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Protective and modulatory effects of Curcumin and L-Carnitine against Methotrexate-induced Oxidative stress in albino rats.

Faten R Abdel-Ghaffar [1]*, Ibrahim A El-Elaimy [1], Khaled A El-Dougdoug [2], Hossam I Nassar[1]

[1] Department of Zoology Faculty of Science, Menoufia University, Shebin El-Kom, Egypt.
[2] Department of Microbiology Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

ABSTRACT

In view of the hepatotoxic side effects associated with methotrexate (MTX) chemotherapy, protective and modulating efficacy of nutritional substances such as curcumin and L-carnitine has been investigated in male albino rats. In this study, a single dose of MTX (20 mg/kg b. wt., i.p.) increased notably the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) liver 5' nucleotidase ,5'NT, (122 %, 101 % and 185 %, respectively). Also, the activities of, the levels of liver protein carbonyl groups (PCO, 53 %) and lipid peroxidation (LPO, 143 %) were elevated compared to their control groups. In contrast, MTX-treated group also exhibited a significant decrease in liver antioxidant machinery represented by reduced glutathione (GSH, -42 %), glutathione peroxidase (GPx, -46 %), glutathione-s-transferase (GST, -52 %), glutathione reductase (GR, -49 %), superoxide dismutase (SOD, -33 %) and catalase (CAT, -48 %). All Those results indicated the oxidative damage caused by MTX compared to control group. The pre- and post-treatment with Curcumin, CUR, (200 mg/kg b. wt., orally, for 7 consecutive days) and L-carnitine, L-CAR, (500 mg/kg b. wt., orally, for 7 consecutive days) showed significant protective and modulatory effects for both liver and serum MTX- induced alterations. In the current study, however, the pre-treatment scored effectiveness more than post-treatments and in concurrent, CUR was effective more than L-CAR in both types of treatment.

Keywords: methotrexate; Curcumin; L-carnitine; oxidative damage; antioxidants; pre-treatment; post-treatment.

Abbreviations: (MTX)methotrexate; (CUR) Curcumin; (L-CAR) L-carnitine.

Corresponding author

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INTRODUCTION

Methotrexate (MTX) is an antineoplastic agent used to fight a number of different cancers, such as acute lymphoblastic leukemia and solid cancers, due to its inhibitory effect on de novo purine and pyrimidine synthesis through dihydrofolate reductase inhibition (Zhang et al.,2009). On the other hand, substantial evidence supports the concept that MTX was mutagenic and carcinogenic in animals. And the efficacy of this compound is often limited by its severe hepatotoxicity (Hassanane et al., 2010; Tunali-Akbay et al., 2010). Moreover,(Penalva et al., 2002) reported acute liver failure in a patient with MTX therapy. The toxic effect of anticancer drugs may be reduced if supplemented with natural anti- oxidants plant products (Hassanane et al., 2010).Turmeric, which contains primarily curcumin and other curcuminoid compounds, is obtained from the roots of the plant *Curcuma longa* that grows in tropical regions (Javvadi et al., 2008). It is known to possess multiple pharmacological properties such as anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-ischemic, hypotensive and finally it acts as free radical scavenger and antioxidant (Somasundaram et al., 2002; Joe et al., 2004; Yadav et al., 2009). Moreover, many studies indicated the protective effect of curcumin in the treatment of various pro-inflammatory diseases (Srivastava et al., 2011). L-Carnitine is an endogenous compound that plays an important physiological role in the transfer of long-chain fatty acids across the inner membrane of the mitochondria for their β-oxidation and energy production (Sayed-Ahmed., 2010). Sener et al. (2006) and Calabrese et al. (2007) assumed that L-CAR may play an important antioxidative system and has an antiperoxidative and anti-apoptotic effects on several tissues. Recent study reported that the possible protective mechanisms of L-carnitine include the inhibition of mitochondrial membrane permeability, as transition, the decrease of oxidative stress, and the prevention of pro-apoptotic protein expression (Chao et al., 2011). In view of the above findings, the present study was designed to evaluate the antioxidant effects of curcumin, as a plant extract, and L-carnitine, as a pharmaceutical medicine, against MTX-induced oxidative stress in male albino rats. We used two types; pre- and post-treatment, of curcumin and L- carnitine.

