# Assessment of the possible protective role of L-carnosine versus L- carnitine on cyclophosphamide - induced renal toxicity in male adult albino rats

**Original Article**

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## ABSTRACT

**Background**: Cyclophosphamide (CPA) is widely used anticancer drug and is associated with renal toxicity. The nephroprotective effect of L-carnosine (CAR) and L-carnitine (LC) is evaluated with other drugs induced renal damage.
**Objective:** To assess the possible protective role of CAR and LC against CPA nephrotoxicity in adult albino rats.
**Materials and Methods**: Sixty adult male wistar rats were divided into five equal groups.Group I as control, group II that divided into: Group IIA received CAR 250 mg/kg/day and group IIB received LC 300 mg/kg/day for 5 days, group III received CPA 150 mg/kg single dose then received physiological saline daily for other 4 days, in the previous groups the rats were sacrificed on the 6$^{th}$ day. Group IV and group V received CAR 250 mg/kg/day and LC 300 mg/kg/day respectively for 5 days then received CPA 150 mg /kg single dose on the 6$^{th}$ day and the rats were anesthetized and sacrificed on the 11$^{th}$ day. All chemicals were given intraperitoneally. Assessment of blood urea and creatinine levels, histological and caspase 3 immunohistochemical studies of the renal cortex were done.
**Results**: In CPA treated group, significant elevation of blood urea and creatinine levels, marked histological changes in the renal cortex with marked increase of the caspase 3 immunoreaction of tubular cells were recorded. In the CAR+CPA group, the previous changes associated with CPA treatment reduced while no protection was recorded in LC+CPA group.
**Conclusion**: CPA renal toxicity was reduced by pretreatment with CAR while pretreatment with LC did not protect against CPA renal toxicity.

**Key Words:** Cyclophosamide, L- carnitine, L-carnosine, toxicity.

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## INTRODUCTION

Unfortunately, the incidence of cancers of different organs was increased and the use of chemotherapeutic drugs has an important role in the cancer therapy beside the surgical treatment[1]. The kidney is a target for multiple chemicals and drugs[2]. Cyclophosphamide (CPA) is one of the alkylating agents (Class of oxazaphosphorins)[3] and is used in treatment of lymphoma, Hodgkin's disease, leukemia, ovarian and breast cancers and severe rheumatoid arthritis[4]. Two active metabolites of CPA (acrolein and phosphoramide mustard) were detected to interfere with the proliferation and growth of malignant cells by prevention of DNA duplication , also these metabolites changed the kidney redox that led to the kidney damage[5]. Various side effects had been detected to be associated with the use of CPA , including nephrotoxicity, cardiotoxicity , hepatotoxicity and myelo-suppression[6]. The antineoplastic drugs were found to destroy the tumors cells or halt the reproduction and growth of them and also they were cytotoxic to the normal cells. High doses of CPA should be administered and this approach is limited due to its multiple side effects[7]. In the recent years there is increase of the use of antioxidants to reduce the side effects of chemotherapeutic drugs[1]. L- carnosine (CAR) is a natural dipeptide present in skeletal muscles and a lot of animal tissues as brain and cardiac muscle[8-10]. The main sources of carnosine are meats and fishes , also variations of its amount depend on the type of fish and meat and method of its cooking[11,12]. Also, CAR is present in different food types as beef (Leg and shoulder), chicken (breast) and lamb[13]. L-carnosine is formed of 2 amino acids that are L- histidine and B-alanine[14]. It has many biological properties including anti-inflammatory, membrane protection, anti-oxidant property and chelation of metal ions[15], so CAR reduced the pathological conditions induced by oxidative stress as in atherosclerosis[16], aging[17], Alzheimer's disease[18] and complication of diabetes mellitus[19]. L-carnitine (LC) is a natural cofactor used as a protective factor for different organs as kidney in different diseases[20,21]. It is formed from lysine and methionine amino acids and is important for production of energy in the body[22]. There was a protective evidence of LC against nephrotoxicity induced by gentamicin[23] and doxorubicin[24]. The current study

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was aimed to assess the protective role of CAR versus LC on the CPA induced renal toxicity in male adult albino rats.

## MATERIALS AND METHODS

### *2.1 Animals*:

Sixty adult male wistar rats with average weight (180-210 gm) were obtained from the animal house center (Faculty of medicine, Zagazig University) and used in this work. Before the beginning of the experiment. all animals were housed 2 weeks for acclimatization. The animals were left in controlled laboratory conditions and housed in suitable cages, at 25°C temperature and under a normal daily 12 hours light/dark cycle. Standard food and fresh water were available. This experimental study was approved in agreement with Zagazig University - IACUC (Institutional Animal Care and Use Committee) with reference number (ZU-IACUC/3/F/168 /2019).

### *2.2 Chemicals and solutions*:

Cyclophosphamide (CPA) in the form of powder was obtained from Baxter Oncology GmbH, Germany and dissolved in physiological saline to reach a concentration 15 mg/ml of CPA solution.

L-Carnosine and L-Carnitine in the form of powder (Sigma – Aldrich Chemical Company and purchased from Egyptian International Center For Import) were dissolved in physiological saline to reach a concentration 25 mg/ml of CAR solution and a concentration 30 mg/ml of LC solution respectively.

### *2.3 Experimental planning*:

The rats were divided into five equal groups (12 rats each) as the following:

-In group (I) or control group , each rat received 2 ml physiological saline, intraperitoneally (IP) per day for 5 days and the rats were sacrificed on the 6$^{th}$ day.

-Group (II) the animals divided into 2 equal subgroups (In group II A, each rat received CAR solution IP (250 mg/kg/day)[15] for 5 days and in group II B, each rat received LC solution IP (300 mg/kg/day)[22] for 5 days and the rats were sacrificed on the 6$^{th}$ day.

-In group (III) or cyclophosphamide group, each rat was given cyclophosphamide solution (150 mg /kg)[25] as a single dose IP then received 2 ml physiological saline (IP) for other 4 days and the rats were sacrificed on the 6$^{th}$ day.

