

# Methotrexate-induced Hepatic and Renal Toxicity: Role of L-carnitine in Treatment

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**Abstract** Methotrexate (MTX) is used as a chemotherapeutic agent used to treat many cancer types. The present study aimed to examine the possible modifying effects of l-carnitine against hepatic and renal toxicity induced by MTX in rats. A total 60 male albino rats were equally divided into six groups; the first and second groups were the control and l-carnitine groups respectively while the 3rd group was MTX rat group; the 4th and 5th groups were co- and post treated MTX rat with l-carnitine respectively and the 6th group was MTX self treated rat group. A significant increase in serum ALT, AST, urea, creatinine, uric acids and MDA levels and significant decrease GSH, catalase and total protein in MTX and self healing groups when compared with control and l-carnitine groups. In contrast, MTX-treated with l-carnitine exhibited a significant decrease in serum ALT, AST, urea, creatinine, uric acids and MDA levels and significant increase GSH, catalase and when compared with total protein in MTX and self healing groups. Histopathological results supported the biochemical results and the ameliorating effect of l-carnitine on liver and kidney toxicities. L-carnitine possessed various protective mechanisms against MTX-induced liver and kidney toxicity throughout co- and post treatment. We can conclude that treatment with l-carnitine during the MTX chemotherapy has beneficial properties and can reduce the liver and kidney toxicity induced by MTX.

**Keywords:** Methotrexate (MTX), L-carnitine, Oxidative damage, Antioxidants, liver, Kidney

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## 1. Introduction

Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, it does not distinguish between a cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including, hair and blood cells. Methotrexate (MTX, 4-amino-N<sup>10</sup>-methyl folic acid), a folic acid dihydrofolate reductase inhibitor, affects primarily the tissues that are growing most rapidly. Toxicity studies with methotrexate highlight the role of oxidative stress in causing toxicity on the liver, kidney, heart and other organs [1,2].

The most serious side effect of MTX therapy is hepatic toxicity [3]. Hersh et al. [4] reported that mild hepatitis, cholestasis, fatty changes, fibrosis and cirrhosis, in patients receiving MTX for malignant disorders. Penalva Polo et al. [5] reported that; acute liver failure in a patient with methotrexate therapy. Gong and Gluud [6] state that the methotrexate increased mortality in patients with primary biliary cirrhosis.

Methotrexate was first used, in high doses, to treat cancer but experience over thirty years has shown that methotrexate at much lower doses is helpful in the

treatment of a number of joint, skin and bowel conditions. Methotrexate is a well established effective treatment for several different types of rheumatic diseases (for example, rheumatoid arthritis, psoriatic arthritis, juvenile idiopathic arthritis), severe psoriasis and for bowel diseases (such as Crohn's disease). It is also used in some other conditions where the body's natural defence system is overactive. With the widespread use of MTX, although hepatic and renal toxicity (grade 3-4) is one of the important potential major side effect [2,7,8,9,10]. Levels of both enzymatic and nonenzymatic antioxidants are inhibited and the levels of oxidants increase in the liver, kidney and gut tissues of laboratory animals given methotrexate [10,14]. Methotrexate enters the cell via active transport across the reduced folate carrier and is effluxes from the cell by several of the ATP-binding cassette transporters [1,2].

L-Carnitine (LC) is a small water-soluble molecule which plays an important role in fat metabolism. It is essential for the normal mitochondrial oxidation of fatty acids and excretion of acyl-coenzyme A (acyl-CoA) esters and affects adenosine triphosphate levels [15]. L-Carnitine has antioxidant activity that combines both free radical scavenging and metal-chelating properties [16,17,18]. L-Carnitine has free radical-scavenging activity and ability to scavenge superoxide anion and inhibit lipid peroxidation, thereby conferring protection against damage induced by hydrogen peroxide [19]. Human

embryos generated from IVF exhibit varying degrees of cytoplasmic fragmentation indicative of apoptosis [20]. Stabilization of the mitochondrial membrane leads to increase in the supply of energy to the organelle and protect the cell from apoptotic death.

L-carnitine is absorbed from foods via both active and passive transport across enterocyte (intestinal cell) membranes [21]. Carnitine that not obtained from food is synthesized endogenously from two essential amino acids, lysine and methionine. This occurs in kidney, liver and brain [22,23]. Administration of l-carnitine is an accepted treatment for mitochondrial myopathy and encephalomyopathy, as well as other states of primary and secondary l-carnitine deficiency [24]. Recently, l-carnitine has been proposed as a potential adjuvant treatment to improve anemia, thrombocytopenia, leukopenia and immunological function [1,25,26]. Based on these evidences, the present study was conducted to examine the possible modifying effects of l-carnitine against hepatic and renal toxicity induced by methotrexate in male rats. This could be fulfilled through the histological, immunohistochemical and biochemical analysis of liver and kidney tissues.

## 2. Materials and Methods

### 2.1. Animals

The experiments were performed on 60 male albino rats (*Rattus norvegicus*) weighting 140-150 g and of 9-10 week's age. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt) and water available *ad libitum*. The temperature in the animal room was maintained at  $23\pm 2^{\circ}\text{C}$  with a relative humidity of  $55\pm 5\%$ . Light was on a 12:12 hr light -dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research.

### 2.2. Animal Treatments

The rats were randomly and equally divided into six groups (10 animals each).

**G<sub>1</sub>:** Control group in which animals did not received any treatment.

**G<sub>2</sub>:** L-carnitine or positive control group in which animals received orally l-carnitine (300 mg/Kg body weight/ week) for four weeks according to Salama et al. [18].

**G<sub>3</sub>:** Methotrexate (MTX) rats group in which rats were injected intraperitoneally with MTX administration (0.5 mg /kg body weight/ twice a week) for four weeks according to Yozai et al. [8].

**G<sub>4</sub>:** Co-treated group in which animals injected intraperitoneally with MTX administration (0.5 mg /kg body weight/ twice a week) and also received orally l-carnitine (300 mg/Kg body weight/ week) for four weeks.

**G<sub>5</sub>:** Post treated group in which animals injected intraperitoneally with MTX administration (0.5 mg /kg body weight/ twice a week) for four weeks and then treated orally with L-carnitine (300 mg/Kg body weight/ week) for another four weeks.

**G<sub>6</sub>:** Self treated rat group in which rats were injected intraperitoneally with MTX administration (0.5 mg /kg body weight/ twice a week) for four weeks and self treated without drugs for another successive four weeks.

