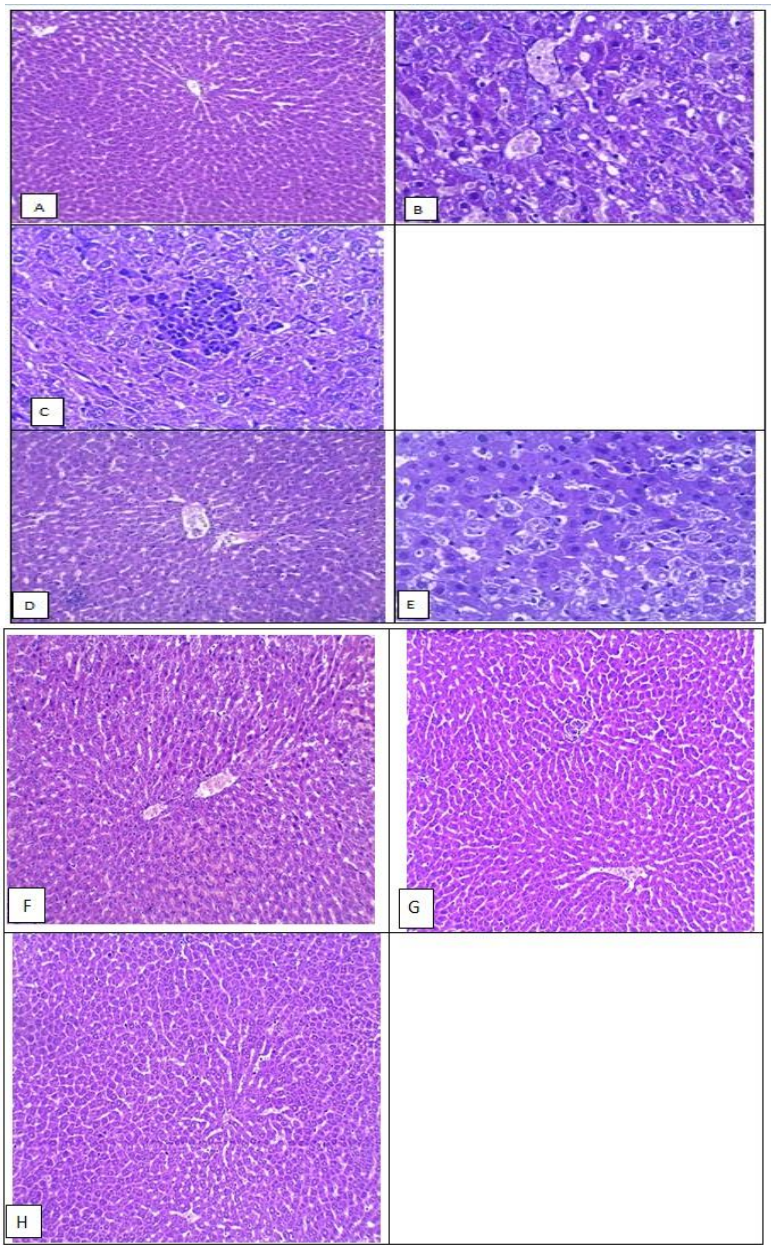

Figure 1: Histopathological changes of ischemia-reperfusion injury of hepatic tissues

(A): Normal hepatic lobular architecture with normal arrangements of hepatic cords, absence of portal inflammation (×200). (B): Grade 3 liver injury showing marked dilatation and congestion of central vein and liver sinusoids with hepatocytes necrosis and drop out (×400).(C): Grade 3 liver injury showing marked portal inflammation (×400). (D): Grade 2 liver injury showing moderate injury in the form of moderate sinusoidal and central vein congestion and moderate inflammation (×200). (E): Grade 2 liver injury showing marked ballooning of hepatocyte, focal hepatocyte necrosis (×400). (F&G): Grade 1 liver injury showing mild injury in the form of cytoplasmic hydropic degeneration with mild sinusoidal and central vein congestion and mild portal inflammation (×1200). (H): Grade 0 minimal or no evidence of liver injury (×200).

