Benha University
Faculty of Veterinary Medicine
Department of Pharmacology

The protective effect of L-carnitine against toxicity of liver, kidneys and testicles induced by cisplatin drug in rats

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Approval Sheet

This is to approve that the dissertation presented by: **Adham Omar Mohamed Sallam** to Benha University entitled "The protective effect of L-carnitine against toxicity of liver, kidneys and testicles induced by cisplatin drug in rats" for Master degree (Pharmacology) has been approved on Tuesday 9/2/2021 by the examining committee:

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بسم الله الرحمن الرحيم

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم الحكيم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Dedication

To My

Father

Mother

Brothers

Sister

الإقرار

DECLARATION

I declare that this thesis has been compiled by myself, and is the results of my own work. It has not been submitted for any other degree and all sources of information have been properly acknowledged.

Name: Adham Omar Sallam

Signature: 

Date: 9/2/2021

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List of Abbreviations

Abbreviations	Full Name
ATP	Adenosine tri-phosphate
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
ASP	Aspartame
AST	Aspartate aminotransferase
ATZ	Atrazine
BUN	Blood Urea Nitrogen
B. Wt	Body weight
CAT	Catalase
℃	Celsius degree
CDDP	Cis-diamminedichloroplatinum
CP	Cisplatin
CIS	Cisplatin
CT	Collecting tubules
CTX	Cyclophosphamide
CK18	Cytokeratin 18
CKs	Cytokeratin Staining
DAB	Diaminobenzene
DCT	Distal convoluted tubules
EDTA	Ethyl Diamine tertra Acetic acid end labeling
GNDF	Glial cell line-derived neurotrophic factor
GSH	Glutathione
g/dl	Gram per deciliter
H&E	Hematoxylin and eosin
HSCs	Hepatic stellate cells
hr.	Hour
HIV	Human Immunodeficiency Virus
H2O2	Hydrogen peroxide
IgG	Immunoglobulin G
IFs	Intermediate filaments
IT	Interstitial tissue
IP	Intraperitoneal
LC	L-carnitine
MDA	Malondialdehyde
mg/kg	Milligram/kilogram
ml	Milliliter
Min	Minute

[O]	Nascent Oxygen
O2	Oxygen
P.O.	Per Os
PBS	Phosphate-buffered saline
pH	Power of Hydrogen (negative logarithm of hydrogen ions concentration)
PCTs	Proximal convoluted tubules
QT	Quercetin
ROS	Reactive Oxygen species
RT	Room Temperature
STs	Seminiferous tubules
SPSS	Statistical package for the social science
SOD	Superoxide dismutase
VIM	Vimentin

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Abstract:المستخلص

Title: A Protective effect of a medicinal plant against organs toxicity in rats
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Abstract

Cisplatin (CP) is one of the most active medications in cancer treatment and has some adverse effects such as hepatotoxicity, nephrotoxicity and testicular toxicity. The present research was planned to determine the ameliorative effects of L-carnitine (LC) against cisplatin (CP) induced hepatic, renal and testicular oxidative stress in rats, via investigating some serum biochemical and tissue oxidative/antioxidant parameters, histopathological alterations, and immunohistochemical expressions of two different intermediate filaments (IFs) proteins; vimentin (VIM) and cytokeratin 18 (CK18). Twenty-eight rats were divided into four groups (7 rats each). Groups I and II were orally administered saline and LC (100 mg/kg body weight), respectively, once daily for 30 consecutive days. Group III received saline orally once daily and a single dose of CP on the 27th day of the experiment (7.5 mg/kg, IP). Group IV received LC and CP. Injection of CP significantly increased serum ALT, AST, ALP, creatinine, and urea, while serum total protein, albumin and serum testosterone level were significantly decreased.

Also, CP induced a dramatic increase in the MDA level along with a substantial decrease in GSH and CAT in the hepatic, renal and testicular tissues. Histologically, all of the liver, kidney and testis of the CP treated group revealed marked degenerative changes. Also, overexpression of both VIM and CK18 in hepatic, renal and testicular tissues, after CP injection, was noted. On the other hand, the administration of LC in the CP injected group (Group IV) restored the biochemical parameters, histological, and immunohistochemical pictures towards the normality.

In conclusion, LC may be given during chemotherapy with CP to ameliorate its oxidative stress and restore the normal organization of Ifs, especially VIM and CK18 within the CP intoxicated hepatic, renal and testicular cells.

Keywords: Cisplatin; L-carnitine; Hepato-renal toxicity; Oxidative stress; Intermediate filaments; Testosterone; Apoptotic markers; Vimentin; Cytokeratin; Malondialdehyde; Immunohistochemistry.

1. Introduction

Increasing numbers of cancer patients as well as the severe side effects of most extensively used chemotherapies, become a global challenge for the scientists to develop novel solutions to ameliorate the induced toxicities (**Abuzinadah and Ahmad, 2020; Un *et al.*, 2020).**

1.1. Cisplatin:

Cisplatin, (cis-diamminedichloroplatinum (II), (CP)), with the molecular formula cis-[Pt(NH3)2Cl2], is inorganic anticancer agent widely used for treatment of childhood tumors, such as Wilms' tumor, as well as multiple human adult solid malignant tumors such as head, neck, lung, bladder, testicular cancers, prostate, neuro- blastoma ,liver cancer and endometrial tumors, as well as hematological malignancies such as sarcoma and lymphoma **(Almeer and Abdel Moneim, 2018; Zhang *et al.*, 2019; Azarbarz *et al.*, 2020).**

CP is a DNA-alkylating molecule that runs its antitumor activity by several mechanisms including DNA damage by interfering with DNA base pair crosslinks and DNA double-strand breaks, these functions inhibit transcription and replication of DNA thus finally lead to programmed cell death of proliferating cells **(Boroja *et al.*, 2018; Abdel-Daim *et al.*, 2019a, b; Azarbarz *et al.*, 2020).**

As well as production of reactive oxygen species (ROS) that cause cellular injury and necrosis through the lipid peroxidation of tissues, DNA damage, and protein denaturation **(Almeer and Abdel Moneim, 2018; Jahan *et al.*, 2018; Abdel-Daim *et al.*, 2019a, b).**

Although highly proven efficacy of CP as potent chemotherapeutic agent, its clinical use has been limited due to two main undesirable side effects: the dose- and duration-dependent cell resistance. In addition, its non-specific target action which cause inevitable severe cytotoxicity to numerous normal tissues, such as nephrotoxicity, ototoxicity, neurotoxicity, cardiomyopathy and reproductive toxicity **(Ekinci Akdemir *et al.*, 2019; Fouad *et al.*, 2019; Köroğlu *et al.*, 2019).**

1.1.1. Cisplatin toxicity:

Hepatorenal toxicity is one of the most prevalent induced side effects of the chemotherapeutics, because these chemotherapeutics are metabolized in the liver and excreted by kidneys and thus increase the susceptibility of these vital organs for the toxic adverse effects of these drugs **(Abdel-Daim *et al.*, 2019; Ge *et al.*, 2019; Un *et al.*, 2020).**

1.1.1.1. Hepatotoxicity:

Hepatotoxicity is another common problem of CP treatment especially when used in high doses. The liver plays a crucial role in the biotransformation of most chemicals, drugs and toxins as well as in the metabolism of carbohydrate, fat and protein so it is the main target of drug-induced damage **(Ekinci Akdemir *et al.*, 2017; Sohail *et al.*, 2019).**

CP induced hepatotoxicity is attributed to oxidative damage and mitochondrial dysfunction which lead to severe liver damage in form of degeneration and necrosis of hepatocytes as well as moderate dilatation of sinusoids **(Ekinci Akdemir *et al.*, 2017; Sohail *et al* 2019; El-Gizawy *et al.*, 2020).**

Cisplatin causes a significant body weight loss and reduction of alkaline phosphatase (ALP) 50 hours after CP injection. Blood urea nitrogen (BUN), creatinine (Cr), and serum nitrite increased significantly 75 hours after CP injection. Also, enhancement of kidney and testis weights, and alkaline aspartate aminotransferase (AST) level; and reduction of alanine aminotransferase (ALT) level and uterus weight occurred significantly 100 hours after the injection, while kidney malondialdehyde level enhanced significantly 75 hours after CP administration. The findings suggested that the CP-induced nephrotoxicity started to develop almost 3 days after administration of the drug in rats. CP surprisingly reduced the serum levels ALP and ALT while AST increased 100 hours after CP injection. CP-induced nephrotoxicity and hepatotoxicity are time-dependent, and the related biomarkers may alter by different trends **(Pezeshki *et al.*, 2017).**

Cisplatin when administered to the rats for 3 days leads to a reduction in the activities of the antioxidant enzymes like lipid peroxidation (LPO) and endogenous

antioxidant systems such as reduced superoxide dismutase (SOD), glutathione (GSH) and catalase in liver homogenate caused to produce the impairment of hepatic functions **(Chandel *et al.*, 2018).**

Cisplatin increases activity of liver enzymes (aspartate aminotransferase and alanine aminotransferase) and kidney function tests (blood urea nitrogen and creatinine). Also, cisplatin induces inflammatory damage by induction of tumor necrosis factor-α, interleukin-1β, and nuclear factor kappa-B and elevation of nuclear factor erythroid 2-related factor 2. Moreover, cisplatin induces oxidative damage indicated by elevation of malondialdehyde and reduced glutathione. Also, cisplatin cause elevation of phosphorylated c-Jun N-terminal kinases (p-JNK) and phosphorylated extracellular signal-regulated kinase (p-ERK) associated with a increase in the expression of caspase-3 **(Bishr *et al.*, 2019).**

It is suggested that cisplatin-associated toxicities are mainly induced by free radicals' production, which result in oxidative organ injury. The evidence is growing over the protective effects of antioxidants on cisplatin induced adverse reactions especially nephrotoxicity **(Hakiminia *et al.*, 2019).**

1.1.1.2. Nephrotoxicity:

The kidneys are one of the most important vital organs that control numerous critical functions for the body such as removing metabolic waste products, cleaning blood and maintaining normal balance of water, salts and minerals **(Ekinci Akdemir *et al.*, 2017; Sohail *et al.*, 2019).**

One of the main side effects of cisplatin is nephrotoxicity. CP induced nephrotoxicity through several ways. one of them that, CP is metabolized to a potent nephrotoxin, unbound free plasma CP is filtered via the glomerulus as most of CP is excreted through filtration via glomerulus and tubular secretion hence most of it is deposited in the renal cortex, ROS generated lead to decrease the renal blood flow as well as apoptosis that caused by Oxidative and DNA damage **(Abdel-Daim *et al.*, 2019; Sohail *et al.*, 2019; El-Gizawy *et al.*, 2020).**

CP also caused a significant increase in the lipid peroxidation marker malondialdehyde (MDA) levels, renal nuclear factor kappa B (NFκB) DNA-binding

activity, protein expression, tumor necrosis factor alpha (TNF-α) and IL-6 levels (**El-Sherbeeny and Attia, 2016).**

Cisplatin induced increase in BUN and serum creatinine levels, renal tubular apoptosis, and oxidative stress. Additionally, it caused tubular degeneration induced renal damage (**Galgamuwa *et al.*, 2016).**

Cisplatin resulted in substantial nephrotoxicity in Wistar rats with significant elevation in serum creatinine and blood urea nitrogen, decline in the concentrations of reduced glutathione and superoxide dismutase, elevation in TNF-α levels in renal tissues (**Karwasra *et al.*, 2016).**

Administration of CP significantly elevated urinary glucose and protein, as well as serum creatinine, urea, and uric acid. Moreover, CP enhanced lipid peroxidation and suppressed the major enzymatic antioxidants in the kidney tissue (**Abdel-Wahab *et al.*, 2017).**

Cisplatin induced cell apoptosis and retarded cell growth. Cisplatin caused significant increase in reactive oxygen species (ROS) accumulation. Furthermore, it induced tumor necrosis factor alpha (TNF-α) expression and NF-κB activation, and stimulated the expression of induced nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (**Wang *et al.*, 2018).**

1.1.1.3 Testicular toxicity:

Testicular dysfunction is the most reported consequence of CP toxicity, due to its high proliferative rate so the adverse effects of chemotherapy on the testis are very intense and irreversible causing death of zoogenic cells in the process of spermatogenesis and sperm DNA damage thus lead to inability to produce a sufficient number of healthy and active sperms injuries (oligozoospermia), azospermia or even cause permanent sterility **(Karimi *et al.*, 2018; Zhang *et al.*, 2019; Azarbarz *et al.*, 2020).**

Testis is one of the most important organs in male reproductive system, whether its structure and function are normal or not can affect the reproductive function as it

considered as organ of androgenesis and spermatogenesis **(Marty *et al.*, 2003 &. Zhang *et al.*, 2019).**

It secretes male sex hormones, testosterone, which plays a critical role in the growth beside its important role in the development and maturation of male reproductive organs, as well as the emergence of male sexual characteristics. Also responsible for spermatogenesis which is a natural process characterized by cell division in the testis **(Almasry *et al.*, 2017; Mercantepe *et al.*, 2018; Azarbarz *et al.*, 2020).**

CP-induced testicular toxicity has not clearly identified however physiological and histopathological disturbances induced by CP treatment thought to be from over production of reactive oxygen species (ROS), exhaustion of plasma or tissue antioxidants, bio-membrane lipid peroxidation, nitration of cellular macromolecules by reactive nitrogen species (RNS) up-regulation of inflammatory pathways, disequilibrium between pro- and anti-inflammatory cytokines , DNA fragmentation and then finally necrosis and apoptosis of gonadal tissue thus affects both endocrine and exocrine compartments resulting in impaired spermatogenesis owing to spermatogenic damage, androgenesis due to an altering Leydig cell functions and finally gonadal dysfunction **(Yaman and Topcu-Tarladacalisir Y, 2018 ; Shati *et al.*, 2019; Zhang *et al.*, 2019).**

At physiological concentrations, the ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals have been shown to play an important role in the defense mechanisms against pathological conditions but excessive generation of ROS in association with reduced generation or activity of antioxidants may cause damage to tissues **(Reddy *et al.*, 2016; Fouad *et al.*, 2019; Köroğlu *et al.*, 2019).**

It results in a reduction of sperm parameters (count, viability, and motility), sperm morphological abnormalities, chromosomal abnormalities in spermatozoa, reduction in DSP (daily sperm production), changes in the structure of the testicular tissue as well as reduction in levels of testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) that are all implicated in pathogenic mechanism of male infertility induced by CP **(Fallahzadeh *et al.*, 2017; Singh *et al.*, 2017; Karimi *et al.*, 2018).**

Cisplatin significantly reduced reproductive organ weight, sperm count and sperm motility, and increased sperm abnormalities and histopathological damage of testicular tissue. In addition, it resulted in a significant decline in serum testosterone as well as levels of testicular enzymatic and non-enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxides, and reduced glutathione). Moreover, cisplatin remarkably augmented malondialdehyde, nitric oxide, tumor necrosis factor-a, and nuclear factor-kappa B contents in testicular tissue. Conversely, carvedilol administration markedly mitigated cisplatin-induced testicular and spermatological injury as demonstrated by suppression of oxidative/nitrosative and inflammatory burden, amendment of antioxidant defenses, enhancement of steroidogenesis and spermatogenesis, and mitigation of testicular histopathological damage (**Eid *et al.*, 2016).**

Cisplatin significantly reduced reproductive organs weight, sperm count and sperm motility, and increased sperm abnormalities and histopathological damage of testicular tissue. In addition, it resulted in a significant decline in serum testosterone as well as levels of testicular enzymatic and non-enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxides, and reduced glutathione). Moreover, cisplatin remarkably augmented malondialdehyde, nitric oxide, tumor necrosis factor-α, and nuclear factor-kappa B contents in testicular tissue (**Singh *et al.*, 2017).**

Cisplatin significantly decreased serum testosterone, and testicular tissue antioxidant status, and significantly increased interleukin-6, interleukin-1β, malondialdehyde, nitric oxide, Bax/Bcl-2 ratio, and caspase-3 in rat testes (**Fouad *et al.*, 2017).**

Cisplatin induced oxidative stress, with a significant increase in testicular malonedialdehyde (MDA), decreased testicular glutathione (GSH), histological testicular damage and body weight loss. Additionally, increased abnormal sperm forms and decreased count and motility were noted. Melatonin and montelukast both rescued GSH concentrations, increased sperm count and motility and decreased abnormal forms (El-**Shafaei *et al.*, 2018).**

Cisplatin treatment resulted in a significant decrease in daily sperm production, decrease in head length and % DNA in head, reduction of epithelial cell height,

tubular diameter, reduction of the number of spermatogonia, spermatocytes and spermatids, increase in the thiobarbituric acid reactive substances (TBARS) and oxidative stress in testicular tissues, and change of the intra-testicular testosterone concentrations (**Jahan *et al.*, 2018).**

1.2. L-carnitine:

L-carnitine is an important water-soluble quaternary amine (βhydroxy-γ-trimethylaminobutyrate), non-protein amino acid. It was first extracted from beef muscles in 1905. Almost about 75% of L-carnitine stored in the body is obtained from the diet, whereas the remaining 25% is synthesized naturally synthesized in human body from lysine and methionine **(Abd-Elrazek and Ahmed-Farid, 2018; Khushboo *et al.*, 2018; Alharthi *et al.*, 2020).**

The major exogenous dietary sources of L carnitine are from mushroom, carrot, bread, rice, tomato, meat, fish, poultry, and milk, and also generated endogenously in the liver by methylation of lysine and methionine in the presence of iron, ascorbic acid, niacin, and vitamin B6. L-carnitine is stored in a high energy demanding tissues such as heart, skeletal muscles, brain and the male reproductive system especially in the epididymis. L-carnitine is highly concentrated in epididymis and spermatozoa and its concentration is about 2000-fold higher than that of the plasma **(Khushboo *et al.*, 2018; Mardanshahi *et al.*, 2019; Alharthi *et al.*, 2020).**

The efficacy of L-carnitine as an antioxidant has been observed in both humans and rats .LC is reported as potent non-enzymatic antioxidant, that protects the cell, mitochondrial membrane, and DNA integrity against free oxygen radicals by acting as a scavenger for free oxygen radicals, protective agent for mitochondria against oxidative stress injury and also enhances antioxidant enzymes such as glutathione peroxidase **(Cabral *et al.*, 2019; Kelek *et al.*, 2019; Mardanshahi *et al.*, 2019).**

Oxidative stress results in generation of ROS and then initiation of nuclear factor-kB signaling pathway which is important for the regulation of many genes implemented in inflammatory responses, as TNF-α, cyclooxygenase-2, inducible nitric oxide synthase, and caspase family of proteases leading to apoptosis, hence L

