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L-carnitine ameliorates methotrexate-induced ovarian dysfunction in female rats

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Abstract

This study aimed to investigate the effect of L-carnitine (L-car) supplementation on methotrexate (MTX) related ovarian dysfunction. Fifty female albino rats weighing 200 –250 g were randomly divided into five groups (10 rats each); the duration of the experiment was four weeks. Control group received saline (IP). MTX group received 1 mg/kg (IP) once weekly for four weeks. Whereas, MTX+L-car50, MTX+L-car100 and MTX+folic acid groups: daily received either L-car (50 or 100 mg/kg) or folic acid 250 µg/kg respectively (orally) plus 1 mg/kg methotrexate (IP) once weekly for four weeks. Treatment of the rats with MTX produced a significant decrease in body and ovarian weights, ovarian index, serum total antioxidant capacity (TAC) and serum anti-Müllerian hormone (AMH). However, serum total oxidant status (TOS), Oxidative stress index (OSI), ovarian tissue malondialdehyde (MDA), nitric oxide (NO) and caspase-3 levels were significantly increased. Besides, there were histological abnormalities in the ovarian tissues with a significant increase in antral follicles count (AFC). Administration of either L-car (50 or 100 mg/kg) or folic acid corrected the abnormal biochemical parameters and improved the histopathological abnormalities and AFC. These data suggested that L-car might protect against MTX-induced ovarian dysfunction in female rats through antioxidant, anti-apoptotic effects and improving the ovarian reserve.

Keywords: Methotrexate; L-carnitine; ovarian dysfunction; oxidative stress; caspase-3; anti- Müllerian hormone.

1. Introduction

Women receive cytostatic agents for the treatment of cancer. Some women are in the prepuberty period and most of them are in the reproductive age. The preservation of ovarian reserve in these women at the reproductive age directly affects their future potential problems of infertility **(Yucebilgin et al., 2004)**. Methotrexate (MTX) a folic acid antagonist is commonly used in the treatment of hematological tumors (lymphoblastic leukaemia) **(de Deus et al., 2012)** and many other types of cancer.

Due to its anti-inflammatory and immunosuppressive effects, it is also effective in the treatment of chronic inflammatory diseases and various autoimmune diseases. Moreover, it is used in ectopic and molar pregnancies **(Furst, 1997)**. MTX acts by reversible inhibition of dihydrofolate reductase (DHFR) enzyme leading to a decrease in the synthesis of folic acid, impairing DNA synthesis and cell multiplication **(Barnhart et al., 2007)**. MXT toxicity is obvious in rapidly turnover cells **(Stika, 2012)**. **Shamberger et al. (1981)** reported that MTX, particularly in high doses could

produce ovarian dysfunction. As the primordial follicles cannot regenerate, ovarian insufficiency and infertility are more likely to occur **(Yucebilgin et al., 2004)**. Folate antagonizing property of MTX is considered the main cause responsible for MXT associated side effects. These undesirable effects of MTX may be theoretically decreased or prevented by folic acid treatment, but this may be associated with decreased effectiveness of MTX therapy **(Whittle and Hughes, 2004)**.

Oxidative stress is involved in MTX-induced tissue damage. In cells, MTX inhibits cytosolic nicotinamide adenosine diphosphate hydrogen (NADPH), which is used by glutathione reductase (GR) to maintain glutathione (GSH) levels, an important cytosolic antioxidant **(Vardi et al., 2010)**. Besides, MTX not only produces a decrease in GSH levels, but also increases malondialdehyde (MDA) a marker of oxidative stress and a metabolite of free radical-induced lipid peroxidation **(Kose et al., 2012)**.