At the end of the experimental period (8 weeks), Animals were euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

### 2.3. Sample Preparation

Animals were fasted overnight and for clinical chemistry blood samples from each rat were collected from the eyes by retro-orbital puncture using blood capillary tubes without heparin as per requirement under mild ether anesthesia blood samples were incubated at room temperature for 10 minutes and left to clot then centrifuged at 3000 r.p.m for 10 min and the serum were collected, serum was separated and kept in clean stopper plastic vial at  $-80^{\circ}\text{C}$  until the analysis of serum parameters. After animals were sacrificed, the liver and kidney were instantly removed, washed three times in ice cold saline and blotted on filter paper, then used for preparation of tissue homogenates for estimation of tissue MDA, total protein, Reduced glutathione (GSH) and catalase enzymes.

### 2.4. Biochemical analysis

Serum ALT and AST activities in serum were assayed by using commercial kit that was supplied by Randox (Egypt) according to the method of Rietman and Frankel [27]. Albumin concentration in serum was assayed by using commercial kit that was supplied by Diamond, from Egypt. Albumin concentration was estimated according to the method of Bowers and Wong [28]. Concentration of total protein was spectrophotometrically determined using commercial diagnostic kits supplied by Diamond (Egypt) according to the method of Bowers and Wong [28]. Urea level in serum was assayed by using commercial kit that was supplied by Diamond, from Egypt. Urea was estimated according to the method of Patton and Crouch [29]. Creatinine concentration in serum was assayed by using commercial kit that was supplied by Diamond, from Egypt. Creatinine was estimated according to the method of Bowers and Wong [28]. Uric acid level in serum was assayed by using a commercial kit that was supplied by SPINREACT from Santa Coloma, Spain. Uric acid was estimated according to the method of Fossati et al. [30].

### 2.5. Preparation of tissue homogenates

Tissue homogenates were prepared as reported by Sakeran et al. [31]. Briefly, specimens were separated into two parts. Each piece was weighted and homogenized separately with a Potter Elvehjem tissue homogenizer. The crude tissue homogenate was centrifuged at 11,739 *ref*, for 15 min in a cold centrifuge, and the resultant supernatant was used for the different estimations.

### 2.6 Enzymatic and Non-enzymatic Antioxidant Assays

**MDA assay:** The MDA is one of terminal products, formed at the time of the decomposition of

polyunsaturated fatty acids mediated by free radicals. MDA was estimated by the method of Mesbah et al. [32].

**Reduced GSH:** GSH content was determined with dithionitrobenzoic acid using the method described by Beutler et al. [33] and was expressed in  $\mu\text{mol}/\text{mg}$  tissue. The method is based on the reduction of DTNB to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm.

**Catalase:** The catalase (CAT) activity was measured by monitoring  $\text{H}_2\text{O}_2$  (The substrate of the enzyme) decomposition at 240 nm according to the method described by Aebi [34].

**Total protein:** The total protein concentration in liver and kidney were detected by the method of Lowry et al. [35] as modified by Tsuyosh and James [36].

## 2.7. Histopathological Examination

Immediately after decapitation animals were dissected, liver and kidney from different groups were quickly removed and fixed in 10 % neutral buffered formalin. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin (mp. 50–58°C). Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. Sections were stained with Ehrlich's haematoxylin and counterstained with eosin as a routine method after Bancroft and Stevens [37]. All stained slides were viewed by using Olympus microscope and images were captured by a digital camera (Cannon 620). Brightness, contrast were adjusted using Adobe Photoshop software.

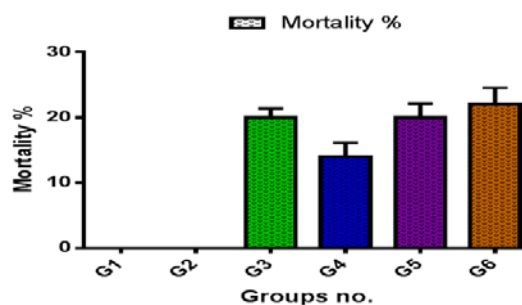
## 2.8. Statistical Analysis

Data were expressed as mean values  $\pm$  SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at  $p < 0.01$  for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

## 3. Results

### 3.1. Toxicity

The animals under practice appeared healthy and did not show clinical signs of disease and no mortality was recorded in control and l-carnitine groups during the experiment duration. On the other hand, various side effects were observed in animals injected with methotrexate such as loosing of body weight, loss of activity, weakness, yellowish body hair and  $20 \pm 1.4\%$  mortality was recorded in methotrexate group, while  $22 \pm 2.5\%$  mortality was recorded in self treated methotrexate group. Mortality percent in co-treated methotrexate with l-carnitine were  $14 \pm 2.1\%$  while about  $20 \pm 2.1\%$  mortality was recorded in post treated methotrexate with l-carnitine group (Figure 1).



**Figure 1.** Changes in mortality% in different groups under study. G1, control group; G2, l-carnitine group; G3, MTX group; G4, Co-treated group with l-carnitine; G5, Post treated group with l-carnitine; G6, MTX self treated group.

## 3.2. Biochemical investigations

### 3.2.1. In vivo hepatic and renal protective effects of l-carnitine

The data summarized in Table 1 indicates that a significant ( $P < 0.01$ ) elevation in ALT, AST, Urea, Creatinine and Uric acid in MTX group when compared with control and l-carnitine groups, this elevation decreased in treated group with l-carnitine and increased in MTX self treated when compared with methotrexate group. ALT, AST, Urea, Creatinine and Uric acid levels in co-treated MTX with l-carnitine group were significantly decreased when compared with post treated MTX group with l-carnitine group. Total protein and Albumin were significantly decreased in MTX and in MTX self treated groups when compared with control and l-carnitine groups. In contrast, Total protein and Albumin were significantly increased in treated group with l-carnitine when compared with MTX and MTX self treated groups (Table 1).