-In group (IV) or CAR+CPA group, each rat was pretreated with CAR solution for 5 days then given on the 6$^{th}$ day cyclophosphamide solution (single dose) as in the previous doses and routes and the rats were sacrificed on the 11$^{th}$ day.

-In group (V) or LC+CPA group, each rat was pretreated with LC solution for 5 days then given on the 6th day cyclophosphamide solution (single dose) as in the previous doses and routes and the rats were sacrificed on the 11$^{th}$ day.

For euthanasia, at the end of the study intramuscular injection of ketamine (250 mg/Kg)[26], blood samples were taken from the tail vein of the rats for biochemical studies then the rats were sacrificed and the both kidneys were fixed in buffered formalin (10%).

### *2.4 Biochemical assessment of kidney function*:

Measurement of BUN (blood urea nitrogen) according to pamphlet of Diamond Diagnostics by Berthelot. Enzymatic colorimetric method and assessment of SC (serum creatinine) according to pamphlet of Spinreact by Jaffe. colorimetric- kinetic method.

### *2.5 Histological study*:

Pieces of the kidneys were processed and paraffin tissue blocks were prepared then thin tissue sections (4-5 µm thick) stained with the following:

-Hematoxylin & Eosin (H&E) to demonstrate the histological changes and mallory's trichrome to demonstrate the collagen fibers (blue in color)[27].

-PAS (Periodic Acid Schiff) for demonstration of brush border of the proximal convoluted tubules, the positive reaction was magenta[28].

Caspase-3 immunohistochemistry of renal sections by avidin biotin peroxidase method for demonstation of apoptosis[29] [Caspase-3 (CPP32) Ab-4], cat # RB-1197-R7, rabbit polyclonal IgG, Thermo Fisher Scientific, Lab Vision Corporation, caspase-3 reaction is brown in color in the cytoplasm with some staining of nuclei. A photomicroscope (OLYMPUS C5060-ADU 5HO1155, Japan) was used for light microscopic examination of the renal stained sections.

### *2.6 Morphometric study* :

Image J software was used for the morphometric study (Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA). The area percentage of collagen fibers from the total area of renal cortex was measured in mallory's trichrome stained renal sections. The optical density (O.D.) of caspase 3 immunoexpression of renal tubular cells was measured in caspase-3 immune stained renal sections. The morphometric measurements were determined in each group at magnification X400 in 6 non overlapping fields of 6 random sections of 6 different rats.

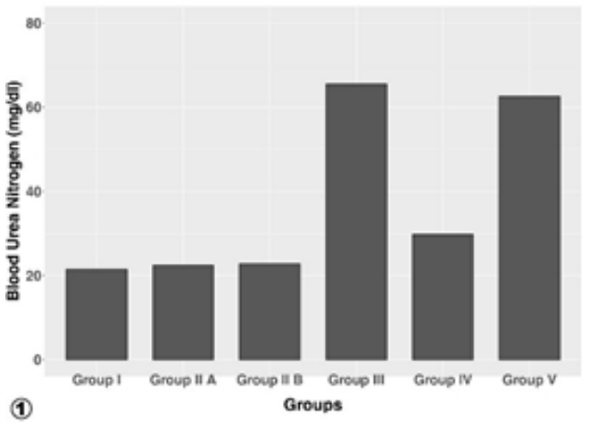
### *2.7 Statistical study*

SPSS (Version 23) program statistical software (IBM Corp. Armonk, NY, USA) was used for calculation of the means and standard deviation (SD) of the results. For calculation of the probability (P) value, one-way analysis of variance (ANOVA) was used followed by Post Hoc test and if (P) value is more than 0.05, the results were insignificant.

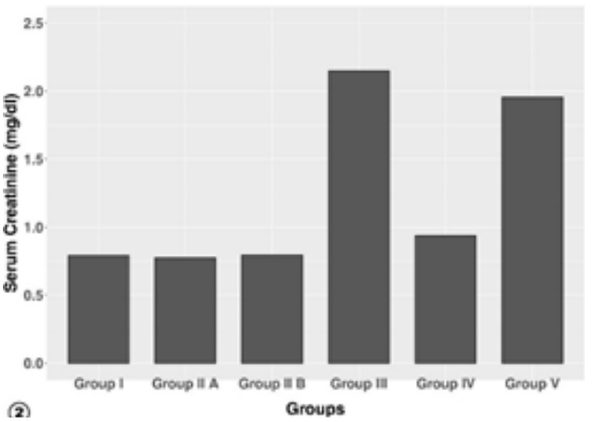
## RESULTS

### *3.1 Biochemical results*:

Biochemical results of the control (group I), CAR (group IIA) and LC (group IIB) demonstrated no significant difference between these groups ($P > 0.05$), so the biochemical results of group I were selected to compare with the results of the remaining groups. In CPA treated group, there was significant increase of BUN and SC levels when compared with the control group. In CAR+ CPA treated group, there was significant decrease of BUN and SC levels when compared with CPA treated group while in LC + CPA treated group there was no significant difference in the levels of BUN and SC when compared with CPA treated group [Graphs 1,2 and Tables 1,2].




**Graph 1:** Showing the levels of BUN (mg/dl) in the studied groups.




Graph 2: Showing the levels SC (mg/dl) in the studied groups.

