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Original Article

A Light and Immunohistological Study on the Protective Effects of Ascorbic Acid (Vit.C) and L-Carnitine on Cisplatin-Induced Renal Impairment in Mice

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ABSTRACT

Background: Despite being efficient anti-neoplastic agent, Cisplatin therapy leads to nephrotoxicity as a major associated complication implicated to be induced via oxidative stress.

Aim of the Work: This study was conducted to compare the nephro-protective effects of vitamin-C or L-carnitine in amelioration of the dramatic toxic effects of Cisplatin on renal tissue.

Material and Methods: A total of thirty male mice were divided into six groups; each included 5 mice. Group (I) used as the control, mice received a single intraperitoneal (IP) injection of 1.5 ml of sterile distilled water. Group (II) is treated mice with a single IP injection of Cisplatin (10 mg/kg BW). In group (III) the animals received Cisplatin as in group II plus ascorbic acid (500 mg/kg BW) orally for 2 days before and 3days after Cisplatin injection. Group (IV) is L-carnitine +Cisplatin-treated mice, received Cisplatin as in group II plus L-carnitine (200 mg/kg BW) orally for 2 days before and 3days after Cisplatin injection. Group (V) is Ascorbic acid-treated animals that received the same dose of ascorbic acid as in group III orally for 5 consecutive days. Group (VI) is L-carnitine-treated animals, which received the same dose of L-carnitine as in group IV orally for consecutive 5 days.

Results: Mice treated with Cisplatin showed extensive tubular necrosis, presence of casts in the cortex, changes in the basement membrane and brush border loss. While, mice treated with both Cisplatin and vitamin C or L-carnitine revealed marked reduction in the apoptotic cells, maintenance of brush border and marked decrease in basement membrane oedema.

Conclusions: L- carnitine supply is a better protection against the nephrotoxic effects of drug Cisplatin.

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Key Words: Cisplatin, nephrotoxicity, vitamin-C, L-carnitine, amelioration, kidney, histology.

INTRODUCTION

Cisplatin (Cis-diammineedichloroplatinym) is an effective chemotherapeutic agent used in the treatment of a wide array of both pediatric and adult malignancies *(Kuhad, et al. 2006)*. Nevertheless, both clinical and experimental studies reported a dose limiting nephrotoxicity which restricted cisplatin's optimal usefulness in cancer chemotherapy *(Lierberthal, et al. 1996; Kintzel, 2001 and Fujieda, et al. 2006)*.

Although several mechanisms have been suggested to account for Cisplatin- induced nephrotoxicity, the precise mechanisms underlying this pathogenesis are not completely understood. It has been reported that Cisplatin elicited oxidative stress in and around mitochondria, thereby inducing apoptosis of renal proximal tubule cells and dysfunction of the kidney *(Kuhlmann, et al. 1997 and Chang, et al. 2002)*. The mechanisms of this toxic effect may involve direct inhibition of specific membrane transport systems, mitochondrial toxicity and depletion of glutathione, finally leading to loss of specific function and / or necrosis of renal tubular cells *(Antunes, et al. 2001; Saad and Al-Rikabi, 2002)*.

L-carnitine is a nutrient, located primarily in mitochondria and possesses potential protective effects against mitochondrial toxic agents *(Schuiz, 1991 ; Arrigoni-Martelli and Caso, 2001)*. *Boon-*

Sanit et al. (2006) reported that L-carnitine can protect renal impairment functionally, biochemically and histopathologically via a corresponding reduction of oxidative stress without decreasing the tumerocidal action of the agent.

Reactive oxygen species and free radicals are involved in the nephrotoxicity induced by Cisplatin *(Ajith, et al. 2007)* and lipid peroxidation products have been identified in vivo and in vitro following Cisplatin treatment and pretreatment with antioxidant decreased Cisplatin-induced lipid peroxidation and injury *(Mayes, 1999)*.

Ajith et al. (2007) showed that administration of Ascorbic acid (Vit.C) modified the nephrotoxicity induced by Cisplatin.

So, this study was carried out to compare between the protective effects of the antioxidant Ascorbic acid (Vit.C) and L-carnitine in Cisplatin-induced oxidative renal damage in mice.

MATERIAL AND METHODS

Thirty male mice of local strains, weighing from 28-30 gm were used in this study. They were acclimatized for one week prior to the experiment. Mice were caged five per cage in fully ventilated room at room temperature. Mice had ad libitum, access to water and semi synthetic balanced diet. Mice were divided into 6 groups, each included 5 mice:

- **Group (I) [Control group]:** The animals received a single intraperitoneal (IP) injection of 1.5 ml of sterile distilled water.

- **Group (II) [Cisplatin-treated group]:** The animals received a single IP injection of Cisplatin (10 mg/kg BW) (Ajith et al., 2007).

- **Group (III) [Ascorbic acid (Vit.C) + Cisplatin-treated group]:** The animals received Cisplatin as in group II plus Ascorbic acid (Vit.C) (500 mg/kg BW) (Ajith et al., 2007) orally by intragastric intubation for 2 days before and 3days after Cisplatin injection.

- **Group (IV) [L-carnitine + Cisplatin-treated group]:** The animals received Cisplatin as in group II plus L-carnitine (200 mg/kg BW) *(Chang, et al. 2002)* orally by intragastric intubation for 2 days before and 3days after Cisplatin injection.