**Table 1.** Changes in the serum ALT(U/l), AST(U/l), Total protein (g/dL), Urea (mg/dl), Creatinine(mg/dl) and Uric acid (mg/dl) levels in different groups under study

	ALT	AST	Total protein	Albumin	Urea	Creatinine	Uric acid
G1	63.5 $\pm$ 2.30 <sup>b</sup>	75.5 $\pm$ 3.20 <sup>a</sup>	6.92 $\pm$ 0.21 <sup>b</sup>	5.58 $\pm$ 0.13 <sup>b</sup>	33.7 $\pm$ 2.28 <sup>b</sup>	0.97 $\pm$ 0.089	5.37 $\pm$ 0.29 <sup>b</sup>
G2	63.9 $\pm$ 3.30 <sup>b</sup>	66.6 $\pm$ 3.50 <sup>b</sup>	6.05 $\pm$ 0.55 <sup>b</sup>	5.73 $\pm$ 0.15 <sup>b</sup>	29.2 $\pm$ 2.72 <sup>b</sup>	0.86 $\pm$ 0.139 <sup>b</sup>	5.13 $\pm$ 0.50 <sup>b</sup>
G3	120.3 $\pm$ 10.7 <sup>a</sup>	166.1 $\pm$ 19.9 <sup>a</sup>	5.65 $\pm$ 0.35 <sup>a</sup>	3.53 $\pm$ 0.06 <sup>a</sup>	57.2 $\pm$ 5.61 <sup>a</sup>	1.12 $\pm$ 0.159 <sup>a</sup>	7.16 $\pm$ 0.85 <sup>a</sup>
G4	75.1 $\pm$ 7.10 <sup>b</sup>	105.9 $\pm$ 8.10	5.81 $\pm$ 0.18 <sup>a</sup>	3.58 $\pm$ 0.25 <sup>a</sup>	39.0 $\pm$ 3.33 <sup>ab</sup>	0.70 $\pm$ 0.075 <sup>b</sup>	5.90 $\pm$ 0.73 <sup>b</sup>
G5	99.6 $\pm$ 1.60 <sup>ab</sup>	97.2 $\pm$ 8.10 <sup>b</sup>	6.20 $\pm$ 0.15 <sup>b</sup>	3.97 $\pm$ 0.16 <sup>ab</sup>	35.8 $\pm$ 2.73 <sup>b</sup>	0.50 $\pm$ 0.069	3.51 $\pm$ 0.37 <sup>b</sup>
G6	105.5 $\pm$ 13.5 <sup>a</sup>	138.5 $\pm$ 13.5 <sup>a</sup>	5.25 $\pm$ 0.16 <sup>a</sup>	3.58 $\pm$ 0.16 <sup>a</sup>	59.0 $\pm$ 2.55 <sup>a</sup>	1.16 $\pm$ 0.155 <sup>a</sup>	5.80 $\pm$ 0.58 <sup>b</sup>

Data are expressed as mean  $\pm$  SE of 10 observations. Superscripts of different letters differ significantly ( $p < 0.01$ ) from each other. G1, control group; G2 l-carnitine group; G3, MTX group; G4, Co-treated group with l-carnitine; G5, Post treated group with l-carnitine; G6, MTX self treated group. bSignificantly different from MTX (G3) group. aSignificantly different from control (G1) group.

### 3.3. L-carnitine effects on oxidative stress markers in MTX toxicity

The data summarized in Table 2 & Table 3 indicates that, a significant ( $P<0.01$ ) increased in the liver and kidney MDA of MTX (G3) and MTX self treated (G6) groups when compared with the control and l-carnitine (G1&G2) groups. In the same time, data declared significant decreased ( $P<0.01$ ) in the liver and kidney GSH, catalase and total protein levels of the MTX (G3) and MTX self treated (G6) groups as compared with the

control and l-carnitine (G1&G2) groups. As well, the importance of treatment with l-carnitine groups (G4&G5) has been shown significant decreased in the MDA and significant increased in the liver and kidney GSH, catalase and total protein levels when compared to the MTX (G3) and MTX self treated (G6) groups (Table 2 & Table 3). Table 2 & Table 3 shows that; Co-treatment with l-carnitine group (G4) has been shown significant increased in the MDA and significant decreased in the liver and kidney GSH, catalase and total protein levels when compared to the post treatment with l-carnitine group (G5).

**Table 2. Changes in MDA (nmole/g tissue), catalase (mmole/min./g tissue), Glutathione (GSH; mg/tissue) and total protein (mg/g tissue) levels on liver tissues in different groups under study**

Liver				
	MDA	GSH	Catalase	Total Protein
G 1	31.32 ± 3.40 <sup>b</sup>	320.22 ± 7.856 <sup>b</sup>	670.3 ± 7.845 <sup>b</sup>	1.416 ± 0.050 <sup>b</sup>
G 2	27.43 ± 2.83 <sup>b</sup>	348.71 ± 4.126 <sup>b</sup>	698.9 ± 9.577 <sup>b</sup>	1.517 ± 0.026 <sup>b</sup>
G 3	48.72 ± 2.58 <sup>a</sup>	200.24 ± 31.379 <sup>a</sup>	319.1 ± 65.422 <sup>a</sup>	0.715 ± 0.059 <sup>a</sup>
G 4	35.23 ± 1.77 <sup>a</sup>	290.13 ± 23.336 <sup>b</sup>	580.4 ± 190.540 <sup>a</sup>	1.210 ± 0.039 <sup>b</sup>
G 5	41.34 ± 12.58 <sup>b</sup>	270.94 ± 8.682 <sup>b</sup>	550.9 ± 117.972 <sup>a</sup>	1.100 ± 0.099 <sup>b</sup>
G 6	44.11 ± 3.67 <sup>b</sup>	260.44 ± 19.409 <sup>b</sup>	410.3 ± 24.034 <sup>b</sup>	0.829 ± 0.059 <sup>a</sup>

Data are expressed as mean ± SE of 10 observations. Superscripts of different letters differ significantly ( $p<0.01$ ) from each other. G1, control group; G2 L-carnitine group; G3, MTX group; G4, Co-treated group with L-carnitine; G5, Post treated group with L-carnitine; G6, MTX self treated group.

<sup>b</sup>Significantly different from MTX (G3) group. <sup>a</sup>Significantly different from control (G1) group.