MATERIALS AND METHODS

Animals:

Male albino rats weighing 100-120 g were obtained from National Research Institute, Cairo, Egypt and acclimatized for one week prior to the experiment. They were maintained under standard laboratory conditions of room temperature (22-25 °C) and a relative humidity (55±5 %) with a light period of 12 h light/dark cycle/day. Animals received standard laboratory balanced commercial diet and water *ad libitum*. Working on rats in this study was performed according to the guidance for care and use of laboratory animals.

Chemicals and reagents:

Methotrexate was obtained from Orion Pharma (Nasr City, Cairo, Egypt). L-carnitine was obtained from Arab Co. for Pharmaceuticals & Medicinal Plants MEPACO- EGYPT (Enshas El

Raml, Sharkeiya, Egypt. The fine chemicals; Sodium nitrite, ammonium molybidate, 1-chloro-2,4-dinitrobenzene(CDNB), ascorbic acid, ammonium acetate, riboflavin, Nitroblue Tetrazolium (NBT), sulfanilamide and trichloroacetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thiobarbituric acid was obtained from Fluka (Berlin, Germany).

Preparation of Curcumin extract:

The rhizomes of *Curcuma longa* were collected from the agricultural Research Centre, Giza, Egypt.Curcumin was powdered and repeatedly extracted in the room temperature with 70% ethanol/water until exhaustion.

Protocol design

48 rats were separated randomly into 8 groups,6 rats each, as follow:-

Group 1: Served as normal control and remained untreated.
Group 2: Rats were treated orally with CUR (200 mg/kg b.w.,) (Cekmen et al., 2009) for 7 consecutive days.
Group 3: Rats were treated orally with L-CAR (500 mg/kg b.w) (sener et al., 2006) for 7 consecutive days.
Group 4: Rats were injected with a single dose of MTX (20 mg/kg b.w., i.p.) (sener et al., 2006).
Group 5: Rats were orally administrated the same dose of CUR (pre-treatment), then injected with the corresponding dose of MTX.
Group 6: Rats were injected with the corresponding dose of MTX, then, 24 hours later, followed by the same dose of CUR (post-treatment).
Group 7: Rats were orally administrated the same dose of L-CAR (pre-treatment), then injected with the corresponding dose of MTX.
Group 8: Rats were injected with the same dose of MTX, then, 24 hours later, followed by the corresponding dose of L-CAR (post-treatment).

Samples collection

Groups 1, 2, 3 were anaesthetized at the end of treatment. **Group 4** was anaesthetized, 24 hours after MTX injection. **Groups 5,6,7,8** were anaesthetized on 9^{th} day from the beginning of their treatment. Then blood samples from hepato-portal vein were withdrawn. Serum was separated by centrifugation at 4000 rpm for 15min, at 4ºC. Liver was discarded, blotted dry, weighed and homogenized in cold 0.15 M KCl to give a final concentration of 10% (w/v) homogenate. All samples were stored at -20^{o}C till biochemical analyses.

Post-mitochondrial supernatant preparation

Samples from the dissected livers were taken, and immediately perfused with ice-cold saline. The liver was homogenized (1:10, w/v) in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%). The homogenate was filtered, centrifuged at 3000 rpm for 10 min to

separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 rpm for 20 min at 4 ^{o}C to obtain post-mitochondrial supernatant (PMS).