DISCUSSION

Hepatic ischemia-reperfusion injury (IRI) is a common and main complication that occurs in many clinical conditions as hepatic surgery, trauma, resection, and implantation (1). The present work aimed to study the protective effect of the combined use of L-carnitine and L-arginine against hepatic IRI in rats.

The results of the current study revealed that pre-treatment of the rats with either LC or LG separately produced a significant reduction in the serum level of the liver enzymes (AST and ALT) compared to the untreated IRI group. Interestingly,

pre-treatment with LG caused a similar but less significant effect than that of LC. Finally, the combined use of both substances caused a more significant decrease than their separate use.

The elevated serum levels of AST and ALT in the untreated IR group indicate the occurrence of liver cell damage while their significant improvement by using both LC and LG denotes the attenuation of liver damage caused by IR.

In agreement with our results, it was reported that LC reduced the elevated serum level of AST and ALT produced by liver IRI (24). Also, they are in accordance with the results of other researchers who reported that the pre-treatment with LG before the performance of liver IRI caused a significant decrease in the elevated serum AST and ALT values (16,25,26,27).

Additionally, the results of our study revealed that the induction of liver IRI caused a significant increase in the level of malonaldehyde (MDA) associated with a significant reduction in the level of SOD, CAT, and GSH-Px antioxidant enzyme activities in the liver tissue indicating the increased oxidative stress state and lipid peroxidation.

The increased level of MDA in liver tissue caused by IRI observed in our study was also reported by other researchers who proved that hepatic IRI produced increased levels of ALT, AST, MDA, and decreased levels of SOD, GSH-Px, and CAT (28,29,30).

It was proved that the cellular damage caused by reperfusion of ischemic tissue is mainly caused by the release of ROS (31) which causes lipid peroxidation with resultant damage of the cell membranes (5).

The results of our work showed that the pre-treatment of the rats with LC produced a significant reduction in the elevated level of MDA associated with a significant reduction in the antioxidant enzymes activities (SOD, CAT, and GSH-Px) in the liver tissue compared to the untreated sham group. Also, the use of LG produced the same but less significant results than that produced by LC. Remarkably, pre-treatment with both LC and LG caused a more significant reduction in MDA and elevation in antioxidant enzymes activities in hepatic tissue than using each of them separately. These results imply the significant improvement of the oxidative stress state which could be due to the protective effects of both LC and LG.

This effect of LC could be attributed to its role in membrane stabilization and its anti-apoptotic effects by suppressing the mitochondrial permeability, stabilizing free radicals, and its anti-ischemic action (8,9,32). LC has an antioxidant effect and protects against cleavage of deoxyribonucleic acid (DNA) (10) and its protective effect against IRI was confirmed to be related to the lessening of oxidative stress as it improves the activity of the antioxidant enzymes (33).

Previous studies showed that changes in MDA level and SOD activity caused by ROS were significantly improved by pre-treatment with LC, strengthening its antioxidant and free radical scavenger effects (34).

As regards the effect of LG on oxidative stress markers, it was demonstrated that LG reduced oxidative stress and scavenged superoxide radicals in hypercholesterolemia rats (27,35).

The results of the present research revealed that the induction of hepatic IRI caused a significant increase in the level of IL-1β and TNF-α in the

liver tissue and that the administration of LC for one week before the induction of IRI produced a significant decrease in their levels. Also, the use of LG resulted in a significant reduction of TNF-α level, but it caused an insignificant reduction of IL-1β tissue level. Furthermore, it was obvious that the concomitant use of both substances made a more significant decrease in the hepatic tissue level of IL- 1β and TNF-α.

Excitingly, the results of the present work agree with the results of previous studies which confirmed that hepatic IRI was associated with increased levels of TNF-α and IL-1β due to their increased synthesis and release by the Kupffer cells of the liver (36,37).

As well, it was reported that the pre-treatment of Sprague-Dawley rats with LC before the induction of intestinal IRI had a protective effect against intestinal IRI and it caused a significant reduction in the serum level of TNF-α, IL-1β, and IL-6 (38).