L-carnitine (L-car) is synthesized mainly in the liver and kidney from lysine and methionine essential amino acids **(Kelly, 1998)**. L-car has an important role in lipid metabolism, where it acts as a cofactor necessary for the transport of long-chain fatty acids into the mitochondrial matrix, where they undergo β-oxidation for cellular energy production, and so it inhibits mitochondrial oxidative stress and apoptosis in different cell types **(Furuno et al., 2001; Barhwal et al., 2007)**. It acts as a free radical scavenger and has an important role in stabilizing anti-oxidative systems. Besides, it has an anti-peroxidative effect on different tissues, which might be responsible for its beneficial effects on oxidant-induced injury **(Rauchova et al., 2002)**. L-car increases follicular survival and function of ovarian grafts via decreasing oxidative stress and preventing follicle apoptosis **(Zhang et al. 2015)**. Another study reported that L-car can improve and repair lead-induced harmful effects on the ovary through the reduction in reactive oxygen species (ROS) production and increasing the antioxidant capacity **(Galal et al., 2016)**. The goal of the current study was to investigate the underlying mechanisms of MTX-related ovarian dysfunction, Moreover, the study aimed to evaluate the efficacy of L-carnitine in comparison to folic acid in ameliorating this effect in female rats.

2. Materials and Methods

2.1. Drugs and chemicals

MTX, L-car and folic acid were obtained from Sigma Aldrich Company, England. Kits for measurements of total oxidant status (TOS), total antioxidant capacity (TAC), MDA, nitric oxide (NO) levels and saline (0.9% NaCl) were obtained from Biodiagnostic Company Pharmaceutical Industries, Egypt. Caspase-3 kit was obtained from Elabscience Co, Egypt, and anti-Müllerian hormone (AMH) kit was obtained from Bioassay Technology Co, Egypt.

2.2. Animals

Fifty female adult albino rats weighing 200 –250 g were used. Animals were obtained from the animal house, Faculty of Science, Sohag University, and housed in the animal house with room temperature being maintained at 22-30°C. Animals were fed on a commercial pellet diet and kept under a normal light/dark cycle. Animals were given food and water ad libitum. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Sohag University (Approval No. Soh-Med 21-12-40).

2.3. Experimental design

Rats were randomly classified into five groups, ten rats each. The duration of treatment was four weeks.

Control group: rats treated with normal saline 0.5 ml/rat IP route for four weeks.

MTX group: rats treated with 1 mg/kg MTX IP once weekly for four weeks (Cetinkaya et al., 2006).

MTX+L-car 50 group: rats treated daily with 50 mg/kg L-car orally for four weeks (Dokmeci et al., 2006) plus 1 mg/kg methotrexate IP once weekly for four weeks.

MTX+L-car 100 group: rats treated daily with 100 mg/kg, L-car orally for four weeks (Dokmeci et al., 2006) plus 1 mg/kg methotrexate IP once weekly for four weeks.

MTX+folic acid group: rats treated daily with 250 µg/kg, folic acid orally for four weeks (Ardeshir and Rezaei, 2003) plus 1 mg/kg methotrexate IP once weekly for four weeks.

Weights of all animals were recorded at the beginning of the experiment and before being sacrificed by decapitation at the end of the study.

Blood samples were collected in prelabeled centrifuge tubes. The serum was separated following centrifugation of blood at 3500 rpm for 15 minutes. Serum was stored quickly at –80ºC until the time of analysis and used for measurement of TOS, TAC and AMH levels. After dissection, both ovaries were rapidly excised and perfused with cold normal saline, then dried with filter paper and weighed. The right ovaries were homogenized in 1-2 ml cold buffer (100 mM potassium, pH 7.4, containing 2 mM EDTA per g tissue. The sample was centrifuged at 4000 rpm for 15 minutes. The supernatant was removed, stored at -80ºC and used for measurement of MDA, NO and caspase 3 levels. The left ovaries were preserved in 10% formalin and used for histopathological examination.