**Table 1:** Showing the mean levels of BUN (mg/dl) and SC (mg / dl) in the studied groups.

| | Group I<br>Mean ± SD | Group II A<br>Mean ± SD | Group II B<br>Mean ± SD | Group III<br>Mean ± SD | Group IV<br>Mean ± SD | Group V<br>Mean ± SD | *P value* |
|---|---|---|---|---|---|---|---|
| Blood urea nitrogen (BUN) mg/dl | 21.47 ± 1.72 | 22.39 ± 1.36 | 22.73 ± 1.80 | 65.49 ± 5.97 | 29.76 ± 2.60 | 62.54 ± 4.20 | 0.000 *** |
| Serum crealinine (SC) mg/dl | 0.79 ± 0.18 | 0.77 ± 0.16 | 0.80 ± 0.16 | 2.14 ± 0.27 | 0.93 ± 0.17 | 1.95 ± 0.37 | 0.000 *** |

SD: standard deviation *** : significant ($P<0.001$)

**Table 2:** Showing comparison of the levels of BUN and SC in the studied groups (post Hoc test – LSD).

| | Group I Versus Group III | Group I Versus Group IV | Group I versus Group V | Group III versus Group IV | Group III Versus Group V |
|---|---|---|---|---|---|
| Blood urea nitrogen (BUN) | 0.000 *** | 0.000 *** | 0.000 *** | 0.000 *** | 0.055 NS |
| Serum creatinine ( SC) | 0.000 *** | 0.171 NS | 0.000 *** | 0.000 *** | 0.072 NS |

NS: non significant *** : significant (p<0.001)

### *3.2 Histological and immunohistochemical results*:

The histological sections of the control (group I), CAR (group IIA) and LC (group IIB) are nearly similar, so figures of group I were used for comparison With other groups.

### *- H&E staining results:*

The H&E stained sections of the renal cortex in the control group showed normal histological appearance, the glomeruli were composed of a tuft of capillaries that surrounded by two layers of Bowman's capsule (Parietal and visceral layers) that were separated by urinary space. The proximal convoluted tubules demonstrated with acidophilic cuboidal epithelium that had vesicular nuclei and the tubules had a narrow lumen. The distal convoluted tubules had a wide lumen and lined with acidophilic cuboidal epithelium that had vesicular nuclei (Plate 1A). In the CPA treated group, there were multifocal histological affection of renal cortex, the renal tubules showed exfoliated nuclei and marked intracellular vacuolar degeneration, also dilatation of the tubules with dark stained nuclei were recorded (Plate 1B). Presence of luminal acidophilic casts were detected in some tubules with peritubular congested capillaries (Plate 1C). Marked infiltration with inflammatory cells and thickening of the wall of blood vessels were noted in the renal cortex of CPA treated group (Plate 1D). Also, the glomeruli were deformed and other glomeruli were shrunken with wide Bowman's space (Plate 1E).

In the renal cortex of CAR + CPA treated group, glomeruli and renal tubules appeared nearly normal with few intracellular vacuoles and some exfoliation of tubular nuclei (Plate 1F). In sections of the renal cortex of LC + CPA group, the tubules showed marked intracellular vacuolar degeneration, exfoliation of nuclei of epithelial cells of renal tubules, dark stained nuclei in some tubules and numerous deformed glomeruli were recorded (Plate 1G).

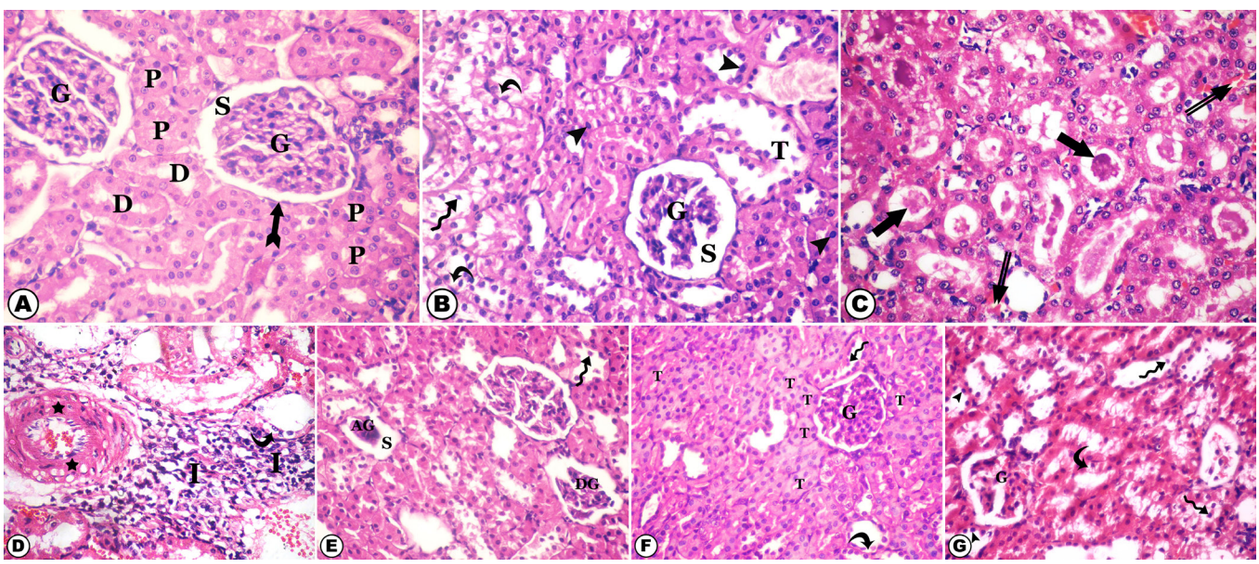



**Plate 1:** Photomicrographs of sections of the renal cortex of the studied groups. (A) control, (B, C, D, E) CPA treated, (F) CAR+CPA treated, (G) LC+CPA treated. (A) showing the glomeruli (G) are composed of a tuft of capillaries. The parietal layer (tailed arrow) of Bowman's capsule is separated from the visceral layer by a space (S). The proximal convoluted tubules (P) and the distal convoluted tubules (D) are showed vesicular nuclei and acidophilic cytoplasm. (B): showing intracellular vacuolar degeneration (curved arrows), dilatation of the tubules (T) with dark stained nuclei (arrow heads), exfoliation of nuclei of tubular cells (zigzag arrow) and deformed glomerulus (G) with wide Bowman's space (S). (C): showing luminal casts (thick arrows) in some tubules with peritubular capillaries congestion (double arrows). (D) : showing marked infiltration with inflammatory cells (I), thickening of the wall of blood vessels (stars) and intracellular vacuolar degeneration of renal tubules (curved arrow). (E) : showing some deformities of glomeruli (DG) and other glomeruli are shrunken (AG) with wide Bowman's space (S), also exfoliation of nuclei of tubular cells (zigzag arrow) is noted. (F) : showing normal appearance of most glomeruli (G) and renal tubules (T) with few intracellular vacuoles (curved arrow) and some exfoliation of nuclei of tubular cells (zigzag arrow). (G) : showing intracellular vacuolar degeneration of the tubules (curved arrow), exfoliation of nuclei of tubular cells (zigzag arrows), dark stained nuclei (arrow heads) and deformed glomeruli (G) are recorded (H&E x400).