**Table 3. Changes in MDA (nmole/g tissue), catalase (mmole/min./g tissue), Glutathione (GSH; mg/tissue) and total protein (mg/g tissue) levels on kidney tissues in different groups under study**

Kidney				
	MDA	GSH	Catalase	Total Protein
G 1	27.6 ± 1.96 <sup>b</sup>	314.03 ± 15.50 <sup>a</sup>	590.2 ± 39.14 <sup>b</sup>	1.030 ± 0.036 <sup>b</sup>
G 2	24.9 ± 1.33 <sup>b</sup>	290.11 ± 10.67 <sup>a</sup>	610.4 ± 46.13 <sup>b</sup>	1.121 ± 0.047 <sup>b</sup>
G 3	47.4 ± 8.49 <sup>a</sup>	150.91 ± 28.98 <sup>b</sup>	290.1 ± 67.02 <sup>a</sup>	0.702 ± 0.087 <sup>a</sup>
G 4	33.4 ± 0.62 <sup>b</sup>	280.42 ± 9.941 <sup>b</sup>	560.4 ± 64.98 <sup>b</sup>	0.940 ± 0.066 <sup>b</sup>
G 5	37.2 ± 1.73 <sup>b</sup>	260.93 ± 14.20 <sup>b</sup>	510.8 ± 140.9 <sup>a</sup>	0.810 ± 0.042 <sup>b</sup>
G 6	29.2 ± 3.39 <sup>ab</sup>	220.04 ± 8.73 <sup>b</sup>	450.4 ± 24.03 <sup>b</sup>	1.760 ± 0.098 <sup>a</sup>

Data are expressed as mean ± SE of 10 observations. Superscripts of different letters differ significantly ( $p<0.01$ ) from each other. G1, control group; G2 l-carnitine group; G3, MTX group; G4, Co-treated group with l-carnitine; G5, Post treated group with l-carnitine; G6, MTX self treated group.

<sup>b</sup>Significantly different from MTX (G3) group. <sup>a</sup>Significantly different from control (G1) group.

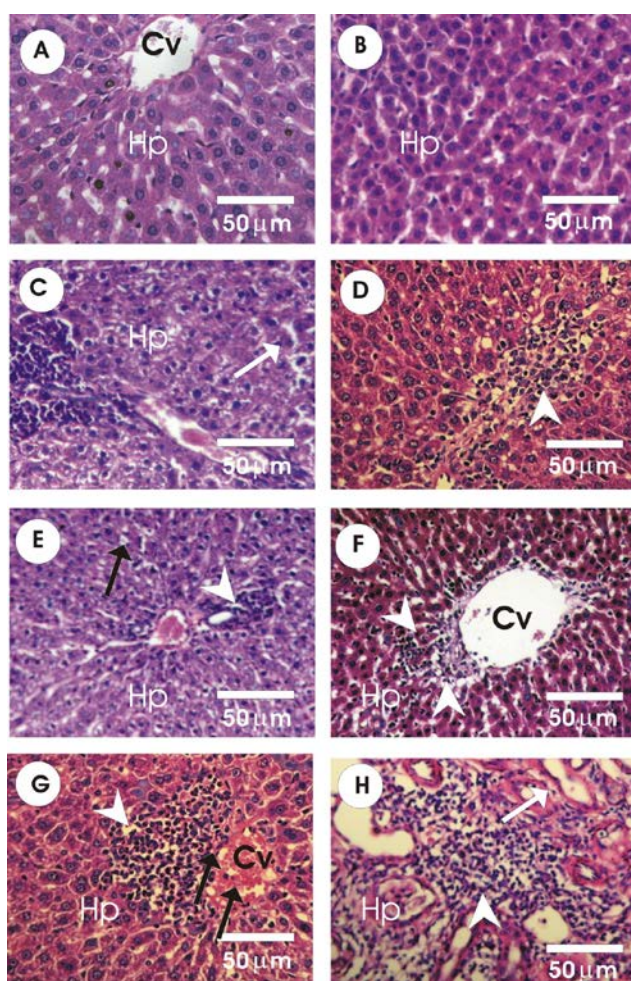
### 3.4. Effect of L-carnitine on liver and kidney histopathology

Rat liver sections in control (G1) and l-carnitine (G2) groups showing normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, and few spaced hepatic sinusoids arranged in-between the hepatic cords with fine arrangement of Kupffer cells (Figure 1A & Figure 1B). Rat liver sections in MTX group showed severe lose of liver architecture as disturbance of the hepatocytes radially arranged cords, moderate vacuolated hepatocytes, mild shrinkage and inflection hepatocytes nucleus with condensation in the structure of chromatin and congestion in central veins (Figure 1C).

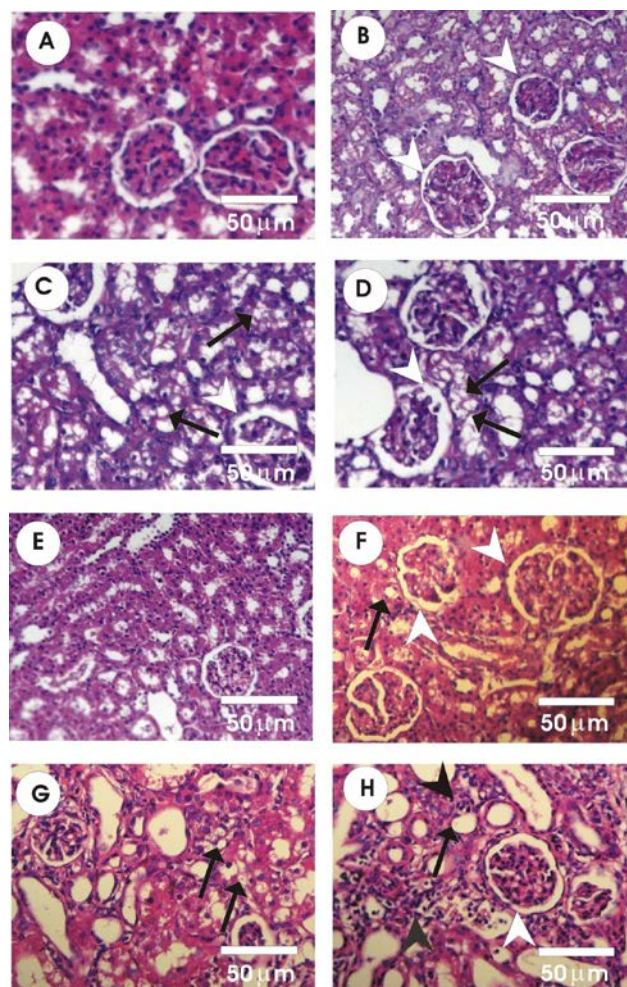
Rat liver sections in co-treated MTX with l-carnitine group (G4) shows a good degree of improvement in hepatocytes where a few vacuolated hepatocytes and mild congestion in central veins were observed, also the apoptotic cells that appeared in MTX group were not observed in this group. (Figure 1D). Rat liver sections in post treated MTX with l-carnitine shows a mild to moderate degree of improvement in hepatocytes where a mild disturbance of the hepatocytes, a few atrophied, infiltrations, congestion in central veins and mild focal necrosis (Figure 1E). Liver section in MTX self healing showed marked disturbance of the hepatocytes with strong marked hepatocellular vacuolation (Figure 1F).