2.4. Biochemical analysis

2.4.1. Measurement of serum TOS level

Total oxidant status (TOS) was measured in serum by a colorimetric method as described by **Fossati et al. (1980)** and **Aebi (1984)**. This method is based on the reaction of H_2O_2 with 3,5-discharge-2-hydroxybenzensulfonic (DHBS) acid and 4-aminopheazone (AAP) in the presence of peroxide (HRP) to form achromphore, its absorbance was measured at 510 nm. TOS was expressed in mM/L.

2.4.2. Measurement of serum TAC level

Total antioxidant capacity (TAC) was measured in serum by a colorimetric method as described by **Koracevic et al. (2001)**. The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro–2–hydroxy benzensulphonate to a colored product. The change in the absorbance was measured at 505 nm. TAC was expressed in mM/L.

2.4.3. Calculation of OSI

Oxidative stress index (OSI) was calculated according to the following formula: (TOC/TAC) ×100 **(Harman, 1965)**.

2.4.4. Measurement of tissue MDA level

Malondialdehyde (MDA) level is an indicator of lipid peroxidation. MDA in ovarian tissue homogenate was detected by a colorimetric method as described by **Ohkawa et al. (1979)**. Thiobarbituric acid reacts with MDA in an acidic medium for 30 min to form a thiobarbituric acid reactive product. The absorbance of the resultant pink product was measured at 534 nm. MDA level was expressed in nmol/g tissue.

2.4.5. Measurement of NO level

Nitric oxide (NO) was determined in ovarian tissue homogenate by a colorimetric method according to the method of **Montgomery and Dymock (1961)**. The level of Nitrite in the sample was expressed in µmol/ g tissue.

2.4.6. Measurement of caspase-3 level

Ovarian tissue caspase-3 was measured using sandwich enzyme-linked immunosorbent assay (ELISA) kits. The concentration of caspase 3 in the samples was determined by comparing the optical density of the samples to the standard curve using an ELISA microplate reader at 450 nm. Caspase 3 level was expressed as ng/ g tissue.

2.4.7. Measurement of AMH level

Serum anti-Müllerian hormone (AMH) level was measured using ELISA kits. AMH level was expressed as ng/ml.

2.5. Histopathological Examination

The left ovary from each rat was excised and washed in sterile saline solution. The tissues were fixed in neutral buffered formalin, processed to paraffin wax sectioned at 5 µm. The slides were stained with haematoxylin and eosin (H&E) **(Bancroft and Gamble, 2002)**. Stained sections were examined and photographed using a digital camera, attached to Olympus CX21 light microscope and connected to a computer. The number of antral follicles in the ovaries were counted by examining five fields of each ovary at a magnification of x200 and then counting the follicles whose plane of section passed through the nucleolus of the oocytes.

2.6. Statistical analysis

Mean ± SE of the values were expressed. Data were statistically analyzed using the one-way analysis of variance (ANOVA). A Tukey post hoc test was performed to compare the changes among individual groups. The difference was regarded as significant when $P<0.05$. All statistical analyses were performed using the SPSS program, version 26 software package.

3. Results

3.1. Changes in body and ovarian weights

As shown in **Table 1**, the final body weight in MTX group decreased significantly (P<0.05) compared to the control group. However, the MTX+L-car50 group, MTX+L-car100 group and MTX+folic acid group produced an insignificant change in the final body weight compared to the MTX group. The ovarian weight in MTX group was significantly (P<0.05) decreased compared to the control group. In MTX+L-car50 group, MTX+L-car100 group and MTX+folic acid group the ovarian weight significantly (P<0.05) increased compared to the MTX group with insignificant difference among the three groups.

3.2. Changes in ovarian Index

The ovarian index showed a significant (P<0.05) decrease in the MTX group compared to the control group. In treatment with MTX+L-car50, the ovarian index showed insignificant change compared to the MTX group. However, upon treatment of the rats with either MTX+L-car100 or MTX+folic acid, the ovarian index increased significantly (P<0.05) compared to the MTX group, with an insignificant difference between them **(Table 1)**.