### *-Mallory's trichrome staining results*:

Sections of the renal cortex of the control group showed few and thin blue collagen fibers around the renal tubules and corpuscles (Plate 2A). In CPA treated rats, sections of the renal cortex showed increasing of blue collagen fibers around the renal tubules and corpuscles, also within the glomeruli and around the large congested blood vessels (Plate 2B and 2C)

In CAR+ CPA treated rats, sections of the renal cortex showed few collagen fibers around the renal tubules and corpuscles (Plate 2D). In LC+CPA treated rats, the renal cortex revealed excess blue collagen fibers around the renal tubules and corpsules, also within the glomeruli and around the blood vessels as in CPA treated group (Plate 2E).

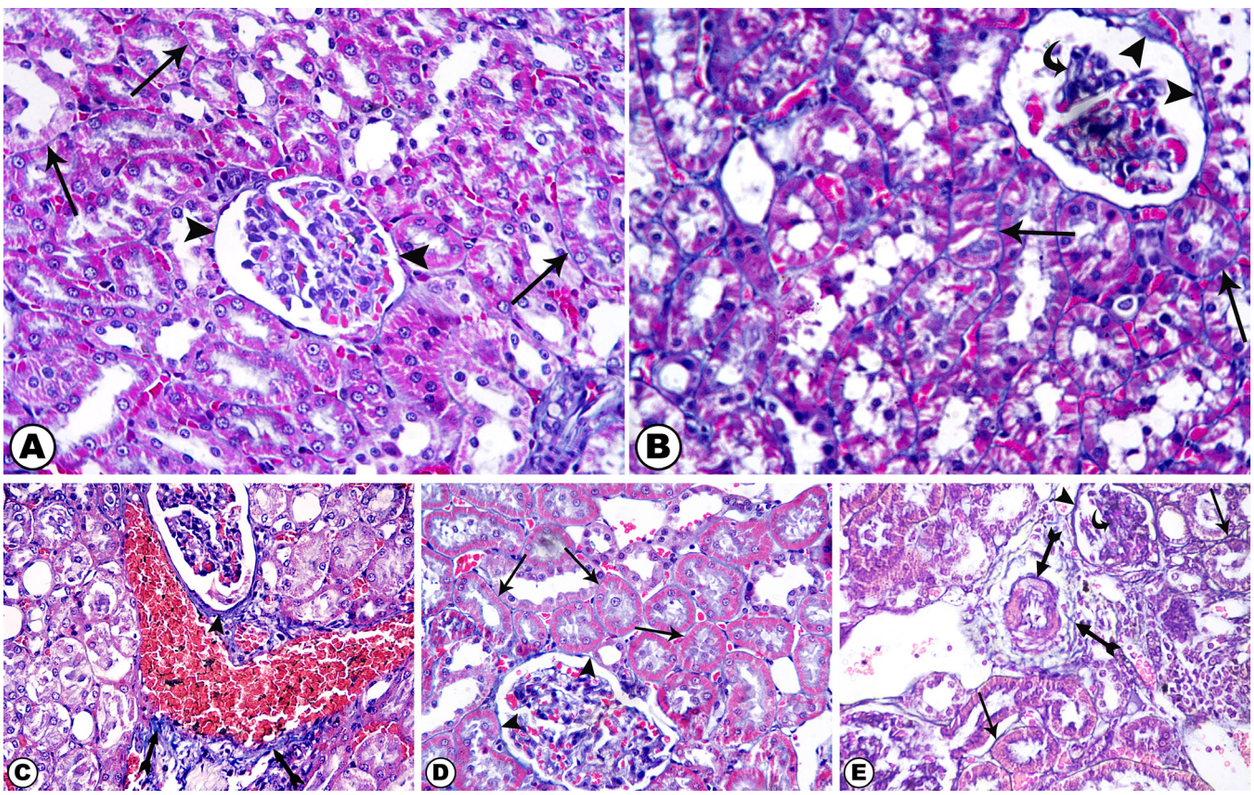



**Plate 2:** Photomicrographs of sections of the renal cortex of the studied groups. (A) control, (B,C) CPA treated, (D) CAR+CPA treated, (E) LC+CPA treated. (A) showing few thin collagen fibers around the renal tubules (arrows) and corpuscles (arrow heads). (B): showing excess blue stained collagen fibers around the renal tubules (arrows) and corpuscle (arrow heads), also within the glomerulus (curved arrow). (C) : showing excess blue stained collagen fibers around the renal corpuscle (arrow head) and around the blood vessels (tailed arrows). (D): showing few thin blue stained collagen fibers around the renal tubules (arrows) and corpuscle (arrow heads). (E) : showing thick blue stained collagen fibers around the renal tubules (arrows) and corpuscles (arrow head), also within the glomeruli (curved arrow) and excess blue stained collagen fibers around the blood vessels (tailed arrows) (Mallory's x400).