- **Group (V) [Ascorbic acid (Vit.C)-treated group]:** The animals received the same dose of Ascorbic acid (Vit.C) as in group III orally by intragastric intubation for 5 consecutive days.

- **Group (VI) [L-carnitine-treated group]:** The animals received the same dose of L-carnitine as in group IV orally by intragastric intubation for 5 consecutive days.

Cisplatin was obtained from Merck Company, Germany while Ascorbic acid (Vit.C) (Cevilene) was obtained from Kahira Pharmaceutical & Chemical Industries, Egypt and L-carnitine was obtained from Mepaco-Arab Company for Pharmaceutical & Medicinal plants, Sharkeiya, Egypt.

Five days after Cisplatin injection, the animals were sacrificed by an overdose of ether *(Ajith, et al. 2007)*. Their kidneys were removed and divided into two halves parallel to the major axis then washed and fixed in 10% neutral buffered formalin solution and processed for paraffin embedding for light microscopic study. The specimens from both kidneys were taken from the middle portion of each kidney in the form of a wedge including the cortex and medulla. Serial sections (5 um thick) were prepared and stained with: H&E to verify microanatomical details; Periodic Acid-Schiff (PAS) staining was used to demonstrate the basement membrane and brush border; Masson's trichrome staining was used to demonstrate the accumulation of connective tissue in the nephrons, and to detect development of areas of tissue degeneration.

Parafin-embedded sections were also analysed by immunostaining using the Bak-protein, present in the cytoplasm of apoptotic cells and the polyclonal anti-Ki-67 antibody, a nuclear non-histone protein present only in cycling cells *(Schluter, et al. 1993)* (dilution, 1: 400; Novocastra, Newcastle, UK) to estimate the degree of cell proliferation.

By using the light microscope, histological sections were examined to assess the degree of glom-

erular and tubular epithelial affection. The degree of affection was recorded as: faint, minimal, mild, moderate and marked. *(Schluter, et al. 1993)*

Morphometric study: By the aid of the Leica Q 500 image system [LICA Microsystem Corporation, England] the degree of morphological involvement in renal failure was determined using light microscopy, as described by *Shih et al. (1988)* and *Megyesi et al. (1998)* with some modifications. The following parameters were chosen as indicative of pathological damage to the kidney: Brush border loss, tubule dilatation, tubule degeneration, tubule necrosis, tubular cast formation, cloudy swelling and hydropic degeneration. These parameters were evaluated on a grade of 0–4, which ranged from absent (0 = normal; 0.5 = small focal areas), mild (1= involvement of less than 10% of the cortices and outer medullae), moderate (2= 10–25% involvement of the cortices and outer medullae), severe (3= 25–75% involvement of the cortices and outer medullae), to very severe (4= extensive damage involving more than 75% of the cortices and outer medullae). Each parameter was determined in five different rats in three sections from each animal.

Statistical Analysis: Data are expressed as means ± SE. One-way ANOVA followed by Bonferroni's t-test was used to compare the values in the Cisplatin group with control values, and an unpaired t-test was used to compare the values in the Cisplatin group with those of the Cisplatin+Vit.C and Cisplatin+L.carnitine groups. The level of statistical significance was set at $P < 0.05$.

All statistical analyses were performed with the aid of SPSS-11 (Chicago, USA) statistical analysis software.

RESULTS

Gross Examination: No changes were observed in the kidneys of the sacrificed animals in groups I. IV, V and VI while in group II and III, the kidneys of the sacrificed animals were swollen and pale.

Histological and Immunohistological results: Sections of the kidney stained by Hematoxylin & Eosin (H&E) in control (I, V&VI) animals showed cortex and medulla with normal histological architecture. The cortex showed the malpighian renal corpuscles surrounded by proximal and distal convoluted tubules. Each malpighian corpuscle was made up of a tuft of blood capillaries (the glomerulus) surrounded by the Bowman's capsule which was lined by a single layer of flat epithelium. Each proximal convoluted tubule was lined by few pyramidal cells. The cytoplasm was acidophilic and the nuclei were rounded and near the base of the cells. The cells had a free striated border. The distal convoluted tubules were lined by a relatively large number of cuboid epithelial cells. The lumens of the distal tubules were wider than the proximal tubules. The cytoplasm was less acidophilic and the nuclei were round and tend to be located in the apical region (Fig. 1). The connective tissue in Masson's trichrome stained sections in control groups showed minimal greenish color in between renal tubules in the cortex and relatively extensive medullary connective tissue (Fig. 2). In PAS stained sections, the proximal tubules showed a bright red coloured basement membrane and deeply stained brush border. The glomeruli showed deeply stained basement membrane of the capillary loops. The distal tubules showed striated basement membrane but not as bright as that of the proximal tubules (Fig. 3). By immunohistological staining; the degree of cell proliferation was analysed using an antibody against Ki-67; in control groups, the proximal tubules, distal tubules and glomerulus showed marked nuclear reaction with Ki67 (Fig. 4) and showed faint diffuse granular cytoplasmic reaction with Bak immunostaining (Fig. 5).