Carnitine neutralize ROS generated by oxidative stress and protect against apoptosis **(Kelek *et al.*, 2019; Alharthi *et al.*, 2020).**

L-carnitine has numerous biological activities including anti-inflammatory, antioxidant neuroprotective, cardioprotective, gastroprotective, cytoprotective, and antiapoptotic properties **(Yaman and Topcu-Tarladacalisir, 2018; Alharthi *et al.*, 2020).**

LC contributes in the metabolism of branched amino acids, energy metabolism via improving β-oxidation of long-chain fatty acids as it acts as a cofactor of enzymes associated with fatty acid metabolism and it also transports the chains of fatty acids into the mitochondria ,thus allowing the cells to burn fat and get energy from the body fat reserves necessary for cellular activities, It is also participates in cellular membranes stabilization, removal of excess acyl groups in various tissues to maintain acyl-CoA/CoA ratio, store energy in the form of acetylcarnitine and prevent the toxic effects of poorly metabolized acyl groups by esterifying them with carnitine and eliminate them as carnitine esters (**Abd-Elrazek and Ahmed-Farid *et al.*, 2018; Khushboo *et al.*, 2018; Cabral *et al.*, 2019).**

L-carnitine is very widely considered as an effective substance to treat infertility. L-carnitine (LC) is another antioxidant and plays an important role in spermatogenesis, sperm maturational changes and metabolism. Moreover, it plays an important role in regulating the male reproductive system and normal testis function **(Abdel Aziz *et al.*, 2018; Cabral *et al.*, 2018; Soliman *et al.*, 2020).**

L-carnitine is found in high concentrations in male gonadal tissues, especially in the epididymis, about 2000-fold as its concentration in plasma. Free L-carnitine is taken up from the blood plasma and then transported into the epididymal fluid and then passively diffuses into the spermatozoa, where it finally stacks as free and acetylated carnitine. The initiation of sperm motility is proportional to the L-carnitine concentraton in the epididymal lumen ; It is involved in male gamete maturation , energy production for sperm respiration and motility by increasing oxidation of mitochondrial fatty acids as it facilitates long-chain fatty acids' β-oxidation in mitochondria and is necessary for energy production L-carnitine also supports germ

cell recovery and Sertoli cell metabolism **(Abd-Elrazek and Ahmed-Farid *et al.*, 2018; Yaman and Topcu-Tarladacalisir *et al.*, 2018; Mardanshahi *et al.*, 2019).**

Also, L carnitine is a potent free radical scavenging agent, particularly to superoxide anion, which is involved in the formation of hydroxyl radicals and hydrogen peroxide, known inducers of oxidative damage in the germ cells and/or sperm membrane, also in the DNA **(Alabi *et al.*, 2018; Mardanshahi *et al.*, 2019).**

1.2.1 Hepatoprotective of L-carnitine:

Acetyl-L-carnitine has prophylactic and therapeutic potential against acetaminophen-induced hepatotoxicity in mice. Acetaminophen significantly increased the markers of liver injury, hepatic reactive oxygen species, and nitrate/nitrite ratio, and decreased hepatic glutathione (GSH) and the antioxidant enzymes. Acetyl carnitine supplementation resulted in reversal of all biochemical parameters toward the normal values. Acetyl-carnitine administration resulted in partial reversal of liver injury only at 2 mmol/kg orally **(Alotaibi *et al.*, 2016).**

Dietary L-carnitine prevents histopathological changes in tilapia (*Oreochromis niloticus*) exposed to cylindrospermopsin (CYN). L-carnitine pretreatment was able to totally prevent those CYN induced alterations from 400 mg LC/kg b.wt fish/day in almost all organs, except in the heart, where 880 mg LC/kg b.wt fish/day were needed **(Guzmán-Guillén *et al.*, 2017).**

Acetyl-l-carnitine (ALC) ameliorated arsenic-induced oxidative damage, mitochondrial dysfunction, apoptosis, inflammation and histological damage. ALC's protective features against arsenic hepatotoxicity might be due to this agent's antioxidant and anti-inflammatory properties as well as its stabilizing effects on mitochondrial function (**Bodaghi-Namileh *et al.*, 2018).**

Administration of L-carnitine and selenium decreased cadmium-induced increase in ALT and AST levels and reduced oxidative stress to normal levels. In addition, L-carnitine combined with selenium had a good synergistic effect and elevated significantly the enzymatic antioxidants and decreased lipid peroxidation levels compared with those in the cadmium-treated group. It is clear from the data that

both L-carnitine and selenium inhibit liver injury and improve the redox state in mice (**Abu-El-Zahab *et al.*, 2019).**

LC prevented ASP-induced liver damage as demonstrated by the enhancement of all the above parameters. Results of histopathological and electron microscopic examination proved the biochemical feedback and the improved LC effect on liver toxicity. The co-treatment of LC showed different improvement mechanisms against ASP-induced liver impairment. So, the intake of ASP should be regulated and taken with LC when it is consumed in different foods or drinks to decrease its oxidative stress, histopathology, and genotoxicity of liver (**Hamza *et al.*, 2019).**

1.2.2. Nephroprotective of L-carnitine:

L-carnitine administration resulted in an improvement in kidney function tests. Antioxidative, antiapoptotic and anti-inflammatory properties of ALCAR were supported by the findings that this agent improves kidney function tests and has the effects of tissue protection and inhibition of apoptosis in cisplatin-induced nephrotoxicity (**Tufekci *et al.*, 2009).**

L-carnitine induced dose-dependent improvement of renal function, inflammation, and fibrosis. Furthermore, the administration of Lcarnitine at a high dose inhibited the expression of caspase-3 and LC3-II. These findings suggest that L-carnitine has a protective effect against CsA-induced pancreatic and renal injuries **(Xiang *et al.*, 2013).**

Cimetidine and L-carnitine have protective effects - almost equally - against mARF. Using both agents together, minimizes the renal injury (**Estaphan *et al.*, 2015).**

L-carnitine improves cognitive and renal functions in a rat model of chronic kidney disease (CKD). All CKD animals exhibited renal function deterioration, as shown by elevated serum creatinine, BUN, and ample histopathological abnormalities. L-carnitine treatment of CKD rats significantly reduced serum creatinine and BUN, attenuated renal hypertrophy and decreased renal tissue damage. In addition, in the two-way shuttle avoidance learning, CKD animals showed cognitive impairment which recovered by the administration of L-carnitine. It is

concluded that in a rat model of CKD, l-carnitine administration significantly improved cognitive and renal functions **(Abu Ahmad *et al.*, 2016).**

Maternal L-carnitine supplementation ameliorates renal underdevelopment and epigenetic changes in male mice offspring due to maternal smoking. These disorders were reversed by maternal LC administration. The effect of maternal SE on renal underdevelopment involves global epigenetic alterations from birth, which can be prevented by maternal LC supplementation **(Stangenberg *et al.*, 2019).**

1.2.3. Testicular protective of L-carnitine:

The sperm analysis of epididymis showed that administration of LC resulted in significant recovery of the sperm count and sperm motility after decrease sperm count and motility induced by CTX administration. The results showed that CTX could damage the spermatogenesis and reduce the expression of occludin and GDNF, and increase the expression of TGF-β3 in testis of mouse, which indicates CTX's damage or efficacy to testis Sertoli cells. LC could protect the Sertoli cells of testis from these damages caused by CTX, and promote or protect the spermatogenesis. This study provided meaningful information about the possible adverse effects on male fertility by chemotherapeutics and potential of LC in the protection of male fertility during chemotherapy (**Cao *et al.*, 2017).**

Co-administration with low dose QT or different doses of LC succeeded to counteract the negative toxic effects of ATZ on serum oxidative stress indicators, serum testosterone levels, testicular IgA level and improved testicular CYP17A1 mRNA expression. In conclusion, QT in low dose and LC in both low and high doses exerted a significant protective action against the reproductive toxicity of ATZ, while higher dose of QT failed to induce immune-stimulant effect against ATZ in adult male Albino rats (**Abdel Aziz *et al.*, 2018).**

L-carnitine and L-arginine have a protective effect against busulfan-induced oligospermia in adult rat. The results showed significant improvement in sperm morphology, motility, velocity and count in the groups treated with L-arginine and L-carnitine and accompanied with an increase in MDA, GSSG and ATP, reduction in GSH, AMP, ADP, NO and 8-OHDG also recorded (**Abd-Elrazek and Ahmed-Farid, 2018).**

Oxidative stress and their related events appear to be a potential mechanism involved in copper testicular toxicity and L-carnitine supplementation significantly modulated the possible adverse effects of copper on seminiferous tubules damage, testes function, spermatogenesis, and sperm quality. It was validated that the use of L-carnitine at doses of 50 and 100 mg/kg protects against copper-induced testicular tissue damage and acts as a therapeutic agent for copper heavy metal toxicity (**Khushboo *et al.*, 2018).**

LC decreased diabetes-induced oxidative stress complications and also improved serum level of FSH, LH, and TH by reducing levels of lipid peroxidation and increasing antioxidant enzymes (**Rezaei *et al.*, 2018).**

L-carnitine counteracts prepubertal exposure to cisplatin induced impaired sperm in adult rats by preventing germ cell apoptosis. L-carnitine treatment reduced testicular damage and the number of TUNEL positive cells significantly, while the number of PCNA positive cells in the cisplatin + carnitine group increased compared to the cisplatin group. During the adult period, epididymal sperm count and viability were improved in rats treated with L-carnitine before prepubertal cisplatin injection. L-carnitine may reduce late testicular and spermatic damage caused by cisplatin administration to prepubertal rats by inducing germ cell proliferation and preventing apoptosis (**Yaman and Topcu-Tarladacalisir, 2018).**

The present study aimed to investigate the possible ameliorative effect of L-carnitine administration against Cisplatin induced multiple organ (liver, kidneys and testicles) toxicity in rats via investigating some serum biochemical and tissue oxidative/antioxidant parameters. In addition, both histological alterations and immunohistochemical expressions of VIM and CK18 proteins were also evaluated.

L-carnitine mitigates oxidative stress and disorganization of cytoskeleton intermediate filaments in cisplatin induced hepato-renal toxicity in rats

ABSTRACT

Cisplatin (CP) is one of the most active medications in cancer treatment and has some adverse effects such as hepatotoxicity and nephrotoxicity. The present research was planned to determine the protective effects of L-carnitine (LC) against cisplatin (CP) induced hepato-renal oxidative stress in rats, via investigating some serum biochemical and tissue oxidative/antioxidant parameters, histological alterations, and immunohistochemical expressions of two different intermediate filaments (IFs) proteins; vimentin (VIM) and cytokeratin 18 (CK18). Twenty-eight rats were divided into four groups (7 rats each). Groups I and II were orally administered saline and LC (100 mg/kg body weight), respectively, once daily for 30 consecutive days. Group III received saline orally once daily and a single dose of CP on the 27th day of the experiment (7.5 mg/kg, IP). Group IV received both LC and CP. Injection of CP significantly increased serum ALT, AST, ALP, creatinine, and urea, while serum total protein and albumin were significantly decreased. Also, CP induced a dramatic increase in the MDA level along with a substantial decrease in GSH and CAT in the hepato-renal tissues. Histologically, both liver and kidney of the CP treated group revealed marked degenerative changes. Also, overexpression of both VIM and CK18 in hepato-renal tissues, after CP injection, was noted. On the other hand, the administration of LC in the CP injected group (Group IV) restored the biochemical parameters, histological, and immunohistochemical pictures towards the normalcy. In conclusion, LC may be supplemented for chemotherapy with CP to ameliorate its oxidative stress and restored the normal organization of Ifs, especially VIM and CK18 within the CP intoxicated hepato-renal cells.

Keywords: Cisplatin; L-carnitine; Hepato-renal toxicity; Oxidative stress; Intermediate filaments.

Introduction

Under normal conditions, the various cell types of both liver and kidney have characteristic cytoskeleton and intermediate filaments (IFs) compositions, which are involved in the cell shape maintenance, mechanical stability, and intracellular organization, and transport (**Ku *et al.*, 1999**). Vimentin (VIM) is the IF of the non-epithelial cells, especially those of mesenchymal origin (**Sen *et al.*, 2010**) that is the only IF protein found in endothelial cells and fibroblasts (**Evans, 1998**). Meanwhile, cytokeratins (CKs) represent the largest and most common epithelial IFs (**Snider, 2016**).

Since chemotherapeutic drugs are metabolized by the liver and excreted through the kidneys, hepato-renal toxicity is a common adverse effect caused by chemotherapy (**Abuzinadah and Ahmad, 2020**).

Although cisplatin (*cis*-diamminedichloroplatinum (II), CP) is potent anticancer medication use to treat a variety of tumors including testicular, ovarian, bladder, and lung (**Karwasra *et al.*, 2016**), several recent studies recorded that the CP induces hepatotoxicity (**Abdel-Daim *et al.*, 2020; Hwang *et al.*, 2020**) and nephrotoxicity (**Abdel-Razek *et al.*, 2020; Sadeghi *et al.*, 2020**). Inflammation, apoptosis, and oxidative stress are mentioned as the most relevant pathways for CP toxicity (**Meng *et al.*, 2017**) as well as, disorganization of the IFs components of the cytoskeleton (**Evans and Simpkins, 1998**). In the last decades, VIM is considered a mesenchymal marker for liver and kidney toxicity (**Matos *et al.*, 2007; Wang *et al.*, 2017**). Also, CKs are known as cellular stress protein, specially CK18, which use as novel markers of liver and kidney injuries (**Yang *et al.*, 2015; Djudjaj *et al.*, 2016**).

L-carnitine (LC) is a natural nutrient, which synthesized from lysine and methionine essential amino acids. It is derived from dietary sources (75%) and endogenous biosynthesis (25%), mainly in the liver and kidney (**Bremer, 1983; Aboubakr *et al.*, 2020**). LC is necessary for the production of ATP by β-oxidation of fatty acids in mitochondria (**Furuno *et al.*, 2001**). Therefore, LC can prevent mitochondrial oxidative stress induces mitochondria damage and apoptosis in different cell types (**Barhwal *et al.*, 2007**). The major regulatory role of LC in antioxidant processes was discussed in various organs like heart, colon, and retina,

and brain (**Al-Majed *et al.*, 2006; Cetinkaya *et al.*, 2006; Sezen *et al.*, 2008**), respectively.

From all the above-mentioned data, the purpose of this research was to investigate the protective effects of LC administration on CP-induced hepato-renal injuries in rats via investigating some serum biochemical and tissue oxidative/antioxidant parameters. Also, both histological alterations and immunohistochemical expressions of VIM and CK18 proteins were evaluated in all experimental groups.

Material and Methods

Chemicals

Cisplatin was obtained from (EIMC United Pharmaceuticals, Egypt). Each vial (50mg/50ml) was dissolved in physiological saline (0.9% sodium chloride). L-carnitine was obtained from MEPACO Company (Inshas Elraml, Egypt). All biochemical analysis kits were purchased from Biodiagnostics Company (Dokki, Giza, Egypt).

Experimental animals

The present study was carried out on a total number of 28 white Albino male rats weighing 175-195 gm. Rats were obtained from the Center of Laboratory Animal, Faculty of Veterinary Medicine, Benha University, Egypt. They acclimatized for two weeks prior to the experiment. All rats received standard laboratory balanced commercial diet and water ad libitum.

Experimental design

In the present study, male albino rats were randomly assigned into 4 equal groups (7 rats each). Group I, served as control, orally administered saline (the vehicle) once daily for 30 consecutive days. Group II received LC (100 mg/kg body weight), orally once daily for 30 consecutive days **(Avsar *et al.*, 2014).** Group III served as CP toxic control and received saline orally once daily and a single dose of CP on the 27th day of the experiment (7.5 mg/kg, IP; **Adeyemi *et al.*, 2017)**. Group IV received both LC and CP as the same previous treatments.

Sampling

After 24 h from the end of the experiment, rats were anesthetized by diethyl ether. Blood samples from each rat were collected by puncturing retro-orbital plexus in sterilized dry centrifuge tubes and kept for 30 min at room temperature (RT) in a slanted position to coagulate before centrifugation at 1200 x g for 20 min to obtain serum, which was preserved at -20 ℃ until further use for biochemical investigations. After blood collection, the animals of all groups were sacrificed by cervical

decapitation then the liver and kidneys were excised from each rat and washed with physiological saline. A gram specimen of each tissue was homogenized in 5 ml phosphate buffer pH 7.4 using an electrical homogenizer and maintaining the sample on ice. Tissue homogenates were centrifuged at 1200 x g for 20 min at 4 ^{O}C. The resulting supernatants were isolated and stored at -20 ^{O}C until further used in the assessment of the oxidative stress biomarkers in hepatic and renal tissues. Remaining liver and kidney tissues were immediately preserved in 10% neutral buffered formalin for histological and immunohistochemical investigations.

Serum biochemical studies

The activities of AST and ALT (**Reitman and Frankel, 1957**), ALP (**Tietz *et al*., 1983**) were determined in collected sera as markers for liver injury. While albumin and total protein were determined according to **Doumas *et al*., (1971) and Doumas *et al*., (1981)**, respectively. In addition, the serum levels of urea (**Coulombe and Favreau,1963)** and creatinine (**Bartels *et al*., 1972**) were determined to evaluate kidney function.

Preparation of liver and kidney homogenates

The tissue was dissected and washed with a phosphate-buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. One gram of each tissue was homogenized in 5 ml of 5-10 ml cold buffer (i.e., 50mM potassium phosphate, pH7.5 1mM EDTA) per gram tissue, using a homogenizer. Aliquots of tissue homogenates were centrifuged by cooling centrifuge 4000 rpm for 20 min then stored at -20 ℃till biochemical analysis.

Detection of oxidative/antioxidant cascades

Oxidative status was done by determination of the activity of glutathione reductase (GSH; **Sedlak and Lindsay, 1968**) and catalase (CAT; **Aebi, 1984**), and concentration of malondialdehyde (MDA; **Ohkawa *et al*., 1979**) by using special diagnostic kits obtained from Bio diagnostic company, Egypt.