3.3. Changes in serum TOS and TAC

Compared to the control group, the MTX group exhibited a significant (P<0.05) increase in serum TOS. Treatment of the rats with MTX+L-car (50, 100) and MTX+folic acid exhibited a significant decrease (P<0.05) in serum TOS compared to the MTX group with insignificant difference among the three groups **(Table 2)**.

As regard TAC, the MTX group produced a significant (P<0.05) decrease in serum TAC compared to the control group. However, MTX+L-car50 group produced an insignificant change in serum TAC compared to MTX group. On the other hand, MTX+L-car100 group and MTX+folic acid group produced a significant (P<0.05) increase in serum TAC compared to MTX group. There was an insignificant difference between MTX+L-car100 group and MTX+folic acid group **(Table 2)**.

3.4. Changes in OSI

As shown in Table (2), treatment of the rats with MXT produced a significant (P<0.05) increase in the OSI compared to the control group. Treatment with MTX+L-car (50, 100) or MTX+folic acid produced a significant decrease (P<0.05) in OSI compared to MTX group.

3.5. Changes in ovarian tissue MDA level

Ovarian tissue MDA level showed a significant (P<0.05) increase in MTX group compared to the control group. L-car supplementation of both groups produced a significant (P<0.05) decrease in ovarian tissue MDA level compared to MTX group, with MTX+L-car100 group near to the control level. In addition, folic acid supplementation produced a significant (P<0.05) decrease in ovarian tissue MDA level compared to MTX group. No significant difference was detected between MTX+L-car50, MTX+L-car100 and MTX+ folic acid groups **(Table 3)**.

3.6. Changes in ovarian tissue NO level

MTX group produced a significant (P<0.05) increase in ovarian tissue NO level compared to the control group. However, MTX+L-car50, MTX+L-car100 and MTX+folic acid groups showed a significant (P<0.05) decrease in ovarian tissue NO level compared to MTX group with insignificant difference among the three groups **(Table 3)**.

3.7. Changes in ovarian tissue caspase-3 level

Ovarian tissue caspase-3 level showed a significant (P<0.05) increase in MTX group compared to the control group. MTX+L-car50 and MTX+folic acid groups showed insignificant change in ovarian tissue caspase-3 level compared to MTX group. As regard MTX+L-car100 there was a significant (P<0.05) decrease in ovarian tissue caspase-3 level compared to MTX group **(Fig. 1)**.

3.8. Changes in serum AMH level

There was a significant (P<0.05) decrease in serum AMH level in MTX group compared to the control group. Either MTX+L-car50, MTX+L-car100 or MTX+folic acid groups showed a significant (P<0.05) increase in serum AMH level compared to MTX group. Besides, there was insignificant difference among the three groups **(Fig. 2)**.

Table 1. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on body weight, ovarian weight and ovarian index in MTX -induced ovarian dysfunction in female rats.

Parameter	Control	MTX	MTX +L-car50	MTX +L-car100	MTX +folic acid
Body weight (g)	244.50 ±2.70	204.20* ±2.36	215.10* ±3.27	214.60* ±3.24	216.20* ±3.58
Ovarian weight (g)	0.140 ±0.004	0.073* ±0.005	0.101*• ±0.008	0.104*• ±0.009	0.106*• ±0.005
Ovarian index	0.0573 ±0.0017	0.0357* ±0.0026	0.0472 ±0.0039	0.0484• ±0.0040	0.0492• ±0.0025

Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine.
***** Significant change at ($p < 0.05$) vs. control group**.**
• Significant change at ($p < 0.05$) vs. MTX group.

Table 2. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on serum TOS, TAC and OSI in MTX -induced ovarian dysfunction in female rats.