Biochemical parameters

The enzymes and metabolites were determined according to the corresponding methods. Serum AST and ALT activities (Reitman and Frankel, 1957) and liver 5´-NT activity (El-Aaser and El-Merzabani, 1975). Liver reduced glutathione (GSH) content (Beutler and Kelley, 1963) and activities of liver antioxidant enzymes ; glutathione-S-transferase (GST)(Habig et al.,1974), glutathione reductase (GR) (Carlberg and Mannervik, 1975), glutathione peroxidase (GPx) (Hafeman et al., 1974), superoxide dismutase (SOD) (Beauchamp and Fridovich,1971) and catalase (CAT) (Goth, 1991) were determined. Also, liver lipid peroxidation (LPO) (Ruiz-Larrea et al., 1994) and protein carbonyl (PCO) (Levine et al., 1990) contents were assayed.

Statistical analysis

Results are expressed as mean ± SE. Data were analyzed by SPSS software, version 19(Chicago, IL, USA). The statistical evaluation of all data was done using analysis of variance (ANOVA) followed by Student's t-test. P value <0.05 was considered statistically significant.

RESULTS

MTX treatment

MTX provoked highly significant elevation in activities of serum AST (122 %), ALT (101 %)and liver 5'-NT (185 %), compared to control group (Table 1).

Table (1): **Liver function enzymes in the different studied groups.**

Treatment groups	Serum aspartate aminotransferase (AST) (U/L)	Serum alanine aminotransferase (ALT) (U/L)	Liver 5'-Nucleotidase (mol Pi/min/gm tissue)
Control	96.00 ±2.37	59.00 ±1.21	13.54 ±0.26
Curcumin (CUR)	104.00 ±2.55	64.00 ±1.34	14.81 ±0.37
L-carnitine (L-CAR)	102.00 ±2.32	60.00 ±1.11	15.44 ±0.42
Methotrexate(MTX)	214.00 ±2.84^{c} (**122%**)	119.00 ±1.84^{c} (**101%**)	38.62 ±0.62^{c} (**185%**)
CUR+MTX (PRE)	151.00 ±5.81^{c,e} (**57%**)	72.00 ± 3.24^{a,f} (**22%**)	21.31 ±1.87^{f} (**57%**)
MTX+CUR (POST)	158.00 ±5.51^{c,e} (**64%**)	75.00 ±3.67^{a,e} (**%26**)	22.28 ±2.42^{f} (**65%**)
L-CAR+ MTX(PRE)	162.00 ±3.50^{c,e} (**68%**)	78.00 ±3.50^{a,e} (**32%**)	23.94 ±2.36^{e} (**77%**)
MTX+L-CAR(POST)	166.00 ±4.10^{c,e}(**72%**)	82.00 ±3.85^{b,e} (**38%**)	24.58 ±2.04^{a,e} (**82%**)

Each value represents mean ± S.E. (n = 6).
(a) P < 0.05, (b) P < 0.01, and (c) P < 0.001, are significant compared to the corresponding control group.
(e) P < 0.01, and (f) P < 0.001, are significant compared to the corresponding MTX -treated group.

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It caused a highly significant (P < 0.001) depletion (42 %) in the level of liver GSH compared to control group. Also, liver antioxidant enzymes GST and GR activities decreased with MTX treatment (52 % and 49 %), respectively, compared to their control values (table2).

Table 2: Liver GSH content, GST and GR activities in the different studied groups.

Treatment groups	Reduced glutathione (GSH) (mg/g tissue)	Glutathione-S-transferase (GST) (nmole CDNB conjugate formed/min/ml)	Glutathione reductase (GR) (nmole NADPH oxidized/min/ml)
Control	72.10 ±0.54	51.60 ±0.44	33.20 ±0.21
Curcumin (CUR)	67.50 ±0.75	50.50 ±0.52	32.30 ±0.32
L-carnitine (L-CAR)	69.00 ±0.62	50.90 ±0.37	32.88 ±0.26
Methotrexate (MTX)	41.60 ±0.61 [c] **(42%)**	24.70 ±0.60 [b] **(52%)**	17.00 ±0.41 [b] **(49%)**
CUR + MTX (PRE)	65.20 ±2.33 [f] **(10%)**	44.80 ±1.16 [e] **(13%)**	30.40 ±1.32 [e] **(8%)**
MTX + CUR (POST)	64.40 ±2.06 [f] **(11%)**	43.20 ±1.43 [e] **(16%)**	29.60 ±1.21 [e] **(11%)**
L-CAR + MTX (PRE)	61.70 ±3.10 [e] **(14%)**	42.20 ±2.11 [e] **(18%)**	28.13 ±1.30 [d] **(15%)**
MTX + L-CAR (POST)	60.50 ±3.30 [e] **(16%)**	41.70 ±2.34 [e] **(19%)**	26.80 ±1.37 [d] **(19%)**
Each value represents mean ± S.E., (n = 6). (b) P < 0.01 and (c) P < 0.001, is significant compared to the corresponding control group. (d) P < 0.05, (e) P < 0.01, and (f) P < 0.001, are significant compared to the corresponding MTX- treated group.			

