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POTENTIAL PROTECTIVE EFFECT OF METFORMIN AND L-CARNITINE IN RAT MODEL OF CARBOPLATIN-INDUCED MYELOSUPPRESSION, COMPARATIVE STUDY

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Background: *Carboplatin (CP), a well-known second-generation platinum compound, is effective against various malignancies including ovarian, lung, neck, breast, cervix and bladder cancer. However, myelosuppression is the most overwhelming toxic effect which results in leucopenia, thrombocytopenia and anemia. L-Carnitine (LCR) is a well-known antioxidant, cardioprotective, neuroprotective, and immunostimulant natural chemical compound. Metformin (MF) is a well-known drug approved for type 2 DM treatment. The aim of this work was to compare the potential protective effect of metformin and L-carnitine in a rat model of CP-induced myelosuppression.* ***Methods and results:*** *This study was performed on 40 adult male albino rats which were divided into four groups: control group, Carboplatin (CP) group, metformin (MF+CP) group and L-Carnitine (LCR+CP) group. Serum was used for measurement of TNF-α alpha, malondialdehyde (MDA), glutathione peroxidase (Gpx), glutathione reductase, catalase and superoxide dismutase (SOD) levels. Histopathological and immunohistochemical changes analyses were done in bone marrow. Carboplatin group showed redox status imbalance which was reversed by metformin and l-carnitine treatment corrected redox status balance. RBCs, WBCs and platelets counts were decreased in carboplatin treated group. Metformin and l-carnitine increased RBCs, WBCs and platelets counts significantly. TGF-β1 and caspase 3 expression were high in carboplatin treated group. Metformin and l-carnitine treatment significantly decreased their expression.* ***Conclusions****: Metformin and L-Carnitine have a possible protective role in myelosuppression induced by carboplatin and this effect is more potent with LCR treatment so we could recommend using L-Carnitine during Carboplatin treatment to prevent developing myelosuppression.*

Keywords: *Carboplatin, Myelosuppression, Metformin, L-Carnitine, TGF-β1, caspase 3.*

INTRODUCTION

Myelosuppression is the most devastating toxic effect of antineoplastic drugs owing to its inhibitory effect of bone marrow cellular replication. This results in leucopenia, thrombocytopenia and anemia leading to severe bacterial and fungal infections, gingival hemorrhage as well as impaired wound healing[1].

Carboplatin (CP) is a well-known second-generation platinum compound. It is classified as alkylating agent that is effective against various types of malignancies including

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ovarian, bladder, breast, cervix lung, neck and central nervous system. Furthermore, CP is used for preparation of the patient for bone marrow and stem cell transplantation. Carboplatin has acquired significant popularity once it's introduced since 1980s because of its fewer side effects compared to cisplatin[2&3].

Carboplatin undergoes intracellular activation forming reactive platinum complexes. These complexes cause inter- and intra-strand cross-linkage of cellular DNA. This leads to DNA mutations and suppression of DNA replication and transcription ending in cell death. CP action affects all phases of cell cycle; therefore, CP is not specific to certain phase unlike other chemotherapeutic agents[4]. CP-induced myelosuppression is a limiting factor of its wide clinical use[5].

Many studies have been performed to evaluate the myeloprotective effect of many agents (e.g. norepinephrine, corticosteroids, amifostine) in a trial to lessen or postpone CP-induced myelosuppression. However, these agents have a limited protection against CP-induced myelotoxicity, so the development of new agents is highly required[6].

L-Carnitine (LCR) is a potent superoxide anion radical scavenger. It shows ability to inhibit the lipoperoxidation[6&7]. Moreover, L-Carnitine may act as a chemical chelator through decreasing cytosolic iron concentration that enhances free radical production[9]. L-Carnitine is a well-known immunostimulant, cardioprotective, neuroprotective and nontoxic natural antioxidant compound. Several studies have demonstrated the valuable effect of L-Carnitine against cancer treatment- associated toxicities[10].

Metformin is a well-recognized biguanide drug that is approved for type 2 diabetes mellitus treatment. Metformin has an anti-inflammatory and antioxidant properties which can decrease lipid peroxidation in many tissues[11&12].

Glucose intolerance is increasingly observed among cancer patients[13]. Unfortunately, insulin resistance and type II diabetes is correlated with increased cancer risk, cancer recurrence, metastasis and mortality[14].

To our knowledge, no previous studies compared the protective effects of metformin and L-Carnitine on Carboplatin-induced bone marrow suppression. Therefore, this work's aim is to compare the potential protective effect of metformin and L-Carnitine in rat model of CP-induced myelosuppression.