Rat kidney sections in control (G1) and l-carnitine (G2) groups showing normal structure of the cortex and medulla (Figure 2A & Figure 2B). kidney sections in

MTX group showed variable pathological changes in glomeruli and some parts of the urinary tubules (Figure 2C). The most severe changes were in the malpighian corpuscles lost their characteristic configuration and cell infiltration atrophied and mild vacuolated (Figure 2C). Rat kidney sections of co-treated MTX with l-carnitine revealed a good degree of improvement glomerular damage with minimal vacuolization in tubular cells (Figure 2D). Rat kidney sections of post treated MTX with l-carnitine showed moderate organized tubular and glomerular structures with well-established epithelia which resembled that of the control group except mild inflammatory infiltration (Figure 2E). Rat kidney sections of MTX self treated showed severe loss of kidney architecture, marked disturbance in glomeruli seemed to have lost their attachments and mesangial stroma and others were atrophied with dilatation in the sub capsular space (Figure 2F).



**Figures 1A-1F.** Photomicrographs of rat Liver sections in the different experimental groups stained with Haematoxylin & Eosin. **A:** Rat Liver sections in control group revealed normal structure of hepatocytes. **B:** Rat Liver section in LC group revealed normal structure of hepatocytes (hp). **C:** Liver sections of MTX group showed moderate to severe loss of liver architecture, disturbance of the hepatocytes, congestion (Black arrows) in central veins (CV), atrophied, and vacuolated hepatocytes (White arrows). **D&E:** Liver sections in co-treated and post treated rat with LC groups revealed a moderate to mild degree of improvement in hepatocytes where a few vacuolated hepatocytes (White arrows) and mild congestion in central veins were observed. **F:** Liver section in self treated showed severe loss of liver architecture, marked disturbance of the hepatocytes and strong marked hepatocellular vacuolation (White arrows)



**Figures 2A-2F.** Photomicrographs of rat kidney sections in the different groups under study stained with Haematoxylin & Eosin. **A&B:** Rat kidney sections in control and LC groups respectively showed normal structure of renal cortex which comprised renal corpuscles (White arrows), proximal and distal convoluted tubules. **C:** Rat kidney sections in MTX group showed glomerular damage (White arrows), vacuolization in tubular cells focal necrosis and cell infiltration (Black arrows). **D:** Kidney section of co-treated group with LC revealed a good degree of improvement glomerular damage with minimal vacuolization (Arrow heads) in tubular cells. **E:** Rat kidney section in post treated group with LC showed moderate organized tubular and glomerular structures except mild inflammatory infiltration (Black arrows). **F:** Kidney section of MTX self treated showed severe loss of kidney architecture, marked disturbance in glomeruli with strong inflammatory infiltration (Black arrows)

## 4. Discussion

This study conducts a biochemical and histopathological investigations into whether l-carnitine has a protective and ameliorated effect on methotrexate-induced hepatic and renal toxicity in male rats. Methotrexate is an antimetabolite and an analogue of folic acid that used to treat autoimmune diseases such as psoriasis, rheumatoid arthritis and as a chemotherapeutic agent to treat many cancer types such as breast, skin, head, neck, lung, lymphoma, osteosarcoma and leukemia [1,2,10-14,38]. Various side effects have been reported. Studies in rheumatoid arthritis, juvenile idiopathic arthritis, sarcoidosis, and psoriasis have reported that vomiting, nausea, diarrhea, and leucopenia are the most common

adverse reactions that usually respond to dose reduction [39,40,41].

Methotrexate enters the cell via active transport across the reduced folate carrier, where it is effluxed from the cell by several of the ATP-binding cassette (ABC) transporters, especially ABCC1-5, ABCG2 and ABCB1 [42,43,44]. Levels of both enzymatic and non-enzymatic anti-oxidants are inhibited and the levels of oxidants increase in the testes, heart, liver, kidney, and gut tissues of laboratory animals given methotrexate [10,45].

In liver, the conversion of MTX to its major extracellular metabolite, 7-hydroxy methotrexate, takes place where it is oxidized by a soluble enzymatic system. In the assessment of liver injury the analysis of enzyme levels such as ALT and AST is largely used and located in the cytosol of hepatocytes. They are involved in the breakdown of amino acids into  $\alpha$ -keto acids [46]. The result of these study indicate that a significant elevation in the levels of serum ALT in methotrexate treated group that agree with Fu et al. [47], Hemeida and Omar [12], Vaghasiya et al. [48] and Vardi et al. [49] who evaluated the effect of polyherbal formulation on methotrexate induced hepatotoxicity in rats.

L-Carnitine, a quaternary amine, has a key role in the metabolism of fatty acids (energy) as a substrate for the reversible acetylation of coenzyme A [50,51] and as a carrier for the transport of long-chain fatty acids from cytosol across the inner mitochondrial membrane [52]. Furthermore, it is suggested that l-carnitine improves the oxygen supply and utilisation of the skeletal and heart muscle [53-57].

In the current study, a significant elevation in ALT and AST in MTX group however, this elevation decreased in treated group with L-carnitine and increased in self treated when compared with MTX group. A significant decreased in ALT and AST levels in co- treated methotrexate group with l-carnitine group when compared with post treated group.

Our results agreed with Fu et al. [47], Hemeida and Omar [12], Vardi et al. [49] and Tousson et al. [2] who reported that Serum ALT and AST were significantly increased in MTX induced liver damage. Elevated levels of serum ALT and AST enzymes are indicative of cellular leakage and loss of functional integrity of cell membranes in the liver [31,58,59]. Our results agreed with Kadikoylu et al. [60], Klukowska et al. [61] and Saad et al. [62], who reported that, MTX administration induced significant increase in serum ALT and AST levels. Our histological observations basically supported the results obtained from serum enzyme assays. There was a significant ( $P<0.01$ ) restoration of these enzyme levels on administration of the l-carnitine extract. The reversal of increased serum enzymes in MTX induced liver damage by the l-carnitine may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [63].

In the current study, a significant ( $P<0.01$ ) elevation in urea, creatinine and uric acid in MTX group when compared with control, this elevation decreased in treated group with l-carnitine and increased in methotrexate self healing when compared with MTX group. Our result is

agree with Kolli et al. [64] who, reported that; MTX increased urea and creatinine activities and also with Tousson et al. [2] who find that MTX increased urea and creatinine activities which induced renal toxicity. On the other hand, our results are disagreement with Cetiner et al. [11].

