Mitigative Effect of L-Carnitine and Simvastatin against Dexamethasone Induced Hepato-renal Damage in Rats: "Biochemical, Antioxidant, Histological and Immunohistochemical Insights"

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Abstract

Background: Prolonged use of dexamethasone (DEX) has been linked to disruptions in liver and kidney function. L-carnitine and simvastatin, known for their antioxidant and anti-inflammatory properties, may help counteract these effects.

Objectives: To evaluate the ameliorative effects of L-carnitine (L-CAR), simvastatin (SIM), and their combined treatment on liver and kidney damage provoked by dexamethasone (DEX).

Materials and methods: Thirty female Wistar rats were randomly split into five groups of six rats each. The control group received only saline, and the second group was given DEX at 7 mg/kg/I.M. once a week for 4 weeks. The third group received L-CAR at 100 mg/kg/day orally for 4 weeks, after 4 weeks of DEX administration. The fourth group was given SIM at 10 mg/kg/day orally for 4 weeks, following 4 weeks of DEX administration. The fifth group received a combination of L-CAR and SIM in the same previous doses for 4 weeks after 4 weeks of DEX administration. The study assessed serum total antioxidant capacity (TAO), and liver and kidney function markers and used immunohistochemical analysis to examine proliferating cell nuclear antigen (PCNA) in liver and kidney tissues.

Results: DEX received group showed disturbance in the liver and kidney function test markers and histological damage to hepatic and renal tissue while with the treatment with antioxidant l-carnitine, simvastatin there was improvement in liver and kidney function and their histological structure and more improvement noticed in group received L-CAR and SIM together.

Conclusion: Treatment with L-CAR, SIM, their combination reduced these DEX-induced biochemical imbalances and histological damage.

Keywords: Dexamethasone; L-carnitine; Simvastatin; Liver; Kidney.

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Introduction

Dexamethasone (DEX) is a part of the glucocorticoid hormones. It is used in dermatology, allergic conditions, endocrine syndromes, gastrointestinal illnesses, hematologic, neoplastic, and rheumatic disorders **(Hammadi et al., 2022)**. Using DEX in high doses and for long periods leads to dyslipidemia, hyperglycemia, lipid peroxidation, and hematological upsets **(Hasona and Morsi, 2019)**. Additionally, it induced irregularities in liver functions **(Abou-seif et al., 2019)**, and nephropathy by enhancing apoptosis and fibrosis of the glomerular cells **(Mohamed et al., 2022).**

L-carnitine (L-CAR) is a combination involved in metabolic rate in most mammals, plants, and some bacteria. It is derived from an amino acid with multiple components, including L-carnitine, acetyl-L-carnitine, and propionyl-L-carnitine **(Talenzhad et al., 2020).** It has multiple effects on the human body, including lowering oxidative stress, improving pro-inflammatory cytokines, and enhancing the dysfunction of mitochondria **(Almannai et al., 2019; Modanloo and Shokrzadeh, 2019).**

The great action of carnitine is the formation of a mitochondrial matrix from long-chain fatty acids, which leads to the creation of β-oxidation and energy. So, carnitine deficiency leads to fatty acid oxidation defects, which appear in the form of hypoglycemia and precipitation of fat in the hepatic, muscular, and cardiac cells (**Longo et al., 2016).**

Statins such as simvastatin (SIM) have antioxidant and anti-inflammatory reactions. So it has a role in the protection of tissue harm, and hepatic and renal toxicity **(Mohammadi et al., 2014).** Also, SIM reduced the degree of renal damage when used in a mouse with chronic renal failure. And found when used in nephrotic syndrome it reduces the harm caused by oxidative stress and reduces glomerular damage and proteinuria **(Goodarzi et al., 2017).**

The objective of this study was to estimate the role of L-CAR and SIM against the damaging effect of Dexamethasone on the liver and kidneys of rats.

Materials and methods

Drugs and chemicals

L-carnitine (L-CAR) and simvastatin (SIM) ($C_{25}H_{38}O_5$) 99 % (HPLC) were purchased from Sigma Aldrich Company, England. Dexamethasone (DEX) ($C_{22}H_{29}FO_5$) 98 % (HPLC) was obtained from Biotech Pharmaceutical Co., Ltd. Egypt. Physiological saline (0.9 % NaCl) was purchased from Nile Company for pharmaceuticals and chemical industries. Egypt.

Animals

The present study was conducted on thirty adult female Wistar rats weighing 200–250. Animals were obtained from the animal house of the Faculty of Medicine, Sohag University, and were housed in the animal house at room temperature (22-27°C). Rats were kept under normal light/dark cycles and given food and water ad libitum. All procedures of this study were approved by the Institutional Animal Care and Use Committee of Sohag University, Sohag, Egypt (approval number: Sohag 5-5-11-2024-01).

Experimental groups

Thirty female Wistar rats were randomly divided into five groups (n=6).

Control group: Rats received normal saline (0.9% NaCl) IM once a week for all time of the experiment.

DEX group: Rats received DEX (7 mg/kg/I.M) once a week for 4 weeks.**(Arafa,2023)**

L-CAR group: Rats received DEX (7 mg/kg/I.M) once a week for 4 weeks followed by administration of L-CAR (100 mg/kg/day) orally for 4 weeks **(Elhemiely, 2024).**

SIM group: Rats received DEX (7 mg/kg/I.M) once a week for 4 weeks followed by administration of SIM (10 mg/kg/day) orally for 4 weeks **(Liang et al., 2018).**

L-CAR+ SIM group: Rats received DEX (7 mg/kg/I.M) once a week for 4 weeks followed by combined administration of L-CAR (100 mg/kg/day) and SIM (10 mg/kg/day) orally for 4 weeks.

Samples collection

Rats were anesthetized with diethyl ether and then sacrificed by decapitation at the end of the experiment, blood samples from neck veins were collected in pre-labeled centrifuge tubes. Blood samples were collected to be centrifuged for 15 min at 3500 rpm using a Heraeus Sepatech centrifuge (Labofuge 200, DJB Lab care Co), serum was stored quickly on -20˚c for biochemical analysis; Liver function tests (AST, ALT, Total protein and albumin), kidney function tests (serum urea and creatinine) and serum TAO. Samples from liver and kidney tissue were taken and fixed in formalin (10%) for further histopathological evaluation and immunohistochemical assessment of proliferating cell nuclear antigen (PCNA) in liver and kidney tissue.