Histological examination

Tissue specimens were taken from the liver and kidney of rats in different groups and fixed in 10% neutral buffered formalin for 48 hours. Then, specimens were dehydrated in serial dilutions of alcohol cleared in xylene and embedded in paraffin. Paraffin sections of 5 microns thickness were cut and collected on glass slides and stained by hematoxylin and eosin for histological examination **(Bancroft *et al*., 2013).**

Immunhistochemical studies:

Liver and kidney paraffin sections of 5 microns thickness were cut and collected on positively charged slides for immunohistochemical localization of VIM and CK18 using a streptavidin-biotin complex (ABC) method. After, dewaxing, rehydration, and blocking endogenous peroxidase activity, the sections were heated at 90°C with citrate buffer pH 6 for 30 min. Nonspecific staining was blocked 10% bovine serum albumin for 30 min. The sections were then incubated with the primary antibodies (rabbit monoclonal anti-vimentin and anti-cytokeratin 18, Abcam, Boston, the USA at 1:200 dilution) for 1 hr at RT. Next, sections were incubated with biotinylated donkey anti-mouse IgG (Abcam, Boston, USA) for 30 min at RT. The visualization of the immunoreactions was observed using a commercial ABC system recommended by the manufacturer (Santa Cruz Biotech, CA, USA). Then the slides were then subjected to diaminobenzene (DAB) as the chromogen and counterstained with hematoxylin. The VIM and CK18 staining in both hepatic and renal tissues of all examined rats were evaluated blindly. At least 5 random high-power fields were checked at a magnification of 400X using Leica DM3000 microscope. Staining features were scored semi-quantitatively according to Liu *et al*., (2007) as follow: negative (-), weak (+), moderate (++), strong (+++) for no stain, < 10% positive cells, 10–50% positive cells and >50% positive cells, respectively.

Statistical analysis:

Statistical analysis was performed using SPSS (Version 20.0; SPSS Inc., Chicago, IL, USA). The significant differences between groups were evaluated by one way ANOVA using Duncan test as a post hoc. Results are expressed as mean ± SEM. $P<0.05$ was considered significant.

Results

Serum biochemical analysis

CP injection significantly increased serum ALT, AST, and ALP activities compared with those in control rats. Similarly, CP significantly increased the levels of creatinine and urea. Conversely, serum total protein and albumin were significantly decreased due to CP injection compared to that in control rats. LC administration with CP restored these parameters towards the normal values (Table 1).

Hepatic and renal oxidative damage parameters

In the present study, there were substantial increases in MDA level along with dramatic decreases in GSH and CAT in the liver and kidney tissues of CP-intoxicated rats. Meanwhile, LC+CP administrated group revealed a decrease in MDA level along with elevations in GSH and CAT in hepatic and renal tissues compared with CP treated group (Table 2).

Histological observations

Liver sections from control and LC treated rats exhibited normal hepatic histo-architecture. Hepatocytes organized in cords radiating from the central vein and separated by regular sinusoids (Figure 1A, B). Otherwise, CP treated rats revealed several histological changes like dilatation of the central vein and sinusoids, inflammatory cells aggregation, kupffer cells proliferation (Figure 1C), swelling of hepatocytes, hydropic degeneration (Figure 1D), and fatty infiltration with signet ring appearance in some hepatocytes (Figure 1E). The liver section from LC+CP treated rats represented almost normal hepatocytes and sinusoids, but mild congested central vein and no signs of fatty changes were noted (Figure 1F).

Kidney sections from both control and LC groups showed regular renal histo-architecture with normal renal corpuscles and renal tubules; proximal (PCT) and distal convoluted tubules (DCT) and collecting (CT) tubules (Figure 2A,B). In CP group, many distinguishing histological changes were noted including an excessive degenerative changes and desquamation of the tubular epithelia were observed (Figure 2C, D, E) with the presence of eosinophilic hyaline casts in some tubules

(Figure 2D, E). Also, deformity of some golumeruli with widening of glomerular space was identified (Figure 2E) as well as, congestion of peritubular blood vessels and capillaries (Figure 2C, D, E). However, kidney from CP+LC group, revealed mild tubular degeneration with minimal interstitial congestion (Figure 2F).

Immunohistochemical observations

A summary of VIM and CK18 immunohistochemical expressions in the livers and kidneys of all examined groups was recorded (Tables 3, 4).

VIM expression

Both control and LC rats expressed weakly to moderate VIM mainly in the hepatic sinusoids and Kupffer cells but the hepatocytes were VIM negative (Figure 3A, B). Meanwhile, CP-injected rats showed overexpression of VIM in the blood sinusoids (Figure 3C) and an increased number of Kupffer cells (Figure 3D, E) as well as, some hepatocytes labeled weak VIM (Figure 3E). But, the LC+CP group showed moderate expression of sinusoidal VIM and fewer Kupffer cells compared with the CP group.

In kidneys, VIM was expressed mainly in the glomeruli, some peritubular blood capillaries, and interstitial fibroblasts of all experimental groups (Figure 4). In both control and LC groups, the renal tubules did not express VIM protein but, the interstitial tissues and glomeruli showed weak and moderate VIM staining, respectively (Figure 4A, B). Otherwise, overexpression of VIM was seen in the damaged renal tubules and interstitial tissues of the CP treated group (Fig. 4C) that tended to be decreased in the interstitium and renal tubules of LC+CP group compared with CP group (Figure 4D).

CK18 expression

Hepatocytes of both control and LC rats showed weak CK18 staining at the periphery of the cells giving reticular appearance (Figure 5A, B). CP group revealed strong, dense, and clumped CK18 staining in the hepatocytes surrounding the central veins, fat cells, and triad area (Figure 5C, D, E). But, the LC+CP group showed lower CK18 immunostaining compared with the CP group (Figure 5F).

Regard to kidneys, both control and LC groups demonstrated weak to moderate CK18 immunostainings in the visceral cells layer of Bowman's capsule as well as the cells of different segments of renal tubules; PCT, DCT, and CT (Figure 6A, B). However, CK18 was overexpressed in the epithelial cells of renal tubules mainly PCT and few DCT after injection of CP (Figure 6C). Meanwhile, the LC+CP group showed a lower expression of CK18 (Figure 6D) compared with the CP group.

Table 1: Effect of CP and/or LC treatment on serum biochemical parameters in rats (n=7).

Parameters	Control	LC	CP	LC+ CP
AST (U/L)	76.36±2.96^{c}	69.74±1.95^{c}	192.46±2.58^{a}	115.57±3.85^{b}
ALT (U/L)	48.19± 4.39^{c}	39.55± 2.89^{c}	107.64 ±3.72^{a}	61.63±5.39^{b}
ALP (U/L)	122.97±3.91^{c}	114.87±3.56^{c}	252.01±8.49^{a}	184.60±3.53^{b}
T. protein (gm/dl)	8.03± 0.06^{a}	7.59±0.20^{ab}	5.29± 0.04^{c}	7.17± 0.28^{b}
Albumin (gm/dl)	4.48± 0.05^{a}	4.35± 0.11^{a}	3.14± 0.04^{c}	3.78± 0.03^{b}
Creatinine (mg/dl)	0.64± 0.01^{c}	0.59± 0.006^{c}	1.22± 0.05^{a}	0.88± 0.01^{b}
Urea (mg/dl)	32.55±1.14^{c}	29.64±1.07^{c}	78.03±2.99^{a}	54.37±1.98^{b}

LC, L carnitine at dose of 100 mg/Kg PO; CP, cisplatin at dose of 7.5 mg/Kg IP; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; T. protein, total protein.

Data are expressed as the mean±SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

Table 2: Effect of CP and/or LC treatment on oxidative stress markers in liver and kidney tissues in rats (n=7).

Parameters	Organ	Control	LC	CP	LC+CP
MDA (nmol/g)	Liver	47.26± 1.13^{c}	44.29± 0.88^{c}	116.34± 3.17^{a}	81.23± 3.37^{b}
GSH (mg/g)	Liver	69.28± 2.09^{a}	64.25± 1.86^{a}	34.81± 2.25^{c}	54.34± 2.17^{b}
CAT (U/g)	Liver	2.06± 0.02^{a}	1.98± 0.04^{a}	1.06± 0.04^{c}	1.52± 0.03^{b}
MDA (nmol/g)	Kidney	78.06± 2.37^{c}	73.19± 5.29^{c}	182.06± 5.16^{a}	121.53± 4.30^{b}
GSH (mg/g)	Kidney	108.98± 4.98^{a}	101.61± 3.47^{a}	58.95± 3.07^{c}	78.65± 1.85^{b}
CAT (U/g)	Kidney	2.43± 0.05^{a}	2.22± 0.06^{b}	1.06± 0.04^{d}	1.69± 0.04^{c}

LC, L carnitine at dose of 100 mg/Kg PO; CP, cisplatin at dose of 7.5 mg/Kg IP; MDA, malondialdehyde; GSH, reduced glutathione; CAT, catalase.

Data are expressed as the mean±SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

Table 3. Summary of VIM and CK18 immunohistochemical expressions in the livers of all examined groups.

Hepatic tissues	VIM				CK18			
	Control	LC	CP	LC+CP	Control	LC	CP	LC+CP
Hepatocytes	-	-	+	-	+	+	+++	++
Sinusoids	+/++	+/++	+++	++	-	-	-	-
Kupffer cells	+/++	+/++	+++	+	-	-	-	-

-, negative; +, weak; ++, moderate; +++, strong

Table 4. Summary of VIM and CK18 immunohistochemical expressions in the kidneys of all examined groups.

Renal tissues	VIM				CK18			
	Control	LC	CP	LC+CP	Control	LC	CP	LC+CP
Renal corpuscles	++	++	++	++	+	+	-	-
Renal tubules	-	-	+++	+	++	++	+++	+
Interstitium	+	+	+++	++	-	-	-	-

-, negative; +, weak; ++, moderate; +++, strong

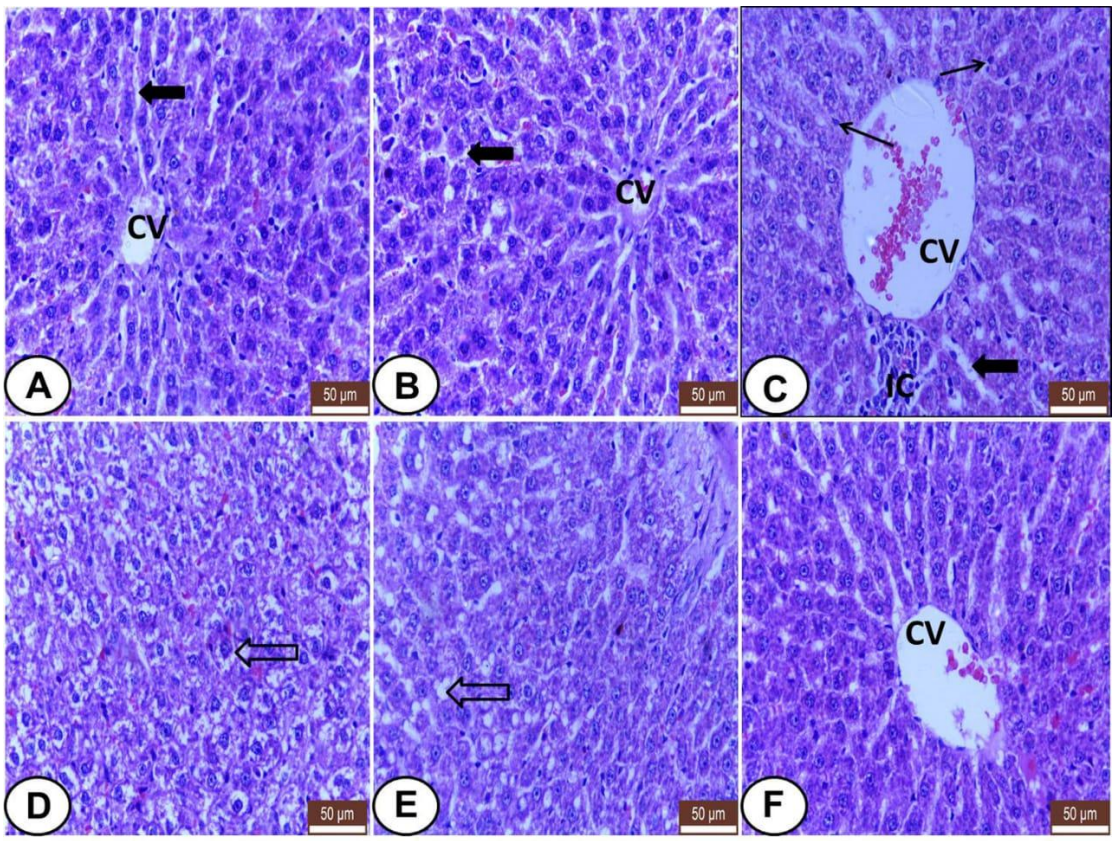

Figure 1

Histological sections of livers from all examined groups. A and B; Control and LC groups showed normal hepatic histo-architecture. Hepatocytes organized in cords radiating from central vein (CV) and separated by regular sinusoids (wide black arrow). C-E; CP treated rats showed several histological changes. C; showed dilatation of the central vein (CV) and sinusoids (wide black arrow), inflammatory cells aggregation (IC) and kupffer cells proliferation (thin arrow). D; showed swelling of hepatocytes, hydropic degeneration (hollow arrow). E; showed fatty infiltration with signet ring appearance in some hepatocytes (hollow arrow). F; LC+CP treated rats showed almost normal hepatocytes and sinusoids, but congested central vein (CV), and no signs of fatty changes were noted. H&E stain, scale bars=50µm.

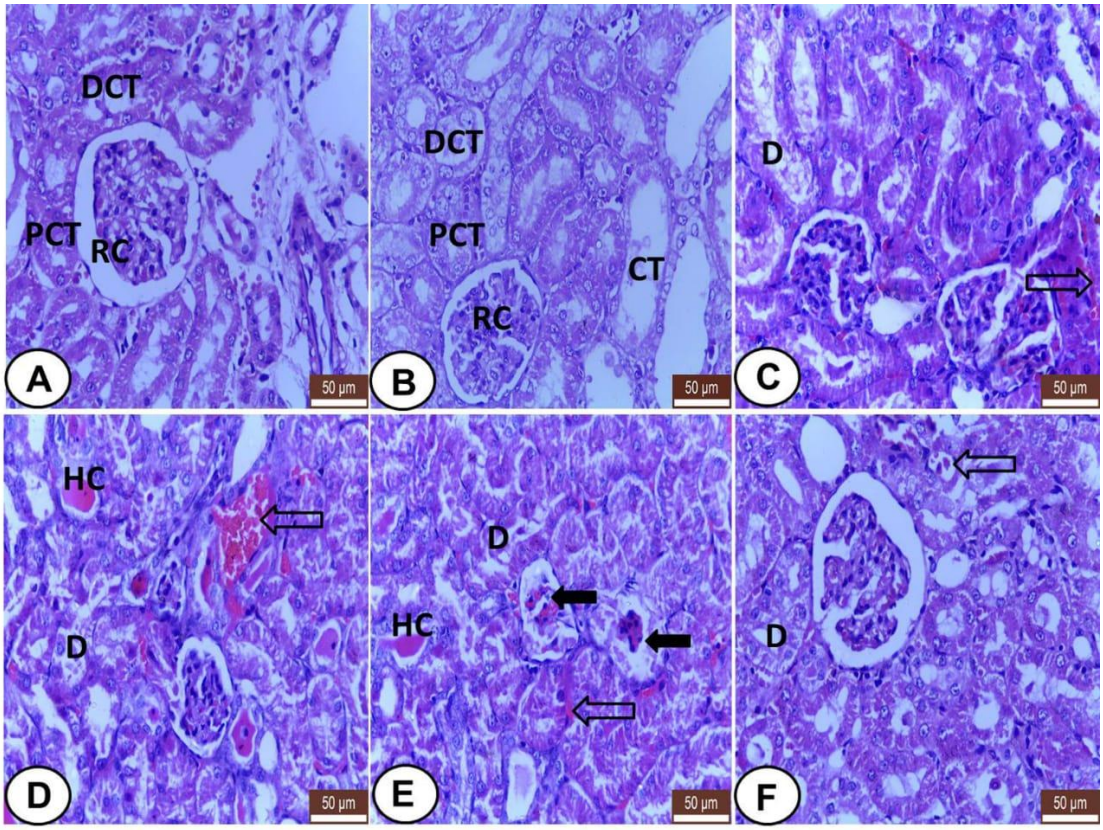

Figure 2

Histological section of kidneys from all examined groups. A and B; Control and LC groups showed regular renal histo-architecture with normal renal corpuscles (RC) and renal tubules; proximal (PCT) distal convoluted tubules (DCT) and collecting tubules (CT). C-E; CP treated rats showed several histological changes. Degenerative changes and desquamation of the tubular epithelia (D), eosinophilic hyaline casts (HC) in some tubules, deformity of some golumeruli with widening of glomerular space (wide black arrows) and congestion of peritubular blood vessels and capillaries (hollow arrows). F; LC+CP treated rats showed mild degenerated tubular epithelium (D) with minimal interstitial congestion (hollow arrow). H&E stain, scale bars=50µm.

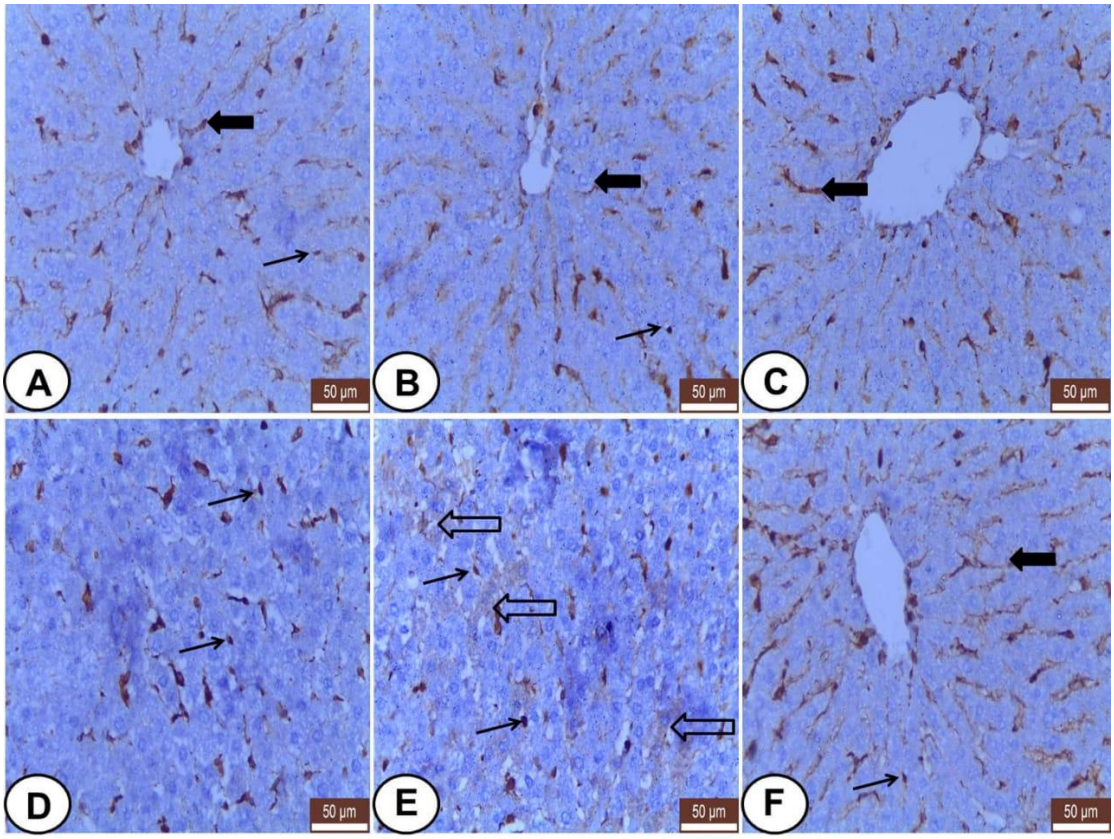

Figure 3

Immunohistochemical staining of VIM in hepatic sections from all examined groups. A and B; Control and LC groups showed weak to moderate hepatic sinusoids (wide arrow) and Kupffer cells (thin arrow) for VIM. C-E; CP group revealed overexpression of VIM in blood sinusoids (wide arrow), Kupffer cells (thin arrows) and some hepatocytes showed weak VIM (hollow arrow). F; LC+CP treated rats showed moderate expression of sinusoidal VIM (wide arrow) and fewer Kupffer cells (thin arrow) compared with CP group. Scale bars = 50µm.