***- PAS staining results :***:

PAS stained sections of renal cortex in the control group showed strong positive reaction at the brush border of the proximal convoluted tubules (PCTs), and the basement membrane of renal tubules, also thin PAS positive reaction of basement membrane of parietal layer of Bowman's capsule was recorded (Plate 3A). In CPA treated rats, sections of renal cortex showed absence of PAS reaction at brush borders of PCTs, strong PAS reaction of thick basement membrane of parietal layer of Bowman's capsule, moderate PAS reaction of the basement membrane of renal tubules (Plate 3B).

Sections of renal cortex in CAR + CPA treated rats showing preserved PAS positive reaction in the brush borders of multiple PCTs, strong thin PAS reaction of basement membrane of the parietal layer of Bowman's capsule and strong positive PAS reaction of basement membrane of renal tubules (Plate 3C). In LC+CPA treated rats, sections of renal cortex showing absence of PAS reaction of the brush borders of PCTs with its preservation in few PCTs, positive PAS reaction of basement membrane of renal tubules and strong thick PAS reaction of basement membrane of parietal layer of Bowman's capsule (Plate 3D).

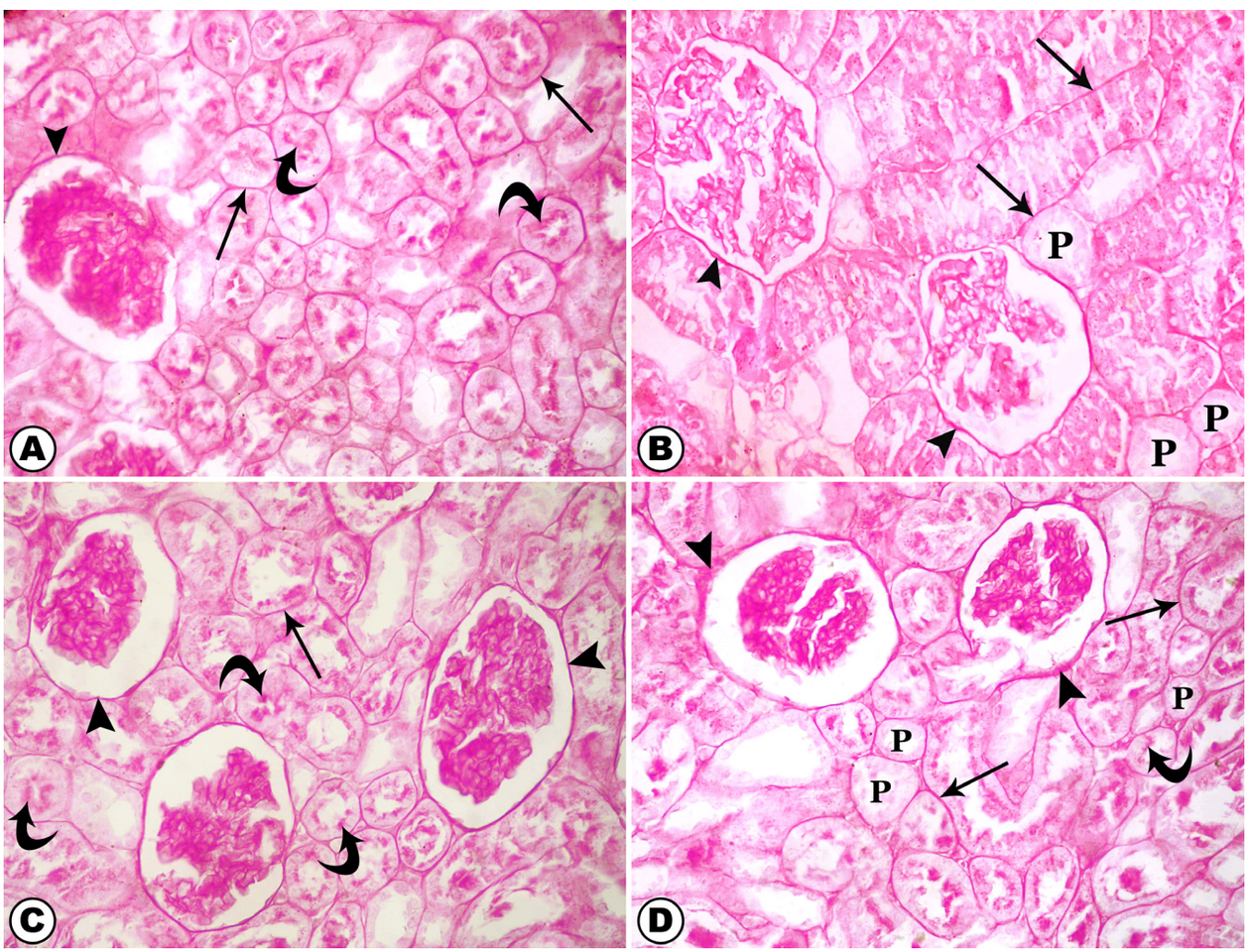



**Plate 3:** Photomicrographs of sections of the renal cortex of the studied groups. (A) control, (B) CPA treated, (C) CAR+CPA treated (D) LC+CPA treated. (A): showing strong positive reaction at the brush border of PCTs (curved arrows) and basement membrane of renal tubules (arrow ), also thin PAS positive reaction of basement membrane of parietal layer (arrow head) of Bowman's Capsule is recorded. (B): showing absence of PAS reaction at brush borders of PCTs (P), strong PAS reaction of basement membrane of parietal layer (arrow heads) of Bowman's capsule and moderate PAS reaction of basement membrane of renal tubules (arrows). (C): showing PAS positive reaction in the brush borders of multiple PCTs (curved arrows), strong thin PAS reaction of basement membrane of the parietal layer (arrow heads) of Bowman's capsule and strong positive PAS reaction of basement membrane of renal tubules (arrow). D: showing absence of PAS reaction of brush borders of PCTs (P) with its preservation in few PCTs (curved arrow), positive PAS reaction of basement membrane of renal tubules (arrow) and strong thick PAS reaction of basement membrane of parietal layer (arrow heads) of Bowman's capsule ( PASx400).