MATERIAL AND METHODS

Chemicals and drugs

Carboplatin was purchased as ampoule (250mg/5ml) (Hikma pharmaceutical company, Egypt). Metformin was purchased as cidophage 500 mg tablets (Amoun company, Egypt). L-Carnitine was purchased as 250 mg capsule (Mepaco company, Egypt). All other chemicals were purchased from Al Gomhoria pharmaceutical company, Egypt.

Animals

This study was performed on 40 adult male albino rats, weighting between 150-220 grams. They were obtained from Faculty of Medicine, Tanta University, Egypt. The rats were then housed for seven days in wire mesh cages. Rats had free access to commercial standard diet and water. Animals were exposed to twelve: twelve hours light/ dark cycles and room temperature between 22 and 25 °C. Before experiment, animals were deprived from food (water was allowed) for 24 hrs to ensure an empty stomach.

The study was performed according to the guide of care and laboratory animals use approved by the Ethical Committee of Faculty of Medicine, Tanta University. Additionally, painless procedures were used with appropriate sedation. This experiment was approved by the Research Ethics Committee of Tanta University (Approval number: 34876/8/21). The experimental procedures were conducted in accordance with international guidelines for care and use of laboratory animals.

Experimental design

Rats were randomly divided into four groups (10 rats/each group): Group I (control): 10 rats were injected intraperitoneally with normal saline (2.5 ml/kg) for 7 days. Group II (Carboplatin (CP group): 10 rats were injected with single intraperitoneal injection of carboplatin at a dose of 125 mg/kg to induce bone marrow suppression on day zero[15]. Group III (metformin group): 10 rats received a single daily oral metformin dose (150 mg/kg/day) 7 days[16], plus the previously mentioned CP dose. Group IV(L-Carnitine group): 10 rats received L-Carnitine intraperitoneally at a dose of

100mg/kg daily for 7 days[17], plus the previously mentioned CP dose.

Sample collection

After the experimental period (7 days from start of the experiment), rats were anesthetized and sacrificed. Intracardiac blood was collected in two different tubes ; one with an anticoagulant for counting RBC's, WBC's and platelets counts in day 7 and another without anticoagulant for serum separation for biochemical tests. Serum was used for estimation of TNF-α, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase (Gpx) and catalase enzyme.

Bone marrow preparation

Femur bone was separated from the body, then was temporarily soaked in 75% ethanol, and rinsed in phosphate buffer solution (PBS) under aseptic conditions. The bone marrow was extracted and divided into three parts The first part was homogenized in phosphate buffered saline for biochemical analyses. The second part was used for real time gene expression analysis. The third part was assigned for histopathological and immunohistochemical studies.

Biochemical assessment

Spectrophotometric assay of serum and bone marrow malondialdehyde (MDA), superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase (Gpx) and catalase by commercial kits (Bio-diagnostic company,Egypt). Serum TNF-α was measured using commercial kits (Chongqing Biospes company, China) catalogue number: BEK1214). All ELISA techniques were performed according to the manufacturer's protocol and read on microplate reader (Stat Fax®2100, Fisher Bioblock Scientific, France), at 450 nm wave length.

Assay of bone marrow relative TGF-beta mRNA expression by real-time PCR

RNA was extracted from bone marrow after processing using Qiagen RNeasy Total RNA isolation kit (Qiagen, Hiden, Germany) according to the protocol provided by the manufacturer. This was followed by synthesis of the first strand using Super-Script III First-Strand Synthesis System for real-time PCR kit (Life Technologies, Carlsbad, California, USA). PCR reactions were performed using Power SYBR Green PCR Master Mix (Life Technologies, Carlsbad, California, USA) following the manufacturer's instructions. TGF-beta mRNA transcripts were quantified relative to the housekeeping gene β-Actin gene, that was used as an internal control. Sequence specific primers were designed as follows: rat TGF-beta : up-stream: 5′-ATA CGT CAG ACA TTC GGG AAG CAG TG-3′, down-stream: 5′-AAT AGT TGG TAT CCA GGG CTC TCC G-3′,and rat β-Actin: up-stream: 5′-GTG CCC ATC TAC GAG GGC TAT GCT-3′ and down-stream:.5′-TAC CCA AGA AGG AAG GCT GGA AAA-3′ Relative gene expression was calculated automatically using the comparative threshold (Ct) method for the values of target and the reference genes using the $2^{-\Delta\Delta CT}$ formula[18].