H&E stained sections of the kidney of Cisplatin-treated animals showed extensive proximal and distal tubular necrosis, mainly in the corticomedullary region and intratubular casts in the outer stripe of the outer medulla and extensive tubular necrosis and casts in the inner cortex and outer medulla which were graded 4 as analyzed morphometrically (Fig. 6). Glomerular changes were observed in the form of capsular adhesions with glomerular tuft. These changes were proved to be statistically highly significant when compared to the control groups ($P<0.0001$). In Masson's trichrome stained sections, there was marked increase in greenish connective tissue in between renal tubules (Fig. 7). In PAS stained sections, there was marked decrease in PAS positive materials in brush border of proximal tubules and basement membrane of renal tubules (Fig. 8). By immunohistological staining, regarding proximal tubules, distal tubules and glomerulus, Ki67 stained sections showed faint nuclear reaction (Fig. 9)

and Bak stained sections showed marked diffuse granular cytoplasmic reaction (Fig. 10).

In Cisplatin-vitamin C treated animals, H&E stained sections showed marked improvement in renal tubules cells (Grade 2 as analyzed morphometrically) (Fig. 11) and these improvements were statistically significant when compared to both control and Cisplatin-treated animals ($P<0.05$)). Mild increase of greenish connective tissue in between renal tubules was observed in Masson's trichrome stained sections (Fig. 12). Minimal decrease in PAS positive materials in brush border of proximal tubules and basement membrane of renal tubules was seen in PAS stained sections (Fig. 13). Ki67 stained sections showed minimal nuclear reaction (Fig. 14) and Bak stained sections revealed mild diffuse granular cytoplasmic reaction (Fig. 15) in proximal tubules, distal tubules and glomerulus.

In Cisplatin-L-carnitin treated animals, H&E stained sections revealed normal cells in most renal tubules epithelium (Grade 0.05 as analyzed morphometrically) (Fig.16) which were statistically highly significant ($P<0.005$) when compared to the Cisplatin-treated group. The connective tissue in Masson's trichrome stained sections showed minimal greenish color in between renal tubules in the cortex and relatively extensive medullary connective tissue that were similar to those of control animals (Fig. 17). In PAS stained sections, the proximal tubules showed a bright red coloured basement membrane and deeply stained brush border. The glomeruli showed deeply stained basement membrane of the capillary loops. The distal tubules showed striated basement membrane which were very similar to those of control animals (Fig. 18).

Ki67 & Bak immuno-stained sections showed that; the proximal tubules, distal tubules and glomerulus showed marked nuclear reaction with Ki67 and faint diffuse granular cytoplasmic reaction with Bak immunostaining that were similar to control sections (Figs.19-20).

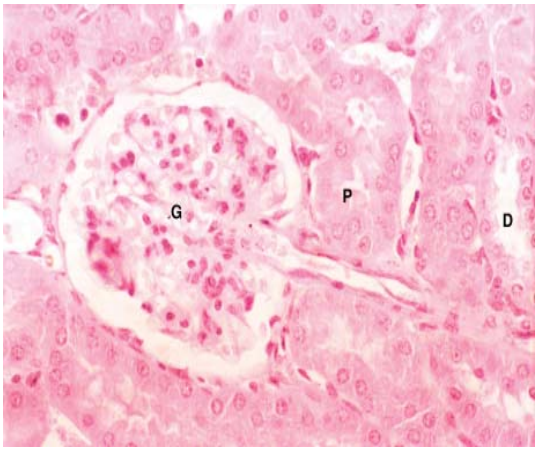

Fig. 1: A photomicrograph of the kidney of the control group showing proximal tubules (P), distal tubules (D) and glomeruli (G) H&E; X400

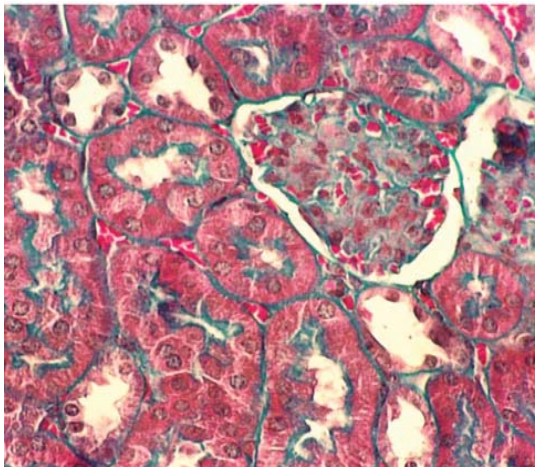

Fig. 2: A photomicrograph of the kidney of the control group showing minimal greenish connective tissue in between the renal tubules. Masson's Trichrome; X400

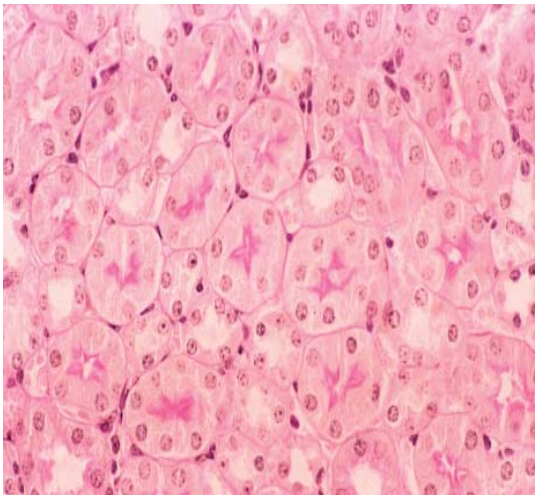

Fig. 3: A photomicrograph of the kidney of the control group showing PAS positive material in brush border of the proximal tubules and the basement membrane of the renal tubules. PAS; X400