Biochemical analysis

- ***Liver functions:*** AST, ALT, total protein, and albumin were evaluated by spectrophotometer (Jenway 6051 colorimeter spectrophotometer). Serum ALT and AST values were described in U/L. Serum total protein and albumins were recoded as gm/dl.
- ***Kidney functions:*** Serum urea and creatinine levels were evaluated by colorimetric method using biochemical kits purchased from Biosystems S.A., Spain, according to the manufacturer’s instructions. Their values were described as mg/dl.
- ***Serum TAO capacity:*** Serum TAO levels were assessed by using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Its result was recorded as mM/L.

Histological analysis

Liver and kidney tissue samples of all rats were kept in 10% formaldehyde solution for 24 hours. Then these tissues were inserted in paraffin, and 5 µm sections were obtained. We used hematoxylin and eosin stains to verify histological features using light microscopy. The section was prepared for Immunostaining which required pretreatment for antigen retrieval, this was done by boiling for 10 minutes in 10Mm citrate buffer at pH 6 and leaving the sections to cool at room temperature for 20 minutes then rinsed 4 times, finally incubated for 1 hour for the following antibody PCNA. **(Tousson, 2024).**

Reading of staining: brownish coloration at the cell is the place of the antigen which is an index of positive reactivity. The control tissue demonstrated the absence of specific staining which denoted no reactivity. Immuno-stained sections were examined using Olympus microscope (CX40). The PCNA was determined by counting the number of PCNA-labeled nuclei in the liver cells and tubules of the kidney.

Statistical analysis

The data were analyzed using SPSS software (IBM-SPSS version 22.0). One-way analysis of variance was used followed by Tukey's post hoc test. Differences were considered significant if $P < 0.05$.

Results

Biochemical evaluations

Liver functions: In this study, our results revealed that administration of DEX induced a rise in liver enzymes (AST and ALT) significantly in comparison to the normal control group, as well as a significant decrease in serum albumin levels when compared to the control group ($P < 0.001$). Treatment with L-CAR and SIM, individually or in combination resulted in a significant reduction in levels of AST and ALT and elevated serum levels of albumin and total protein significantly ($P < 0.05$) when in comparison to the DEX group (**Table.1**).The L-CAR administration induced a significant improvement in serum AST, ALT, albumin, and total protein parameters ($P < 0.05$) when compared to the SIM group. The combined group induced a significant improvement in all parameters when compared to the L-CAR group and SIM group. (**Table.1**).

Table 1. Effects of DEX, L-CAR, SIM, and their combination on liver functions

Groups	ALT (U/L)	AST (U/L)	Albumin (gm/dl)	Total protein (gm/dl)
I (Normal control)	131.24±5.51	44.25± 2.45	5.22± 0.34	7.25± 0.94
II (DEX)	233.22± 3.6 [a]	94.11±5.33 [a]	3.11±0.76 [a]	4.32±0.54
III (L-CAR+ DEX)	155.12 ± 6.8 [b d]	46.34±2.54 [bd]	4.72±0.54 [b d]	5.20±0.34 [b d]
IV (SIM+ DEX)	205.01±8.5 [b]	77.5±3.61 [b]	3.72±0.44 [b]	4.45±0.20 [b]
V (L-CAR + SIM+ DEX)	154.28± 6.33 [bc]	59.15± 3.2 [b cd]	4.83± 0.82 [bc]	5.85±0.23 [b cd]

Data represent mean ± SEM of 6 observations. DEX: dexamethasone, L-CAR: L- carnitine, SIM: simvastatin, ALT: alanine aminotransferase. AST: aspartate aminotransferase. A refers to significant differences from normal control groups, [b] from the DEX group, [c] from L-CAR groups, and [d] from SIM groups.

Kidney functions: The acquired results in (**Table.2)** indicated a significant elevation of serum level of creatinine and urea after the administration of DEX, where ($P< 0.001$) when compared to the normal control group. Treatment with L-CAR produces significant improvement in serum creatinine and urea compared to the DEX group where ($P < 0.001$). In the SIM group, there was a significant improvement in serum creatinine and urea levels in relation to the DEX group ($P < 0.001$). Treatment with L-CAR improves kidney function where there was a significant difference ($P < 0.001$) when differentiating from the SIM group. Combined administration of L-CAR and SIM induced a mild significant decrease in serum levels of creatinine ($P < 0.05$) when correlated with the L-CAR group and induced an insignificant change when differentiated from the SIM group. (**Table. 2**).

Table 2. Effects of DEX, L-CAR, SIM, and their combination on kidney functions

Groups	Serum creatinine level (mg/dl)	Serum Urea level (mg/dl)
I (Normal control)	0.29 ± 0.037	39.52 ± 2.39
II (DEX)	2.24 ± 0.197 [a]	96.90± 3.31 [a]
III (L-CAR+ DEX)	0.40 ± 0.028[bd]	51.51 ± 3.47 [bd]

IV (SIM+ DEX)	1.35 ± 0.075 [b]	57.70 ± 1.96 [b]
V (L-CAR + SIM+ DEX)	0.35 ± 0.019 [bc]	37.98 ± 2.56 [bcd]

Data represent mean ± SEM of 6 observations. DEX: dexamethasone, L-CAR: L- carnitine, SIM: simvastatin. A refers to significant differences from normal control groups, [b] from the DEX group, [c] from L-CAR groups, and [d] from SIM groups.

Serum TAO capacity: Administration of DEX significantly reduced the serum level of TAO when compared to a normal control group ($P < 0.001$). In the L-CAR group and SIM group, there was an improvement in the serum level of TAO where there was a mild significant difference between them and the DEX group ($P < 0.05$). Also in combined administration of L-CAR and SIM, there was a mild significant improvement in the serum level of TAO when compared to the DEX group ($P < 0.05$).

Treatment with L-CAR induced a significant increase in serum levels of TAO ($P < 0.05$) when compared to the SIM group, so it had priority in improvement. Combined administration of L-CAR and SIM induced a significant elevation of serum TAO level ($P < 0.05$) when compared to separate administration of L-CAR or SIM (**Fig.1**).