### *-Immunohistochemical caspase -3 staining results*:

Sections of the renal cortex of the control group showed negative or weak cytoplasmic reaction of tubular cells (Plate 4A). Moreover, sections of renal cortex of CPA treated group revealed strong dark brown caspase-3 reaction in tubular cells ( mainly cytoplasmic and some nuclear reaction), also this reaction demonstrated in the glomeruli (Plate 4B).

In CAR+CPA treated group , sections of renal cortex revealed mild caspase-3 reaction in most tubules and few tubules showed strong positive reaction, also moderate reaction present within the glomeruli (Plate 4C). In LC+CPA treated group, sections of renal cortex showed strong caspase-3 reaction in tubular cells in most tubules and within the glomeruli (Plate 4D).

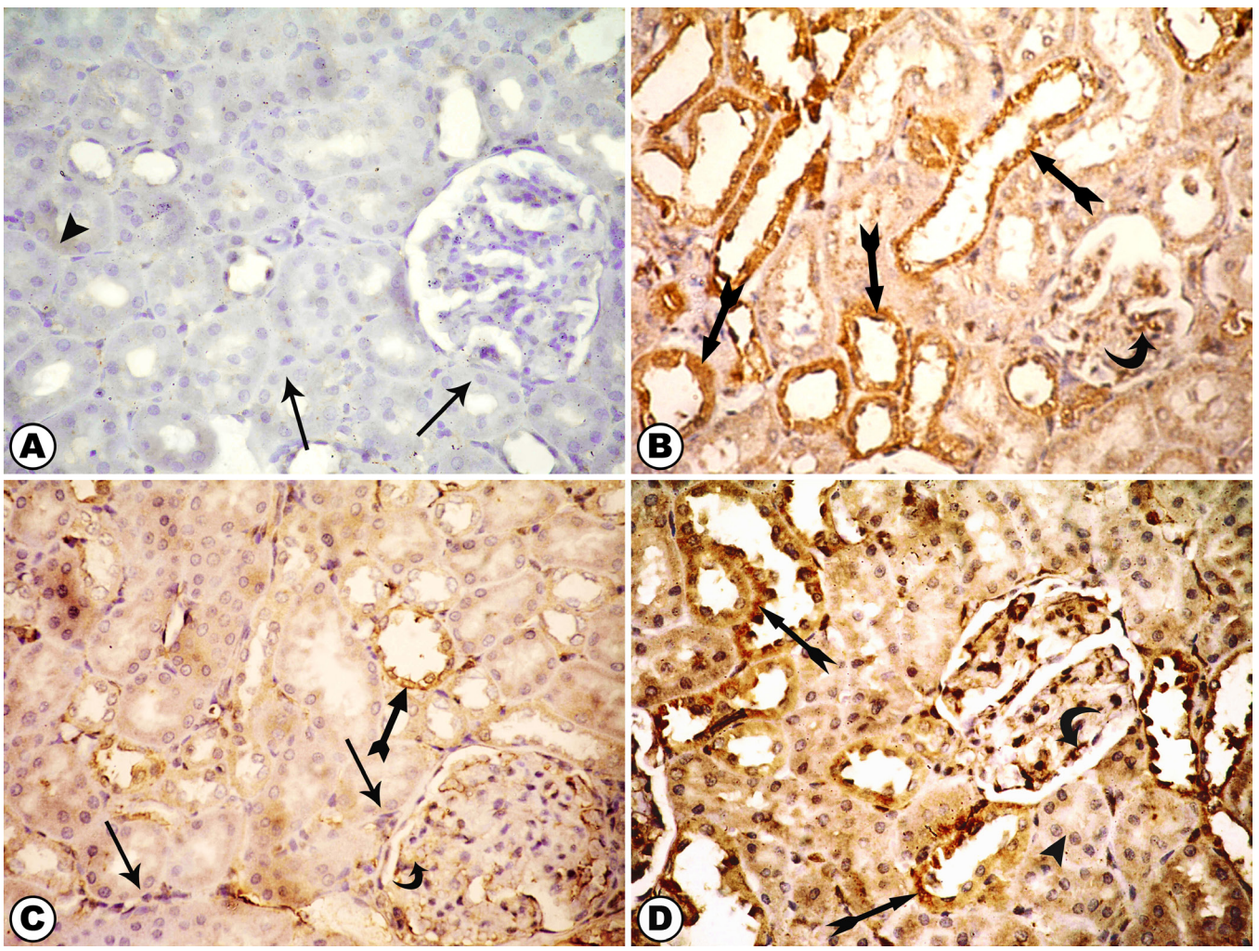
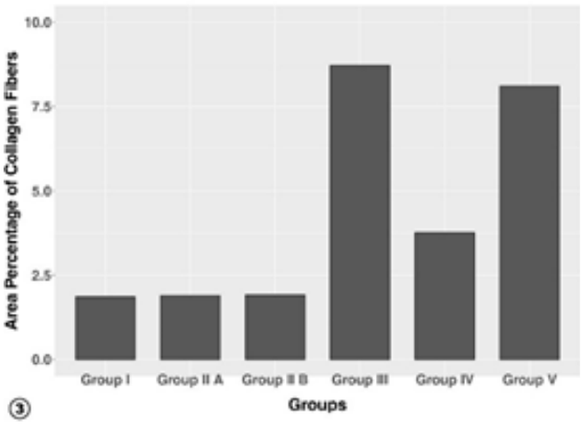