Additionally, Taha et al. proved that the administration of LG in hepatic IR resulted in a significant reduction in TNF-α expression in hepatic tissue (26). These results are in accordance with the results of our study.

However, contrary to our results, it was reported that the use of LG as a supplement with the diet reduced the expression of IL-1β in the spleen of burned rats (39).

Moreover, the results of the current study showed that the occurrence of hepatic IRI caused a significant decrease in NO levels in hepatic tissue. Also, it showed that the pre-treatment of LC before induction of IRI caused a significant elevation in NO level in liver tissue. Additionally, the use of LG caused a more significant increase in hepatic NO level than that produced by LC. Interestingly, the combined use of both LC and LG produced more significant results.

These results agree with that of the previous study which showed that the liver IRI is associated with the diminished release of endothelial cell-derived nitric oxide (eNO) and this was due to a reduction in the formation of NO and increased its deactivation by the increased production of superoxide (40).

A previous study showed that ATP depletion and stimulation of several cellular enzyme systems such as proteases, phospholipases, and nitric oxide synthases are responsible for the cell organs dysfunction and finally cell death in cases of IRI (41).

Also, it was reported that the supplementation of LC had a strong antioxidant action by increasing the plasma levels of NO and glutathione and decreasing thiobarbituric acid reactive substance (TBARS) plasma levels (42).

Besides, LC was proved to increase NO levels in isolated small mesenteric arteries from spontaneously hypertensive rats (43). This effect was approved in experiments done in rats subjected to warm IR in which an increase in the blood flow of the liver was detected after LC treatment (44).

Additionally, the results of our study exhibited that liver IRI caused a significant elevation in the levels of VCAM-1 and ICAM-1 in the liver tissue and the administration of LC resulted in a significant reduction in their levels as compared to the IRI group. As well, the use of LG produced a similar but less significant effect than that produced by LC. Excitingly, the combined use of both substances produced a more significant reduction level of VCAM-1 and ICAM-1 in the

hepatic tissue than that produced using each of them separately.

These results agree with the results of other studies which proved that liver IRI caused a significant elevation in the VCAM-1 and ICAM-1 levels in the liver tissue (36,45). They demonstrated that during IRI, the KCs secrete ROS, TNF-α, and IL-1β which activate the hepatic sinusoidal endothelial cells, increase the expression of ICAM-1 and VCAM-1. The increased level of these adhesion molecules stimulates the adhesion, migration, chemotaxis, and activation of neutrophils with subsequent damage of the hepatocytes (36).

Previous studies showed that neutrophils activation can damage the hepatic tissue directly through the release of oxidants and proteases after reperfusion or it inactivates the endogenous anti-protease system with resultant protease-mediated injury (46,47).

It was stated that the use of LC reduced the plasma levels of adhesion molecules as VCAM-1, ICAM-1, and E-selectin which were increased by acute exercise in patients with intermittent claudication (48). Also, it was proved that Propionyl-l-carnitine enhanced peripheral vascular blood flow and diminished the plasma levels of adhesion molecules (ICAM-1, VCAM-1 and E, L, and P-selectin) that cause increased damage in ischemic tissues (48,49,50).

Also, it was proved that LG reduced the endothelial expression of VCAM-1and ICAM-1 and decreased human monocyte adhesion to endothelial cells (51).

Moreover, the results of the present work showed that the induction of liver IRI in group II caused grade 3 liver injury, while in group III, the pretreatment of the rats with LC produced grade 1 liver injury. This means that LC protected the liver cells from the extensive injury that occurred in group II (IRI group). Also, the use of LG in group IV before the induction of IR resulted in grade 2 liver injury. Interestingly, it is obvious that the LG protected the liver cells from the massive injury caused by IR, but its effect is less than that produced by LC. In group V, the combined use of LC and LG showed no evidence of liver injury (nearly normal liver) or minimal injury (grade 0). This means that the combined use of both substances effectively protected the hepatocytes from the harmful effect of the ischemia-reperfusion injury.

It was proved that cases of severe liver IRI are associated with pericentral liver hypoxia, with ischemic hepatitis, manifested by a remarkable elevation in serum aminotransferase levels in the absence of other causes of hepatic necrosis (47).