Histopathologic study

The femur was taken from sacrificed rats, fixed in 10% neutral buffered formalin solution for 24 hrs, followed by decalcification in ethylenediaminetetraacetic acid (EDTA) solution (pH 7) for 5 days. Specimens were then dehydrated and embedded in paraffin wax. Sections (5µm thick) were prepared and stained with hematoxylin and eosin. Slides were examined by two blinded pathologists to assess the bone marrow cellularity.

Immunohistochemical staining

Sections of 5 µm, on positively charged slides, were deparaffinized in xylene, rehydrated in descending grades of alcohol and washed in phosphate buffer solutions. Antigen retrieval was achieved using heat induced epitope retrieval in microwave for 10 min. Blocking of endogenous peroxidase activity was performed by immersion of sections in 3% hydrogen peroxidase. Sections were then incubated with anti-caspase 3 antibody (Rabbit monoclonal, clone number EPR18297, Abcam, USA) in 1/1000 dilution for 30 minutes. Immunoreaction was visualized by streptavidin- biotin immunoperoxidase technique (ABC kit; Vector Laboratories, Burlingame, CA, USA). Sections were incubated with biotinylated secondary antibody followed by streptavidin peroxidase solution for 10 min at room temperature. Diaminobenzidine (DAB) was applied as

chromogen and then sections were counterstained with hematoxylin.

Evaluation of Caspase 3 immunostaining

Caspase 3 staining intensity was determined using image analysis software Fiji (ImageJ bundled with plugins)[19]**.** Three random images from every slide were captured at x400 magnification using Leica DM500 microscope with built-in Leica ICC50 digital camera (Leica Microscopy and Scientific Instruments Group Germany). Caspase 3 intensity was determined in the three captured images from each slide. The average of the resulting three values was used as a representative for the mean caspase 3 intensity in each slide.

Statistical analysis

Data was analyzed using SPSS (Statistical package for social studies) program, version 23. Mean ± standard deviation was used to express data. Kruskal-Wallis test was used to compare means of all groups followed by post-hoc test to detect significant difference between groups. Results were considered statistically significant with P value <0.05.

RESULTS AND DISCUSSION

Results

Effect of metformin and L-Carnitine on redox status and inflammatory biomarkers in serum

Carboplatin group showed redox status disturbance as evidenced by marked increase in serum MDA level with decrease in SOD enzyme activity, catalase enzyme activity and GSH level as compared to the control group (P< 0.01). Metformin and

L-Carnitine treatment corrected redox status disturbance, but it was still significantly higher than the control group (P< 0.01) as presented in Table 1. Also, Carboplatin group showed marked inflammatory state as evidenced by marked increase in serum TNF-α level as compared to the control group (P< 0.01). Metformin and L-Carnitine treatment corrected inflammatory state, but it was still significantly higher than the control group (P< 0.01) (Table 1).

Table 1: Effect of metformin and l-carnitine on oxidant/antioxidant biomarkers and inflammatory biomarkers in serum among the studied groups.

	Control group (n=10)	(CP) group (n=10)	(CP+MF) group (n=10)	(CP+LCR) group (n=10)
TNF-α (pg/ml)	19.4 ± 0.8^{bcd}	43.8 ± 1.3^{acd}	35.7 ± 0.7^{abd}	26.8 ± 0.9^{abc}
MDA (nmol / ml)	15.8 ± 1.1^{bcd}	32.3 ± 1.5^{acd}	24.2 ± 0.9^{abd}	19 ± 0.7^{abc}
SOD (U/ml)	3.94 ± 0.49^{bcd}	0.98 ± 0.14^{acd}	1.91 ± 0.36^{abd}	3.01 ± 0.1^{abc}
Catalase (U / L)	7.81 ± 0.46^{bcd}	2.59 ± 0.85^{acd}	5.04 ± 0.18^{abd}	6.45 ± 0.45^{abc}
GSH (mmol/L)	71.57 ± 1.36^{bcd}	15.59 ± 1.43^{acd}	34.84 ± 2.44^{abd}	45.32 ± 2.22^{abc}

Data are represented as mean± SD (n= 10 rats in each group). Statistical analysis was carried out using Kruskal-Wallis test followed by post-hoc test , SPSS computer program $^{a–d}$ Significant difference between groups at $*p < 0.05$. ^{a}: significance from Control group; ^{b}: significance from CP group; ^{c}: significance from (CP+MF) group; $^{d:}$ significance from (CP+LCR) group. Abbreviations: Carboplatin (CP), Metformin(MF), L carnitine(LCR), Tumor necrosis factor-alpha (TNF- α), Malondialdehyde (MDA), Superoxide dismutase (SOD), Reduced glutathione (GSH).