Oxidative stress is an indicator of the damage that results from a change in the balance between oxidants and antioxidants in favor of oxidants. If the delicate balance between oxidants and anti-oxidants cannot be maintained in tissues, many pathological changes extending to cellular damage occur. Oxidative stress or oxidative cellular damage with its dual of free radical generation and profound lipid peroxidation are hallmarks of MTX toxicity [66]. In the current study, MDA levels in MTX group were significantly increased while, glutathione, catalase and total protein levels were significantly decreased when compared with control group. The current results were similar previously reported by other investigators [9,11,45].

In the current study; a significant ( $P<0.01$ ) decreased in the MDA and significant increased in GSH, catalase and total protein levels in treated MTX with l-carnitine in rat liver and kidney when compared to the MTX and MTX self healing groups. Also, a significant ( $P<0.05$ ) increased in the MDA and significant decreased in the liver and kidney GSH, catalase and total protein levels in co-treatment with l-carnitine when compared to post treatment with l-carnitine groups. GSH is considered to be one of the most very important components of the antioxidant defense of living cells [14]. Our current results agree with [2,49,66,67,68]. who reported that, MTX administration induced significant increase in GSH levels. This results is in agreement with several studies demonstrated that MTX induces oxidative stress in tissues companied with decreased antioxidants levels [38,69].

The present study agree with Vardi et al. [49] who reported that, oxygen radicals and hydrogen peroxides have been associated with the many side effects of MTX and these free radicals trigger cell damage through binding to cellular macromolecules, particularly membrane lipids leading to releasing of ALT and AST from cells to serum. The increased in oxidative stress may cause shape and structural changes of the nucleus by causing DNA fragmentation and denaturation, which play a critical role in the initiation of apoptosis [70]. Our histopathological result is agreed with O'Rourke and Eckert [71] and Ros et al. [72] who stated that such hepatic fibrosis seemed to be due to direct toxic effects of MTX which induced proliferation of the hepatic fibrous connective tissue. Also, Hytioglou et al. [73] found that the methotrexate is known to cause hepatic fibrosis in some patients, which might progress to cirrhosis. Our results showed that; using l-carnitine along with methotrexate administration show improving oxidative stress parameters in these groups (G4, G5). MDA level in G4, G5 was significantly decreased while, GSH, CAT and total protein levels were increased. These effects of l-carnitine may result directly from antioxidant effects against free oxygen radicals or from enhanced biosynthesis of enzymatic antioxidants, such as GSH, CAT [23]. L-carnitine is known to have good antioxidant activity and has the ability to detoxify ammonia, a metabolic by-product associated with early fatigue [74]. This results agree with johovic et al. [66],

cetin et al. [11], vardi et al. [38], Ozogu et al. [10], Tousson et al. [1], Tousson et al. [2].

Our histopathological results showed that, treatment of liver and kidney with l-carnitine showed moderate to good degree of improvement in hepatocytes and malpighian corpuscles. So; methotrexate led to oxidative stress in the rat liver and kidney, while l-carnitine significantly prevented methotrexate- induced oxidative stress. Data so far obtained from this study would suggest that administration of l-carnitine after MTX challenge may have beneficial effects that could possibly be ascribed, in part, to its regulation of the oxidant/anti-oxidant balance. So, it is therefore possible that l-carnitine could scavenge free radicals and produce beneficial effects against MTX damage in liver and kidney. The mechanism by which MTX causes hepatotoxicity results from binding to the enzyme dihydrofolic reductase, thus preventing conversion of folic acid to its active form, folinic acid. This in turn blocks the synthesis of nucleic acids, certain amino acids and indirectly proteins. This might lead to damage of organelles and plasma membranes of hepatic parenchymal cells interfering with their function and allowing leakage of enzymes [1,4,31,45,58,60].

## Conflicts of Interest

The authors had no conflicts of interest to declare in relation to this article.