The results of our study are parallel to that of previous studies which proved that the induction of liver IRI caused necrosis of the liver cells (52,3).

Also, it was reported that carnitine protected the skeletal muscle histopathology against damage by ischemia-reperfusion injury in rabbit hindlimb skeletal muscles (53).

Additionally, L-carnitine plays a very valuable role in the stabilization of the membrane and inhibition of permeability of the mitochondria which contributes to its anti-apoptotic effect (8,9). Moreover, it has an antioxidant, anti-ischemic effect, and protects against cleavage of deoxyribonucleic acid (DNA) (10).

It was proved that the increased production of NO in the liver tissue through the administration of LG reduced necrosis and apoptosis by upregulation of

Bcl-2gene in IRI. So, LG protected the liver's ultrastructure and functions in cases of hepatic IRI (18).

Nitric oxide has an important role in the protection of the liver cells during hepatic IR due to its vasodilator, antiplatelet effects, and its ability of scavenging ROS (46,54). It increases the formation of cGMP which relaxes the vascular smooth muscles, inhibits platelet aggregation and adhesion, and blocks the adhesion of white cells to the blood vessel wall (55).

In addition, it was proved that LG could ameliorate hepatic IRI, and the possible protective mechanism is inhibition of hepatocyte apoptosis via inhibition of caspase-3 activity by nitric oxide synthesis (56).

The effects produced by the pre-treatment with LC in our study could be explained by its strong antioxidant activity (42) which reduces the production of ROS, and this could be considered the key for its protective effect against IRI. ROS can directly cause liver cell damage (5) and affects the KC, damage the endothelial cells, and destroy the integrity of the liver microvasculature (45). The KC will synthesize and release more ROS, TNF-α, and IL-1β which promotes the expression of adhesion molecules which in turn stimulate the migration and adhesion of leukocytes and platelets that affect the microcirculatory blood flow and cause liver cell damage (36). Accordingly, LC through its potent antioxidant effects could prevent all the previously mentioned events with the net result being the protection of the hepatic cells from the damage caused by IR. Moreover, LC was proved to increase the level of NO in rat liver exposed to warm IRI and NO, in turn, increased hepatic blood flow (42)

Interestingly, the results of our study showed the protective effect of LC on the hepatic cells manifested by the improvement of the serum level of liver enzymes, hepatic tissue antioxidant enzymes activity, oxidative stress parameters, proinflammatory cytokines (TNF-α and IL-1β), NO, adhesion molecules and liver histopathology.

Also, the results of the present study revealed that the use of LG before the induction of IR protects the liver cells from its harmful effects. This protective effect was manifested by the ability of LG to improve the serum level of hepatic enzymes, the liver tissue NO, antioxidant enzyme activity, oxidative stress markers, inflammatory cytokines, adhesion molecules, and hepatic pathology.

It was proved that LG enhanced liver regeneration after 70% rat hepatectomy and attributed this effect to its ability to stimulate the formation of NO (57).

Excitingly, it was reported that LG-induced production of NO was the most important factor that explains LG's protective effect against hepatic IRI (58). Preceding studies evidenced that the modulation of the LG/NO pathway may influence the tissue injury caused by IR as LG as a precursor of NO increases NO bioavailability. Nitric oxide, in turn, stimulates microvasculature vasodilatation and increases hepatic arterial blood flow which helps regeneration of the liver cells (59), hepatocytes proliferation (60), and regulates sinusoidal endothelial cell proliferation (61). Also, NO inhibits platelet aggregation and adhesion and reduces the interaction between neutrophils and endothelial surface, and inhibits the inflammatory process (16,17,28). Moreover, LG attenuated the leukocyte infiltration in liver IR in rats (62). Also,

it was demonstrated that the use of LG in liver IRI reduced the histological injury rate (63).

Accordingly, the stimulatory effect of LG on liver NO level could be considered the key to its protective effect against hepatic IRI.

Excitingly, we can see clearly from the results of the present study that the effect of LC was more significant on all the tested parameters than LG except on NO hepatic tissue level where LG effect on NO tissue level was more significant than that produced by LC.