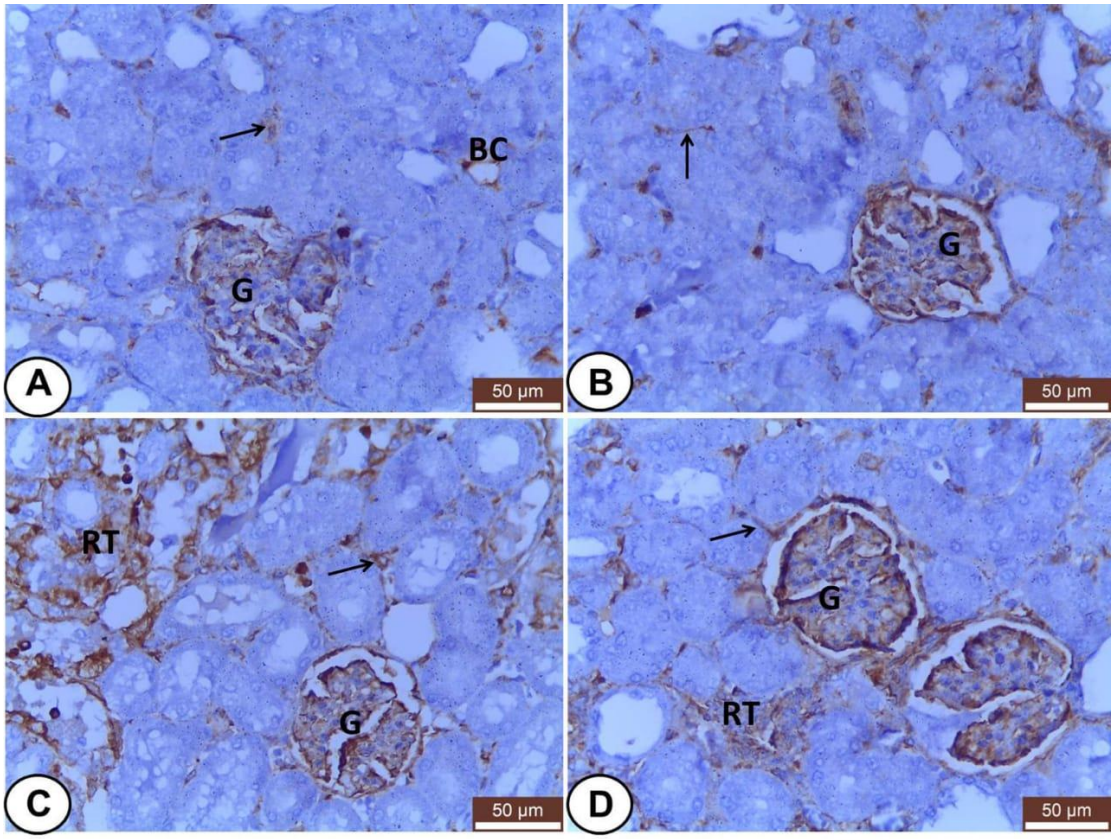

Figure 4

Immunohistochemical staining of VIM in renal sections from all examined groups. A and B; Control and LC groups showed weak to moderate VIM staining in the interstitial tissues (thin arrow), blood capillaries (BC) and glomeruli (G). C; CP group revealed moderate glomerular (G) and strong tubular (RT) and interstitial (thin arrow) VIM staining. D; LC+CP groups showed less VIM reactivity in comparison to CP group especially the interstitium (thin arrow) and renal tubules (RT). Scale bars = 50µm.

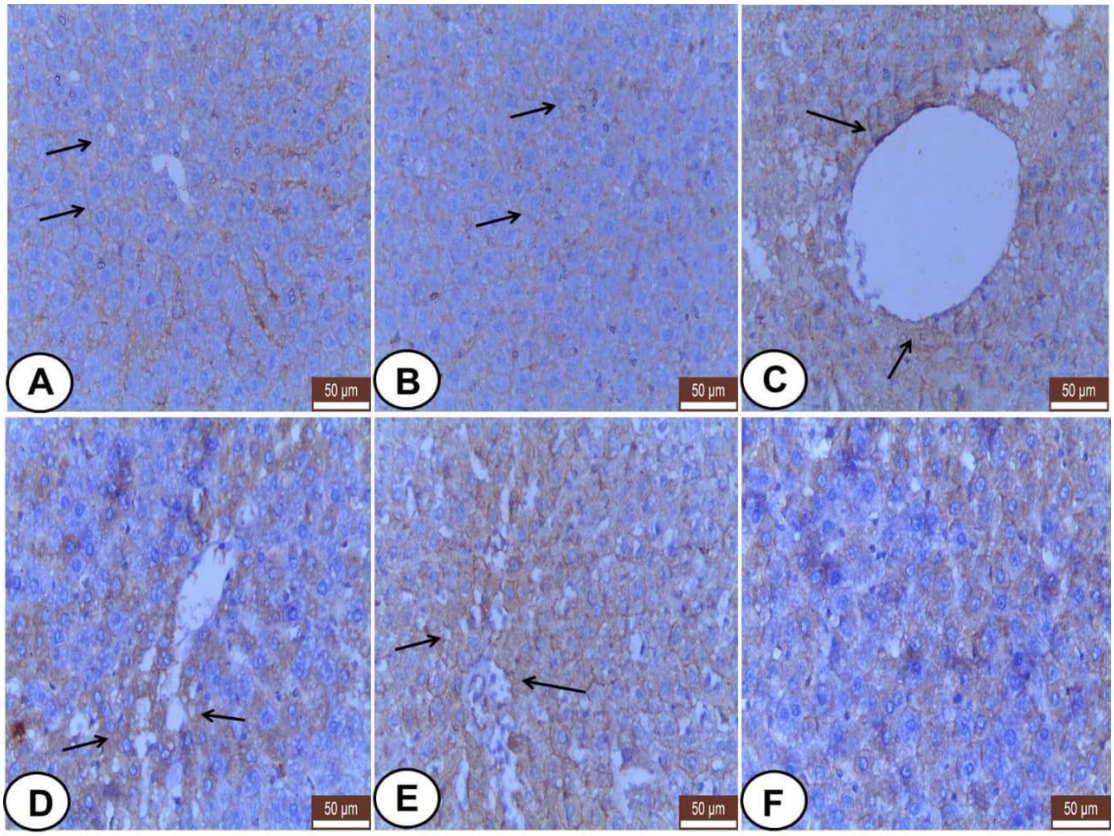

Figure 5

Immunohistochemical staining of CK18 in hepatic sections from all examined groups. A and B; Control and LC groups showed weak CK18 staining at the periphery of the hepatocytes in reticular pattern (thin arrows). C-E; CP group revealed strong, dense and clumped CK18 staining in hepatocytes surrounding the central veins (thin arrows), fat cells (thin arrows) and triad area (thin arrows). F; LC+CP group showed less CK18 staining compared with CP group. Scale bars = 50µm.

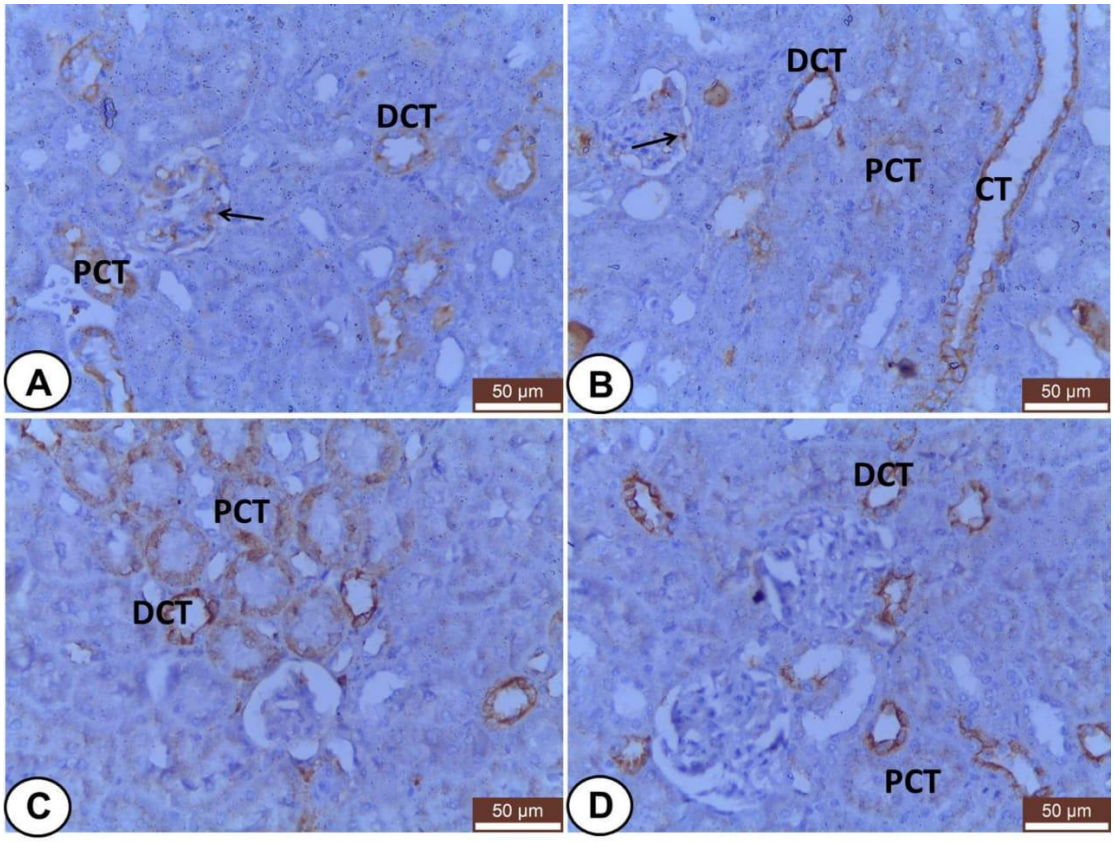

Figure 6

Immunohistochemical staining of CK18 in renal sections from all examined groups. A and B; Control and LC groups showed weak to moderate cytoplasmic immunostainings in the visceral cells layer of Bowman's capsule (thin arrow) as well as the cells of different segments of renal tubules; proximal convoluted (PCT), distal convoluted (DCT) and collecting (CT) tubules. C; CP group revealed overexpression of CK18 in the epithelial cells of renal tubules mainly proximal convoluted (PCT) and few distal convoluted (DCT) tubules. D; LC+CP group displayed lower expression of CK18 in PCT and DCT compared with CP group . Scale bars = 50µm.

Discussion

Hepatic and renal toxicity is the most common dose-limiting side effects of CP-induced chemotherapy (**Neamatallah *et al.*, 2018**). Reducing the potential side effects of CP by LC can be helpful during chemotherapy.

Elevated activities of liver enzymes are known to be markers of cellular leakage and loss of functional integrity of hepatocytes because they are released into the bloodstream when the hepatocyte plasma membrane is impaired (**Jia *et al.*, 2018; Farid *et al.*, 2019; Fadl *et al.*, 2020**). In this work, CP-induced hepatotoxicity was evidenced by significant alternation in serum liver enzymes (AST, ALT, and ALP). This result may be attributed to the metabolism of CP as **Mohamed and Badawy, (2019)** reported CP is significantly taken up by the liver and accumulates in the hepatocyte causing its damage leading to an increase of liver enzymes activities. In addition, CP elevated creatinine and urea levels, which are confirmed by **Sadeghi *et al.*, (2020)** and indicated nephrotoxicity induced by CP. On the other hand, **Cayir *et al.*, (2009)** attributed the hepatotoxicity and nephrotoxicity induced by CP to the free radical generation in the renal and hepatic cells results in lipid peroxidation and oxidative stress that responsible for cellular damage.

CP administration produced a significant decrease in total proteins and albumin. This result supported by **Abuzinadah and Ahmad, (2020)**, these results indicate disturbances in protein metabolism induced by CP intoxication due to reduction in protein synthesis following liver damage and alteration of functional integrity in the kidney leading to proteinuria so, their plasma level decrease in hepatotoxic/nephrotoxic conditions (**Sen *et al.*, 2013**).

Concerning oxidative stress/ antioxidant parameters, CP treated group revealed significant an increase of MDA level (increased lipid peroxidation) and decreased activities of the antioxidants (CAT and GSH) in the liver and kidney tissues. These results are compatible with **El-Shitany and Eid, (2017) and Abd El-Kader and Taha, (2020) and Abdel-Razek *et al.*, (2020)** in the liver and kidney tissues, respectively. These results owed to oxidative stress that is mediated through the generation of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical and depletion in plasma antioxidant levels (**Abuzinadah and Ahmad, 2020**).

So, our results are in harmony with several experimental and clinical studies suggesting that the oxidative stress through the formation of free radicals is one of the mechanisms of CP-induced hepato-renal toxicity (**Neamatallah *et al.*, 2018; Abuzinadah and Ahmad, 2020**).

On the other side, LC is a natural nutrient and necessary for the oxidation of fatty acid in the mitochondria to produce ATP (**Tunez *et al.*, 2007**) so, it has antioxidant properties and plays protective roles against oxidative stress in various tissues, including liver and kidney (**Cayir *et al.*, 2009**). In this study, LC+CP treated group showed an improvement in the liver and kidney function tests as well as oxidative stress/antioxidant parameters compared with the CP group. Our findings are in accordance with **Tunez *et al.*, (2007) and Aboubakr *et al.*, (2020)** who reported that LC reduces liver enzyme activities, oxidative stress, and damage in thioacetamide and tilmicosin, respectively, induced hepatotoxicity in rats. Also, LC can improve the antioxidant enzyme activities including CAT and GSH and reduces the MDA concentration in renal tissues in acute renal failure induced by myoglobinuric in rats (**Aydogdu *et al.*, 2006**).

The results of the present study demonstrated that the daily oral administration of LC markedly ameliorates CP induced hepato-renal damage as shown in the histological examination and biochemical parameters. The ability of LC to significantly improve liver and kidney biochemical parameters may be due to its antioxidant effects and its capability to act as a free radical scavenger, leading to the protection of membrane permeability (**Augustyniak and Skrzydlewska, 2009**). LC prevents oxidative stress and exerts a protective role against mitochondrial toxic agents (**Barhwal *et al.*, 2007**). Additionally, they reduce the harmful effects of free fatty acids by enabling β-oxidation (**Furuno *et al.*, 2001**).

The histological and immunohistochemical observations of the current study were in harmony and confirmed the alterations of the biochemical and oxidant/antioxidant parameters among the experimental groups.

Several histological changes in the livers among CP treated group were noted as recently mentioned by **Abuzinadah and Ahmad, (2020)**. The central vein and sinusoidal dilatation and congestion are in accordance with **Cagin *et al.*, (2015)** and

Omar *et al*., (2016). Also, swelling of hepatocytes, hydropic degeneration, and prominence of kupffer cells are demonstrated in similarity to **El-Shitany and Eid, (2017) and Neamatallah *et al*., (2018)**. In addition, fatty infiltration with signet ring appearance in some hepatocytes was seen as noted by **Omar *et al*., (2016) and El-Shitany and Eid, (2017)**. Otherwise, the pretreatment of CP treated rats with LC can potentially protect the liver against CP-induced histological changes and significantly improved and normalized liver histology that represented by almost normal hepatocytes and sinusoids, but with mild congested central vein with regression of the fatty changes. These findings are similar to **El-Shitany and Eid, (2017)** indicating that LC can attenuate the hepatotoxic effect of CP (**Cayir *et al*., 2009)**.

Like the recent finding of **Abd El-Kader and Taha, (2020), Abdel-Razek *et al*., (2020) and Sadeghi *et al*., (2020)**, the current study exhibited many distinguishing degenerative changes in the kidney of the CP group including degeneration and desquamation of the tubular epithelium, congestion, and dilatation of interstitial blood vessels and capillaries as reported by **Neamatallah *et al*., (2018)**. Also, the presence of eosinophilic hyaline casts in some renal tubules was in agreement with **Abd El-Kader and Taha, (2020) and Abdel-Razek *et al*., (2020)**. Additionally, the deformity of some glomeruli with a widening of glomerular spaces was detected in accordance with **Abd El-Kader and Taha, (2020)**. On the other hand, LC administration ameliorates the histological effects of CP on the kidney, but with mild histological findings evidenced by tubular injury in some renal tubules, and minimal interstitial congestion. This finding confirms that LC can attenuate the nephrotoxic effect of CP (**Yürekli *et al*., 2011**).

The current study focused on immunohistochemical localization of VIM and CK18 in hepato-renal specimens since VIM and CK18 expressions in the liver and kidney had provided a valuable insight into their microanatomy in both health and disease conditions. A co-expression of VIM and CK in areas of damaged tissues was reported (**Moll *et al*., 1991; Stefanovic *et al*., 1996**).

Concerning VIM protein, it has been linked with several pathophysiological conditions such as cancer, rheumatoid arthritis, and HIV (**Danielsson *et al*., 2018**).