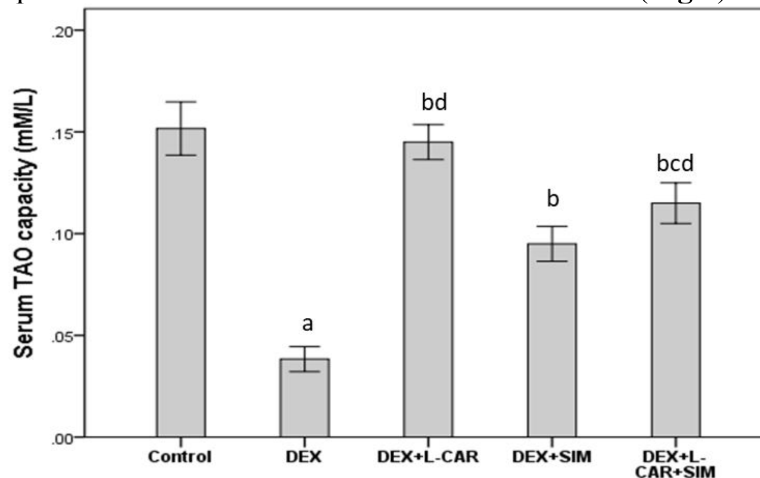

Fig.1. Effects of DEX, L-CAR, SIM, and their combination on serum TAO capacity: Data represent mean ± SEM of 6 observations. DEX: dexamethasone, L-CAR: L- carnitine, SIM: simvastatin, TAO: total antioxidant. A refers to a significant difference from normal control groups, [b] from the DEX group, [c] from L-CAR groups, and [d] from SIM groups.

Correlation coefficient: There were negative correlation results between TAO capacity and serum ALT, AST where a decrease in total TAO was associated with an increase in ALT, AST where ($r = –0.856$, $–0.949$ respectively ,$p< 0.001$) and a positive correlation between TAO capacity and albumin, total protein level where ($r =0.816$ and0.688), respectively with ($p < 0.001$)an increase in the TAO capacity was associated with an improvement of albumin and total protein level . **(Fig. 2).**

Also, there was a negative correlation between TAO capacity and kidney functions (creatinine and urea) where a decrease in TAO capacity was associated with an increase in creatinine and urea. Where ($r = –0.850$ and $–0.816$), respectively with ($p < 0.001$), **(Fig.3).**

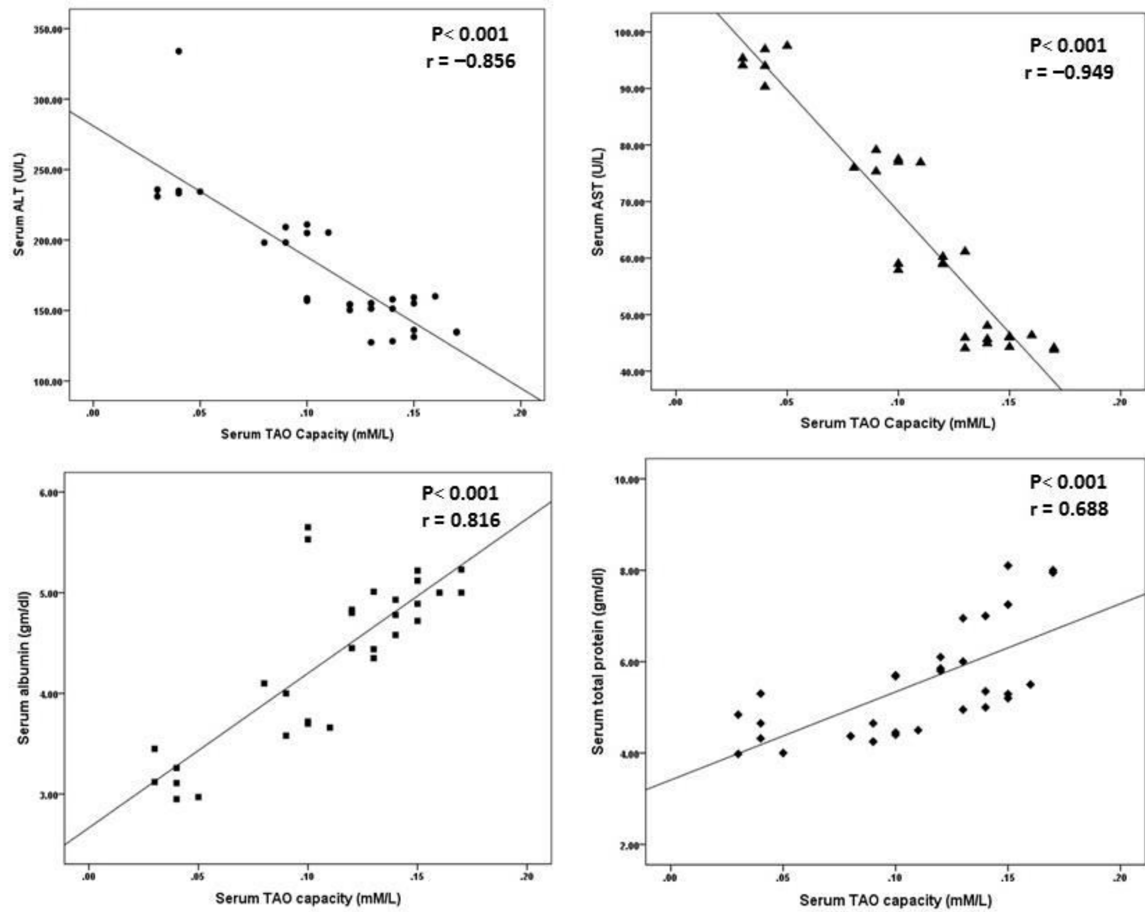

Fig. 2. **The correlation coefficient between TAO capacity with serum ALT, AST, albumin, and total protein levels**. TAO = total antioxidant. ALT (alanine aminotransferase). AST(aspartate aminotransferase).