The more significant effects produced by the combined use of both LC and LG could be explained by the additive effects of both substances in improving the oxidative stress markers, proinflammatory cytokines (TNF-α and IL-1β), NO, adhesion molecules, and liver pathology.

Conclusion:

From the results of the present study, we could conclude that the combined use of both LC and LG could effectively protect the liver cells from the harmful effects of IRI mainly because of their antioxidant and anti-inflammatory effects. We recommend that both substances should be taken by the patients who are at risk of developing hepatic IRI, especially before major liver surgery as they can be considered as an easy-to-use weapon against the ischemia-reperfusion injury harmful effects.

Limitations of the study:

The small number of rats in each group could be a limiting factor for the study and it is better for further studies to be performed on a larger number of animals.

Conflict of interest:

The authors declare no conflict of interest.

Funds:

The authors declare that this research was not funded by any institution.

Acknowledgment:

The authors would like to thank Dr. Marwa A. Abdelazeem; Assistant Professor of Pathology, Faculty of Medicine, Tanta University; for her great help in the preparation and examination of the histopathology slides and her valuable assessment and diagnosis.

REFERENCES

1. **Konishi T, Lentsch AB.** Hepatic Ischemia/Reperfusion: Mechanisms of Tissue Injury, Repair, and Regeneration. Gene Expression 2017;277-278.
2. **Diesen DL, Kuo PC.** Nitric oxide and redox regulation in the liver: Part I. General considerations and redox biology in hepatitis. J Surg Res. 2010;162(1):95-109. DOI: 10.1016/j.jss.2009.09.019.
3. **Guan LY, Fu PY, Li PD, Li ZN, Liu HY, Xin MG, et al.** Mechanisms of hepatic ischemia-reperfusion injury and protective effects of nitric oxide. World J Gastrointest Surg 2014; 6(7): 122-128.
4. **Paulson DJ, Shug AL.** Experimental evidence of the anti-ischemic effect of L-carnitine. In: de Jong JW, Ferrari R, eds. The Carnitine System: A New Therapeutical Approach to Cardiovascular Diseases. 1st ed. Dordrecht, The

Netherlands: Kluwer Academic Publishers; 1995:183–197.

5. **Jaeschke H.** Role of reactive oxygen species in hepatic ischemia-reperfusion injury and preconditioning. J Invest Surg. 2003; 16:127–140.

6. **Abe Y, Hines I, Zibari G, Grisham MB**. Hepatocellular protection by nitric oxide or nitrite in ischemia and reperfusion injury. Arch Biochem Biophys. 2009; 484:232-7.

7. **Furuno T, Kanno T, Arita K, Asamib M, Utsumi T, Doi Y, et al.** Roles of long-chain fatty acids and carnitine in mitochondrial membrane permeability transition. Biochem Pharmacol 2001; 62:1037-46.

8. **Canbaz H, Akca T, Tataroglu C, Caglikulekci M, Dirlik M, Ayaz L, et al.** The Effects of Exogenous L-Carnitine on Lipid Peroxidation and Tissue Damage in an Experimental Warm Hepatic Ischemia-Reperfusion Injury Model. CURRENT THERAPEUTIC RESEARCH. 2007; 68(1):32-46.

9. **Izgut-Uysal VN, Agac A, Derin N.** Effect of carnitine on stress-induced lipid peroxidation in rat gastric mucosa. J Gastroenterol 2001; 36:231-6.

10. **Vanella A, Russo A, Acquaviva R, A Campisi A, Di Giacomo C, Sorrenti V, et al.** L-propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. Cell Biol Toxicol. 2000; 16:99–104.

11. **Atila K, Coker A, Sagol O, Coker I, Topalak O, Astarcioglu H, et al.** Protective effects of carnitine in an experimental ischemia-reperfusion injury. Clin Nutr. 2002; 21:309–313.

12. **Schwedhelm E, Maas R, Freese R, Jung D, Lukacs Z, Jambrecina A, et al.** Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: Impact on nitric oxide metabolism. Br. J. Clin. Pharm. 2008, 65, 51–59.