Effect of metformin and L-Carnitine on redox status and inflammatory biomarkers in bone marrow

Carboplatin group showed redox status disturbance in bone marrow as evidenced by marked increase in bone marrow MDA level with decrease in SOD enzyme activity, catalase enzyme activity, GPx enzyme activity and GSH level as compared to the control group ($P < 0.01$). Metformin and l-carnitine treatment corrected redox status disturbance, but it was still significantly higher than the control ($P < 0.01$) (Table 2).

Effect of metformin and L-Carnitine on some hematological parameters (RBCs, WBCs and platelets counts)

RBCs, WBCs, and platelets counts were significantly decreased (anemia, leucopenia, and thrombocytopenia respectively) in carboplatin group as compared to the control group ($P < 0.05$). Metformin and L-Carnitine treatment significantly increased RBCs, WBCs and platelets counts as compared to carboplatin group. ($P < 0.05$) (Table 3).

Table 2: Effect of metformin and l-carnitine on redox status and inflammatory biomarkers in bone marrow among the studied groups.

	Control group (n=10)	(CP) group (n=10)	(CP+MF) group (n=10)	(CP+LCR) group (n=10)
MDA (mmol/ g tissue)	10.57 ± 0.93^{bcd}	23.68 ± 0.98^{acd}	18.33 ± 0.82^{abd}	14.67 ± 0.57^{abc}
SOD (u/ g tissue)	247.8 ± 24.4^{bcd}	88 ± 6.68^{acd}	110.7 ± 6.27^{abd}	184.7 ± 6.3^{abc}
Catalase (u/ g tissue)	2.26 ± 0.09^{bcd}	0.3 ± 0.03^{acd}	0.91 ± 0.03^{abd}	1.42 ± 0.2^{abc}
GSH (mmol/g tissue)	2.19 ± 0.04^{bcd}	0.39 ± 0.04^{acd}	0.79 ± 0.05^{abd}	1.36 ± 0.16^{abc}
Gpx(u/ g tissue)	3.54 ± 0.3^{bcd}	0.94 ± 0.11^{acd}	1.19 ± 0.09^{abd}	2.64 ± 0.2^{abc}

Data are represented as mean$\pm$ SD (n=10 rats in each group). Statistical analysis was carried out using Kruskal-Wallis test followed by post-hoc test , SPSS computer program. [a–d] Significant difference between groups at $*p < 0.05$. [a]: significance from Control group ; [b]: significance from CP group; [c]: significance from (CP+MF) group; [d:] significance from (CP+LCR) group. Abbreviations: Carboplatin(CP), Metformin(MF), L carnitine(LCR), Malondialdehyde (MDA), Superoxide dismutase (SOD), Reduced glutathione (GSH), Glutathione peroxidase (GPx).

Table 3: Effect of metformin and l-carnitine on some hematological parameters (RBCs, WBCs and platelets counts) among the studied groups**.**

	Control group (n=10)	(CP) group (n=10)	(CP+MF) group (n=10)	(CP+LCR)group (n=10)
RBCs(10^6 cells/m m3)	6.82 ± 0.51^{bcd}	2.83 ± 0.39^{acd}	4.39 ± 0.27^{abd}	6.16 ± 0.18^{abc}
WBCs(10^3 cells/mm3)	12.2 ± 0.42^{bcd}	4.18 ± 0.38^{acd}	6.2 ± 0.25^{abd}	8.8 ± 0.37^{abc}
Platelets (10^3/mm3)	575 ± 42^{bcd}	159 ± 33^{acd}	299 ± 17^{abd}	419 ± 10^{abc}

Data are represented as mean$\pm$ SD (n= 10 rats in each group). Statistical analysis was carried out using Kruskal-Wallis test followed by post-hoc test , SPSS computer program [a–d] Significant difference between groups at $*p < 0.05$. [a]: significance from Control group; [b]: significance from CP group; [c]: significance from (CP+MF) group; [d:] significance from (CP+LCR) group. Abbreviations: Carboplatin (CP), Metformin (MF), L carnitine (LCR), Red blood cells (RBCs), White blood cells (WBCs).

Effect of metformin and L-Carnitine on TGF-β mRNA gene expression

TGF-β mRNA gene expression showed a significant increase in carboplatin treated group when compared to control group (P< 0.05). Metformin and L-carnitine treatment significantly decreased TGF-β m RNA gene expression (*P*< 0.05) (Figure 1).