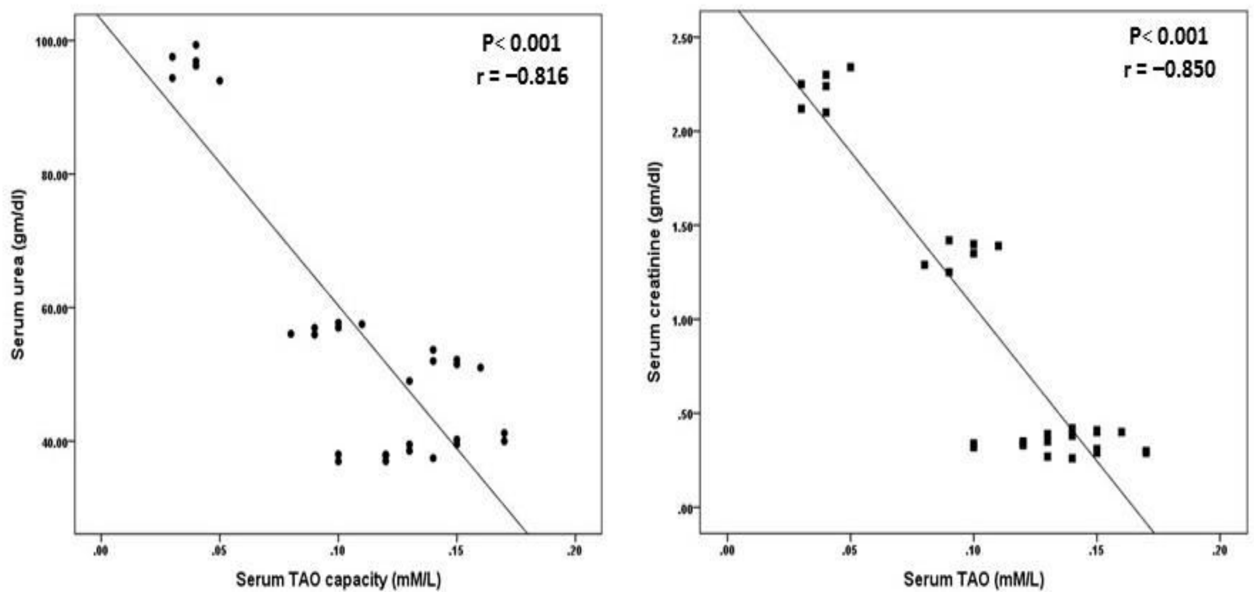

Fig.3. The correlation coefficient between TAO capacity with serum creatinine and urea. TAO = total antioxidant. ALT (alanine aminotransferase). AST (aspartate aminotransferase).

Histomorphological evaluation

1. Hematoxylin and eosin stain

In the liver: In the control group the hepatocyte had standard architecture with a central nucleus and normal arrangement around the central vein which had normal size and shape, the hepatic sinusoid also appeared normal (**Fig. 4 A**).

In the DEX group the portal vein appeared thick and dilated with a thick wall and surrounded by inflammatory cell infiltration also an inflammatory cell between hepatocytes, the bile duct proliferation around the portal vein, and some hepatocytes appeared with faint cytoplasm and shrunken nuclei. (**Fig. 4 B**), in the SIM group central vein appeared normal but some surrounding hepatocytes had vacuolated cytoplasm and shrunken nucleus, there was a dilated hepatic sinusoid and an area of bleeding and inflammatory cell infiltration. (**Fig.4 C**). In the L-CAR group there was a dilated congested central vein, some hepatic sinusoids appeared dilated, area of destructed hepatocytes also appeared. (**Fig. 4 D**). In the L-CAR and SIM groups, liver cells appeared near normal hepatocytes and had a normal arrangement around the central vein (**Fig. 4 E**).

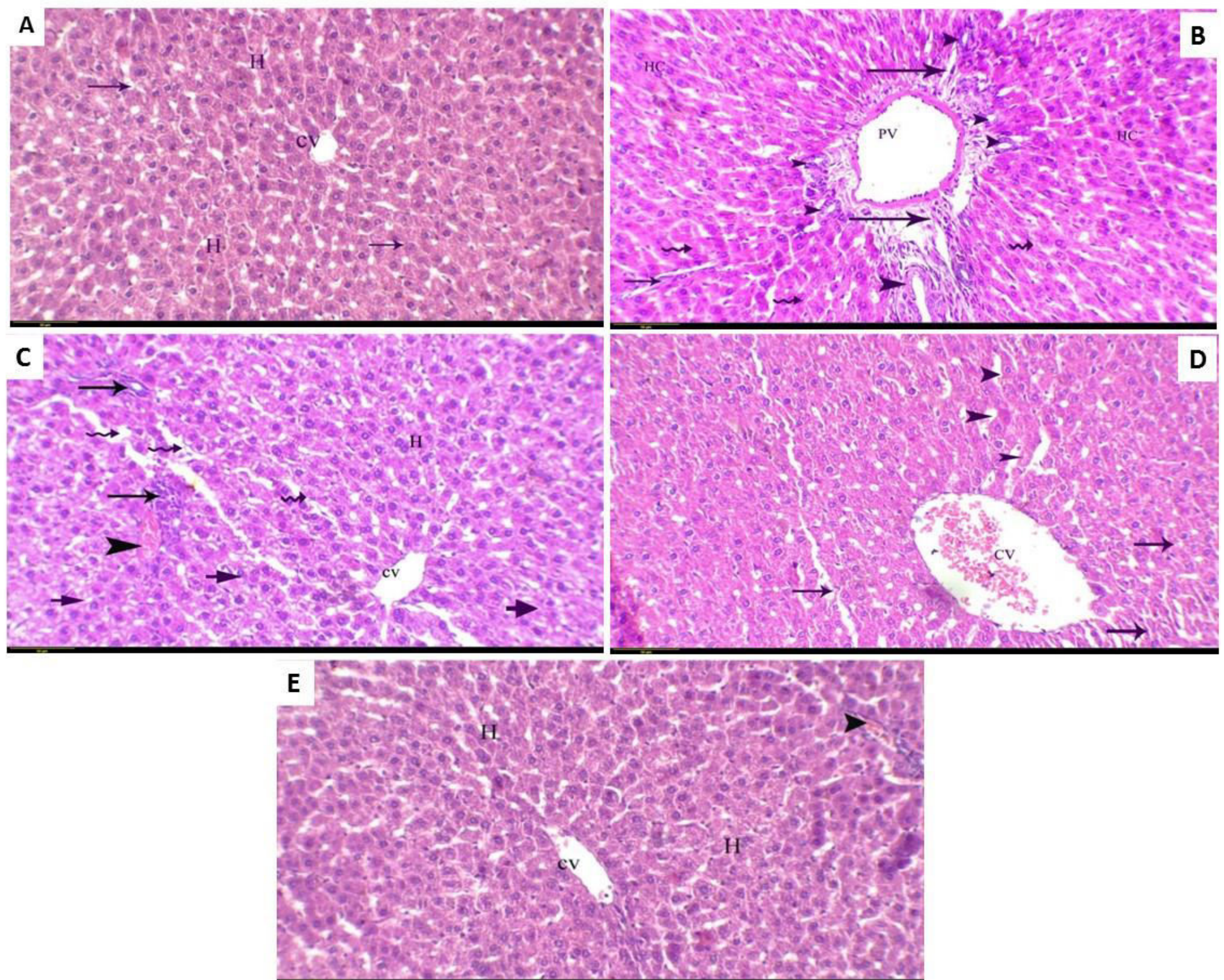

Fig.4. (A) : Hematoxylin &Eosin stained liver section from a control rat showing normal liver histology hepatocytes (H), sinusoid (arrow), and normal central vein (cv).**(B):** a DEX-treated rat dilated portal vein (PV) and inflammatory cell infiltrations (arrow head), bile duct hyperplasia (arrowhead) ,some affect hepatocytes (irregular arrow). **(C):** a DEX & SIM treated rat shows affected hepatocyte (short arrow), area