13. **Giovanardi RO, Rhoden EL, Cerski CT, Salvador M, Kalil AN.** Pharmacological preconditioning using intraportal infusion of l-arginine protects against hepatic ischemia reperfusion. Inj J Surg Res. 2009; 155:244-53.

14. **Tsuchihashi S, Kaldas F, Chida N, Sudo Y, Tamura K, Zhai Y, et al.** FK330, a novel inducible nitric oxide synthase inhibitor, prevents ischemia and reperfusion injury in rat liver transplantation. Am J Transplant. 2006; 6:2013-22.

15. **Yagnik GP, Takahashi Y, Tsoulfas G, Reid K, Murase N, Geller DA.** Blockade of the L-arginine/NO synthase pathway worsens hepatic apoptosis and liver transplant preservation injury. Hepatology (Baltimore). 2002; 36:573-81.

16. **Çekin AH, Gur G, Turkoglu S, Aldemir D, Yilmaz U, Gursoy M, et al.** The protective effect of L-carnitine on hepatic ischemia-reperfusion injury in rats. Turk J Gastroenterol 2013; 24 (1):51-56.

17. **Chattopadhyay P, Verma N, Verma A, Kamboj T, Khan NA, Wahi AK.** L-Arginine Protects from Pringle Manoeuvere of Ischemia-Reperfusion Induced Liver Injury. Biol. Pharm. Bull. 2008;31(5):890-892.
18. **Chattopadhyay P, Shukla G, Wahi AK.** Protective Effect of L-arginine Against Necrosis and Apoptosis Induced by Experimental Ischemic and Reperfusion in Rat Liver. The Saudi Journal of Gastroenterology 2009; 15(3):156-62.
19. **Ohkawa H, Ohishi N, Yagi K.** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351–358.
20. **Marklund S, Marklund G.** Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47:469–474.
21. **Aebi H.** Catalase in vitro. Methods Enzymol 1983; 105:121–126.
22. **Lawrence A, Burk F.** Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 1976; 71:952–958.
23. **Ozturk H, Gezici A, Ozturk H.** The effect of celecoxib, a selective COX-2 inhibitor, on liver ischemia/reperfusion-induce oxidative stress in rats. *Hepatol Res.* 2006; 34:76–83.
24. **Atila K, Coker A, Sagol O, Coker I, Topalak O, Astarcioglu H, et al.** Protective effects of carnitine in an experimental ischemia-reperfusion injury. Clin Nutr. 2002; 21:309–313.
25. **Taha MO, Simões MJ, Haddad MA, Capelato RC, Budny N, Matsumoto AH, et al.** L-Arginine Supplementation Protects Against Hepatic Ischemia–Reperfusion Lesions in Rabbits. Transplantation Proceedings. 2009; 41:816–819.
26. **Taha MO, Caricati-Neto A, Ferreira RM, Simões MJ, Monteiro HP, Fagundes DJ.** L-arginine in the ischemic phase protects against liver ischemia-reperfusion injury. Acta Cirúrgica Brasileira. 2012; 27(9):616-623.
27. **Oleshchuk OM, Posokhova KA, Mudra AYe.** L-ARGININE, BUT NOT L-NAME PROTECTS AGAINST LIVER INJURY INDUCED BY EXPERIMENTAL ISCHEMIA-REPERFUSION. International Journal of Medicine and Medical Research. 2015; 1(1):58-62.
28. **Su JF, Guo CJ, Wei JY, Yang JJ, Jiang YG, Li YF.** Protection against hepatic ischemia-reperfusion injury in rats by oral pre-treatment with quercetin. Biomed Environ Sci. 2003; 16:1–8.
29. **Cimen O, Eken H, Cimen FK, Cekic AB, Kurt N, Bilgin AO, et al.** The effect of Liv-52 on liver ischemia reperfusion damage in rats. BMC Pharmacology and Toxicology. 2020; 21(2):9 pages.
30. **Aydogan MS, Yucel A, Erdogan MA, Polat A, Cetin A, Ucar M, et al.** Effects of oral β- glucan on liver ischemia/reperfusion injury in rats. Transplant Pro. 2013; 45(2):487-91.