Histopathologic assessment of bone marrow cellularity

Bone marrow sections from carboplatin treated group revealed marked myelosuppression with distinct increase in the percentage of fat in relation to hematopoietic component (Figure 2, b). Administration of metformin resulted in mild improvement of bone marrow cellularity with increase hematopoietic component (Figure 2, c). Whereas treatment with L-Carnitine resulted in marked improvement of myelosuppression with enhancement of hematopoietic component and near normal bone marrow cellularity (Figure 2, d).

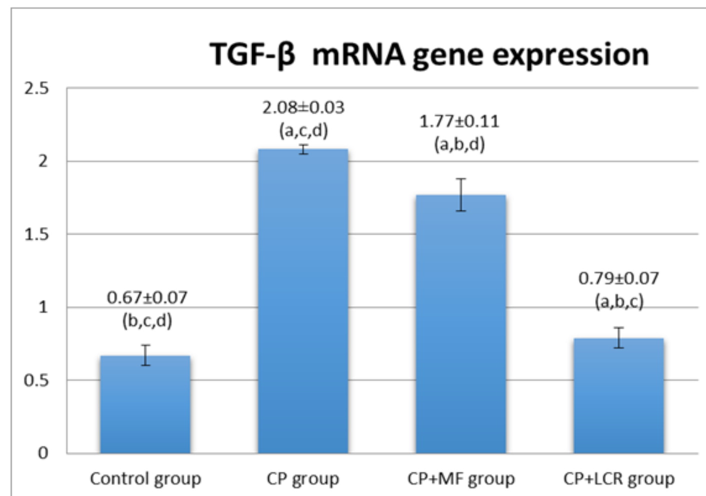

Fig. 1: Effect of metformin and l-carnitine treatment on TGF-β mRNA gene expression. values are represented as mean ± SD.

Data are represented as mean± SD (n= 10 rats in each group). Statistical analysis was carried out using Kruskal-Wallis test followed by post-hoc test , SPSS computer program. [a–d] Significant difference between groups at $*p < 0.05$. [a]: significance from Control group; [b]: significance from CP group; [c]: significance from (CP+MF) group; [d:] significance from (CP+LCR) group. Abbreviation: Carboplatin (CP), Metformin (MF), L carnitine (LCR), Transforming growth factor beta (TGF-β).

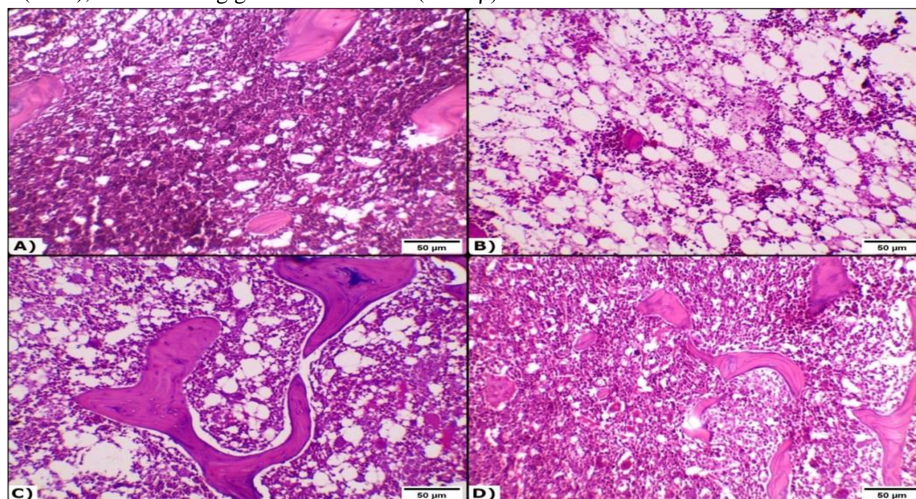

Fig. 2: Histopathologic assessment of the bone marrow biopsies of the studied groups (H and E x200): (A) control group, (B) carboplatin treated group showing marked reduction of hematopoietic component of bone marrow, (C) metformin treated group showing mild improvement of bone marrow cellularity, (D) l-carnitine treated group showing marked improvement of hematopoietic component with near normal bone marrow cellularity .

Caspase 3 immunostaining

Carboplatin treatment significantly increased caspase 3 intensity compared to the control group reflecting activation of apoptosis. Treatment with metformin and L-Carnitine showed lesser intensity of caspase 3 expression than in the carboplatin group. However, it was still higher than the control group (p< 0.001) (Table (4) and Fig (3)).