The detectable VIM in hepatic sinusoids of the present study confirmed the report of **Evan, (1998)** where VIM is widely expressed IF proteins in endothelial cells and fibroblasts. According to **Wang *et al.*, (2017)**, VIM expression in the hepatic sinusoids may reflect its regulatory role of hepatic sinusoidal flow. At the beginning of this decade, both **Golbar *et al.*, (2011) and Aiad *et al.*, (2012)** regarded the sinusoidal VIM expression as hepatic stellate cells (HSCs), which are normally localized at the space of Disse. Also, Kupffer cells in the current study are VIM positive as mentioned by **Sharifi *et al.*, (2000) and Golbar *et al.*, (2011)**. After the injection of CP, an overexpression of VIM in the liver was demonstrated in similarity to the lipopolysaccharide intoxicated liver (**Lee *et al.*, 2014**). The numbers of VIM positive Kupffer cells were increased (**Golbar *et al.*, 2011; Aiad *et al.*, 2012**), and some individual hepatocytes became VIM positive (**Aiad *et al.*, 2012**). As well as, sinusoidal VIM expression was increased in response to CP toxicity where HSCs are activated into myofibroblasts, which characterized by higher VIM expression and more secretion of the extracellular matrix so, they considered as the major contributor to hepatic fibrosis (**Shang *et al.*, 2018**).

In the normal and diseased kidney, VIM is abundantly present in glomerular mesangial and epithelial cells (**Gonlusen *et al.*, 2001; Matos *et al.*, 2007**). Our study revealed VIM expression in the glomeruli of all examined rats confirming the abovementioned data. Also, VIM was expressed in the interstitial blood vessels and fibroblasts of all examined rats that agree with **Stefanovic *et al.*, (1996)** and **Sen *et al.*, (2010)** suggesting that the pivotal regulating role of the renal interstitium for vessels-tubules interaction as well as its involvement in the etiology of renal pathologies (**Becker and Hewitson, 1997**). It was interesting that the current results revealed undetectable VIM in the renal tubular epithelium of the control rats that agree with **Skinnider *et al.*, (2005)** and **Sen *et al.*, (2010).** Otherwise, the overexpression of VIM in renal tubules and interstitial tissue of the CP group was noted may be attributed to glomerulonephritis or tubule-interstitial injury (**Gonlusen *et al.*, 2001; Matos *et al.*, 2007**), respectively.

CKs of hepato-renal tissues increase in response to toxicants, oxidative stress, inflammation, and other damaging insults (**Toivola *et al.*, 2010**). Hepatocytes, as one of the epithelial cells of the liver, have been known to express CKs (**Ku *et al.*, 2007**).

The hepatocytes of both control and LC treated rats showed faint CK18 at the cell periphery forming reticular staining patterns that agree with **Zatloukal *et al*., (2004).** Upon injury by CP, the intoxicated hepatocytes exhibited strong, dense, and clumped CK18 staining that agree with **Omary and Coulumbe, (2004)**. Also, immunohistochemical overexpression of CK18 in hepatocellular carcinoma was noted (**Sawan, 2009**). These findings point to the structural role of CK18 to hepatocytes providing them mechanical stability. Another role of CK18 is a target and modulator of toxic stress (**Zatloukal *et al*., 2004**) therefore; the current study reported that the CK18 expression is significantly up-regulated by oxidative stress induced by CP. That confirms CK-18 levels as a predictor for hepatitis progression (**Yang *et al*., 2015**).

CKs are often used as disease markers in renal pathology and experimental research (**Djudjaj *et al*., 2016**). Expression of CK18 in the parietal cells of Bowman's capsule as well as the different segments of renal tubules of the normal rats agreed with the finding of **Stefanovic *et al*., (1996) and Djudjaj *et al*., (2016)**. CK18 staining was stronger in the collecting ducts as mentioned by **Sen *et al*., (2010)**. Like **Snider, (2016)**, our finding showed a pronounced overexpression of CK18 in the damaged renal tubules in CP treated group indicating that CK18 is upregulated by tubular injury. Therefore, CK18 can be used as a marker and regulator of renal tubular epithelial injury (**Djudjaj *et al*., 2016**).

It was noteworthy to record that the pretreatment of LC can decrease expressions of VIM and CK18 in hepato-renal tissues compared with CP group indicating the ameliorative role of LC against CP toxicity especially in restoring the organization of IFs.

CONCLUSIONS

CP induces hepato-renal toxicity associated with oxidative damage, lipid peroxidation, histological changes, and disorganization of the cytoskeleton IFs; VIM and CK18. A daily LC treatment at 100 mg/kg exerts ameliorative effects against CP-induced hepato-renal toxicity. The antioxidant effect of LC was evidenced by the restoration of activities of oxidative/antioxidant markers, histological pictures, and VIM and CK18 organization in hepato-renal tissues. Therefore, this study suggests LC as a supplement for cancer patients under CP treatment.

Ameliorative effects of L-carnitine against cisplatin-induced gonadal toxicity in rats

ABSTRACT

Even though Cisplatin (CP) is highly efficient remedy in cancer treatment it adversely affected the testicular tissue. This work assesses the ameliorative efficacy of L-carnitine (LC) against CP induced oxidative stress in rat testis, via investigating testosterone level and tissue oxidative/antioxidant parameters, histological alterations and immunohistochemical expressions of two different intermediate filaments (IFs) proteins; vimentin (VIM) and cytokeratin 18 (CK18). Twenty-eight rats were assigned into four groups /7 rats each/ as follows; groups I and II were received saline and LC (100 mg/kg b.wt.) respectively orally once daily for 30 days in a row; group III were injected with a single dose of CP (7.5 mg/kg, IP), 27 days after starting the experiment. Group IV was received both LC and CP. Injection of CP significantly decreased serum testosterone and glutathione reductase and catalase in the testicular tissues and elevated malondialdehyde. Histologically, testes of the CP treated group revealed marked degenerative changes. Also, overexpression of both VIM and CK18 in testicular tissues. However, the administration of LC with CP restored the biochemical parameters, histological and immunohistochemical pictures towards the normalcy. Accordingly, LC is recommended as a supplement with chemotherapy to ameliorate its oxidative stress. This is the first study that investigated the immunohistochemical expressions of IFs proteins, VIM and CK18, following administration of LC as a protective agent against CP induced testicular toxicity in rats.

Keywords: Testosterone; Apoptotic markers; Vimentin, Cytokeratin, Malondialdehyde, Immunohistochemistry.

INTRODUCTION

The testis play a vital role in male reproductive function as it secretes testosterone, the male hormone, and responsible for androgenesis and spermatogenesis **(Marty *et al.*, 2003).** Testosterone plays a critical role in growth, the appearance of sexual characteristics, maturation of male reproductive organs, and spermatogenesis **(Azarbarz *et al.*, 2020).**

Cisplatin (CP) is a potent anti-cancer medication used to treat a variety of tumors of the testes, ovary, bladder, and lungs **(Karwasra *et al.*, 2016).** However, several studies recorded that it induces testicular toxicity **(Afsar *et al.*, 2017; Almeer and Abdel Moneim, 2018; Azab *et al.*, 2020; Azarbarz *et al.*, 2020),** inflammation, apoptosis, and oxidative stress **(Meng *et al.*, 2017)** as well as, disorganization of the intermediate filaments (IFs) components of the cytoskeleton **(Evans and Simpkins, 1998).** Recently, vimentin (VIM) is considered as mesenchymal marker for testicular toxicity. And, cytokeratins (CKs) are known as cellular stress protein specially CK18 which used as novel markers of testicular injuries **(Banco *et al.*, 2016).**

Testicular dysfunction is the most reported consequence of CP toxicity, due to its high proliferative rate so the adverse effects of chemotherapy on the testis could be intense and irreversible causing death of spermatogenic cells in the process of spermatogenesis and alterations in the sperm DNA, thus leading to the inability to generate a sufficient number of viable sperms (oligozoospermia), azospermia or even prolonged sterility **(Ekinci Akdemir *et al.*, 2019; Azarbarz *et al.*, 2020).**

L-carnitine (LC) is a natural nutrient that is synthesized from lysine and methionine essential amino acids. It is derived from dietary sources (75%) and endogenous biosynthesis (25%). It presents in the epididymis in high levels and plays a vital function in spermatogenesis, spermatozoa maturation as well as metabolism **(Abdel Aziz *et al.*, 2018).** LC is necessary for the production of ATP by β-oxidation of fatty acids in mitochondria **(Aboubakr *et al.*, 2020).** Therefore, LC could prevent mitochondrial oxidative stress-induced by mitochondrial damage and apoptosis in different cell types **(Barhwal *et al.*, 2007).** Accordingly, this work assesses the ameliorative efficacy of L-carnitine (LC) against CP induced oxidative stress in rat' testis via investigating testosterone and tissue oxidative/antioxidative parameters and

revealing the histopathological alterations and immunohistochemical expressions of VIM and CK18 proteins.

MATERIALS AND METHODS

Chemicals

Cisplatin was obtained from EIMC United Pharmaceuticals (Badr City, Egypt); each vial (50mg/50ml) was dissolved in physiological saline (0.9% sodium chloride). L-carnitine was obtained from MEPACO Company (Inshas Elraml, Egypt). Kits used for biochemical analysis (MDA, GSH, and CAT) were obtained from Biodiagnostics Company (Dokki, Giza, Egypt).

Experimental animals

The present study was carried out on 28 white Albino male rats weighing 175-195 gm. Rats were obtained from the Center of Laboratory Animal at the Faculty of Veterinary Medicine, Benha University, Egypt. They adapted in the Laboratory of the Department of Pharmacology for two weeks before conducting the experiment. Animals received balanced commercial diet and water *ad libitum*. The study protocol was approved by the ethical committee of the Faculty of Veterinary Medicine, Benha University, Egypt.

Experimental design

Male albino rats were randomly separated into four equal groups /seven each, the control group, group I, received saline (the vehicle) orally, once daily for 30 days in a row. Group, II, LC group, received LC (100 mg/kg b.wt.), orally once daily for 30 days in a row **(Avsar *et al.*, 2014).** Group III, the CP group, were injected with a single dose of CP 7.5 mg/kg, via IP route on the 27th day of the experiment **(Boroja *et al.*, 2018).** Group IV, the LC+CP group, received combination of treatments as both group II and III

Sampling

Twenty- four hours post-treatments; rats were anesthetized by inhalation of ether. Blood samples were collected by puncturing retro-orbital plexus in a sterilized dry centrifuge tube then left for 30 min at room temperature in a slanted position for coagulation before centrifugation at 1200 x g for 20 min to separate serum, which was stored at -20 ^{o}C until use for biochemical studies. Following blood collection, the animals of all groups were euthanized by cervical dislocation then both testicles were removed from each rat and thoroughly washed with physiological saline, then tissue homogenates were prepared (mentioned below) and centrifuged. The supernatants were isolated and used for evaluation of oxidative stress markers in testicular tissues; whereas the rest of the testicular tissues were preserved in neutral buffered formalin (10%) for histopathological and immunohistochemical investigations.

Serum biochemical studies

The serum testosterone level was quantified using an enzyme-linked immunosorbent assay (ELISA) kits (Immunometrics Ltd., London, UK).

Preparation of testicular homogenates

The tissue was dissected and washed with phosphate-buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin for removal of any and cloted red blood cells. Using a homogenizer, a gram of each testicular tissue was homogenized in 5 ml of 5-10 ml cold buffer, 50 mM potassium phosphate, pH7.5 1mM EDTA. Aliquots of tissue homogenates were centrifuged by cooling centrifuge 4000 rpm for 20 min and stored at -20^{o}C till used for biochemical analysis.

Detection of oxidative/antioxidant cascades

Oxidative status was done by determination of the activity of glutathione reductase (GSH), catalase (CAT), and malondialdehyde (MDA) levels using special kits purchased from Bio diagnostic company, Egypt.

Histological examination

Testicular tissues werer fixed in neutral buffered formalin (10%) for 48 hours. Then, specimens were dehydrated using ascending grades of alcohol, cleared in

xylene, and embedded in molten paraffin. Five-micron thickness paraffin sections were cut, and stained by hematoxylin and eosin for histological examination **(Bancroft *et al.*, 2013).**

Immunohistochemical studies:

A streptavidin-biotin complex (ABC) method was used to localize CK18 and VIM immunohistochemically. Antigen retrieval then blocking of nonspecific staining was carried out after dewaxing, rehydration, and blocking of endogenous peroxidase activity. The testicular sections were incubated with the primary antibodies, rabbit monoclonal anti-cytokeratin 18 and anti-vimentin, Abcam, Boston, the USA at 1:200 dilution, for 1 hr at RT. Next, sections were incubated with biotinylated donkey anti-mouse IgG (Abcam, Boston, USA) for 30 min at RT. A commercial ABC system (Santa Cruz Biotech, CA, USA) was used for visualization of the reactions. The sections were then subjected to diaminobenzene (DAB) as the chromogen and counterstained with hematoxylin.

Statistical analysis

Statistical analysis was done using one-way ANOVA using the Duncan test, SPSS Version 20.0; SPSS Inc., Chicago, IL, USA, The data were expressed as mean ± SEM and $P<0.05$ was considered significant.

RESULTS

The biochemical parameters post-treatments were revealed (Table 5). Rats in the CP group had a significant decrease in the serum testosterone level when compared to those of the other groups. The data revealed a significant (P<0.05) increase in the MDA level along with decrease in GSH and CAT in the testicular tissues of CP-intoxicated rats. Meanwhile, animals in the LC+CP group showed a significant (P<0.05) decrease in MDA level along with elevations in GSH and CAT in renal and hepatic tissues when compared to that of the CP treated group.

Histopathologically, both control and LC groups revealed normal histo-architecture of the seminiferous tubules and interstitial tissues. Normal arrangements of spermatogenic cells and Leydig cells were seen (Figs.7A, B). Meanwhile, CP treated group showed massive degeneration in some seminiferous tubules (Fig.7C), cytoplasmic vacuolization, reduction of germ cell layers, congestion of blood vessels in other tubules (Fig.7D), desquamation, and shedding of spermatogenic cells into tubular lumen (Figs.7C, D) as well as widening of interstitial space with eosinophilic edema material (Fig.7E). However, LC+CP treated group showed some improvements in the histological structure of both seminiferous tubules and interstitial tissues (Fig.7F).

Immunohistochemically, most of the Leydig cells in both control and LC groups showed moderate CK18 immunolabeling (Figs.8A, B). While very weak CK18 immunolabeling was seen in few Leydig cells of the CP treated group (Fig.8C). An increase in the number and intensity of CK18 positive Leydig cells was identified in LC+CP treated group (Fig.8D) compared with the CP group. On other hand, strong VIM staining was observed in spermatogonia, spermatozoa, and Leydig cells in both control and LC treated group (Figs.9A, B) ,but CP treated group revealed weaker response to VIM staining (Fig.9C) compared of that control and LC groups. VIM staining nearly returned to normalcy in LC+CP treated group (Fig.9D) compared with that of Fig.9C.

Table 5: Effect of CP and/or LC treatment on serum testosterone and oxidant/antioxidant status in testicular tissues in rats (n=7).

Parameters	Control	LC	CP	LC+CP
Testosterone (ng/ml)	2.38±0.09^{b}	2.29±0.03^{b}	1.23±0.06^{a}	2.04±0.19^{b}
MDA (nmol/g)	57.84±1.51^{c}	55.74±1.37^{c}	92.46±3.40^{a}	79.21±2.38^{b}
GSH (mg/g)	4.85±0.21^{a}	4.78±0.05^{a}	3.26±0.14^{c}	4.03±0.05^{b}
CAT (U/g)	29.26±0.79^{a}	28.88± 0.46^{a}	17.80±0.52^{c}	25.74±1.42^{b}

LC, L- carnitine at dose of 100 mg/Kg PO; CP, cisplatin at dose of 7.5 mg/Kg IP; MDA, malondialdehyde; GSH, reduced glutathione; CAT, catalase.

Data are expressed as the mean ± SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

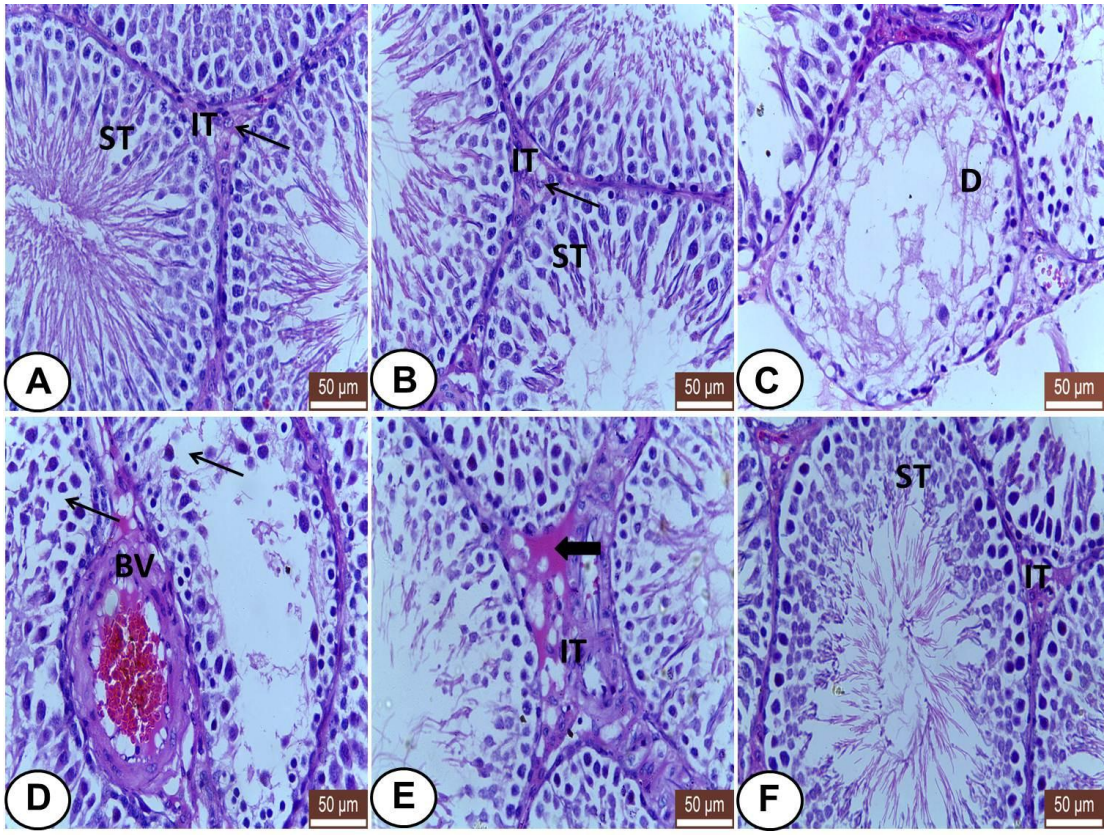

Figure 7:

Photomicrograph of testicular sections from all examined groups. A and B; Control and LC groups showed normal histo-architecture of the seminiferous tubules (ST) and interstitial tissues (IT). Notice, normal arrangements of spermatogenic cells, and Lydig cells (arrow). C-E; CP treated rats showed several histological changes. C; Massive degeneration in some seminiferous tubules (D). D; cytoplasmic vacuolization (arrows), reduction of germ cell layers, congestion of blood vessels (BV). Notice, desquamation, and shedding of spermatogenic cells into the tubular lumen of Figs (C and D). E; widening of interstitial tissue (IT) with eosinophilic edema material (thick arrow). F; LC+CP treated rats showed some improvements in the histological structure of both seminiferous tubules (ST) and interstitial tissues (IT). H&E stain, scale bars=50µm.