31. **Jaeschke H.** Molecular mechanisms of hepatic ischemia reperfusion injury and preconditioning. American Journal of Physiology—Gastrointestinal and Liver Physiology. 2003; 284(1):G15–G26.
32. **Paulson DJ, Shug AL.** Experimental evidence of the anti-ischemic effect of L-carnitine. In: de Jong JW, Ferrari R, eds. The Carnitine System: A New Therapeutical Approach to Cardiovascular Diseases. 1st ed. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1995:183–197.
33. **Gulcin I.** Antioxidant and antiradical activities of L -carnitine. Life Sci 2006; 78:803–811.
34. **Liu Y, Yan S, Ji C, Dai W, Hu W, Zhang W, et al.** Metabolomic Changes and Protective Effect of L-Carnitine in Rat Kidney Ischemia/Reperfusion Injury. Kidney Blood Press Res 2012; 35:373–381.
35. **Darwin E, Elfi EF, Hamidi D.** Effect of Arginine on IL-6, IL-17 and TGF-β levels in high-fat Diet-Induced Hypercholesterolemia Rat. J Young Pharm, 2017; 9(1):83-86.
36. **Zhou W, Zhang Y, Hosch MS, Lang A, Zwacka RM, Engelhardt JF.** Subcellular site of superoxide dismutase expression differentially controls AP-1 activity and injury in mouse liver following ischemia/reperfusion. Hepatology 2001; 33:902-914.
37. **Llacuna L, Mar´M, Lluis JM, Garc´ıa-Ruiz C, Fern´andez-Checa JC, Morales A.** Reactive oxygen species mediate liver injury through parenchymal nuclear factor- κB inactivation in prolonged ischemia/reperfusion. American Journal of Pathology 2009; 74(5):1776–1785.
38. **Yuana Y, Guob H, Yi Zhanga, Dong Zhoua, Ping Gana, et al.** Protective Effects of L-Carnitine on Intestinal Ischemia/Reperfusion Injury in a Rat Model. J Clin Med Res 2011;3(2):78-84.
39. **Cui XL , Iwasa M, Iwasa Y, Ogoshi S.** Arginine-supplemented diet decreases expression of inflammatory cytokines and improves survival in burned rats. Journal of Parenter Enteral Nutr. 2000;24(2):89-96.
40. **Abe Y, Hines I, Zibari G, Grisham MB**. Hepatocellular protection by nitric oxide or nitrite in ischemia and reperfusion injury. Arch Biochem Biophys. 2009; 484:232-7.
41. **Goligorsky MS, Brodsky SV, Noiri E, et al.** Nitric oxide in acute renal failure: NOS versus NOS. Kidney Int 2002; 61: 855–861.
42. **Atalay Guzel N, Erikoglu Orer G, Sezen Bircan F, Coskun Cevher S.** Effects of acute L-carnitine supplementation on nitric oxide production and oxidative stress after exhaustive exercise in young soccer players. The Journal of Sports Medicine and Physical Fitness 2015;55(1-2):9-15.
43. **Alvarez de Sotomayor, M., Bueno, R., Pérez-Guerrero, C., and Herrera, M. D.** Effect of L-Carnitine and Propionyl-L-Carnitine on endothelial function of small

mesenteric arteries from SHR. J. Vasc. Res. 2007; 44, 354–364.