Table 4: Effect of metformin and l-carnitine on caspase 3 staining intensity in bone marrow among the studied groups.

	Caspase 3 staining intensity (au)Mean ± SD	P value	
Control Group (n=10)	184.12±2.18	<0.001*	P1<0.001*, P2<0.001* P3<0.001*, P4<0.001*, P5<0.001*, P6<0.001*
CP Group (n=10)	246.55±1.17		
CP+MF Group (n=10)	231.42±1.52		
CP+LCR Group (n=10)	223.79±1.73		

*significant (p value <0.05), P1: Control vs CP group, P2: Control vs CP+MF groups, P3: Control vs CP+LCR groups, P4: CP vs CP+MF groups; P5: CP vs CP+LCR groups; P6: CP+MF vs CP+LCR groups. Abbreviations: Carboplatin (CP), Metformin (MF), L carnitine (LCR),

	Control Group (n=10)	CP Group (n=10)	CP+MF Group (n=10)	CP+LCR Group (n=10)
Caspase 3 staining intensity (au) Mean ± SD	184.12 ± 2.18^{bcd}	246.55 ± 1.17^{acd}	231.42 ± 1.52^{abd}	223.79 ± 1.73^{abc}

Data are represented as mean±SD (n=10 rats in each group). Statistical analysis was carried out using Kruskal-Wallis test followed by a post hoc test, SPSS computer program. $^{a–d}$ Significant difference between groups at $*p < 0.05$. ^{a}: significance from Control group; ^{b}: significance from CP group; ^{c}: significance from (CP+MF) group; $^{d:}$ significance from (CP+LCR) group. Abbreviations: Carboplatin (CP), Metformin (MF), L carnitine (LCR), arbitrary unit (au).

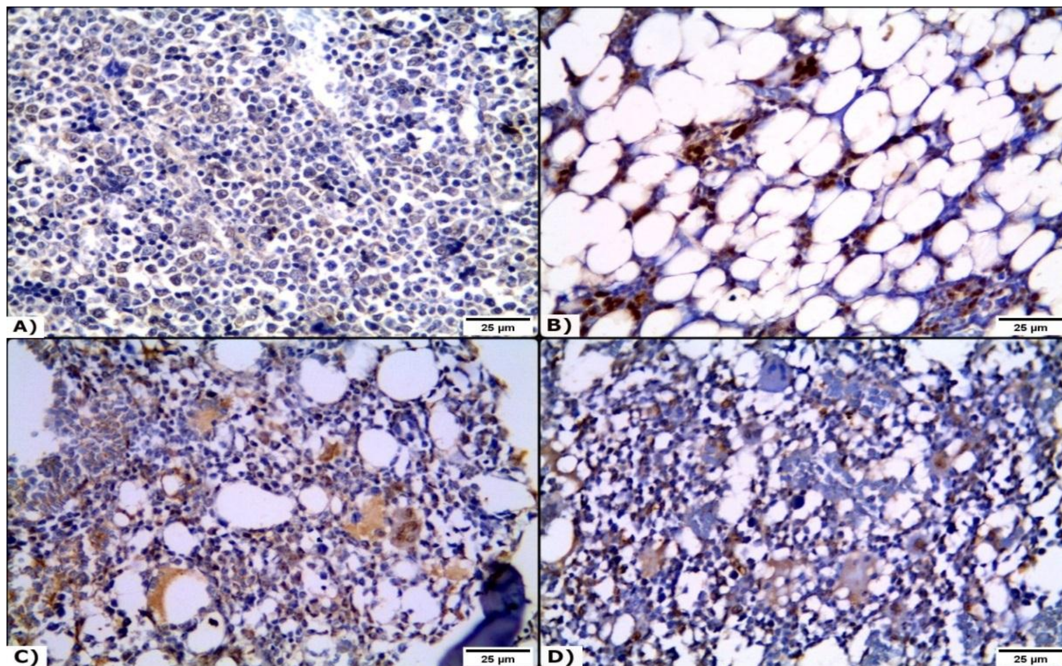

Fig 3: Caspase 3 immunohistochemical expression in bone marrow biopsies of the studied groups (x400): Caspase staining intensity was highest in (B) carboplatin treated group, followed by (C) metformin then (D) l-carnitine treated groups and least in (A) the control group.