Parameter	Control	MTX	MTX +L-car50	MTX +L-car100	MXT + folic acid
TOS (mM/L)	0.12 ±0.007	0.31* ±0.014	0.14• ±0.013	0.14• ±0.008	0.13• ±0.008
TAC (mM/L)	1.50 ±0.086	0.64* ±0.021	0.65* ±0.020	1.07*•[a] ±0.018	0.91*•[a] ±0.024
OSI	8.59 ±0.75	49.22* ±2.64	21.83*• ±2.06	12.84•[a] ±0.73	14.80•[a] ±0.80

Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine, TOS= Total oxidant status, TAC= Total antioxidant capacity, OSI= Oxidative stress index.
* Significant change at ($p < 0.05$) vs. control group.
• Significant change at ($p < 0.05$) vs. MTX group.
[a] Significant change at ($p < 0.05$) vs. MTX+ L-car50 group

Table 3. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on ovarian tissue MDA and NO levels in MTX -induced ovarian dysfunction in female rats.

Parameter	Control	MTX	MTX +L-car50	MTX +L-car100	MXT +Folic acid
MDA nmol/g	134.40 ±6.90	213.55* ±11.99	142.89• ±9.28	135.04• ±10.11	136.85• ±13.57
NO µmol/g	11.790 ±0.504	26.965* ±1.223	19.156*• ±1.138	18.685*• ±1.378	14.958• ±1.124

Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine, MDA= Malondialdehyde, NO= Nitric oxide. * Significant change at ($p < 0.05$) vs. control group. • Significant change at ($p < 0.05$) vs. MTX group.

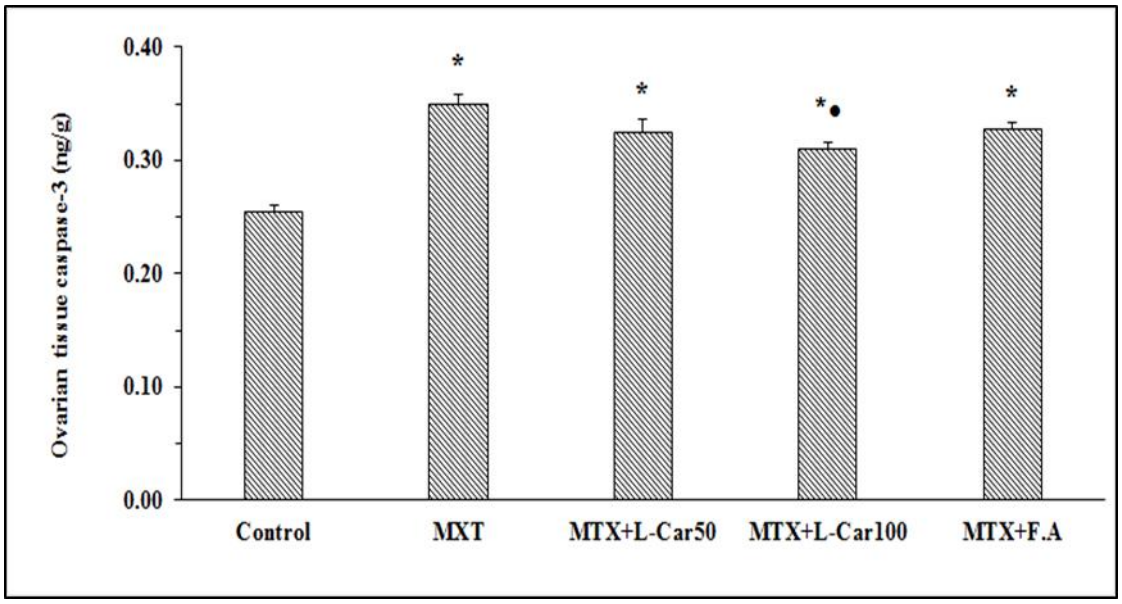

Figure 1. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on ovarian tissue caspase-3 level in MTX -induced ovarian dysfunction in female rats. Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine.
* Significant change at ($p < 0.05$) vs. control group.
• Significant change at ($p < 0.05$) vs. MTX group.