of bleeding (arrowhead), dilated hepatic sinusoid (irregular arrow) **.(D):** DEX and L-CAR treated group destructed hepatocyte (arrow) and dilatated hepatic sinusoid (arrowhead) dilated central vein (CV)**.(E)**: DEX &simvastatin &l-carnitine treated rat with normal hepatic arrangment but there is an area of bleeding appears (arrowhead) **(× 200).**

***In the kidney*:** the control group showed standard renal glomeruli which appeared normal in shape and surrounded by distal and proximal convoluted tubules (**Fig.5A**), in the DEX group renal glomeruli, appeared shrunken with dilated bowmen space renal tubules appeared with destructed wall and bleeding & cast within their lumen (**Fig.5 B**), while in SIM group, some glomeruli appeared normal while other appeared shrunken, the epithelium of tubules with brush border and darkly stained nuclei and there was an area of bleeding and inflammatory cell infiltration (**Fig.5C**). In the L-CAR group, the renal glomeruli and renal tubules but some tubules appeared with ciliated borders and cast within their lumen (**Fig.5D**), in L-CAR and SIM groups, the improvement in renal tissue was performed in kidney glomeruli and tubules excluding some distal convoluted tubules appeared with thin destructed epithelium and some other tubules appeared with cast and hemorrhage. (**Fig. 5E**).

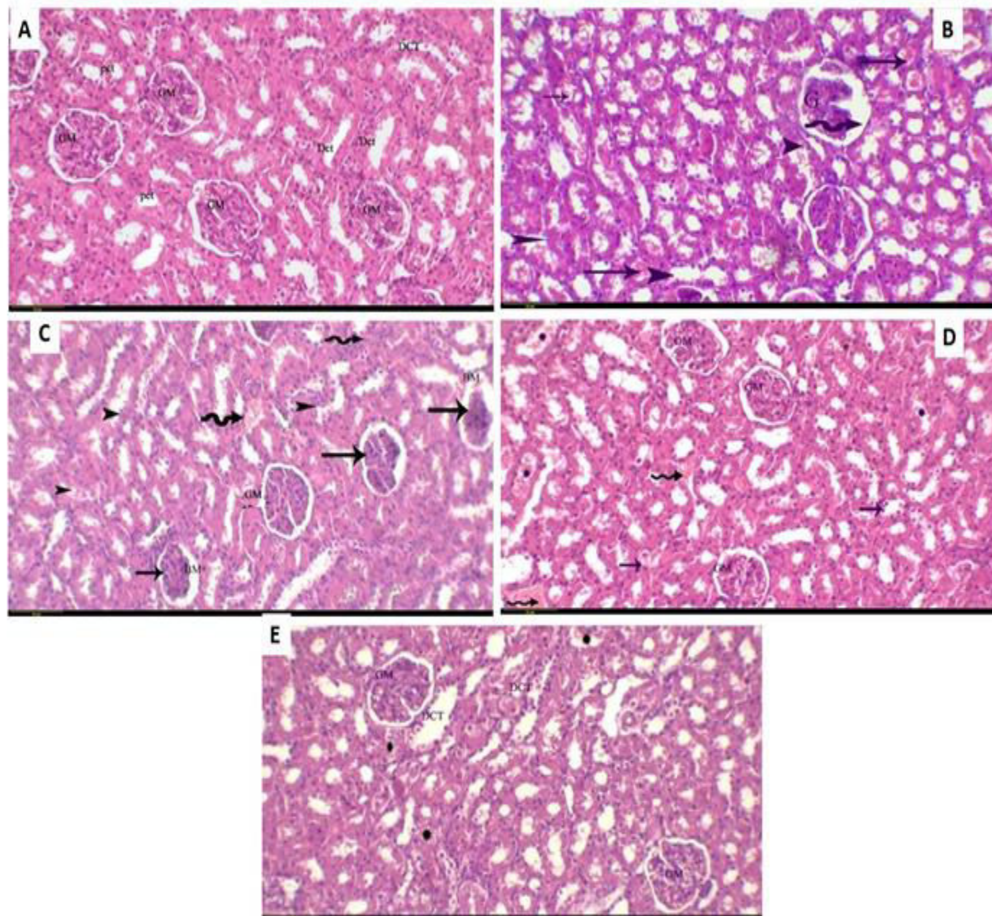

Fig.5. (A): Hematoxylin & Eosin-stained kidney sections from control rats show normal renal tissue were glomeruli surrounded by Bowman's space (GM), normal Proximal and Distal convoluted tubules (PCT), (DCT) .**(B)**:DEX-treated rats show shrunken renal glomeruli(G), widened bowmen capsule(irregular arrow), cast within the lumen of tubules (arrow), destructed tubular epithilium (arrowhead). **(C)**: DEX

and SIM treated rats shrunken renal glomeruli (arrow) wide bowmen capsule(BM), area of bleeding (irregular arrow), in the renal tubular epithelium with brush border (arrowhead). **(D)**:a DEX and L-CAR treated rats, area of bleeding (irregular arrow), destructed renal tubular epithelium (arrow) , cast present within the tube(✹). **(E) :**a DEX &SIM and l-carnitine treated rats thin wall of some distal convoluted tubules (DCT), also cast and hemorrhage present within some tubules(✹).
(×200).