Discussion

As myelosuppression diminishes the therapeutic effects of carboplatin, prevention of CP induced myelosuppression is a major target in chemotherapy field. To our knowledge, this is the first study to compare the protective effects of metformin and L-Carnitine in rat model of CP induced myelosuppression.

This study shows that metformin and L-Carnitine treatment has improved the redox status disturbance and inflammation. Treatment with L-Carnitine showed significant decrease in TNF- α and MDA compared to treatment with metformin however treatment with metformin showed significant decrease in catalase, SOD and GSH compared to treatment with L-Carnitine.

These results suggest the potential protective role of both metformin and L-carnitine in CP induced myelosuppression which may be mediated by their antioxidant effects. These results are in harmony with Aurigena *et al.*,[20] who showed that metformin treatment had reduced MDA and TNF-α levels and increased GSH level. Metformin activates the AMP-activated protein kinase (AMPK). AMPK had been proved to exert a significant immunosuppressive and anti-inflammatory effects in inflammatory/autoimmune disease model[21].

Ranjbar Kohan *et al.,*[22] revealed that TNF-α levels were reduced in diabetes mellitus rats two weeks after L-Carnitine treatment. It was evidenced that L-Carnitine could improve cellular oxidative stress by activation of mitochondrial superoxide dismutase enzyme. Moreover, Acetyl carnitine increased reduced GSH level in multiple sclerosis patients[23] Also, Arafa and Sayed-Ahmed[24] demonstrated that carnitines increased the gastric reduced glutathione level and increased gastric superoxide dismutase activity. L- Carnitine restored CP-induced reduced glutathione depletion and prevented MDA elevation in bone marrow cell culture. Thus, L-Carnitine was able to protect against CP-induced myelosuppression.[25]

Present study shows that Metformin and L-Carnitine treatment significantly increased RBCs, WBCs and platelets counts. This confirms their possible protective effects against CP-induced myelosuppression. L-Carnitine treatment was superior to metformin treatment which may be linked to its antioxidant, immunostimulant and neuroprotective effects. Moreover, it enhances the function of bone marrow progenitors through increasing colony-forming units as a response to granulocyte/macrophage colony stimulating factors[25].

Carboplatin was proved to interfere with DNA repair, which is a fundamental mechanism to suppress cancer cells. However, the normal cell growth may also be affected by carboplatin. As a result, there was a great link between carboplatin and hematological disorders including anemia, neutropenia and thrombocytopenia.[26]

In ovarian cancer cell lines and xenograft models, metformin treatment was found to suppress cell growth, activate apoptosis, suppress angiogenesis and metastasis[27]. Metformin treatment has been associated with decreased ovarian cancer risk and diminished cancer mortality in diabetic patients, compared to non-use and use of other anti-diabetic drugs.[28]

In an in vitro study using bone marrow cell cultures, it was proven that L-Carnitine treatment was effective not only in preventing carboplatin-induced nephrotoxicity, but also was effective in protecting against carboplatin-induced myelosuppression. Moreover, L-Carnitine was found to diminish CP-induced apoptosis.[25]

TGF-β mRNA gene expression showed a significant increase in the carboplatin treated group when compared to the other groups. Metformin and L-Carnitine treatment significantly decreased TGF-β m RNA gene expression and this effect was superior in L-Carnitine treatment.

Transforming growth factor-β (TGF-β) is involved in the pathogenesis of many diseases, including cardiovascular, fibrotic, immunological diseases and cancer[29]. TGF-β has a pro-metastatic effect in cancer. This makes it an attractive target for the development of new drugs aiming at blocking its pro-metastatic signaling pathway. [30]

In accordance with these results, metformin was estimated for its ability to

inhibit TGF-β induced signaling[31]. It was evidenced that metformin is able to inhibit TGF-β signaling and therefore can be used as a treatment for many diseases other than diabetes mellitus.[32]

L-carnitine was shown to decrease renal fibrosis, plasma TGF-β levels, oxidative stress and proinflammatory status[33]. Another study revealed that L-Carnitine treatment markedly decreased Tubulointerstitial fibrosis (TIF) by suppression of the expression of profibrotic cytokines e.g., TGF-β1, oxidative damage, inflammation and subsequent apoptosis[34]. Many researches have highlighted the ability of L-Carnitine to suppress free radical generation, which maintains mitochondrial fatty acid β-oxidation and protects tissues from oxidative damage of membrane lipids. Moreover, L-Carnitine is a direct scavenger of hydrogen peroxide and superoxide radical. Therefore, L-Carnitine has a powerful antioxidant property beyond its action in lipid metabolism[35].