## References

- Tousson E, Hafez E, Zaki S, Gad A. P53, Bcl-2 and CD68 expression in response to amethopterin-induced lung injury and ameliorating role of L-carnitine. 2014; BIOPHA 3409 1-9.
- Tousson E, Atteya Z, El-Atrash A, Jeweely OI. Abrogation by *Ginkgo Byloba* Leaf Extract on Hepatic and Renal Toxicity Induced by Methotrexate in Rats. Journal of Cancer Research and Treatment 2014; 2 (3): 44-51.
- Mardini H, Record C. Detection assessment and monitoring of hepatic fibrosis: biochemistry or biopsy? Ann Clin Biochem 2005; 42 (6): 441-447.
- Hersh EM, Wong VG, Handerson ES, Freireich EJ. Effects of methotrexate. Cancer 1966; 19 (4): 600-606.
- Penalva Polo JC, Gomez Andres A, Vela P, Niveiro M. (2002): Acute liver failure in a patient with methotrexate therapy. Rev Esp Enferm Dig 2002; 94 (7): 440-441.
- Gong Y, Gluud C. Methotrexate for primary biliary cirrhosis. Cochrane Database Syst Rev 2005; 20 (3): CD004385
- West SG. Methotrexate hepatotoxicity. Rheum. Dis Clin North Am 1997; 23: 883-915.
- Yozai K, Shikata K, Sasaki M, Tone A, Ohga S, Usui H. Methotrexate prevents renal injury in experimental diabetic rats via anti-inflammatory actions. J Am Soc Nephrol 2005; 16: 3326-3338.
- ALL C, Ertan B, Ergul BK, Bulent K. N-acetylcysteine ameliorates methotrexate-induced oxidative liver damage in rats. Med Sci Monit 2006; 12 (8): 247-248.
- Ozogula B, Kisaoglua A, Turanb MI, Altunerc D, Senerd E, Cetine N, Ozturk C. The effect of mirtazapine on methotrexate-induced toxicity in rat liver. Science Asia 2013; 39: 336-356.
- Cetin A, Kaynar L, Kocyigit I, Hacioglu SK, Saraymen R, Ozturk A, Chan ES, Montesinos MC, Fernandez P. Adenosine A (2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol 2006; 148 (8): 1144-1155.
- Hemeida AR, Omar MM. Curcumin Attenuates Methotrexate-Induced Hepatic Oxidative Damage in Rats. Journal of the Egyptian Nat Cancer Inst 2008; 20 (2): 141-148.
- Vardi N, Parlakpınar H, Ates B, Cetin A, Otlu A. Anti apoptotic and antioxidant effects of beta-carotene against methotrexate-induced testicular injury. Fertil Steril 2009; 92: 2028-2033.
- Iyyaswamy A, Rathinasamy S. Effect of chronic exposure to aspartame on oxidative stress in the brain of albino rats. J Biosci 2012; 37: 679-688.
- Vanella A, Russo A, Acquaviva R, Campisi A, Di Giacomo C, Sorrenti V, et al. L-Propionyl-carnitine as superoxide scavenger, antioxidant, and DNA cleavage protector. Cell Biol Toxicol 2000; 16: 99-104.
- Srinivas SR, Prasad PD, Umapathy NS, Ganapathy V, Shekhawat PS. Transport of butyryl-L-carnitine, a potential prodrug, via the carnitine transporter OCTN2 and the amino acid transporter ATB (0,+). Am J Physiol. Gastrointest. Liver Physiol 2007; 293: 1046-1053
- Salama AF, Kasem SM, Tousson E. and Elsisy MK. Protective role of L-carnitine and vitamin E on the kidney of atherosclerotic rats. Biomedicine & Aging Pathology 2012; 2: 212-215.
- Salama A, Kasem S, Tousson E. and Elsisy MK. (2013): L-carnitine and vitamin E alleviate reproductive toxicity caused by triton WR 1339 in male albino rats. Toxicology and Industrial Health 2013; DOI: 10.1177/0748233712472523.
- Panneerselvam KS, Kumaran S. L-Carnitine and alpha-lipoic acid improves mitochondrial function during ageing process. Clin Nutr, 2006; 21: 48-60
- Juriscova A, Varmuza S. Casper RF. Programmed cell death and human embryo fragmentation. Mol Hum Reprod 1996; 2: 93-98.
- Flanagan JL, Simmons PA, Vehige J, Willcox MDP, Garrett Q. Role of carnitine in disease. Nutrition & Metabolism 2010; 7: 30.
- Cave MC, Huit RT, Frazier TH, Matheson PJ, Grrison RN, McClave SA. Obesity, inflammation and the potential application of pharmacconutrition. Nutr Clin Pract 2008; 23: 16-34.
- Yildirim R, Yildirim A, Dane S, Aliyev E, Yigitoglu R. Dose-Dependent Protective Effect of L-Carnitine on Oxidative Stress in the Livers of Hyperthyroid. EAJM 2013; 45: 1-6.
- Agarwal V., Chauhan S., Singh R., Sачev A. and D'Cruz S. (2005): Pancytopenia with the first dose of methotrexate in a patient with psoriatic. J. Indian Assoc., 13: 60-61.
- Weinhandl ED, Rao M, Gilbertson DT, Collins AJ, Pereira BJ. Protective effect of intravenous levocarnitine on subsequent-month hospitalization among prevalent hemodialysis patients, 1998 to 2003. Am J Kidney Dis 2007; 50: 803-812.
- Saluk-Juszczak J, Olas B, Wachowicz B, Glowacki R, Bald E. L-carnitine modulates blood platelet oxidative stress. Cell Biol Toxicol 2010; 26: 355-365.
- Reitman S, Frankel SA. colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer J Clin Pathol 1957; 28: 56-63.
- Bowers LD, Wong ET. Kinetic serum creatinine assays. II. A critical valuation and review. Clin Chem 1980; 26 (5): 555-561.
- Patton C, Crouch S. Determination of serum urea. Anal Chem 1977; 49: 464-469.
- Fossati P, Prencipe L, Berti G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/ 4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem 1980; 26: 227-231.
- Sakeran MI, Zidan N, Rehman H, Aziz AT, Saggi S. Abrogation by Trifolium alexandrinum root extract on hepatotoxicity induced by acetaminophen in rats. Redox Rep 2014; 19 (1): 26-33.
- Mesbah L, Soraya B, Narimane S, Jean PF. Protective effect of flavonoides against the toxicity of vinblastine cyclophosphamide and paracetamol by inhibition of lipid- peroxidation and increase of liver glutathione. Haema 2004; 7: 59-67.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-888.
- Aebi H. Catalase in vitro. Methods in Enzymology 1984; 105: 121-126.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265-275.
- Tsuyosh O, James KB. A simplified method of quantitating protein using the biuret and phenol reagents. Anal Biochem 1978; 86: 193-200.
- Bancroft JD, Steven A. Theory and Practice of Histological Technique (3<sup>rd</sup> ed.) NY: Churdchill living stone. 1990; 42: 107-110.