44. **Yonezawa K, Tolba RH, Setter A, Yamamoto Y, Yamaoka Y, Minor T.** L-carnitine could not improve hepatic warm ischemia reperfusion injury despite ameliorate blood flow. J. Surg. Res. 2005; 125, 16–22.
45. **Mendes-Braz M, Elias-Miro M, Jimenez-Castro MB, Casillas-Ramirez A, Ramalho FS, Peralta C.** The current state of knowledge of hepatic ischemia-reperfusion injury based on its study in experimental models. Journal of Biomedicine and Biotechnology. 2012; 2012, 20 pages.
46. **Lentsch A, Kato A, Yoshidome H, McMasters K, Edwards M.** Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. Hepatology 2000; 32:169–173.
47. **Teoh NC, Farrell GC.** Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. J Gastroenterol Hepatol 2003; 18:891–902.
48. **Silvestro A, Schiano V, Bucur R, Brevetti G, Scopacasa F, Chiariello M.** Effect of propionylcarnitine on changes in endothelial function and plasma levels of adhesion molecules induced by acute exercise in patients with intermittent claudication. Angiology. 2006; 57:145–154.
49. **Signorelli SS, Malaponte G, Di Pino L, Digrandi D, Pennini G, Mazzarino MC.** Effects of ischemic stress on leukocyte activation processes in patients with chronic peripheral occlusive arterial disease: role of L-propionyl carnitine administration. Pharmacol. Res. 2001; 44, 305–309.
50. **Loffredo L, Marcoccia A, Pignatelli P, Andreozzi P, Borgia MC, Cangemi R, et al.** Oxidative-stress-mediated arterial dysfunction in patients with peripheral arterial disease. Eur. Hearth J. 2007; 28, 608–612.
51. **Adams MR, Jessup W, Hailstones D, Celermajer DS.** l-Arginine Reduces Human Monocyte Adhesion to Vascular Endothelium and Endothelial Expression of Cell Adhesion Molecules. Circulation 1997; 95(3):662-668.
52. **Massip-Salcedo M, Roselló-Catafau J, Prieto J, Avila M, Peralta C.** The response of the hepatocyte to ischemia. Liver Int 2007; 27:6–16.
53. **Akara H, Sarac A, Konuralp C, Yildiz L, Kolbakir F.** Comparison of histopathologic effects of carnitine and ascorbic acid on reperfusion injury. European Journal of Cardio-thoracic Surgery 2001; 19(4):500-506.
54. **Serracino-Inglott F, Virlos IT, Habib NA, Williamson RCN, Mathie R.** Differential nitric oxide expression during hepatic ischemia reperfusion. Am J Surg. 2003; 185:589-95.
55. **Kubes P, Suzuki M, Granger DN.** Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991; 88:4651-5.

56. **Li SQ, Liang LJ**. Protective mechanism of L-arginine against liver ischemic-reperfusion injury in rats. Hepatobiliary & Pancreatic Diseases International: HBPD INT, 01 Nov 2003, 2(4):549-552.
57. **Kurokawa T, An J, Tsunekawa K, Shimomura Y, Kazama S, Naohisa Ishikawa, et al.** Effect of L-arginine supplement on liver regeneration after partial hepatectomy in rats. World Journal of Surgical Oncology. 2012; 10(99):6 pages.
58. **Lucas ML, Rhoden CR, Rhoden EL, Zettler CG, Mattos AA**. Effects of L-arginine and L-NAME on ischemia-reperfusion in rat liver. Acta Cirúrgica Brasileira. 2015; 30(5):345-52.
59. **Cantre D, Schuett H, Hildebrandt A, Dold S, Menger MD, Vollmar B, et al.** Nitric oxide reduces organ injury and enhances regeneration of reduced-size livers by increasing hepatic arterial flow. Br J Surg 2008, 95:785–792.
60. **Mei Y, Thevananther S.** Endothelial nitric oxide synthase is a key mediator of hepatocyte proliferation in response to partial hepatectomy in mice. Hepatology 2011, 54:1777–1789.
61. **Yoshida D, Akahoshi T, Kawanaka H, Yamaguchi S, Kinjyo N, Taketomi A, et al.** Roles of vascular endothelial growth factor and endothelial nitric oxide synthase during revascularization and regeneration after partial hepatectomy in a rat model. Surg Today 2011, 41:1622–1629.
62. **Katsumi H, Nishikawa M, Yamashita F, Hashida M.** Prevention of hepatic ischemia-reperfusion injury by prolonged delivery of nitric oxide to the circulating blood in mice. Transplantation. 2008; 85:264-9.
63. **Tuncer MC, Ozturk H, Buyukbayram H, Ozturk H.** Interaction of L-arginine-methyl ester and sonic hedgehog in liver ischemia-reperfusion injury in rats. World J Gastroenterol. 2007;13(28):3841- 6.