MTX administration caused highly significant (P < 0.001) decrease (46 %, 33 % and 48 %) in the activities of the enzymatic antioxidants GPx, SOD and CAT, respectively, compared to the corresponding control groups(Table 3).Finally, MTX significantly increased the levels of LPO and PCO (143 % and 53 %), respectively when compared to their control groups (Figs. 1 & 2)

Table 3: **Liver antioxidant enzymes activities in the different studied groups.**			
Treatment groups	**Glutathione peroxidase (GPx) (nmoles GSH oxidized/min/mg tissue)**	**Superoxide dismutase (SOD) (% inhibition)**	**Catalase enzyme (CAT) (kU/L)**
Control	37.60 ±0.28	79.00 ±0.58	375.20 ±4.31
Curcumin (CUR)	38.43 ±0.31	76.40 ±0.69	345.10 ±4.87
L-carnitine (L-CAR)	38.13 ±0.25	77.00 ±0.61	352.50 ±4.66
Methotrexate (MTX)	20.43 ±0.62 [c] **(46%)**	53.00 ±0.75 [c] **(33%)**	187.20 ±3.96 [c] **(48%)**
CUR + MTX (PRE)	29.86 ±1.13 [c,f] **(21%)**	70.10 ±2.27 [b,e] **(12%)**	299.40 ±13.95 [b,f] **(16%)**
MTX + CUR (POST)	29.22 ±0.97 [c,f] **(22%)**	69.10 ±2.15 [b,e] **(13%)**	293.20 ±12.39 [b,f] **(18%)**
L-CAR + MTX (PRE)	27.95 ±1.84 [c,e] **(26%)**	66.00 ±3.23 [b,d] **(17%)**	285.60 ±10.25 [b,e] **(20%)**
MTX + L-CAR (POST)	27.72 ±1.93 [c,e] **(26%)**	65.50 ±3.14 [b,d] **(18%)**	277.90 ±11.64 [b,e] **(22%)**
Each value represents mean ± S.E., (n = 6). (b) P < 0.01 and (c) P < 0.001, are significant compared to the corresponding control group. (d) P < 0.05, (e) P < 0.01, and (f) P < 0.001, are significant compared to the corresponding MTX treated group.			

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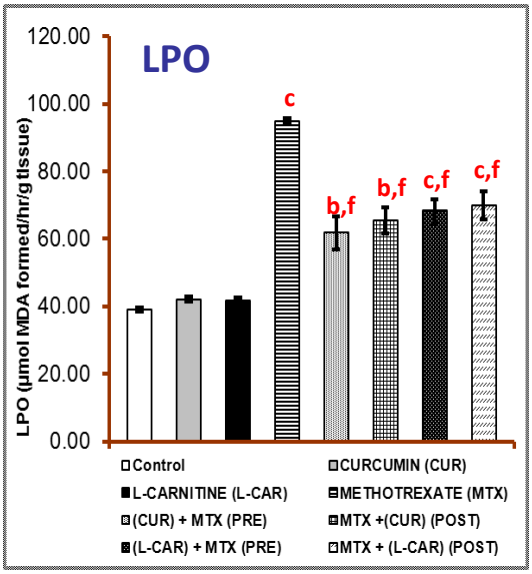