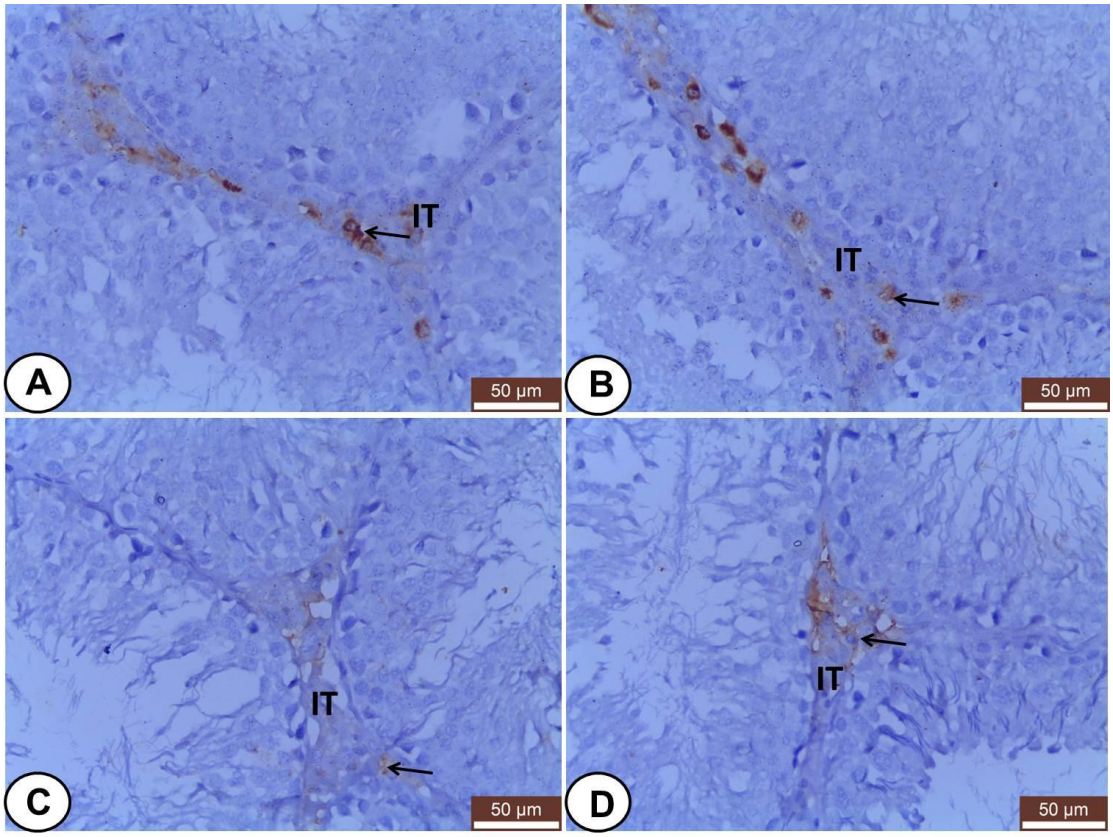

Figure 8:

Photomicrograph of CK18 immunostaining in testicular sections from all examined groups. A and B; Control and LC groups showed moderate CK18 staining. C; CP group revealed very weak and few positive CK18 cells. D; LC+CP group showed an increased number and intensity of CK18 positive interstitial cells. Leydig cells (thin arrow); interstitial tissue (IT). Scale bars = 50µm.

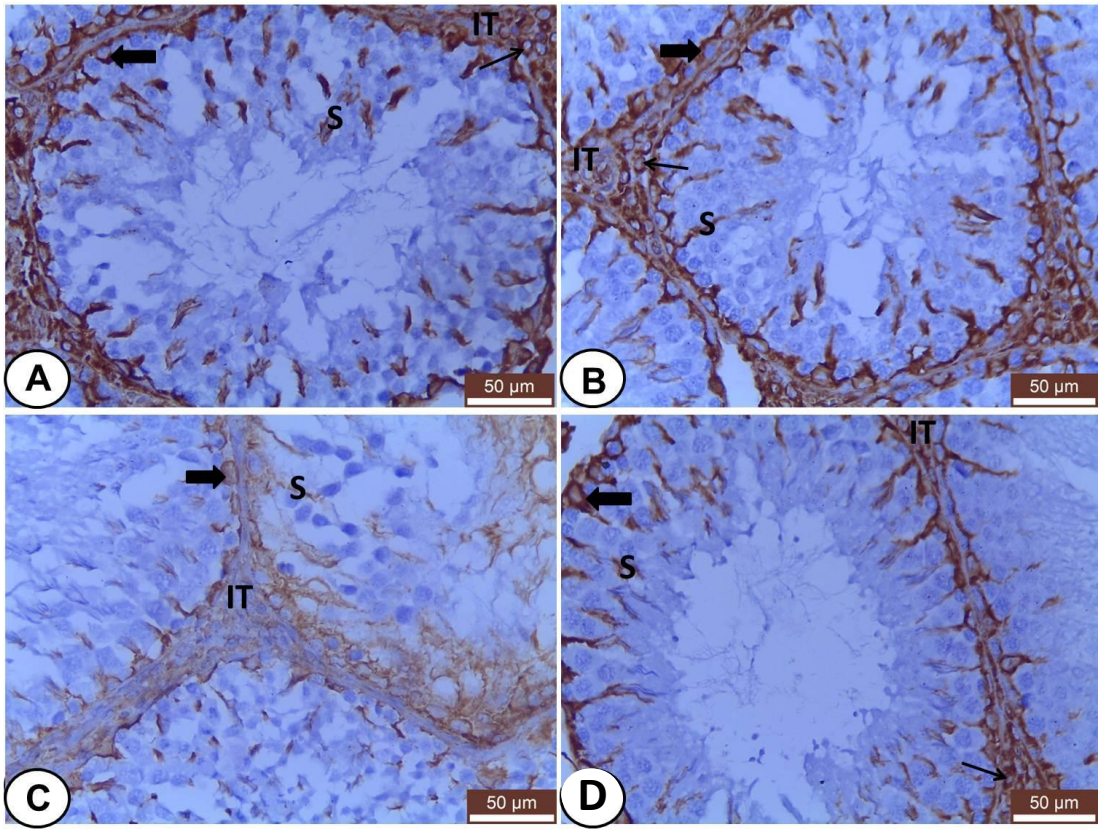

Figure 9:

Photomicrograph of VIM immunostaining in testicular sections from all examined groups. A and B; Control and LC groups showed strong VIM staining in spermatogonia, spermatozoa, and Leydig cells. C; CP group revealed weak and few positive VIM cells. D; LC+CP group showed the increased intensity of VIM positive cells. Leydig cells (thin arrow); interstitial tissue (IT). Spermatogonia (thick arrow), spermatozoa (S), and interstitial tissue (IT). Scale bars = 50µm

DISCUSSION

Most chemotherapeutics used for treating cancer induce toxicity and oxidative injury in different organs as testes **(Azarbarz *et al.*, 2020).** In the present work, CP significantly lowered serum testosterone levels. Such result could be explained as the Leydig cells dysfunction, which produce gonadotropin as well as decreasing the activity of both mitochondrial side-chain cleavage as well as cytochrome P_{450} **(García *et al.*, 2012).** Also, CP cause adverse effects on the function of Sertoli cells and lowers the androgen-binding protein expression. Furthermore, hormonal disorders caused by CP are mediated by its effects on the hypothalamic-pituitary-gonadal axis **(Almeer and Abdel Moneim, 2018).** A similar finding was recorded **(Afsar *et al.*, 2017; Almeer and Abdel Moneim, 2018; Azab *et al.*, 2020).** Moreover, CP-induced reduction in testosterone levels was significantly reverted by L-carnitine administration in the current work. The positive impact of L-carnitine on the level of testosterone may be explained as its anti-oxidative activity which counteracts the oxidative stress-induced Leydig cell damage **(Ghanbarzadeh *et al.*, 2014).**

In this study, CP considerably elevated MDA and depleted GSH, CAT, and SOD activities in the testicular tissue. A similar imbalance was recorded **(Anand *et al.*, 2015)** indicating that the levels of antioxidant enzymes were insufficient for eliminating free radicals produced by CP **(Azarbarz *et al.*, 2020).** Such reduction of antioxidant enzymatic molecules might be because of an uncontrollable generation of H_2O_2, which impairs antioxidant defense systems of the testis. Results of the present work come along with those of previous investigations **(Asfar *et al.*, 2017; Ekinci Akdemir *et al.*, 2019; Yadav, 2019).** However, treatment with LC counteracted the oxidative stress of testes and enhanced the testicular antioxidant defense system, representing that LC suppresses oxidative stress in testes **(Ghanbarzadeh *et al.*, 2014).** Also, LC reduces lipid availability for peroxidation through transporting fatty acids to the mitochondria for β-oxidation and consequently mitigates the production and accumulation of lipid peroxidation products **(Aboubakr *et al.*, 2020).** LC is a natural antioxidant acting as free radical scavenger **(Abdel Aziz RL *et al.*, 2018).** Furthermore, LC could regulate carbohydrate metabolism and preserve the structure

of the cell membrane, cellular vitality, and it is considered as an essential cofactor in the process of long-chain fatty acids oxidation **(Caloglu *et al.*, 2009).**

Histologically, the testicular specimens of both control and LC groups showed normal histo-architecture for the seminiferous tubules and interstitial tissues. Similar findings were reported **(Eid *et al.*, 2016; Aktoz *et al.*, 2017).** CP administration causes massive degeneration, cytoplasmic vacuolization, and reduction of spermatogenic cell layers, congestion of blood vessels, desquamation, and shedding of spermatogenic cells into the tubular lumen as well as edema of interstitial space. such findings were reported **(Almeer and Abdel Moneim 2018; Gevrek and Erdemir 2018; Prihatno *et al.*, 2018).** Such impairment of spermatogenesis might be due to a remarkable reduction of the testosterone level in addition to, the increased production of free radicals due to severe damages of Leydig cells **(Tousson *et al.*, 2014; Kaya *et al.*, 2015).** After administration of LC to CP treated group, structural improvement of the seminiferous tubules and interstitial tissue was observed indicating tissue repair which was similar to findings of **Eid *et al.*, (2016).** These findings could be attributed to the anti-oxidative property of LC that prevents oxidative-stress induced Leydig cell impairment; consequently, LC can restore testosterone level **(Ghanbarzadeh *et al.*, 2014).** Noteworthy, LC improves histopathological changes in the ipsilateral testis of albino rats **(Gawish *et al.*, 2011).** Also, **Ahmed *et al.*, (2014) and Yuncu *et al.*, (2015)** reported that LC prevents spermatic changes after CP exposure.

Immunohistochemically, low expressions of both CK18 and VIM in the testicular tissue after CP administration were observed in the current study. A similar finding is observed by **Prihatno *et al.*, (2018).** Additionally, this study reported the restoration of CK18 and VIM in the testicular tissue of the LC+CP group indicating the protective role of LC against CP induced testicular histopathological changes.

CONCLUSIONS

The present study revealed the adverse effect of CP on rat's testis as inflammation and structural alterations through induction of oxidative stress determined by increased generation of MDA and reduced activity of antioxidant enzymes. This is the first study, according to our knowledge, to investigate the immunohistochemical expressions of IFs proteins, VIM, and CK18, following administration of LC as a protective agent against CP induced testicular toxicity in rats. It is recommended to supplement LC to protect the testes against CP induced toxicity due to its antioxidant and anti-inflammatory properties.

4. GENERAL DISCUSSION

Most chemotherapeutics used for treating cancer induce toxicity and oxidative injury in different organs such as liver, kidneys and testes etc (**Azarbarz *et al*., 2020).** Thus, developing new solutions to ameliorate the induced toxicities become a global challenge (**Abuzinadah and Ahmad, 2020; Un *et al*., 2020).**

Hepatic and renal toxicity is the most common dose-limiting side effects of CP-induced chemotherapy **(Neamatallah *et al*., 2018).** Reducing the potential side effects of CP by LC can be helpful during chemotherapy. Elevated activities of liver enzymes are known to be markers of cellular leakage and loss of functional integrity of hepatocytes because they are released into the bloodstream when the hepatocyte plasma membrane is impaired **(Jia *et al*., 2018; Farid *et al*., 2019; Fadl *et al*., 2020).** In this work, CP-induced hepatotoxicity was evidenced by significant alternations in serum liver enzymes (AST, ALT, and ALP). This result may be attributed to the metabolism of CP. **Mohamed and Badawy, (2019)** reported CP is significantly taken up by the liver and accumulated in the hepatocyte, causing its damage leading to an increase of the liver enzymes activities. In addition, CP elevated creatinine and urea levels, which are confirmed by **Sadeghi *et al*., (2020)** and indicate nephrotoxicity induced by CP. On the other hand**, Cayir *et al*., (2009)** attributed the hepatotoxicity and nephrotoxicity induced by CP to the free radicals generation in the renal and hepatic cells results in lipid peroxidation and oxidative stress that responsible for cellular damage.

Cisplatin administration produced a significant decrease in the total proteins and albumin, this result is supported by the result of **Abuzinadah and Ahmad, (2020).** The above-mentioned results indicate disturbances in protein metabolism induced by CP intoxication due to a reduction in protein synthesis, following liver damage and alteration of functional integrity in the kidney leading to proteinuria, so, their plasma level decreases in hepatotoxic/nephrotoxic conditions **(Sen *et al*., 2013).**

Concerning oxidative stress/antioxidant parameters, the results of these parameters in the CP treated group is compatible with **El-Shitany and Eid, (2017) and Abd El-Kader and Taha, 2020, and Abdel-Razek *et al*., (2020)** in the liver and kidney tissues, respectively. These results own to oxidative stress that is mediated

through the generation of reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical and depletion in plasma antioxidant levels **(Fadl *et al.*, 2019; Abuzinadah and Ahmad, 2020)**. So, our results are in harmony with several experimental and clinical studies suggesting that the oxidative stress through the formation of free radicals is one of the mechanisms of CP-induced hepato-renal toxicity **(Ibrahim *et al.*, 2018; Neamatallah *et al.*, 2018; Abuzinadah and Ahmad, 2020).**

On the other side, LC is a natural nutrient and necessary for the oxidation of fatty acid in the mitochondria to produce ATP **(Tunez *et al.*, 2007).** So, it has antioxidant properties and plays protective roles against oxidative stress in various tissues, including liver and kidney **(Cayir *et al.*, 2009).** In this study, the result of the LC+CP treated group is in accordance with **Tunez *et al.*, (2007) and Aboubakr *et al.*, (2020),** who reported that LC reduces liver enzyme activity, oxidative stress, and damage caused by thioacetamide and tilmicosin in rats. Also, LC can improve the antioxidant enzyme activity, including CAT and GSH, and reduces the MDA concentration in renal tissues in acute renal failure induced by myoglobinuric in rats **(Aydogdu *et al.*, 2006)**. These results were confirmed by the results of histopathology. The ability of LC to significantly improve liver and kidney biochemical parameters may be due to its antioxidant effect and its capability to act as a free radical scavenger, leading to the protection of membrane permeability **(Augustyniak and Skrzydlewska, 2009)**. LC prevents oxidative stress and exerts a protective role against mitochondrial toxic agents **(Barhwal *et al.*, 2007).** Additionally, they reduce the harmful effects of free fatty acids by enabling β-oxidation **(Furuno *et al.*, 2001).**

The histological and immunohistochemical observations of the current study are in harmony and confirmed the alterations of the biochemical and oxidant/antioxidant parameters among the experimental groups. Several histological changes in the liver among CP treated group were noted as recently mentioned by **Abuzinadah and Ahmad, (2020).** The central vein and sinusoidal dilatation and congestion are in accordance with **Cagin *et al.*, (2015) and Omar *et al.*, (2016).** Also, swelling of hepatocytes, hydropic degeneration, and prominence of Kupffer cells are demonstrated in similarity to **El-Shitany and Eid, (2017) and Neamatallah *et al.*,**

(2018). In addition, fatty infiltration with signet ring appearance in some hepatocytes was seen as noted by **Omar *et al.*, (2016) and El-Shitany and Eid, (2017).** Otherwise, the pretreatment of CP treated rats with LC can potentially protect the liver against CP-induced histological changes and significantly improve and normalize liver histology that represents by almost normal hepatocytes and sinusoids, but with mild congested central vein with regression of the fatty changes. These findings are similar to **El-Shitany and Eid, (2017),** indicating that LC can attenuate the hepatotoxic effect of CP **(Cayir *et al.*, 2009).**

Like the recent finding of **Abd El-Kader and Taha, (2020); Abdel-Razek *et al.*, (2020) and Sadeghi *et al.*, (2020),** the current study exhibited many distinguishing degenerative changes in the kidney of the CP group including degeneration and desquamation of the tubular epithelium, congestion, and dilatation of interstitial blood vessels and capillaries as reported by **Neamatallah *et al.*, (2018).** Moreover, the presence of eosinophilic hyaline casts in some renal tubules agrees with **Abd El-Kader and Taha, (2020) and Abdel-Razek *et al.*, (2020).** Additionally, the deformity of some glomeruli with a widening of glomerular spaces was detected in accordance with **Abd El-Kader and Taha, (2020).** On the other hand, LC administration ameliorated the histological effects of CP on the kidney, but with mild histological findings evidenced by tubular injury in some renal tubules, and minimal interstitial congestion. This finding confirmed that LC could attenuate the nephrotoxic effect of CP **(Yürekli *et al.*, 2011).**

The current study focused on immunohistochemical localization of VIM and CK18 in hepato-renal specimens since VIM and CK18 expressions in the liver and kidney had provided a valuable insight into their microanatomy in both healthy and diseased conditions. A co-expression of VIM and CK in areas of damaged tissues was reported **(Moll *et al.*, 1991; Stefanovic *et al.*, 1996).**

Concerning VIM protein, it has been linked with several pathophysiological conditions such as cancer, rheumatoid arthritis, and HIV **(Danielsson *et al.*, 2018).**

The detectable VIM in hepatic sinusoids of the present study confirmed the report of Evans (1998) where VIM is widely expressed IF proteins in endothelial cells and fibroblasts. According to **Wang *et al.*, (2017),** VIM expression in the hepatic

sinusoids may reflect its regulatory role of hepatic sinusoidal flow. At the beginning of this decade, both **Golbar *et al.*, (2011) and Aiad *et al.*, (2012)** regarded the sinusoidal VIM expression as hepatic stellate cells (HSCs), which are normally localized at the space of Disse. Moreover, Kupffer cells in the current study were VIM positive as mentioned by **Sharifi *et al.*, (2000) and Golbar *et al.*, (2011).** After the injection of CP, an overexpression of VIM in the liver was demonstrated in similarity to the lipopolysaccharide intoxicated liver **(Lee *et al.*, 2014).** The numbers of VIM positive Kupffer cells were increased **(Golbar *et al.*, 2011; Aiad *et al.*, 2012),** and some individual hepatocytes became VIM positive **(Aiad *et al.*, 2012).** As well as, sinusoidal VIM expression was increased in response to CP toxicity where HSCs are activated into myofibroblasts, which characterize by higher VIM expression and more secretion of the extracellular matrix so, they consider as the major contributor to hepatic fibrosis **(Shang *et al.*, 2018).**

In the normal and diseased kidney, VIM is abundantly present in glomerular mesangial and epithelial cells **(Gonlusen *et al.*, 2001; Matos *et al.*, 2007).** Our study revealed VIM expression in the glomeruli of all examined rats confirming the above-mentioned data. In addition, VIM was expressed in the interstitial blood vessels and fibroblasts of all examined rats that agree with **Stefanovic *et al.*, (1996) and Sen *et al.*, (2010)** suggesting that the pivotal regulating role of the renal interstitium for vessels-tubules interaction as well as its involvement in the etiology of renal pathologies **(Becker and Hewitson, 1997).** It was interesting that the current results revealed undetectable VIM in the renal tubular epithelium of the control rats that agree with **Skinnider *et al.*, (2005) and Sen *et al.*, (2010).** Otherwise, the overexpression of VIM in renal tubules and interstitial tissue of the CP group was noted may be attributed to glomerulonephritis or tubule-interstitial injury **(Gonlusen *et al.*, 2001; Matos *et al.*, 2007)**, respectively.