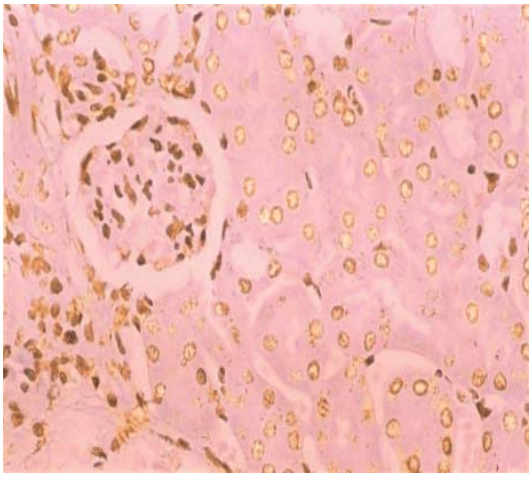

Fig. 4: A photomicrograph of the kidney of the control group showing marked nuclear reaction in proximal and distal tubules and in glomeruli. Ki67; X400

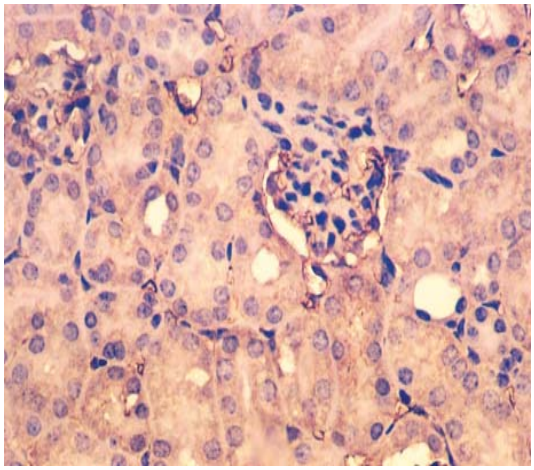

Fig. 5: A photomicrograph of the kidney of the control group showing faint diffuse granular cytoplasmic reaction in proximal and distal tubules. Bak; X 400

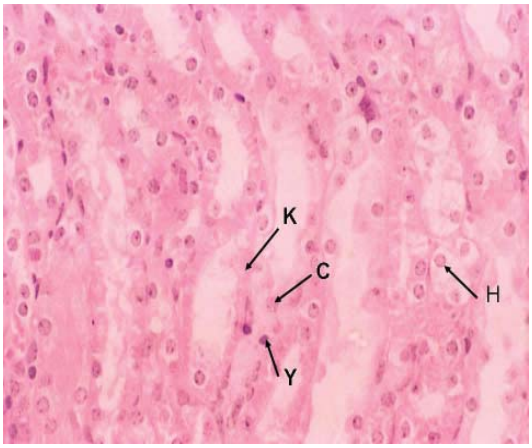

Fig. 6: A photomicrograph of the kidney of a Cisplatin-treated group showing marked cellular necrosis in the form of vacuolated cytoplasm with pyknotic (Y) and karyolytic (K) nuclei. Also hydropic degeneration (H) and cloudy swelling (C) are seen in some cells. H&E; X400

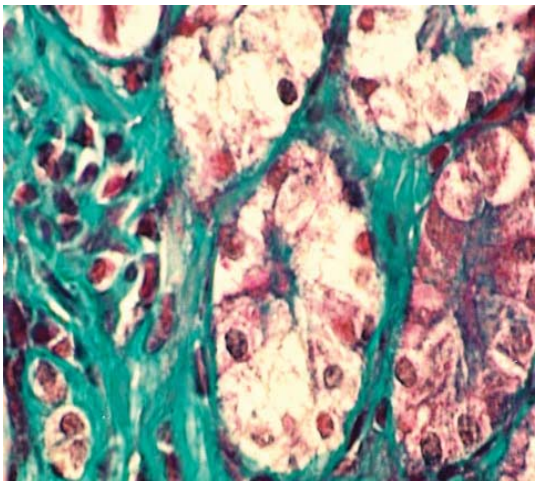

Fig. 7: A photomicrograph of the kidney of a Cisplatin-treated group showing marked increase in the greenish connective tissue in between renal tubules.
Masson's Trichrome; X400

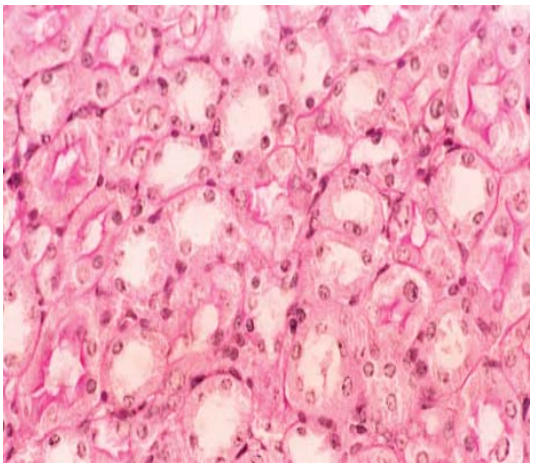

Fig. 8: A photomicrograph of the kidney of a Cisplatin-treated group showing marked decrease in PAS positive material in the brush border of proximal tubules and basement membrane of renal tubules. PAS; X400