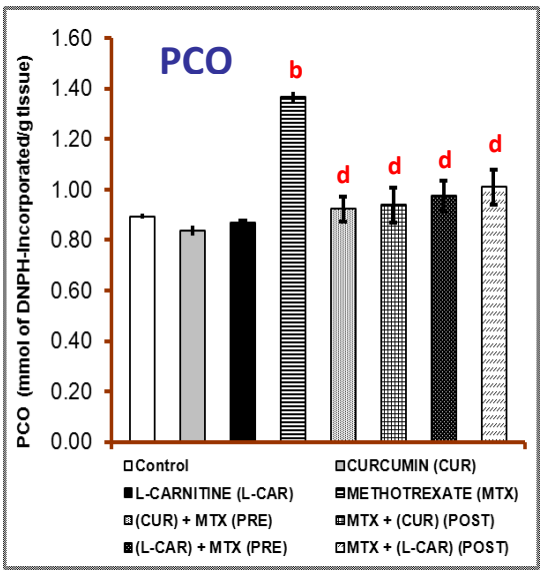

Fig 1: liver LPO level in rat treated with; MTX, CUR and L-CAR. Each value represents mean ± S.E.(n = 6). (b) P < 0.01 and (c) P < 0.001, are significant compared to the control group. (f) P < 0.001, is significant compared to the MTX -treated group

Fig 2: liver PCO level in rat treated with; MTX, CUR and L-CAR. Each value represents mean ± S.E. (n = 6). (b) P < 0.01, is significant compared to the control group. (d) P < 0.05, is significant compared to the MTX -treated group.

Pre-treatment with CUR and L-CAR

Pre-treatment with either CUR or L-CAR moderately protected the serum activities of AST (57 % and 68 %), ALT (22 % and 32%) and Liver 5'-NT (57 % and 77 %), respectively, from the alterative MTX effect, compared to their control groups (Table 1). Similarly, Pre-treatment, significantly protected the level of liver GSH (10 % and 14 %) and liver antioxidant enzymes activities of GST (13 % and 18 %) and GR (8 % and 15 %), with CUR and L-CAR respectively when they were compared to control groups (Table 2).

On the same line, CUR and L-CAR significantly protected the liver activities of GPx (21 % and 26 %), SOD (12 % and 17 %) and CAT (16 % and 20 %), respectively, each was compared to its control group.

On the other hand, liver levels of LPO (58 % and 75 %) and PCO (3 % and 9 %) were protected with CUR and L-CAR, respectively, compared to their control groups(Figs.1&2).

Post-treatment with CUR and L-CAR

CUR and L-CAR significantly ameliorated the serum AST (64 % and 72 %), ALT (26 % and 38 %) and Liver 5'-NT (65 % and 82 %) activities, respectively when compared to their control groups (Table 1). Also, post-treatment significantly restored the level of liver GSH (11 % and 16 %) and liver antioxidant enzymes activities of GST (16 % and 19 %) and GR (11 % and 19 %), with

CUR and L-CAR respectively, all compared to control group. On the same line, CUR and L-CAR significantly improved liver GPx (22 % and 26 %), SOD (13 % and 18 %) and CAT (18 % and 22 %) activities, respectively, compared to normal control (Table 3).

On the other side, liver levels of LPO (68 % and 79 %) and PCO (5 % and 13 %) have been restored with CUR and L-CAR post-treatment, respectively, compared with control group(Figs. 1& 2).

DISCUSSION

Substantial evidence supports the concept that methotrexate was mutagenic and carcinogenic in animals (Hassanane et al., 2010; Tunali-Akbay et al.2010). 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. While inside hepatic cells, MTX is stored in a polyglutamated form. It is well documented that long-term MTX administration can cause accumulation of MTX polyglutamates and consequently decreased folate level (Johovic et al. 2003).

AST and ALT are cytosolic enzymes of the hepatocytes. They are involved in the breakdown of amino acids into α-keto acids (Maiti et al., 2004). Any increase in their activities reflects a leakage in plasma membrane permeability, which in turn, is associated with cell death. They are best indicators of liver necrosis (Hemeida and Mohafez, 2008).