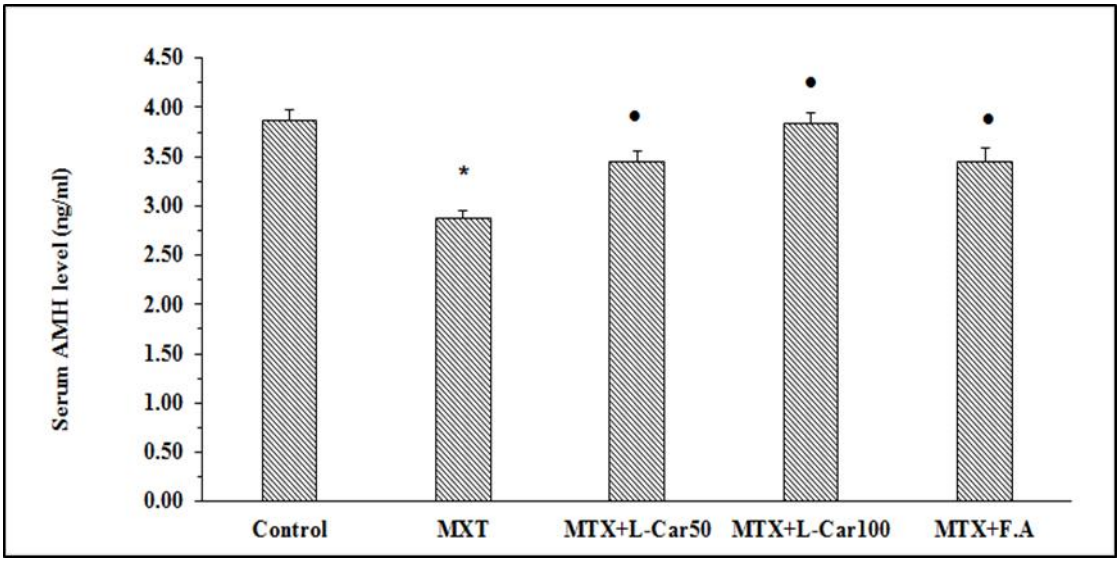

Figure 2. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on serum AMH level in MTX -induced ovarian dysfunction in female rats. Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine, AMH= Anti-mullerian hormone.
* Significant change at ($p < 0.05$) vs. control group.
• Significant change at ($p < 0.05$) vs. MTX group.

3.9. Histopathological changes

Examination of the ovaries of the control group revealed normal ovarian architecture **(Fig. 3A)**, there was intact germinal epithelium and ovarian follicles at different stages of maturation including primordial, primary, secondary, antral and Graafian follicles. The corpora lutea were also noticed in the ovarian stroma. The medulla consists of a richly vascularized loose connective tissue containing blood vessels. However, in the MTX group, the ovaries showed detachment of germinal epithelium, the cortex contained ovarian follicles at different stages of maturation but with degenerative changes in the nucleus of Graafian and antral follicles. The medulla contained multiple dilated blood vessels

(Fig. 3B,C). Regarding, ovaries of MTX+L-car50 group, there was intact epithelium covering fibrous stroma, the ovarian follicles were observed at different stages of development including primary, secondary and Graafian follicles with average lining and containing oocyte **(Fig. 3D)**. MTX+L-car100 group **(Fig. 3E)** revealed intact epithelium covering fibrous stroma. The ovarian follicles at different stages of development were observed including primary, secondary and Graafian follicles with average lining and containing oocytes. Respecting the ovaries of MTX+folic acid group **(Fig. 3F,G)**, the epithelium covering fibrous stroma was intact, the ovarian follicles were observed at different stages of development within the fibrous stroma of the ovary, including primary, secondary and Graafian follicles with average lining and containing oocyte. There were less congested blood vessels compared to MTX group.