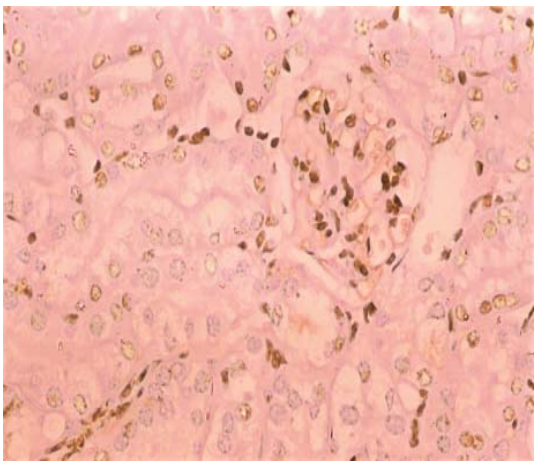

Fig. 9: A photomicrograph of the kidney of a Cisplatin-treated group showing faint nuclear reaction in proximal and distal tubules and in glomeruli. Ki67; X400

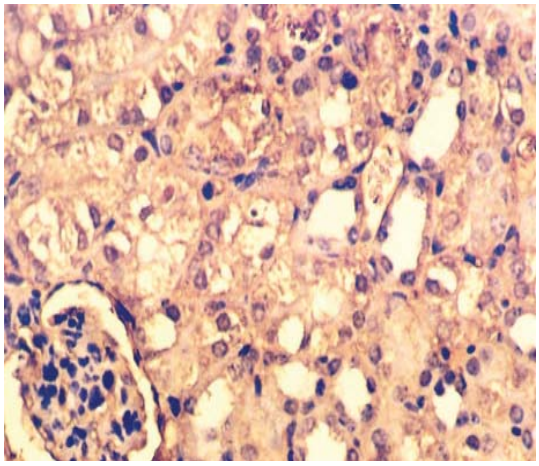

Fig. 10: A photomicrograph of the kidney of a Cisplatin-treated group showing marked diffuse granular cytoplasmic reaction in proximal and distal tubules. Bak; X 400

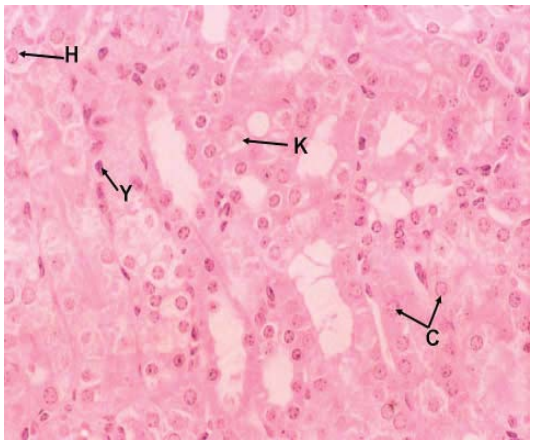

Fig. 11: A photomicrograph of the kidney of the Cisplatin-vitamin C group showing hydropic degeneration (H) and cloudy swelling (C). Cellular necrosis in the form of vacuolated cytoplasm with pyknotic (Y) and karyolytic (K) nuclei also are seen. H&E; X400

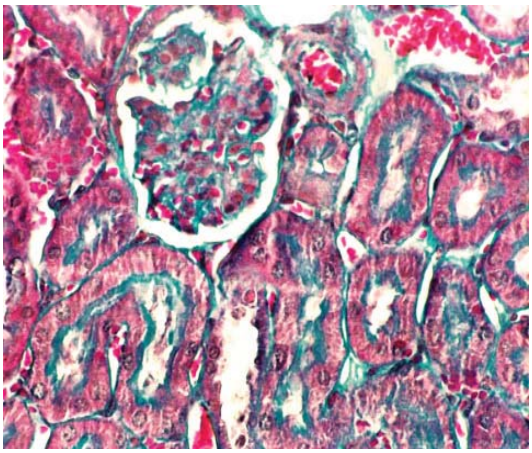

Fig. 12: An A photomicrograph of the kidney of the Cisplatin-vitamin C groupshowing moderate increase in the greenish connective tissue in between renal tubules. Masson's Trichrome; X400

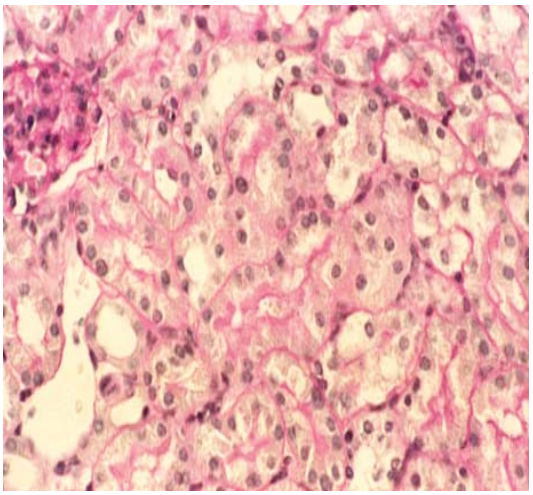

Fig. 13: A photomicrograph of the kidney of the Cisplatin-vitamin C group showing moderate decrease in PAS positive materials in the brush border of proximal tubules and basement membrane of renal tubules. PAS; X400