Bone marrow sections from carboplatin treated group showed marked myelosuppression and significant increase in caspase 3 intensity compared to the control rats. Administration of metformin and L- Carnitine resulted in lesser intensity of caspase 3 expression than in the carboplatin treated group. This effect was more potent with L-Carnitine treatment.

The present study confirms the results obtained by Chan *et al.,*[36] who evaluated the effect of L-Carnitine treatment on offspring's of smoke exposure (SE) in long-term maternal SE. L-Carnitine was shown to normalize the elevated caspase 3 in the offspring's. Caspase 3 was accessed for apoptosis and DNA damage. Another study proved the antiapoptotic effect of L-Carnitine; Administration of LCR protected γ-irradiated mice from damage induced by γ-irradiation through the amelioration of mRNA gene expression of pro-apoptotic and anti-apoptotic genes[37].

Sarıbal *et al.,*[38] provided that metformin decreased cleaved caspase-3 expression and decreased testicular damage by alleviating apoptosis in adolescent rat testis.

Conclusions

L- Carnitine and metformin exert a possible protective role in myelosuppression induced by carboplatin through their antioxidant, anti-inflammatory and anti-apoptotic mechanisms. Our findings might provide evidence for the potential use of L-Carnitine or metformin during the treatment of cancer with carboplatin to protect against myelosuppression. Further research can be done after induction of cancer to assess the ability of both drugs to protect against myelosuppression and enhance carboplatin anticancer effect.

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Conflict of interests

Authors declare that they have no conflict of interests.

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Data Availability

All data generated this study are included in this published article (and its supplementary information files, available upon request from authors).

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نشرة العلوم الصيدليـــة
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التأثير الوقائي المحتمل لميتفورمين وإل كارنيتين في نموذج الفئران لتثبيط النخاع الناجم عن كاربوبلاتين ، دراسة مقارنة

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محتوي الخلفية: كاربوبلاتين (CP) ، مركب بلاتيني معروف من الجيل الثاني ، فعال ضد الأورام الخبيثة المختلفة بما في ذلك سرطان المبيض والرئة والرقبة والثدي وعنق الرحم والمثانة. ومع ذلك ، فإن تثبيط نخاع العظم هو التأثير السام الأكثر خطورة الذي ينتج عنه قلة الكريات البيض ونقص الصفيحات وفقر الدم (LCR) وإل كارنيتين.هو مركب كيميائي طبيعي مضاد للأكسدة ، وقائي للقلب ، وقائي للأعصاب ، ومنبه للمناعة. الميتفورمين (MF) هو دواء مشهور معتمد للعلاج من النوع ٢. DM. كان الهدف من هذا العمل هو مقارنة التأثير الوقائي المحتمل للميتفورمين وإل كارنيتين في نموذج الفئران لتثبيط النخاع الناجم عن كاربوبلاتين.

الطريقة والنتائج: أجريت هذه الدراسة على ٤٠ من ذكور الجرذان البيضاء التي تم تقسيمها إلى أربع مجموعات: مجموعة التحكم ، مجموعة الكربوبلاتين (CP) ، مجموعة الميتفورمين (MF + CP) ومجموعة وإل كارنيتين (LCR + CP). تم استخدام المصل لقياس مستويات TNF-α alpha و (malondialdehyde (MDA و (glutathione peroxidase (Gpx و glutathione reductase و catalase و (superoxide dismutase (SOD. أجريت تحاليل التغيرات النسيجية المرضية والكيميائية المناعية في نخاع العظم. أظهرت مجموعة الكاربوبلاتين عدم توازن حالة الأكسدة والاختزال والتي تم عكسها بواسطة علاج الميتفورمين و وإل كارنيتين لتصحيح عدم توازن حالة الأكسدة والاختزال. انخفض عدد كرات الدم الحمراء ، كرات الدم البيضاء والصفائح الدموية في المجموعة المعالجة بالكربوبلاتين. زاد الميتفورمين والكارنيتين من عدد كرات الدم الحمراء ، كرات الدم البيضاء والصفائح الدموية بشكل ملحوظ. كان تعبير TGF-β1 و caspase 3 مرتفعًا في المجموعة المعالجة بالكربوبلاتين. قلل العلاج بالميتفورمين وال كارنتين بشكل كبير من تعبيرهم.

الاستنتاجات: الميتفورمين و وإل كارنيتين لهما دور وقائي محتمل في تثبيط نخاع العظم الناجم عن كاربوبلاتين وهذا التأثير يكون أكثر فعالية مع علاج وإل كارنيتين.