2. **Immunohistochemical stain (PCNA)**

***In the liver*:** In the control group, there was a minimal reaction to PCNA stain appeared in some hepatocyte nuclei (**Fig.6 A**), while in the DEX group, there was a significant positive reaction to PCNA in differentiation from the control group ($p<0.0001$) appeared in the nuclei (had brown coloration) of hepatocyte and in the wall of portal vein which appeared dilated and in the wall of bile ducts (**Fig.6B**), in the SIM and L-CAR group in separate and in combined there was a mild positive reaction to PCNA appeared in the nuclei of hepatocyte which appeared with brown coloration express significant difference when differentiating from DEX group where ($p<0.001$) **(Fig.6 CDE**, **Table. 3).**

Table 3. Effects of DEX, L-CAR, SIM, and their combination on the PCNA expression of liver &kidney

group / parameter	Control group	DEX group	L-CAR group	SIM group	L-CAR + SIM group
PCNA nuclei in the liver	28.3± 1.2	182.6±6.98[a]	36.3±2.75[bd]	49.5±1.5[bc]	35.8±2.034[bd]
PCNA nuclei in the kidney	50.6 ±5.2	361.3± 16.22[a]	69.2±1.07[b]	70.6±2.6[b]	67.5 ±2.98 [b]

Data represent mean ± SEM of PCNA stain in nuclei of the liver and kidney. DEX: dexamethasone, L-CAR: L-carnitine, SIM: simvastatin. [A] refers to a significant difference from normal control groups, [b] from the DEX group, [c] from L-CAR groups, and [d] from SIM groups.

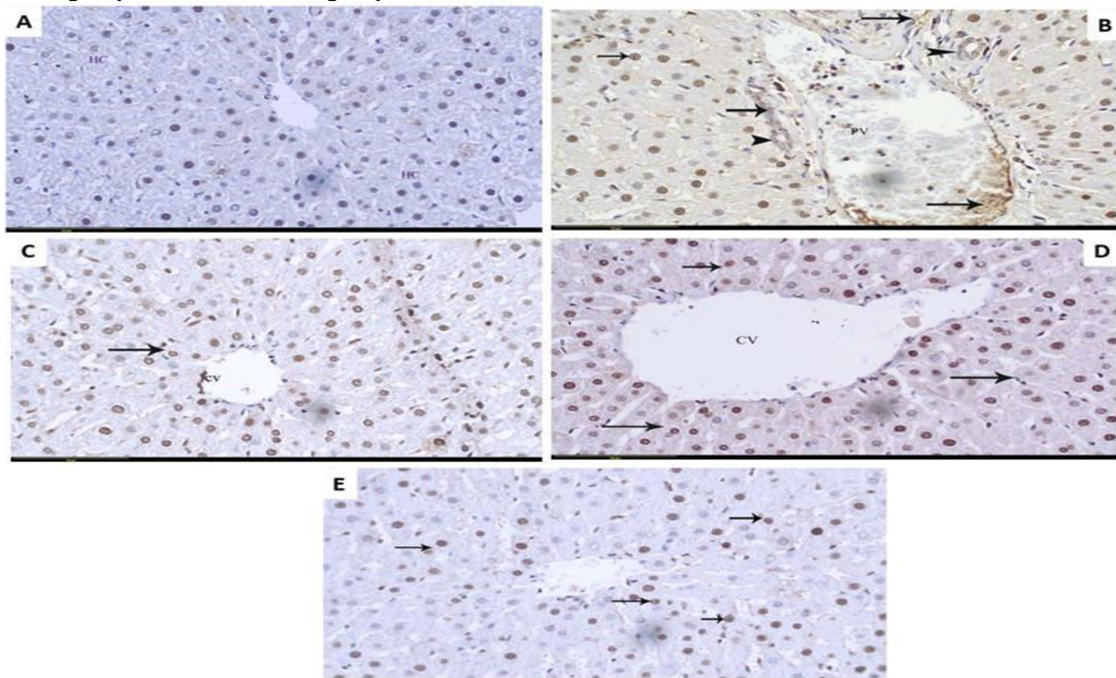

Fig.6. (A): PCNA stained photomicrographic picture in the liver of control rats shows minimal immune positive reaction appears in some hepatocyte (HC) nucleus. **(B)**:

DEX-treated rats show immune positive reactions appearing in the hepatocyte nucleus , portal vein (arrow) and the bile duct (arrowhead).**(C):** DEX &SIM treated rats show moderate immune positive reaction appears in hepatocyte nucleus (arrow). **(D)**: DEX &LCAR treated rats show mild immunopositive reactions that appear in the hepatocyte nucleus, (arrow).**(E)**: DEX &SIM & l-carnitine-treated rats show a mild immunopositive reaction that appears in some hepatocyte nuclei (arrow)**. (×400)**

When comparing the effect of L-CAR, SIM, and their combination. L-CAR induced significant improvement in PCNA expression as compared to the SIM group ($p<0.05$). The combined group induced significant improvement in PCNA expression as compared to either the L-CAR or SIM group ($p<0.001$) **(Table. 3, Fig.7).**

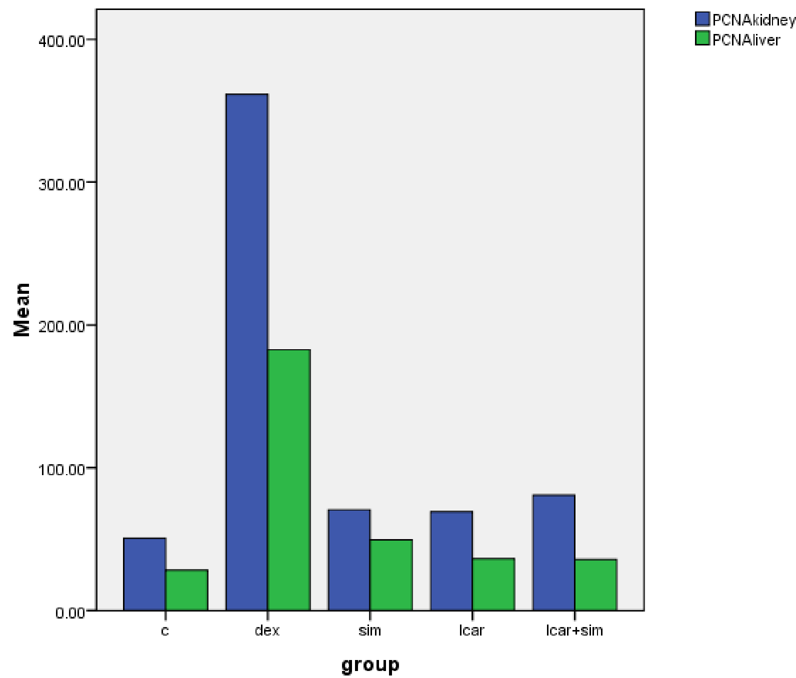

Fig.7. Expression of PCNA stain in the nuclei of liver and kidney cells in different studied groups.