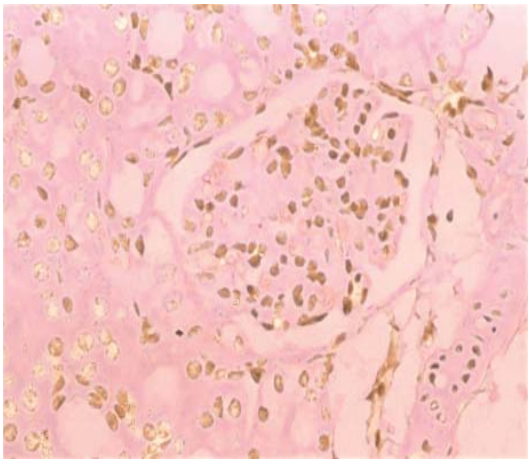

Fig. 14: A photomicrograph of the kidney of the Cisplatin-vitamin C group showing moderate nuclear reaction in proximal and distal tubules and in glomeruli. Ki67; X400

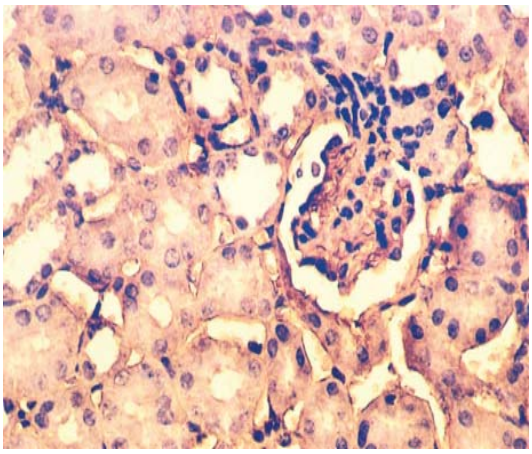

Fig. 15: A photomicrograph of the kidney of the Cisplatin-vitamin C group showing moderate diffuse granular cytoplasmic reaction in proximal and distal tubules. Bak; X 400

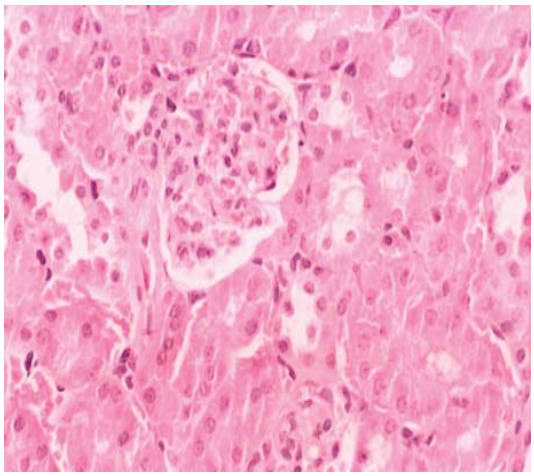

Fig. 16: A photomicrograph of the kidney of the Cisplatin-L-carnitine group showing normal architecture similar to that of control group. H&E; X400

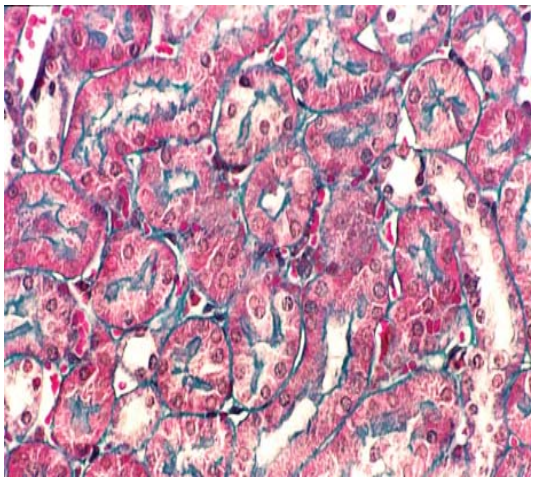

Fig. 17: A photomicrograph of o the kidney of the Cisplatin-L-carnitine group showing minimal greenish connective tissue in between the renal tubules. Masson's Trichrome; X400

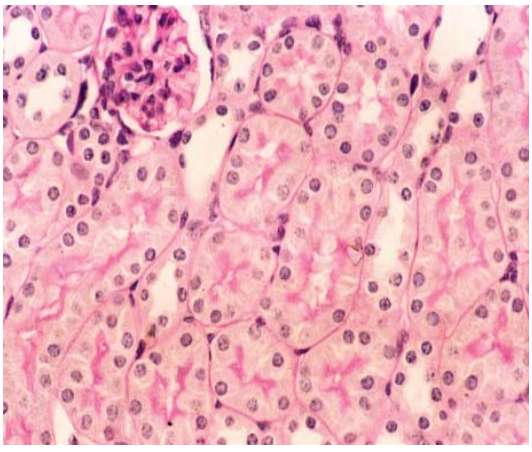

Fig. 18: A photomicrograph of the kidney of the Cisplatin-L-carnitine group showing PAS positive material in brush border of the proximal tubules and the basement membrane of the renal tubules nearer to control. PAS; X400

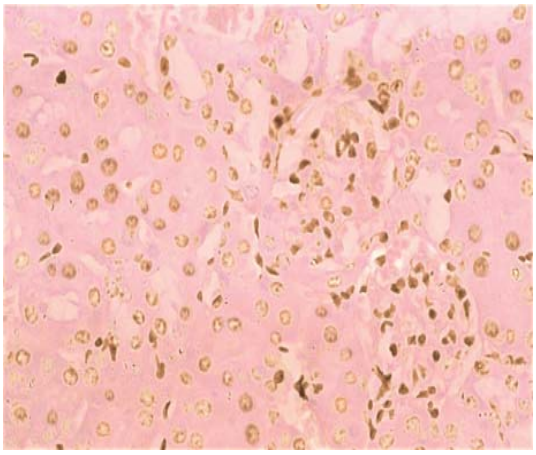

Fig. 19: A photomicrograph of the kidney of the Cisplatin-L-carnitine group showing marked nuclear reaction in proximal and distal tubules and in glomeruli. Ki67; X400