- [38] Vardi N, Parlakpinar H, Ozturk F, Ates B, Gul M, Cetin A, Erdogan A, Otlu A. Potent protective effect of apricot and  $\beta$ -carotene on methotrexate induced intestinal oxidative damage in rats. *Food and Chemical Toxicology*. 2008; 46: 3015-3022.
- [39] Roenigk HH Jr, Auerbach R, Maibach HI, Weinstein GD. Methotrexate guidelines revised. *J Am Acad Dermatol*. 1982; 6: 145-155.
- [40] Baughman RP, Lower EE. A clinical approach to the use of methotrexate for sarcoidosis. *Thorax* 1999; 54: 742-746.
- [41] Cheng KKF. Association of plasma methotrexate, neutropenia, hepatic dysfunction, Nausea/vomiting, and oral mucositis in children with cancer. *European J Cancer Care* 2008; 17: 306-311.
- [42] Genestier L, Paillot R, Quemeneur L, Izeradjene K, Revillard JP. Mechanisms of action of methotrexate. *Immunopharmacology*. 2000; 47: 247-257.
- [43] Wielinga P, Hooijberg JH, Gunnarsdottir S. The human multidrug resistance protein MRP5 transports folates and can mediate cellular resistance against antifolates. *Cancer Res* 2005; 65: 4425-4430.
- [44] Walling J. From methotrexate to pemetrexed and beyond. A review of the pharmacodynamic and clinical properties of antifolates. *Investigational New Drugs* 2006; 24: 37-77.
- [45] Johovic N, Cevik H, Sehirlil OA, Yegen BÇ, Şener G. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. *Journal of Pineal Research* 2003; 34: 282-287.
- [46] Maiti R, Jana D, Das UK, Ghosh D. Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 2004; 92: 85-91.
- [47] Fu Y, Zheng S, Lin J, Ryerse J, Chen A. Curcumin protects the rat liver from CCl<sub>4</sub>-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. *Mol Pharmacol*, 2008; 73 (2): 399-409.
- [48] Vaghasiya J, Bhalodia Y, Rathod S. Drug induced hepatotoxicity: effect of polyherbal formulation. *Phcog Mag* 2009; 5: 232-237.
- [49] Vardi A, Bosviel R, Rabiau N, Adjakly M, Satih S, Dechelotte P. Soy phytoestrogens modify DNA methylation of GSTP1, RASSF1A, EPH2 and BRCA1 promoter in prostate cancer cells. *In Vivo* 2010; 24: 393-400.
- [50] European Food Safety Authority (EFSA). Scientific opinion on the safety and efficiency of L-carnitine and L-tartrate as feed additives for all animal species based on a dossier submitted by Lonza Benelux. *EFSA Journal* 2012; 10 (5): 2676.
- [51] Friedmann S, Fraenkel G. Reversible enzymatic acetylation of carnitine. *Arch Biochem Biophys* 1955; 59, 491-501.
- [52] Fritz IB, Marquis NR. The role of acylcarnitine esters and carnitine palmityltransferase in the transport of fatty acyl groups across mitochondrial membranes. *Biochemistry* 1965; 54: 1226-1233.
- [53] Liedtke AJ, Nellis SH, Whitesell LF, Mahar CQ. Heart and Circulatory Physiology 1982; 243: 691-697.
- [54] Shug AL. Protection from adriamycin-induced cardiomyopathy in rats. *Zeitschrift für Kardiologie* 1987; 76 (5): 46-52.
- [55] Pierpont ME. Carnitine and myocardial function. In: Carter AL (ed.), *Current concepts in carnitine research*. Boca Raton, FL: CRS Press 1992; 192-212.
- [56] Broderick TL, Quinney HS, Lopaschuk GD. L-carnitine increases glucose metabolism and mechanical function following ischaemia in diabetic rat heart. *Cardiovasc Res* 1995; 29: 373-378.
- [57] Neu H. Carnitin: Chemie, Funktion und klinische Bedeutung bei Herzerkrankungen (Kardiomyopathien) des Hundes-eine Literaturübersicht. *Kleintierpraxis* 1995; 3: 197-220.
- [58] Drotman R, Lawhan G. Serum enzymes are indications of chemical induced liver damage. *Drug Chem Toxicol* 1978; 1: 117-163.
- [59] Sagu S, Divekar HM, Gupta V, Sawhney RC, Banerje PK, Kumar R. Adaptogenic and safety evaluation of Seabuckthorn (*Hippophae rhamnoides*) leaf extract: a dose dependent study. *Food and Chemical Toxicology* 2007; 45: 609-617.
- [60] Kadikoylu G, Bolaman Z, Demir S, Balkaya M, Akalin N, Enli Y. The effects of desferrioxamine on cisplatin-induced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys. *Hum Exp Toxicol* 2004; 23: 29-34.
- [61] Klukowska L, Nadulska A, Dyba S. The influence of cisplatin and goserelinum on the magnesium and calcium level in rat serum. *Ann. Univ. Mariae Curie Skłodowska [Med]* 2001; 56: 483-486.
- [62] Saad MF, Greco S, Osei K, Lewin AJ, Edwards C, Nunez M, Reinhardt RR. Ragaglitazar improves glycemic control and lipid profile in type 2 diabetic subjects: a 12-week, double-blind, placebo controlled dose-ranging study with an open pioglitazone arm *Diabetes*. 2004; 27: 1324-1329.
- [63] Thabrew M, Joice P. A comparative study of the efficacy of *Pavetta indica* and *Osbeckia octanda* in the treatment of liver dysfunction. *Planta Med* 1987; 53 (3): 239-241.
- [64] Kolli VK, Abraham P, Rabi S. Methotrexate-induced nitrosative stress may play a critical role in small intestinal damage in the rat. *Archives of Toxicology*. 2008; 82 (10): 763-770.
- [65] Cetiner M, Sener G, Sehirlil AO, Eksioglu-Demiralp E, Ercan F, Sirvanci S, Gedik N, Akpulat S, Tecimer T, Yegen BC. Taurine protects against methotrexate-induced toxicity and inhibits leucocyte death. *Toxicol Appl Pharmacol* 2005; 209 (1): 39-50.
- [66] Jahovic N, Sener G, Cevic H, Ersoy Y, Arbak S, Yegen BC. Amelioration of methotrexate-induced enteritis by melatonin in rats. *Cell Biochem Funct* 2004; 22-28.
- [67] Ciralik H, Bulbuloglu E, Cetinkaya A, Kurutas EB, Celik M, Polat A. Effects of N acetylcysteine on methotrexate induced small intestinal damage in rats. *The Mount Sinai Journal of Medicine* 2006; 73: 1086-1092.
- [68] Sener G, Eksioglu-Demiralp E, Cetiner M, Ercan F, Yegen BC. Beta-glucan ameliorates methotrexate-induced oxidative organ injury via its antioxidant and immunomodulatory effects. *Eur J Pharmacol* 2006; 542 (33): 170-178.
- [69] Lone IA, Kaur G, Athar M, Alam MS. Protective effect of *Rumex patientia* (English Spinach) roots on ferric. *Food Chem Toxicol* 2007; 45: 1821-1829.
- [70] Prahalathan C, Selvakumar E, Varalakshmi P. Protective effect of lipoic acid on adriamycin-induced testicular toxicity. *Clinica Chimica Acta*, 2005; 360: 160-166.
- [71] O'Rourke RA, Eckert GE. Methotrexate-induced hepatic injury in an adult. A case report. *Arch Int Med* 1964; 113: 191-194.
- [72] Ros S, Juanol X, Condom E, Canas C, Riera J, Guardiola J, Del Blanco J, Rebasa P, Valverde J, Roig-Escofet O. Light and electron microscopic analysis of liver biopsy samples from rheumatoid arthritis patients receiving long-term methotrexate therapy. *Scand J Rheumatol* 2002; 31 (6): 330-336.
- [73] Hytioglou P, Tobias H, Saxena R, Abramidou M, Papadimitriou CS, Theise ND. The canals of hering might represent a target of methotrexate hepatic toxicity. *Am J Clin Pathol*. 2004; 121 (3): 324-329.
- [74] Amin KA, Nagy MA. Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetology & Metabolic Syndrome* 2009; 1: 17.