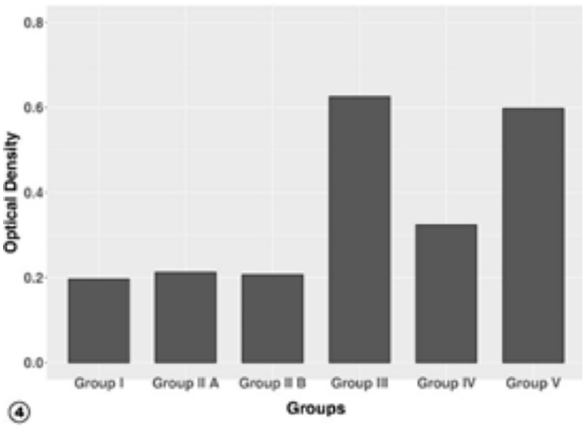

**Plate 4:** Photomicrographs of sections of the renal cortex of the studied groups. (A) control, (B) CPA treated, (C) CAR+CPA treated, (D) LC+CPA treated. (A): showing negative (arrows) or weak (arrow head) cytoplasmic reaction of tubular cells. B: Showing strong dark brown caspase-3 reaction in tubular cells (tailed arrows), also this reaction demonstrated in the glomerulus (curved arrow). (C): showing mild faint caspase-3 reaction in most tubules (arrows) and few tubules showed strong dark brown positive reaction (tailed arrow), also moderate reaction present within the glomerulus (curved arrow). (D): showing strong dark brown caspase-3 reaction in tubular cells in many tubules (tailed arrows), weak reaction in few tubules (arrow head) and strong reaction within the glomerulus (curved arrow) (caspase-3x400).

### *3.3.Morphometrical results*:

Regarding to area percentage of collagen fibers and optical density of caspase-3 immunoexpression, no significant differences present between the control (group I), CAR (group II A) and LC (group II B), so the morphometrical results of group I were used to compare with the results of the remaining groups. There was significant elevation of both morphometrical parameters in CPA treated group when compared with the control group while in CAR + CPA treated group, there was significant reduction of the above mentioned parameters when compared with CPA treated group. In the LC + CPA treated group when compared with CPA treated group, a significant difference of the collagen fibers area percentage and no significant difference of O.D. of caspase-3 immunoreaction were observed (Graphs 3,4; Tables 3,4).




**Graph 3:** showing the values of area percentage of collagen fibers in the studied groups.




**Graph 4:** showing the values of the optical density of caspase-3 immuno expression in the studied groups.

**Table (3) :** Showing the mean values of area percentage of collagen fibers and optical density of caspase-3 immuno expression in the studied groups.

| | Group I<br>Mean ± SD | Group II A<br>Mean ± SD | Group II B<br>Mean ± SD | Group III<br>Mean ± SD | Group IV<br>Mean ± SD | Group V<br>Mean ± SD | *P value* |
|---|---|---|---|---|---|---|---|
| Mean area percentage of collagen fibers | 1.86 ± 0.124 | 1.89 ± 0.133 | 1.92 ± 0.135 | 8.72 ± 0.70 | 3.75 ± 0.74 | 8.10 ± 0.90 | 0.000 *** |
| Mean optical density of caspase-3 immuno expression | 0.19 ± 0.011 | 0.21 ± 0.016 | 0.20 ± 0.013 | 0.63 ± 0.034 | 0.32 ± 0.052 | 0.59 ± 0.043 | 0.000 *** |

SD: standard deviation *** : significant (P<0.001)

**Table 4:** showing comparison of the area percentage of collagen fibers and optical density of caspase-3 immunoexpression in the studied group (Post Hoc test – LSD)

| | Group I Versus Group III | Group I Versus Group IV | Group I versus Group V | Group III versus Group IV | Group III Versus Group V |
|---|---|---|---|---|---|
| Area percentage of collagen fibers | 0.000 *** | 0.000 *** | 0.000 *** | 0.000 *** | 0.019 * |
| Optical density of caspase-3 immunoexpression | 0.000 *** | 0.000 *** | 0.000 *** | 0.000 *** | 0.059 NS |

***NS: non significant*** **** : significant 0.05 > P value > 0.01***

****** : significant (p<0.001)***

## DISCUSSION

The renal toxicity is one of the major complications occurs in the cancer patients treated with CPA[6]. Many studies demonstrated that oxidative stress is the key factor in drug - induced renal dysfunction[30]. Oxidative stress may be involved in CPA induced renal damage because its active metabolites form reactive oxygen species (ROS) which are renotoxic and impairs the renal function. So, the antioxidants can be used as a treatment[6,31].

Metabolism of CPA by enzyme system (hepatic cytochrome P-450) results in production of 2 metabolites that are phosphoramide mustard and acrolein[32]. The phosphoramide mustard has cytotoxic effect on the tumour cells and acrolein has a cytotoxic effect on normal cells[33]. Oxidative stress caused by CPA occurs through depletion of glutathione (GSH) that results in reduction of the cellular defense against the free radicals leading to necrosis and cell death[34]. L-carnosine has a strong antioxidant effect by inactivation of ROS and free radical scavenging[15]. The authors added that CAR has anti-fibrotic and anti-inflammatory effect to reduce the drug or toxin induced hepatic damage. L-carnosine (even in high doses) in safe and its administration does not result in adverse effects. It has a protective effect against hepatic injury induced by antimalarial drugs[8] and reduces the nephrotoxic effect induced by amikacin[35]. Also, CAR plays an important protective role against titanium dioxide nanoparticles induced - cardiac toxicity[14]. L-carnitine has antioxidant effect by multiple mechanisms as a free radical scavenger, its ability of reduction of the formation of ROS and its anti-inflammatory activity[32], but there is contraverse about the potency of the protective effect of LC. some studies reported that it can minimize the nephrotoxic effect of gentamicin[22] while other studies postulated that LC did not prevent the cisplatin nephrotoxicity[26].

In this study, there was significant increase of both SC and BUN in the CPA treated rats when compared with the control group ($P < 0.001$). These results supported by the results of other researchers[25]. In CAR + CPA treated group, there was significant decrease of BUN and SC when compared with CPA treated group and these results were in accordance with other studies of CAR pretreatment with other chemotherapeutic drugs as cisplatin[36]. While in LC + CPA treated group, there was no significant difference in the levels of BUN and SC when compared with CPA treated group and these results were in accordance with other studies that revealed that LC did not reduce the high levels of BUN and SC induced by cisplatin therapy[26].