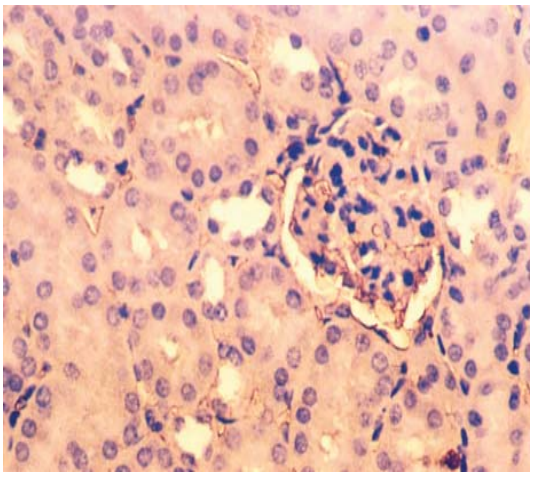

Fig. 20: A photomicrograph of the kidney of the Cisplatin-L-carnitine group showing faint diffuse granular cytoplasmic reaction in proximal and distal tubules. Bak; X 400

DISCUSSION

Immunosuppressive drugs represent a challenge for both patients and governmental resources; trials to prevent side effects are of particular interest *(Sabry, et al. 2006)*. Cisplatin is one of the most effective chemotherapeutics, but its usefulness is limited by its toxicity to normal tissues, including cells of the proximal kidney tubules *(Jisha and Cherupally, 2008).*

Dose dependant and accumulative nephrotoxicity are the major toxicity of this compound, sometimes requiring a reduction in dose or discontinuation of treatment *(Kuhad, et al. 2006)*.

Baliga and Liu (2004) reported that Cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney.

Recent evidences have implicated oxidative stress in Cisplatin-induced nephrotoxicity *(Fujieda, et al. 2006)*.That was also mentioned by *Ajith et al. (2007)* who claimed the involvement of reactive oxygen species and free radicals in nephrotoxicity induced by anti-cancer drug Cisplatin .

A large number of studies have reported the beneficial effects of a variety of antioxidants with Cisplatin-induced nephrotoxicity *(Baek, et al. 2006)*.

This study was performed to examine which has a superior nephroprotective effect in amelioration the dramatic toxic effects of Cisplatin on renal tissue; vitamin C or L-carnitine.

The results in the present study revealed that mice treated with Cisplatin showed extensive tubular necrosis, casts in the cortex, changes in the basement membrane and brush border loss. These results were also demonstrated by *Lee et al. (2006)* and *Kuhad et al. (2006)* who showed that; rats treated with Cisplatin showed marked renal tubular necrosis, apoptosis, intracellular oedema, glomerular and basement membrane alterations.

Megyesi et al. (1998) described the changes in rat kidneys following Cisplatin injection in the form of brush border loss, tubular dilatation, tubular necrosis and tubular cast formation.

The more prominent effect of Cisplatin on the proximal tubules found in this study is consistent with the study of *Ries and Klastersky (1986)* who reported that the nephrotoxic effects of Cisplatin primarily involved the proximal tubules. This can be explained on the basis of the studies made by *Kroning et al. (2000)* and *Gonzalez et al. (2001)* who reported that a difference existed in the uptake rates of Cisplatin among cell lines derived from the proximal tubules and early distal convoluted tubules.

Many authors explained the renal nephrotoxicity following the induction of Cisplatin. *Brady et al. (1990)* mentioned that mitochondrial injury in proximal tubules is an early event following in vitro and in vivo exposures to Cisplatin and may result in adenine nucleotide depletion and cell death.

Park et al. (2002) added that Cisplatin inhibits Ca+2 uptakes by mitochondria and decreases mitochondrial potential which may be responsible for Cispaltin-induced kidney damages.

Kruidering et al. (1997) provided evidence that Cisplatin-induced mitochondrial dysfunction is caused by inhibition of complexes I to IV of the respiratory chain, which results in decreased intracellular levels of ATP. This selectivity for mitochondria is probably caused by accumulation of Cisplatin in the negatively charged inner space of the mitochondria because of the positive charge of aquated complexes of Cisplatin.

Ajith et al. (2007) also mentioned that oxidative stress resulting from an imbalance between prooxidant and antioxidant system, largely contributes to complications observed in patients treated with Cisplatin; there is marked involvement of reactive oxygen species and free radicals in the nephrotoxicity induced by the synthetic anti cancer drug Cisplatin. While *Kwon et al. (2001)* revealed that excessive formation of reactive oxygen species as well as depletion of cellular antioxidants resulted in apoptosis, *Cummings and Schnellman (2002)* mentioned in their study that although it has been well documented that apoptosis plays a crucial role in the pathogenesis of Cisplatin- induced acute renal failure, the intracellular pathway remains unknown.

Several methods had been introduced to reduce Cisplatin nephrotoxicity. The use of antioxidants to destroy free radicals and/or protective agents against mitochondrial toxic effects are some of these methods *(Chandel, et al. 2001)*.