Cytokeratins of hepato-renal tissues increase in response to toxicants, oxidative stress, inflammation, and other damaging insults **(Toivola *et al.*, 2010)**. Hepatocytes, as one of the epithelial cells of the liver, have been known to express CKs **(Ku *et al.*, 2007).** The hepatocytes of both control and LC treated rats showed faint CK18 at the cell periphery forming reticular staining patterns that agree **with Zatloukal *et al.*, (2004).** Upon injury by CP, the intoxicated hepatocytes exhibited strong, dense, and

clumped CK18 staining that agrees with **Omary and Coulumbe, (2004).** Moreover, immunohistochemical overexpression of CK18 in hepatocellular carcinoma was noted **(Sawan, 2009).** These findings point to the structural role of CK18 to hepatocytes providing them mechanical stability. Another role of CK18 is a target and modulator of toxic stress **(Zatloukal *et al.*, 2004)** therefore; the current study reported that the CK18 expression was significantly upregulated by oxidative stress induced by CP. That confirmed CK-18 levels as a predictor for hepatitis progression **(Yang *et al.*, 2015).**

Cytokeratins are often used as disease markers in renal pathology and experimental research **(Djudjaj *et al.*, 2016).** Expression of CK18 in the parietal cells of Bowman's capsule as well as the different segments of renal tubules of the normal rats agree with the finding of **Stefanovic *et al.*, (1996) and Djudjaj *et al.*, (2016).** CK18 staining was stronger in the collecting ducts as mentioned by **Sen *et al.*, (2010).** Like **Snider, (2016),** our finding showed a pronounced overexpression of CK18 in the damaged renal tubules in CP treated group indicating that CK18 was upregulated by tubular injury. Therefore, CK18 can be used as a marker and regulator of renal tubular epithelial injury **(Djudjaj *et al.*, 2016).**

It was noteworthy to record that the pretreatment of LC could decrease expressions of VIM and CK18 in hepato-renal tissues compared with CP group indicating the ameliorative role of LC against CP toxicity, especially in restoring the organization of IFs.

In addition, most chemotherapeutics used for treating cancer induce toxicity and oxidative injury in testes also **(Azarbarz *et al.*, 2020).**

In our study, CIS administration remarkably decreased testosterone hormone level. This can be because of interference of CIS with luteinizing hormone (LH) receptor expression, dysfunction of Leydig cells **(Fallahzadeh *et al.*, 2017, Singh *et al.*, 2017)**, which produce gonadotropin due to deformed cell membranes as a result of increased ROS levels and decrease activity of mitochondrial side-chain cleavage activity and inhibition of cytochrome P-450 **(Beytur *et al.*, 2012; García *et al.*, 2012)**. Moreover, CIS negatively affects the function of Sertoli cells and reduces the expression of androgen-binding proteins. Actually, hormonal disorders induced by

CIS are mediated by its impacts on the hypothalamic-pituitary gonadal **axis (Almeer and Abdel Moneim, 2018).** A similar finding was recorded **(Afsar *et al*., 2017; Almeer and Abdel Moneim, 2018; Azab *et al*., 2020)**. These negative impacts of cisplatin may be a cause of infertility in males undergoing chemotherapy However; it could be reverted by L-carnitine administration in the current work. The positive impact of L-carnitine on the level of testosterone may be explained as its anti-oxidative activity which counteracts the oxidative stress-induced Leydig cell damage **(Ghanbarzadeh *et al*., 2014).**

In this study, CP considerably elevated MDA and depleted GSH, CAT, and SOD activities in the testicular tissue. This imbalance indicating that the levels of antioxidant enzymes were insufficient for eliminating free radicals produced by CP **(Azarbarz *et al*., 2020).** As CP disturbs oxidant / antioxidant balance of the testicular tissue **(Anand *et al*., 2015)** as a result of massive generation of ROS and reduced generation or activity of antioxidant enzymes **(Aksu *et al*., 2016; Ekinci Akdemir *et al*., 2017; Sadeghi *et al*., 2018).** In this research, CP considerably elevated MDA and depleted SOD, CAT, and GSH-Px activities in the testes tissue, representing that the molecules of enzymatic antioxidant were insufficient for scavenging free radicals generated by CP. MDA acts as an indicator for oxidative stress which is the end product of free radical initiated lipid peroxidation of polyunsaturated fatty acids **(Chirino and Pedraza-Chaverri, 2009)**. MDA can cause cross-linked polymerization of macromolecules such as proteins and nucleic acids, which results in cause cytotoxicity **(Han *et al*., 2014).** On the other hand SOD acts as the first preventive antioxidant enzyme, which scavenges singlet oxygen (1 O2) and spontaneously dismutates superoxide radicals (O− 2) to H2O2. Then H2O2 is effectively decomposed by CAT, thus results in suppressing lipid peroxidation. GSH-Px along with GSH catalyzes depletion of H2O2 and lipid peroxides **(Zhao *et al*., 2014).** Reduction of these antioxidant enzymes and molecules may be related with an uncontrollable accumulation of H2O2, which inhibits antioxidant defense systems of testis inducing gentotoxicity. Results of the present work come along with those of previous investigations **(Asfar *et al*., 2017; Ekinci Akdemir *et al*., 2019; Yadav, 2019).** However, treatment with LC counteracted the oxidative stress of testes and enhanced the testicular antioxidant defense system, representing that LC suppresses oxidative stress in testes **(Ghanbarzadeh *et al*., 2014).** Also, LC reduces lipid

availability for peroxidation through transporting fatty acids to the mitochondria for β-oxidation and consequently mitigates the production and accumulation of lipid peroxidation products **(Aboubakr *et al.*, 2020).** LC is a natural antioxidant acting as free radical scavenger **(Abdel Aziz *et al.*, 2018).** Furthermore, LC could regulate carbohydrate metabolism and preserve the structure of the cell membrane, cellular vitality, and it is considered as an essential cofactor in the process of long-chain fatty acids oxidation **(Caloglu *et al.*, 2009).**

Histologically, CP administration causes reduction in diameter and numbers of seminiferous tubules, massive degeneration, cytoplasmic vacuolization, and reduction of spermatogenic cell layers, congestion of blood vessels, desquamation, and shedding of spermatogenic cells into the tubular lumen as well as edema of interstitial space. Such findings were reported in previous studies **(Fallahzadeh *et al.*, 2017; Almeer and Abdel Moneim, 2018; Gevrek and Erdemir, 2018; Prihatno *et al.*, 2018).**

Such impairment of spermatogenesis might be due to a remarkable reduction of the testosterone level in addition to, direct and indirect impact of CIS. Direct impact of CIS trough interaction with damaged DNA synthesis in stem cells and Sertoli cell dysfunction **(Nambu and Kumamoto, 1995)**, in addition to the increased production of free radicals that induced severe damages of Leydig cells **(Tousson *et al.*, 2014; Kaya *et al.*, 2015).** Another indirect impact occurred as a result of induction of lipid peroxidation which is a chemical mechanism that can impair testicular structure and function.

Thus might be the cause of necrosis, intense degeneration and finally the declines in the diameter of seminiferous tubules and thickness of germinal cell layer. After administration of LC to CP treated group, structural improvement of the seminiferous tubules and interstitial tissue was observed indicating tissue repair which was similar to findings of **(Eid *et al.*, 2016).** Such findings could be attributed to the anti-oxidative property of LC that prevents oxidative-stress induced Leydig cell impairment; consequently, LC could restore testosterone level **(Ghanbarzadeh *et al.*, 2014)**. Noteworthy, LC improves histopathological changes in the ipsilateral testis of albino rats **(Gawish *et al.*, 2011).** Also, **Ahmed *et al.*, (2014) and Yuncu *et al.*, (2015)** reported that LC prevents spermatic changes after CP exposure.

Immunohistochemically, low expressions of both CK18 and VIM in the testicular tissue after CP administration was observed in the current study.There are three major cytoskeletal proteins: Microfilament, microtubules and intermediate filament. Vimentin and cytokeratin that were observed in this study are intermediate filaments.In normal condition, during regeneration and developmental stages vimentin was detected in both fetuses and adults sertoli cells **(Paranko *et al.*, 1986; Frojdman *et al.*, 1992; Rogatsch *et al.*, 1996; Sasaki *et al.*, 1998)**.The maturation of Sertoli cells was supported by the presence of vimentin in the cells. It is also implemented in membrane formation, which is known as the desmosome-like, that responsible for connection of Sertoli cells with the adjacent germinal cells in the seminiferous tubules. Its distribution depends on the cycle of spermatogenesis; also they would be distributed along with the head of the sperms. Vimentin also implemented in maintenance of the integrity of the spermatogenic cells and their connection as well **(Kume *et al.*, 2017).** Previous studies also reported that vimentin was also found in the Leydig cells of mature and /or immature testes **(Gerber, 2015).**thus very strong vimentin immunoreactivity indicated a regenerative process. Cytokeratin in rats was observed in the Sertoli, spermatogenic and Leydig cells **(Paranko *et al.*, 1986; Frojdman *et al.*, 1992; Rogatsch *et al.*, 1996; Sasaki *et al.*, 1998).** Cytokeratin was found in the Sertoli cells during the development stage only. However, it was noted during the pre-meiotic, meiotic, and post-meiotic stages **(Kierszenbaum *et al.*, 1996).**The dynamic increase and decrease in the immunoreactivity of vimentin and cytokeratin might be caused by high inflammation reaction by endogenous cytokines.A similar finding is observed by **Prihatno *et al.*, (2018).** Additionally, this study reported the restoration of CK18 and VIM in the testicular tissue of the LC+CP group indicating the protective role of LC against CP induced testicular histopathological changes.

Conclusions

From the present study it was concluded that:

- Extensive utilization of chemotherapeutics as Cisplatin, (cis-diamminedichloroplatinum (II), (CP)), induces multiple organ toxicity that is sometimes irreversible and mostly affects the vital organs causing death in many cases.
- L-carnitine is an important water-soluble quaternary amine (βhydroxy-γ-trimethylaminobutyrate); non-protein amino acid that has a potent anti-inflammatory and anti-oxidant property.
- L-carnitine has been shown to be effective against Cisplatin induced toxicity as it showed significant improvement in biochemical, oxidative damage, tissue histological and immunohistochemical alterations in liver, kidney and testis.
- L-carnitine brings all the parameters altered by cisplatin near again to normalcy.
- L-carnitine supplementation for cancer patients counteracts Liver, Kidneys and testes toxicity induced by CP due to its both potent antioxidant and anti-inflammatory properties, thereby suggesting its use as a potent hepatic, nephro and testicular protective agent.

So, we recommend cancer patients treated with chemotherapy to take L-carnitine as concurrent treatment to avoid and ameliorate the possible induced organ injury associated with chemotherapy treatment.

5. Summary

The purpose of this study was to investigate the protective effects of LC administration on CP-induced hepato-renal and testicular injuries in rats via investigating some serum biochemical and tissue oxidative/antioxidant parameters. Both histological alterations and immunohistochemical expressions of VIM and CK18 proteins were also carried out. Twenty-eight male albino rats weighing 175-195 gm. were randomly assigned into 4 equal groups (7 rats each).

Group (I): served as control and it includes seven rats, they were orally administered saline (the vehicle) once daily for 30 consecutive days.

Group (II): received LC (100 mg/kg body weight), orally once daily for 30 consecutive days.

Group (III): served as CP toxic control and received saline orally once daily and a single dose of CP on the 27th day of the experiment (7.5 mg/kg, IP).

Group (IV): received both LC (100 mg/kg body weight), orally once daily for 30 consecutive days and a single dose of CP on the 27th day of the experiment (7.5 mg/kg, IP).

In this study, CP injection significantly increased serum ALT, AST, and ALP activities compared with those in control rats. Similarly, CP significantly increased the levels of creatinine and urea. Also, CP significantly decreased the level of serum testosterone level when compared to those of the other groups. Conversely, serum total protein and albumin were significantly decreased due to CP injection compared to that in control rats. LC administration with CP restored these parameters towards the normal values.

In the present study, there were substantial increases in MDA level along with dramatic decreases in GSH and CAT in the liver, kidney and testicular tissues of CP-intoxicated rats. Meanwhile, LC+CP administrated group revealed a decrease in MDA level along with elevations in GSH and CAT in hepatic and renal tissues compared with CP treated group.

Histologically, liver sections from control and LC administered rats exhibited normal hepatic histo-architecture. Hepatocytes organized in cords radiating from the central vein and separated by regular sinusoids. Otherwise, CP treated rats revealed several histological changes like dilatation of the central vein and sinusoids, inflammatory cells aggregation, kupffer cells proliferation, swelling of hepatocytes, hydropic degeneration, and fatty infiltration with signet ring appearance in some hepatocytes. The liver section from LC+CP treated rats represented almost normal hepatocytes and sinusoids, but mild congested central vein and no signs of fatty changes were noted.

Histologically, Kidney sections from both control and LC groups showed regular renal histo-architecture with normal renal corpuscles and renal tubules; proximal (PCT) and distal convoluted tubules (DCT) and collecting (CT) tubules. In CP group, many distinguishing histological changes were noted including an excessive degenerative changes and desquamation of the tubular epithelia were observed with the presence of eosinophilic hyaline casts in some tubules. Also, deformity of some golumeruli with widening of glomerular space was identified as well as, congestion of peritubular blood vessels and capillaries. However, kidney from CP+LC group, revealed a mild tubular degeneration with minimal interstitial congestion.

Histologically, testicular specimens from both control and LC groups revealed normal histo-architecture of the seminiferous tubules and interstitial tissues. Normal arrangements of spermatogenic cells and Leydig cells were seen. Meanwhile, CP treated group showed massive degeneration in some seminiferous tubules, cytoplasmic vacuolization, reduction of germ cell layers, congestion of blood vessels in other tubules, desquamation, and shedding of spermatogenic cells into tubular lumen as well as widening of interstitial space with eosinophilic edema material. However, LC+CP treated group showed some improvements in the histological structure of both seminiferous tubules and interstitial tissues.

Immunohistochemically, a summary of VIM and CK18 immunohistochemical expressions in the livers and kidneys of all examined groups was recorded.

Regarding VIM expression, in liver, both control and LC rats expressed weakly to moderate VIM mainly in the hepatic sinusoids and Kupffer cells but the hepatocytes were VIM negative. Meanwhile, CP-injected rats showed overexpression of VIM in the blood sinusoids and an increased number of Kupffer cells as well as, some hepatocytes labeled weak VIM. But, the LC+CP group showed moderate expression of sinusoidal VIM and fewer Kupffer cells compared with the CP group.

In kidneys, VIM was expressed mainly in the glomeruli, some peritubular blood capillaries, and interstitial fibroblasts of all experimental groups. In both control and LC groups, the renal tubules did not express VIM protein but, the interstitial tissues and glomeruli showed weak and moderate VIM staining, respectively. Otherwise, overexpression of VIM was seen in the damaged renal tubules and interstitial tissues of the CP treated group that tended to be decreased in the interstitium and renal tubules of LC+CP group compared with CP group.

In testis, strong VIM staining was observed in spermatogonia, spermatozoa, and Leydig cells in both control and LC treated group, but CP treated group revealed weaker response to VIM staining compared of that control and LC groups. VIM staining nearly returned to normalcy in LC+CP treated group compared with that of CP treated group.

Regarding CK 18 expression, hepatocytes of both control and LC rats showed weak CK18 staining at the periphery of the cells giving reticular appearance. CP group revealed strong, dense, and clumped CK18 staining in the hepatocytes surrounding the central veins, fat cells, and triad area. But, the LC+CP group showed lower CK18 immunostaining compared with the CP group.

Regard to kidneys, both control and LC groups demonstrated weak to moderate CK18 immunostainings in the visceral cells layer of Bowman's capsule as well as the cells of different segments of renal tubules; PCT, DCT, and CT. However, CK18 was overexpressed in the epithelial cells of renal tubules mainly PCT and few DCT after injection of CP. Meanwhile, the LC+CP group showed a lower expression of CK18 compared with the CP group.

Regarding the testis, most of the Leydig cells in both control and LC groups showed moderate CK18 immunolabeling. While very weak CK18 immunolabeling

was seen in few Leydig cells of the CP treated group. An increase in the number and intensity of CK18 positive Leydig cells was identified in LC+CP treated group when compared with that of the CP group.

From this study, it could be concluded that LC supplementation for cancer patients counteracts liver, kidneys and testes toxicity induced by CP due to its both antioxidant and anti-inflammatory properties.