In our study, MTX provoked significant elevation in serum AST and ALT activities, reflecting impaired liver function. This result is in harmony with the previous studies which reported that MTX increased AST and ALT activities (Fu et al., 2008).The authors reported that presence of higher level of MTX poly glutamates inside liver cells causes a longer intracellular presence of the drug, and this has been suggested as a mechanism for MTX hepatotoxicity (Johovic et al. 2003).In addition, 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 AST and ALT from cells to serum (Vardi et al., 2010).

5' Nucleotidase (5'-NT) is a phosphatase that specifically catalyses hydrolysis of nucleotides such as 5' AMP and 5' IMP (Pagani and Panteghini, 2001). In liver, this enzyme is located primarily in the canaliculi and sinusoidal membranes and it is known as a liver damage indicator. An advanced result indicated high 5' NT level in infected hepatitis (Pratibha et al., 2004). In our study, liver 5' NT activities increased as an indication of MTX toxicity, whereas this high activity may be due to intrahepatic obstruction of bile canaliculi as a result of liver cell injury and due to increase in fluidity of the cell membrane (Subhani et al., 2009).

All aerobic organisms have antioxidant defenses to remove or repair the damaged molecules. These antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects. They retard the progress of many chronic diseases as well as lipid

peroxidation (Lai et al., 2001; Gulcin et al., 2002).GSH is one of the most common biologic non-enzymatic antioxidant. Its function includes removal of free radicals such as H2O2 and superoxide anions, maintenance of membrane protein thiols and acting as a substrate for GPx and GR (Naik and Panda, 2007). As several studies suggested that MTX-treatment depletes GSH levels in many tissues (Johovic et al., 2004; Sener et al., 2006), the present study showed a highly significant reduction in GSH level after MTX administration. Recent study reported that GSH forms the first line of defense against oxidative stress, by direct interaction of its sulfhydryl group with ROS and/or it can be involved in the enzymatic detoxification reaction of ROS as a cofactor or as a coenzyme (Tarasub et al., 2012). It is well demonstrated that the cytosolic (NADP)–dependent dehydrogenases and NADP malic enzyme are inhibited by MTX, suggesting that the drug could decrease the availability of NADPH in cells (Johovic et al. 2003). Normally, NADPH is used by glutathione reductase to maintain the reduced state of cellular glutathione (GSH). Thus, this was the main reason for significant reduction in reduced glutathione level promoted by MTX and could lead to the reduction of antioxidant enzyme defense system effectiveness (Vardi et al., 2010). Our data confirmed the concept that oxidative stress plays a role in MTX – induced tissue damage, whereas GSH reduction was accompanied by reduction in the antioxidant enzyme defense system represented as depletion in the levels of GST, GR, GPx, SOD and CAT. This is in agreement with several studies demonstrated that MTX induces oxidative stress in tissues companied with decreased antioxidants levels (Lone et al., 2007; Vardi et al., 2008).It is well documented that GST is known to detoxify carcinogenic metabolites by thiol conjugation while GPx catalyses the reduction of H2O2 and hydroxyl peroxides into nontoxic products. Their decreased level was remarkable after MTX challenge and this may be one reason for high lipid peroxidation (Ikediobi et al., 2004). On the other hand, liver glutathione reductase (GR) used NADPH to maintain the reduced state of cellular glutathione, GSH (Vardi et al., 2008).The authors added that SOD catalyses the dismutation of superoxide anions and protect cells against oxidative stress (Saradha and Mathur, 2006 ; Coleshowers et al., 2010). Catalase (CAT) acts as a preventative antioxidant whereas it catalyses the reduction of H_2O_2 and plays an important role in protection against the deleterious effects of lipid peroxidation, ROS and hydroxyl radicals caused by MTX administration (Dinkova-Kostova, 2002; Vardi et al., 2008). Herein, decrease in the activities of antioxidant enzymes can be explained either with their induction during the conversion of free radicals into inactive metabolites or secondarily with the direct inhibitory effect of MTX on enzymes activities.