Histologically, CPA treated rat kidneys showed tubular and vascular changes. The vascular changes were observed as congestion, thickening of the blood vessels walls, infiltration with inflammatory cells and atrophic glomeruli. While, the tubular changes were demonstrated as vacuolations of tubular epithelial cells, dark stained nuclei with exfoliation of other nuclei and loss of PAS reaction at the brush borders of PCTs. Similar results reported by other researchers and these changes suggested increase in the oxidative stress with reduction of antioxidant factors[37, 38]. In CPA treated rats, the Mallory's trichrome sections of the renal cortex showed increasing of collagen fibers around the blood vessels and the renal tubules. These results were confirmed by significant increase in the area percentage of collagen fibers when compared with the control group. The caspase-3 stained renal cortex showed strong dark brown immuno-reaction in the tubular cells and it was a sign of apoptosis that may explained by CPA induced oxidative stress. These results in agreement with other studies[38] and confirmed by significant increase in the optical density of caspase-3 reaction when compared with the control group.

When the rats were pretreated with CAR before IP injection of CPA, the histological and immunohistochemical changes associated with CPA therapy were minimized and these results were confirmed by significant reduction of the optical density of caspase-3 immunoreaction of tubular epithelial cells and reduction of the amount of collagen fibers around renal tubules and blood vessels when compared with the CPA treated group. These results in agreement with Noori and Mahboob[36] results who reported that pretreatment of CAR before cisplatin therapy counteracted the renal pathological effects of cisplatin. While in LC + CPA treated group, LC did not prevent the renal histological changes of CPA, also no significant reduction of the optical density of caspase-3 immunoreaction of tubular epithelial cells while there was significant reduction of the amount of collagen fibers around renal tubules and blood vessels when compared with the CPA treated group ($0.05 > P\ value > 0.01$). These results in agreement with Uzunoglu *et al*.[26] results who reported that LC did not prevent the renal histological changes of cisplatin chemotherapy. L-carnitine has an important role in metabolism of the lipids and production of ATP[39]. In conclusion various pharmacological interventions may help in reducing the renal damage that induced by oxidative stress. CAR ameliorates the CPA renal toxicity by its potent antioxidant effect while LC did not protect against the CPA induced nephrotoxicity, so supplementation of CAR before the beginning of CPA therapy is highly recommended.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

There are no conflicts of interest.

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## الملخص العربى

# تقييم الدورالوقائي المحتمل ل إل- كارنوزين مقابل إل- كارنيتين على السمية الكلوية المحدثة بالسيكلوفوسفاميد في الجرذان البيضاء الذكور البالغة

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السيكلوفوسفاميد من الادويه المضاده للسرطان و يرتبط استخدامه بالسمية الكلوية وقد كان هدف البحث تقييم الدور الوقائي الممكن لمادة إل- كارنوزين مقابل ماده إل- كارنيتين في تحسين السمية الكلوية المحدثة بالسيكلوفوسفاميد في الجرذان البيضاء البالغة و قد اشتمل البحث علي ٦٠ من ذكور الجرذان البيضاء البالغة وقسمت إلي ٥ مجموعات متساوية (١٢ لكل مجموعة) المجموعة الاولي ضابطة و المجموعة الثانية والتي قسمت الي مجموعتين فرعيتين بالتساوي وتم حقن احداها بمادة إل- كارنوزين (٢٥٠ مجم/ كجم / في اليوم) وحقن الأخرى بمادة إل- كارنيتين (٣٠٠ مجم/ كجم / في اليوم ) واستمرت المعالجة لمدة ٥ أيام وتم ذبح الجرذان في اليوم السلدس وفي المجموعة الثالثة تم حقن الجرذان بالسيكلوفوسفاميد (١٥٠ مجم/ كجم) كجرعه واحده ثم حقنها بمحلول ملح فسيولوجي لمدة ٤ أيام اخري وتم ذبح الجرذان في اليوم السادس بينما في المجموعة الرابعة والخامسة فتم معالجة الجرذان بمادة إل- كارنوزين (٢٥٠ مجم/ كجم / في اليوم) وحقن الأخرى بمادة إل - كارنيتين (٣٠٠ مجم/ كجم / في اليوم) علي التوالي واستمرت المعالجة لمده ٥ أيام ثم تم حقن السيكلوفوسفاميد (١٥٠ مجم/ كجم) كجرعة واحدة في اليوم السادس وتم ذبح الجرذان في اليوم الحادي عشر و قد تمت المعالجات السابقه جميعها بالحقن البروتوني وتم تخدير جميع الجرذان قبل ذبحها وعمل دراسات هيستولوجية وهستوكيميائية مناعية للكسبيز-٣ واخذ عينات من دم الجرذان ودراسه وظائف الكلي وقد لوحظ في المجموعة المعالجة بالسيكلوفوسفاميد ارتفاع ذو اهمية احصائية في مستوي اليوريا والكريتيانين مع وجود تغيرات هيستولوجية قي القشرة الكلوية كما لوحظ زيادة كبيرة في تفاعل الكسبيز-٣ للخلايا المبطنة للانيبيبات الكلوية وفي المجموعة التي تمت معالجتها بماده إل- كارنوزين قبل حقن السيكلوفوسفاميد ظهر تراجع ذو اهمية احصائية في التغييرات الكيميائية و الهيستولوجية والهستوكيميائية المناعية للكسبيز-٣ والتي تحدث مع استخدام عقار السيكلوفوسفاميد ولم يحدث هذا التراجع في المجموعة المعالجة بمادة إل- كارنيتين قبل حقن السيكلوفوسفاميد و نستخلص من النتائج السابقة ان مادة إل- كارنوزين كمضاد اكسدة ذو كفاءة عالية لها دور وقائي ضد الاعتلال الكلوي السمي الناتج من استخدام عقار السيكلوفوسفاميد في الجرذان البالغة علي عكس مادة إل- كارنيتين.