In the kidney: In the control group the renal glomeruli and tubules showed minimal PCNA reaction appeared in some renal tubules epithelium (**Fig.8 A**), while in DEX treated group high reaction to PCNA appeared as brown coloration in the nuclei of renal tubules and in renal glomeruli and show a significant difference when differentiating from a control group where ($p<0.001$) (**Fig. 8 B**), in the SIM and L-CAR group separate and combination showed moderate reaction appeared in the renal tubular epithelium and had a significant difference compared to DEX group ($p<0.001$) (**Table 3, Fig.7, Fig.8 C, D, E**).

When comparing the effect of L-CAR, SIM, and their combination there was no significant difference between groups their effect was nearly similar**. (Table 3, Fig.7).**

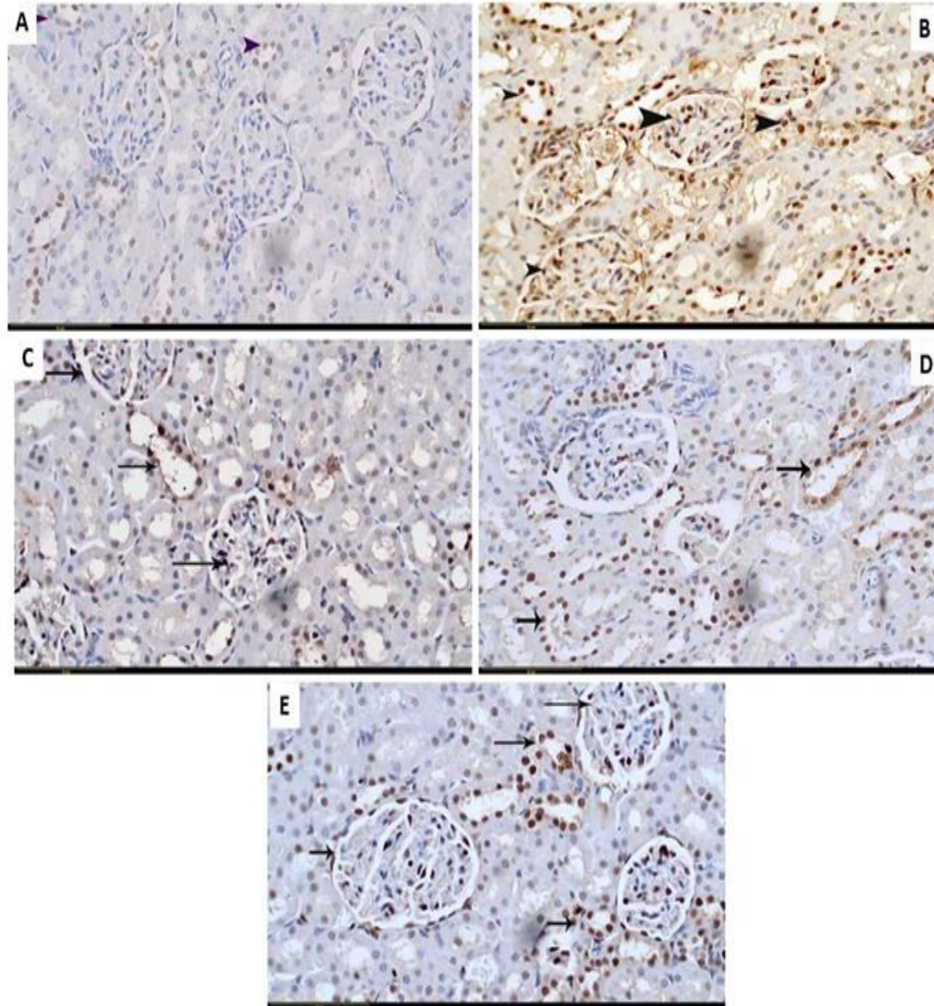

Fig.8.(A): PCNA stained photomicrographic picture in the kidney in the control rats shows minimal immune positive reaction appears the renal tubules (arrowhead). **(B)**: In DEX-treated rats, severe immune-positive reactions appear in nuclei of epithelial cells lining the renal tubules and in renal glomeruli (arrowhead**). (C)**: DEX and SIM-treated rats show moderate reactions in the renal tubules (arrowhead**). (D):** DEX and LCAR-treated rats show moderate reaction appears in nuclei of epithelial cells lining the renal tubules and in renal glomeruli (arrowhead). **(E):** DEX and SIM and l-CAR treated rats show moderate reaction appears (arrowhead) . **(× 400).**

Discussion

While other studies demonstrate individual effects of L-carnitine and simvastatin our study highlights their synergistic effect on dexamethasone-induced organ damage. The findings of this study demonstrate that both L-carnitine (L-CAR) and Simvastatin (SIM) offer significant ameliorative effects against dexamethasone (DEX)-induced liver and kidney damage in rats. Dexamethasone is known to cause oxidative stress and inflammation, which can result in organ dysfunction, particularly in the liver and kidneys. Our study supports previous research highlighting the detrimental effects of DEX on these organs and suggests that both L-CAR and SIM can mitigate these effects through their antioxidative and anti-inflammatory properties.

Administration of DEX led to a significant rise in serum ALT and AST levels both well-established markers of

liver injury, while also significantly reducing serum albumin and total protein levels in relation to a normal control group. These findings were consistent with **Björnsson et al. (2022)** demonstrating that DEX induces hepatotoxicity via oxidative stress and inflammatory pathway within hepatic tissues, also **Danboyi et al. (2022)** demonstrated that DEX-induced instability in hepatic enzymes and serum proteins.

Treatment with L-CAR significantly upgraded these biochemical results by reduction of liver enzymes and elevation of serum albumin and total protein levels when differentiated from the DEX group, likely due to its ability to scavenge free radicals and enhance mitochondrial function, as noted by **Bene et al. (2018).** SIM also ameliorated liver damage by worth drop of liver enzymes and a significant elevation of serum albumin level when compared to the DEX group, which can be attributed to its pleiotropic effects, including its antioxidant, anti-inflammatory, and lipid-lowering properties, as observed by **Liang et al. (2018).** Additionally, the combination of L-CAR and SIM resulted in the greatest improvement in liver function, which is consistent with **Foster (2011),** who noted enhanced protection with the collective use of antioxidants and statins in the treatment of nonalcoholic fatty liver and their augmenting effect.