With MTX, superoxide and reactive hydroxyl radicals attack polyunsaturated fatty acids in cell membranes and lead to lipid peroxidation (LPO) and membrane disruption. (Saluk-Juszczak et al., 2010).This increase in LPO levels suggests enhanced peroxidation leading to tissue damage and failure of the antioxidant defense mechanism to prevent the formation of excessive free radicals (Vardi et al., 2008; Cetin et al., 2011).

Exposure of proteins to reactive oxygen species can alter the physical and chemical structure of the target protein causing consequent oxidation leading to oxidation of hydrophobic amino acyl residues to hydroxy and hydroperoxy (P-OOH) derivatives, protein carbonylation (PCO), oxidation of total sulfhydryl groups (T-SH), nitrotyrosine (NT) formation, and many others (Beal, 2002 ; Stadtman, 2004). Protein carbonyl groups (PCO), aldehydes and

ketones, are produced on protein side chains when they are oxidized by almost all types of ROS. Our data showed that MTX administration increased PCO level in liver. It has been suggested that detection of elevated levels of PCO is generally a sign not only of oxidative stress but also of a protein dysfunction (Dalle-Donne et al., 2003).

The current study clarified that pre-treatment with CUR and L-CAR protected plasma membranes from the attacking of free radicals and prevented their damage. Consequently, they reduced releasing of AST and ALT. Also the hepatotoxicity was limited for a degree that 5'NT activity still closed to normal value with MTX injection. While the post-treatment improved AST, ALT and 5'NT activities and ameliorated MTX cytotoxic effect this is in agreement with other studies(Sriganth and Premalatha, 1999; Hemeida and Mohafez, 2008).

The results showed that, CUR and L-CAR treatment impeded and consequently improved GSH level. Their pre-treatment limited GSH reduction and the depletion in the activities of antioxidants GST, GR, GPx, SOD and CAT. This regulation documented the roles of CUR and L-CAR in protecting cells against oxidative damage caused by MTX, in liver tissue, throughout their antioxidant and free radical scavenging activities (Sener et al., 2006; Tuba and Gulcin, 2008). On the other side, as shown in our results, post-treatment minimized the toxic effects caused by MTX which is in agreement with several studies that recorded CUR and L-CAR activities against oxidative damage. (Mahfouz et al., 2009; Kandemir et al., 2011).

So in general, CAR may acts as antioxidant either having a primary antioxidant activity, inhibiting free radical generation, scavenging the initiating free radicals, and terminating the radical propagation reactions, or more likely, functioning as a secondary antioxidant, repairing oxidized polyunsaturated fatty acids esterified in membrane phospholipids (sener et al., 2006; Mahfouz et al., 2009). Also, the antioxidant mechanism of CUR may include one or more of; cavenging or neutralizing of free radicals, interacting with oxidative cascade and preventing its outcome, oxygen quenching and making it less available for oxidative reaction,as well as inhibition of oxidative enzymes (Tuba and Gulcin, 2008; Kandemir et al.,2011).

CONCLUSION

Curcumin and L-carnitine possessed various protective and modulatory mechanisms against MTX-induced liver toxicity throughout pre- and post- treatment. We can conclude that pre-treatment prevented oxidative damage by inhibiting ROS production and improving antioxidant enzymes. While post-treatment regulated the oxidant/antioxidant balance and neutralized the toxic side effects caused by MTX-induced oxidative stress. Furthermore, along with their antioxidant and free radical scavenging activities, they are expected to have no side effects.

Our study showed that CUR possessed protective and ameliorative effects more,for some extent, than L-CAR. Concurrently, pre-treatment acted with more effectiveness than post-treatment. Thus, our study provides important evidences for experimental and clinical

investigations about the role of the pre- and post- ways of treatment against the toxic side effects of MTX therapeutics.

DISCLOSURE: The authors declare that there is no conflict of interest.

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