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VITA

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Appendix 1 I

1.1 Tested substances:

Cisplatin: it was obtained from (EIMC United Pharmaceuticals, Egypt). Each vial (50mg/50ml) was dissolved in physiological saline (0.9% sodium chloride).

L-carnitine: it was obtained from MEPACO Company (Inshas Elraml, Egypt).

1.2 Experimental animals:

The present study was carried out on a total number of 28 white albino male rats male rats weighing 175-195 gm. Rats were obtained from the Center of Laboratory Animal, Faculty of Veterinary Medicine, and Benha University, Egypt. They acclimatized for two weeks prior to the experiment. All rats received standard laboratory balanced commercial diet and water ad libitum.

1.3 Kits used for biochemical study

Commercial diagnostic kits were used for estimation of different biochemical parameters:

- The diagnostic kits for estimating alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, total protein and albumin, in plasma, were supplied from Centronic GmbH Company, Am Kleinfeld, Wartenberg, Germany.
- The diagnostic kits for estimating lipid parameter, cholesterol, triglycerides, LDL and HDL in plasma were supplied from BioDiagnostic Company, Dokki, Giza, Egypt.
- The diagnostic kits of estimating oxidative stress markers, Malondialdehyde (MDA), superoxide dismutase (SOD) and Catalase (CAT) in liver tissue homogenate were also from BioDiagnostic Company, Dokki, Giza, Egypt.

1.4 Reagents used for oxidative stress assessment in liver tissue:

1. Phosphate buffered saline (PBS) solution, pH 7.4 containing 0.16 mg/ml heparin.
2. Cold buffer (100 ml potassium phosphate, pH 7.0, containing 2 ml EDTA per gram tissue).

1.5 Chemicals used for histopathological examination:

1. Formalin (10 %): from Middle East Company, Cairo, Egypt.

2. Hematoxylin and Eosin (H&E) stain: from Middle East Company, Cairo, Egypt.

1.6 Chemicals used for immunohistochemistry Examination:

2 Formalin (10 %): from Middle East Company, Cairo, Egypt.
3 DAB, citrate buffer saline, H2O2 and Mayer's hematoxylin.
4 Rabbit monoclonal anti-vimentin and anti-cytokeratin 18, Abcam, Boston, the USA at 1:200 dilution.
5 Biotinylated donkey anti-mouse IgG (Abcam, Boston, USA)
6 ABC kit (Santa Cruz Biotech, CA, USA).

1.7 Other standard laboratory chemicals and solutions were also used as: 70% hydroethanolic alcohol, distilled water, normal saline solution (sodium chloride 0.9 %) … etc.

1.8 Laboratory equipment:

1.8.1. Apparatus for serum Biochemical studies:

1- Spectrophotometer, JASCO 7800, un/vis, JAPAN.

2- Clean and dry Eppendorf labeled tubes for serum preservation.

1.8.2 Apparatus for Oxidative cascade:

1- Sonicator homogenizer (COLOMBIA INTERNATIONAL)

2- Clean and dry Eppendorf labeled tubes for liver, kidney and testis tissues preservation.

3- Cooling centrifuge heraeus, W. GERMANY.

4- Refrigerator for preservation of samples.

1.8.3 Apparatus for histological examination:

1- Slide microtome.

2- Light microscope: NOVEL, model XSZ-N107-1.

18.4 Apparatus for immunohistochemistry imaging:

1- Slide microtome.

2- Light microscope: NOVEL, model XSZ-N107-1.

Publications

1- Paper 1

Title: L-carnitine mitigates oxidative stress and disorganization of cytoskeleton intermediate filaments in cisplatin induced hepato-renal toxicity in rats

Authors: Ashraf Elkomy, Mahmoud Abdelghaffar Emam, Fatma Abdel Monem Gad, Adham Sallam, Sabreen Ezzat Fadl, Ehab Yahya Abdelhiee, Mohamed Aboubakr

Journal name: Frontiers in Pharmacology

Volume and pages: doi: 10.3389/fphar.2020.574441.

2- Paper 2

Title: Ameliorative effects of L-carnitine against cisplatin-induced gonado-toxicity in rats

Authors: Adham Omar Sallam, Hanan A. Rizk, Mahmoud Abdelghaffar Emam, Sabreen Ezzat Fadl, Ehab Yahya Abdelhiee, Hanem Khater, Ashraf Elkomy, Mohamed Aboubakr.

Journal name: Pakistan Veterinary Journal

Volume and pages: doi.org/10.29261/pakvetj/2020.082

الملخص العربي

استهدفت هذه الدراسة معرفة التأثيرات الوقائية لإعطاء ال ل-كارنتين على سمية الكبد والكلى والخصية التي يسببها عقار السيسبلاتين في الجرذان. وذلك من خلال فحص بعض العوامل البيوكيميائية في الدم وعوامل الأكسدة / المضادة للأكسدة في الأنسجة. أيضًا، كل من التغيرات الهستولوجيه والتغيرات الهستوكيميائية المناعية لبروتينات فايمنتين وسيتوكيراتين(VIM و CK18).

في هذه الدراسه تم اجراء التجربه على ثمان وعشرين ذكرا من الجرذان البيضاء من نوع الألبينو تتراوح اوزانها ما بين (175-195 جم). وقد تم تقسيم الجرذان الي أربع مجموعات متساوية (7 جرذان لكل مجموعة).

المجموعة الاولي (الضابطه): تم إعطائها محلول ملحي عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا.

المجموعة الثانيه: تم إعطائها (100 مجم إل-كارنتين / كجم من وزن الجسم) عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا

المجموعة الثالثه: لملاحظه التسمم للسيسبلاتين وتم اعطاءها محلول ملحي عن طريق الفم مرة واحدة يوميًا وجرعة واحدة من ال سيسبلاتين في اليوم السابع والعشرين من التجربة (7.5 مجم / كجم، عن طريق الحقن البروتوني).

المجموعة الرابعه: تم اعطائها كل من) 100مجم إل-كارنتين / كجم من وزن الجسم)، عن طريق الفم مرة واحدة يوميًا لمدة 30 يومًا متتاليًا وجرعة واحدة من السيسبلاتين في اليوم السابع والعشرين من التجربة (7.5 مجم / كجم، عن طريق الحقن البروتوني).

أدى حقن السيسبلاتين في هذه الدراسة إلى زيادة كبيرة في أنشطة انزيم الالنين امينو ترانسفيريز واسبرتات امينو ترانسفيريز وانزيم الفوسفاتيز القلوي في الدم مقارنة بتلك الموجودة في الجرذان الضابطة. وبالمثل، زاد السيسبلاتين بشكل ملحوظ كل من مستويات الكرياتينين واليوريا. أيضًا، أدي حقن السيسبلاتين الي انخفاض ملحوظ في مستوى هرمون التستوستيرون في الدم بالمقارنة مع المجموعات الأخرى. على العكس من ذلك، انخفض البروتين الكلي والألبومين في الدم بسبب حقن السيسبلاتين مقارنة بالفئران الضابطة. اعطاء عقار إل-كارنتين مع عقار سيسبلاتين أدي الي عودة هذه المعدلات نحو قيمها العادية.

و كانت هناك زيادات كبيرة في مستوى المالونالدهيد(MDA) إلى جانب انخفاض كبير في جلوتاثيون والكتاليز(GSH وCAT) في خلايا الكبد والكلى والخصية للفئران التي تعاني من تسمم ال سيسبلاتين وفي الوقت نفسه ، المجموعة التي تم اعطاؤها إل-كارنتين مع السيسبلاتين ظهر فيها انخفاض في مستوى المالونالدهيد (MDA) جنبًا إلى جنب مع ارتفاع في جلوتاثيون والكتاليز(GSH وCAT) في خلايا الكبد والكلى مقارنة بالمجموعة المعالجة بـسيسبلاتين.

اما بالنسبه للتغيرات الهستولوجيه للكبد من الفئران المعالجة ب إل-كارنتين فقد اظهرت خلايا كبد طبيعية. تنتظم خلايا الكبد في حبال تشع من الوريد المركزي وتفصل بينها جيوب دموية طبيعية، وبخلاف ذلك أظهرت الفئران المعالجة بالـسيسبلاتين عدة تغيرات نسيجية مثل اتساع الوريد المركزي و الجيوب الدموية وتجمع الخلايا الالتهابية وتكاثر خلايا كوبفر(kupffer) وتضخم الخلايا الكبدية والتنكس المائي والدهني مع ظهور حلقة الخاتم في بعض خلايا الكبد.

اما بالنسبة للتغيرات ف الكبد لمجموعه الفئران المعالجة بـ إل-كارنتين مع السيسبلاتين اظهرت خلايا كبدية وأكياس دموية طبيعية تقريبًا، ولكن تم ملاحظة احتقان خفيف في الوريد المركزي ولم يلاحظ أي علامات على حدوث التنكس الدهني بها.

أما بالنسبه للتغيرات الهستولوجية في الكلى في كل من المجموعتين الضابطة والمجموعه المعالجه ب إل-كارنتين، اظهرت خلايا كلوية طبيعيه ،مع كبيبة كلوية ، قنوات قريبة ، قنوات بعيدة ، و قنوات جامعة طبيعية.

في المجموعة المعالجه ب بالـسيسبلاتين، لوحظت العديد من التغيرات الهستولوجيه المميزة متضمنة تغيرات مفرطة وتقشر في الخلايا الانبوبية مع وجود قوالب الهيالينية ايوزينية في بعض الأنابيب. كما تم تحديد تشوه في بعض الكبيبات الكلوية مع اتساع المسافه بين الكبيبيات وكذلك احتقان الأوعية الدموية والشعيرات الدموية حول الأنبوب.

اما بالنسبه للمجموعه المعالجه بـ إل-كارنتين + السيسبلاتين لوحظ تنكس بسيط في الانابيب مع الحد الأدنى من الاحتقان الخلالي.

اما بالنسبه للتغيرات الهستولوجية في الخصية من كلا المجموعتين الظابطة والمعالجة ب إل-كارنتين فقد اظهرت خلايا طبيعيه وترتيب طبيعي للأنابيب المنوية والخلايا المنوية وأيضا ترتيب طبيعي للخلايا المولدة للحيوانات المنوية وخلايا لايدج.

اما بالنسبه للمجموعة المعالجة بالـسيسبلاتين لوحظ تغير واضح في بعض الأنابيب المنوية، وخلايا فارغه من السيتوبلازم، وتقليل طبقات الخلايا الجرثومية، واحتقان الأوعية الدموية في الأنابيب الأخرى، وانفصال الخلايا المولدة للحيوانات المنوية في التجويف الأنبوبي بالإضافة إلى اتساع الحيز الخلالي مع تجمع مواد الوذمة اليوزينية.

اما بالنسبه للمجموعة المعالجة بـ إل-كارنتين مع السيسبلاتين فلقد لوحظ بعض التحسن في التركيب الهستولوجي لكل من الأنابيب المنوية والأنسجة الخلالية.

من الناحية الهستوكيميائية المناعية، تم تسجيل ملخص لتعابير فايمنتين و سيتوكيراتين (VIM و CK18) المناعية في الكبد والكلى لجميع المجموعات التي تم فحصها.

فيما يتعلق بتعبير فايمنتين (VIM) ، في الكبد ، أظهر كل من الفئران الضابطة و المعالجة بـ إل-كارنتين فقد ظهر بشكل ضعيف إلى معتدل بشكل رئيسي في الجيوب الكبدية وخلايا كوبفر(Kupffer) ولكن خلايا الكبد كانت سلبية التعبير ل فايمنتين (VIM). وفي الوقت نفسه، أظهرت الفئران المحقونة بالسيسبلاتين زيادة في التعبير ل فايمنتين (VIM) في الجيوب الكبدية الدموية ، وزيادة عدد خلايا كوبفر (kupffer) وكذلك بعض خلايا الكبد ذات التعبير الضعيف ل فايمنتين(VIM). ولكن، أظهرت مجموعة المعالجة بـ إل-كارنتين مع السيسبلاتين تعبيرًا معتدلًا عن فايمنتين (VIM) في الجيوب الدموية وفي عددًا أقل من خلايا كوبفر (kupffer) مقارنة بمجموعة ال سيسبلاتين.

في الكلى، تم التعبير عن VIM بشكل رئيسي في الكبيبة، وبعض الشعيرات الدموية حول النبيبات ، والأورومات الليفية الخلالية لجميع المجموعات التجريبية. في كلتا المجموعتين الضابطة و المعالجة بـ إل-كارنتين ، لم تعبر الأنابيب الكلوية عن بروتين فايمنتين (VIM) ولكن ، أظهرت الأنسجة الخلالية والكبيبات تصبغ ب فايمنتين (VIM) ضعيفًا ومتوسطًا ، على التوالي. بخلاف ذلك، شوهد الإفراط في التعبير عن فايمنتين (VIM) في الأنابيب الكلوية التالفة والأنسجة الخلالية للمجموعة المعالجة بالسيسبلاتين التي تميل إلى الانخفاض في الأنابيب الكلوية والانسجة الخلاليّة للمجموعة المعالجة بـ إل-كارنتين مع السيسبلاتين مقارنة مع مجموعة المعالجة بسيسبلاتين .

في الخصية، لوحظ تصبغ قوي ب فايتمنين (VIM) في الحيوانات المنوية ، والخلايا المولدة للحيوانات المنوية ، وخلايا لايدج في كل من المجموعة الضابطة ومجموعة المعالجة ب إل-كارنتين ، لكن المجموعة المعالجة بالسيسبلاتين أظهرت استجابة أضعف لتصبغ فايتمنين(VIM) مقارنة بمجموعات الظابطة والمعالجة ب إل-كارنتين .عاد تصبغ فايتمنين (VIM) تقريبًا إلى طبيعته في المجموعة المعالجة بـ إل-كارنتين مع ال سيسبلاتين مقارنة مع المجموعة المعالجة ب سيسبلاتين منفردا.

فيما يتعلق بتعبيرسيتوكيراتين (CK 18) ، أظهرت الخلايا الكبدية لكل من جرذان المجموعة الضابطة والفئران المعالجة ب إل-كارنتين تلطيخًا ضعيفًا لـ سيتوكيراتين (CK 18) في المحيط الخارجي الخلايا مما يعطي مظهر شبكي.كما اظهرت المجموعة المعالجة بالسيسبلاتين عن تصبغ سيتوكيراتين (CK 18) قويًا وكثيفًا ومتكتلًا في خلايا الكبد المحيطة بالأوردة المركزية والخلايا الدهنية ومنطقة الثالوث. ولكن، أظهرت المجموعة المعالجة بـ إل-كارنتين مع السيسبلاتين انخفاض التصبغ المناعي بسيتوكيراتين (CK18) مقارنة بالمجموعة المعالجة ب سيسبلاتين منفردا.

فيما يتعلق بالكلى، أظهرت كل من المجموعتين الضابطة و المعالجة ب إل-كارنتين تصبغ مناعي ضعفً إلى معتدل بسيتوكيراتين (CK18) في طبقة الخلايا الحشوية من كبسولة بومان وكذلك خلايا الأجزاء المختلفة من الأنابيب الكلوية ؛ القريبة و البعيدة و الجامعة. ومع ذلك تم التعبير عن سيتوكيراتين (CK18) بشكل مفرط في الخلايا الطلائية للأنابيب الكلوية بشكل رئيسي في الانابيب الكلوية القريبة وعدد قليل من الانابيب الكلوية

البعيدة بعد حقن السيسبلاتين وفي الوقت نفسه ، أظهرت المجموعة المعالجة بـ إل-كارنتين مع السيسبلاتين تعبيرًا أقل لسيتوكيراتين (CK18) مقارنة بالمجموعة المعالجة بسيسبلاتين منفردا.

فيما يتعلق بالخصية، أظهرت معظم خلايا لايدج في كل من المجموعتين الضابطة والمعالجة ب إل-كارنتين علامات مناعية معتدلة لسيتوكيراتين (CK18). في حين لوحظ ضعف شديد في التصبغ المناعي بسيتوكيراتين (CK18) في عدد قليل من خلايا لايدج من المجموعة المعالجة بـسيسبلاتين منفردا . بالاضافة انه تم تحديد زيادة في عدد وكثافة خلايا لايدج الإيجابية لـسيتوكيراتين (CK18) في المجموعة المعالجة بـ إل-كارنتين + السيسبلاتين عند مقارنتها مع مجموعة المعالجة بسيسبلاتين منفردا.

من هذه الدراسة، يمكن استنتاج أن اعطاء إل- كارنتين لمرضى السرطان يقلل من سمية الكبد والكليتين والخصيتين التي يسببها عقار السيسبلاتين. ويمكن ان يكون هذا بسبب خصائص إل-كارنتين المضادة للأكسدة والمضادة للالتهابات.

جامعة بنها
كلية الطب البيطري
قسم الفارماكولوجيا

قرار لجنة الحكم والمناقشه

قررت لجنة الحكم والمناقشة بجلستها في يوم الثلاثاء الموافق ٢٠٢١/٢/٩م منح **ط.ب/ أدهم عمر محمد سلام** دكتوراه الماجستير في العلوم الطبية البيطرية تخصص "الفارماكولوجيا" بعنوان (تأثير ال- كارنيتين الواقى ضد تسمم الكبد والكليتين والخصيتين المُحدَث بدواء السيسبلاتين فى الجرذان).

اعضاء اللجنة :

١- أ.د/ مصطفي عباس مصطفي شلبي (رئيسا ومحكماً)

أستاذ الأدوية- كلية الطب البيطرى- جامعة القاهرة.

٢- أ.د/ أشرف عبد الحكيم أحمد الكومي (عضواً ومشرفاً)

أستاذ ورئيس قسم الفارماكولوجيا- كلية الطب البيطري- جامعة بنها.

٣- أ.د/ خالد أبوالسعود محمود إبراهيم (عضواً ومحكماً)

أستاذ ورئيس قسم الأدوية- كلية الطب البيطرى- جامعة القاهرة.

٤ - أ.م.د/ محمد حافظ محمد أبوبكر (عضواً ومشرفاً)

أستاذ مساعد الفارماكولوجيا - كلية الطب البيطري- جامعة بنها.

جامعة بنها
كلية الطب البيطري
قسم الفارماكولوجيا

تأثير إل-كارنيتين الواقي ضد تسمم الكبد والكليتين والخصيتين المُحدَث بدواء السيسبلاتين في الجرذان

رسالة مقدمة من

ط.ب / أدهم عمر محمد سلام
بكالوريوس العلوم الطبية البيطرية كلية الطب البيطري جامعة بنها (2017)

للحصول على

ماجستير العلوم الطبية البيطرية تخصص (الفارماكولوجيا)

تحت إشراف

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