This is similar to DEX-induced nephrotoxicity, which is evident in the significantly elevated serum urea and creatinine levels. This agrees with **Choi et al. (2013)**, who demonstrated the nephrotoxic effects of glucocorticoids. **Kumar et al. (2014)** demonstrate that DEX increased serum urea and creatinine levels in experimental models by glomerular filtration reduction**.**

Also, our result comes in the same line with **Hasona et al. (2017),** who approved that DEX stimulates an increase in the level of reactive oxygen species (ROS) and reduces the action of antioxidant systems, leading to lipid peroxidation, protein oxidation, and DNA damage in renal cells.

The administration of L-CAR significantly reduced creatinine and urea levels as compared to the DEX group, which may be due to its role in reducing oxidative damage to renal tissues, as suggested by **Sharma and Yadav (2023).** Also, **Koohpeyma et al. (2021)** found that L-carnitine helps to eliminate the products of fatty acid metabolism and other toxic compounds from kidney cells. It also scavenges free radicals and protects the cells from oxidative stress**.** Similarly, SIM improved kidney function by decreasing inflammation and oxidative stress in the kidneys (**Yao et al., 2010; Satirapoj, 2015).**

A key finding of this study was the significant reduction in TAO capacity following DEX administration when compared to a normal control group, which highlights the oxidative stress induced by DEX. Both L-CAR and SIM significantly increased TAO levels in relation to the DEX group, with the combination therapy providing the most substantial improvement.

Oxidative stress plays a key role in the initiation and progression of liver diseases. It causes inflammation and cell death by activating redox-sensitive transcription factors and inflammatory agents, so antioxidant agents have a crucial task in preventing the induction and provocation of liver injury **(Pashayee-Khamene et al.,2024).** Also, **Omid et al 2024** observed a link between DTAC(dilatory total antioxidant capacity) and urea levels where Individuals who have high DTAC had

a lower risk of elevated serum urea levels by improving kidney function.

L-CAR is known to enhance endogenous antioxidant defenses, involving the activity of enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) as described by previous studies (**Mingorance et al., 2011; Boyacioglu et al, 2014).** SIM has also ameliorated antioxidative function by enhancing the activity of antioxidant enzymes and decreasing the generation of reactive oxygen species (ROS), as noted by **Yuqian et al. (2017).** The greater improvement observed with the combined treatment could be explained by the complementary mechanisms of action of L-CAR and SIM, as each drug has anti-inflammatory and antioxidant abilities, so its combination induced more effective results.

In histological examination of the current study, we found that DEX induced a damaging effect in the hepatocyte and also produced bile duct proliferation and portal vein dilatation. Additionally, it induced destruction in renal tubules and glomeruli. **Hammadi et al. (2022)** noticed that administration of dexamethasone in rabbits led to degeneration and increased pigmentation of tubular cells, also proximal tubules showed necrosis and diminished lumen, while there was degeneration and congestion of the central vein in the liver had Hypertrophied cell. Also, **Amr et al. (2013)** stated that the administration of DEX induced fibrosis, necrosis, and fatty liver cells; this effect resulted from the change in lipid metabolism, increased oxidative stress, and production of free radicals. Additionally, a previous study approved that the l-CAR reduced the toxic effect of cadmium on the liver and kidney (**Ögütçü et al., 2024).** Long periods of high doses of DEX administration induced observable changes in hepatocytes, including degeneration of glycogen and subsequent steatosis and excessive Glycogen deposition in hepatocytes (**Kuo et al., 2015).**

In the current study, administration of L-CAR and SIM showed mild reduction in the degree of liver destruction, while there was some sort of destruction in hepatocytes and dilatation in the central vein, renal tissue appeared with intact glomeruli & little degree of destruction in the tubules observed. While combined administration of L-CAR & SIM induced improvement and preservation of hepatocyte architecture and renal tubules & glomeruli that appeared near to normal.

L-carnitine as an antioxidant plays a notable role in the management of energy and lipid metabolism and has an antioxidant system that protects hepatocytes and reduces mitochondrial dysfunction (**Abu-El-Zahab et al., 2019).** In addition, **Nakamura et al. (2018)** reported that L-CAR controlled inhibition of eNOS in IL-1β-stimulated hepatocytes and may have a potential therapeutic effect on multiple organs as liver injury.

Simvastatin is a protective agent for the liver and glomerular toxicity induced by cisplatin in rats, this occurred by a reduced inflammatory factor as -α, nuclear -κB, cyclooxygenase, and tumor necrosis factor (**Koubaa-Ghorbel et al., 2020). Mohammadi et al. (2014)** demonstrated the protective effects of SIM on kidneys in mice exposed to the toxicity of lead. SIM due to its antioxidant effect; has a role in the protection of renal and hepatic tissue also it neutralizes free radicals, has anti-inflammatory properties, and reduces oxidative stress (**Aryanpour et al., 2014).**

In our study, we found highly significant expression of PCNA stain

in the nuclei of the hepatocyte and renal tubular epithelium in the DEX group in differentiation from the control group. While PCNA expression is less in the group that received L-CAR and SIM more improvement appeared in the combined group. PCNA selectively accumulated at damaged regions during early cell cycle stages and increased PCNA expression indicates arrest of cell regeneration and this was supported by **Babaeenezhad et al. (2023)** who found that treatment with gentamycin may be associated with cell cycle arrest that leads to PCNA overexpression. **Ahmed et al. (2021)** demonstrated that PCNA strong expression indicated liver cell damage when rats were treated with methotrexate. PCNA is a marker of cell proliferation, but, some later studies showed that PCNA is also stated in non-proliferating cells which are repairing their DNA **(Essers et al., 2005)**, additionally, **Domitrović et al. (2014)** reported that treatment with cisplatin induces renal injury and increased PCNA expression may represent a crack of DNA repair.

In the present study, there was a reduction of PCNA expression after treatment with L-carnitine and SIM which is considered an indicator for the improvement of liver and kidney cell and their return to the normal state of proliferation. When comparing the efficacy of L-CAR and SIM, Notably, the effect of L-carnitine was superior to SIM in improving both biochemical and histological assessments of the liver and kidney that deteriorated after administration of DEX and their combination therapy is the most effective result. This suggests a synergistic interaction between the two compounds, with L-CAR primarily targeting mitochondrial oxidative stress and SIM modulating systemic inflammatory responses. The combination of an antioxidant and a statin has been proposed by **Bitto et al. (2008)** as a promising therapeutic strategy for managing oxidative stress-induced organ damage.

Conclusion

The combined administration of L-CAR and SIM demonstrated superior protective effects against DEX-induced liver and kidney damage compared to individual treatments. This highlights the potential of combination therapy as a more effective strategy for mitigating glucocorticoid-induced organ dysfunction.

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