In the present study we used vitamin C and L-carnitine as two potent antioxidants to compare between their effects in reducing the nephrotoxicity induced by Cisplatin.

The results obtained from the group of mice treated with both Cisplatin and vitamin C revealed marked reduction in the apoptotic cells, maintenance of brush border and marked decrease in basement membrane oedema. Our results are matching with those obtained by *Ajith et al. (2007)*, who reported that vitamin C protected against oxidative renal damage induced by Cisplatin. They also mentioned that the protection was mediated by preventing the decline of renal antioxidant status.

Tarladacalisir et al. (2008) performed a study to investigate the protective effect of vitamin C on Cisplatin-induced renal damage. They found that administration of vitamin C reduced most of the structural damage caused by Cisplatin. They also suggested that administration of vitamin C was of therapeutic benefit on Cisplatin nerphrotoxicity.

In the present study the results obtained from the group of mice treated with both Cisplatin and L-carnitine revealed marked reduction in the apoptotic cells, maintenance of brush border and marked decrease in basement membrane oedema. These results match with those reported by *Boonsanit et al. (2006)* who demonstrated that carnitine administration prevented the development of Cisplatin induced renal injury in rats.

A study done by *Sayed-Ahmed et al. (2004)* to examine the progression of Cisplatin nephrotoxicity in a carnitine-depleted rat model revealed that carnitine deficiency is a risk factor in Cisplatin-induced renal dysfunction and that L- carnitine supplementation attenuated Cisplatin-induced nephrotoxicity.

Also, *Boonsanit et al. (2006)* claimed that L-carnitine improved renal function of rats with induced nephritic syndrome. These results are matching with results of *Kopple et al. (2002)* who concluded that L-carnitine can protect renal impairment, with a corresponding reduction of oxidative stress.

By comparing the results obtained from the group treated with vitamin C and from that treated with L-carnitine; it was concluded that L-carnitine supply is a better protection against the nephrotoxic effects of drug Cisplatin..

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دراسة ضوئية هستولوجية مناعية تبين دور فيتامين ج و دور ل-كارنيتين فى الحماية من الاعتلال الكلوى الناتج عن العلاج بمادة السيسبلاتين فى الفئران

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ملخص البحث

السيسبلاتين مادة علاجية كيماوية فعالة فى علاج كثير من الاورام السرطانية. و قد اثبتت هذه المادة كفاءة عالية فى العلاج الا ان مضاعفاتها الصحية ادت الى عدم الاستفادة القصوى منها, بل و ايقاف العلاج فى كثير من ا لحالات. و اهم هذه المضاعفات و اكثرها حدوثا هو تدمير الانسجة الكلوية و الاخلال الوظيفى للكلى.

و كان الهدف من هذه الدراسة هو مقارنة بين دور فيتامين ج و دور ل-كارنيتين فى الحماية من الاعراض الجانبية التى تحدث فى الكلى اثناء العلاج بمادة السيسبلاتين. و قد تم استخدام عدد ثلاثين فارا ذكرا خلال هذه الدراسة و تم تقسيمهم كالاتى:

- مجموعة (١) هى المجموعة الضابطة.
- مجموعة (٢) معالجة بمادة السيسبلاتين جرعة واحدة (١٠ ملليجرام من الوزن) عن طريق الحقن البريتونى.
- مجموعة (٣) معالجة بمادة السيسبلاتين جرعة واحدة (١٠ ملليجرام من الوزن) عن طريق الحقن البريتونى بالاضافة الى مادة فيتامين ج (٥٠٠ ملليجرام) عن طريق الفم لمدة يومان قبل الحقن بمادة السيسبلاتين وثلاثة ايام بعد الحقن.
- مجموعة (٤) معالجة بمادة السيسبلاتين جرعة واحدة (١٠ ملليجرام من الوزن) عن طريق الحقن البريتونى بالاضافة الى مادة ل-كارنيتين(٢٠٠ ملليجرام) عن طريق الفم لمدة يومان قبل الحقن بمادة السيسبلاتين وثلاثة ايام بعد الحقن.
- مجموعة (٥) معالجة بمادة فيتامين ج (٥٠٠ ملليجرام)يوميا عن طريق الفم لمدة خمسة ايام متتالية.
- مجموعة (٦) معالجة بمادة ل-كارنيتين (٢٠٠ ملليجرام)يوميا عن طريق الفم لمدة خمسة ايام متتالية.

وقد تم اعطاء فيتامين ج و ل-كارنيتين لمدة يومين قبل و ثلاثة ايام بعد العلاج بمادة السيسبلاتين.

و فى اليوم السادس من العلاج تم التضحية بالحيوانات و اخذ عينات من الكلى و تحضيرها للصبغة بالصبغات المختلفة و من ثم الفحص بالميكرو سكوب الضوئى.

و قد وجد بالفحص ان المجموعة المعالجة بمادة السيسبلاتين تميزت بوجود تدمير للنسيج الضام بين الخلايا و تدمير شديد للخلايا بالقنوات الكلوية.

اما بالنسبة للمجموعتين المعالجتين بمادة فيتامين ج و ل-كارنيتين وكان هناك تحسن ملحوظ فى النسيج الكلوى.ولكن بمقارنة تأثير المادتين وجد ان التحسن ذو نسبة اكبر و اوضح فى المجموعة المعالجة بمادة ل-كارنيتين.