Moreover, the antral follicles count (AFC) in MTX group was significantly ($P<0.05$) decreased compared to the control group. In MTX+L-car (50,100) and MTX+folic acid groups, there was a significant ($P<0.05$) increase in AFC compared to the MTX group with an insignificant difference between them **(Fig. 4)**.

4. Discussion

Concurrent with the growing incidence of cancer, antineoplastic drugs usage increased. Methotrexate is one of the widely used anti-cancer agents. Together with its beneficial effects, it has various side effects on many body organs. Oxidative stress, inflammation, and apoptosis are proposed to be the mechanisms implicated in MTX toxicity **(Çakır et al 2011; Morsy et al., 2013; Mukherjee et al., 2013; Vardi et al., 2013; Ali et al., 2014; Ibrahim et al., 2014)**. Accordingly, this study was designed to investigate the underlying mechanisms of MTX-related ovarian dysfunction. Moreover, to evaluate the efficacy of L-car in ameliorating this effect in female rats.

In the present study, treatment of rats with MTX displayed a significant decrease in the body, ovarian weights and ovarian index compared to the control group. These results are comparable with the findings of **Karri (2011)**. MTX is a DHFR inhibitor, which alters folate coenzyme pattern, and in turn alters cell differentiation and tissue growth leading to a decrease in body and ovarian weights **(Aarsaether et al., 1988)**. L-car and folic acid treatment could not ameliorate the decrease in the body weight. However, they significantly ameliorated the decrease in ovarian weight induced by MTX administration. These findings agree with **Boonsanit et al. (2006)**, who studied the effect of L-car in ameliorating doxorubicin-induced nephrotic syndrome in rats and reported no effect of L-car on the body weight of the rats. Moreover, **Eid (2016)** mentioned that L-car could not alleviate the decrease in the body weight, but it normalized the decrease in testicular weight in cisplatin-induced testicular toxicity in rats. In addition, **Mutavdzin et al. (2019)** reported no change in the body weight of diabetic rats treated with folic acid in comparison to diabetic rats without treatment.

Oxidative stress occurs due to increased production of ROS and the imbalance between oxidants and antioxidants levels **(Asvadi et al., 2011)**. Oxidative stress takes part in MTX-induced tissue damage. Cells usually try to retain MTX in the cytosol in the form of polyglutamate. Prolonged administration of MTX leads to elevated intracellular polyglutamate levels and decreased folic acid levels **(Vardi et al., 2010)**. Furthermore, MTX suppresses cytosolic nicotinamide adenosine diphosphate (NADP)-dependent dehydrogenases and NADP malic enzyme, and so decreases NADPH and GSH levels in the cells **(Johovic et al., 2003)**, this leads to reduction of the antioxidant enzyme defense system effectiveness and sensitizing the cells to ROS **(Babiak et al., 1998)**. Moreover, MTX causes lipid peroxidation that is indicated by a significant increase in MDA levels. Lipid peroxidation, arbitrate by oxygen-free radicals, is considered to be the main cause of damaging the cell membranes and contributes to MTX-induced tissue damage **(Pınar et al., 2018)**. In addition, MTX increases the activity of inducible nitric oxide synthase (iNOS) prompting increased production of NO; resulting in oxidative damage **(Leitão et al., 2011)**.

In the present study, MTX administration led to an increase in serum TOS and a decrease in serum TAC. Moreover, OSI was significantly increased. In addition, ovarian tissue MDA and NO levels significantly increased with MTX treatment. This result was similar to the findings of **Daggulli et al. (2014)** and **Soylu Karapinar et al. (2017)**. In addition, **Gunyeli et al. (2021)** reported a significant increase in TOS and OSI levels in ovarian tissue due to the administration of MTX.

In the present study, L-car and folic acid treatments seem to be efficient in alleviating MTX- induced oxidative stress effect. They significantly decreased

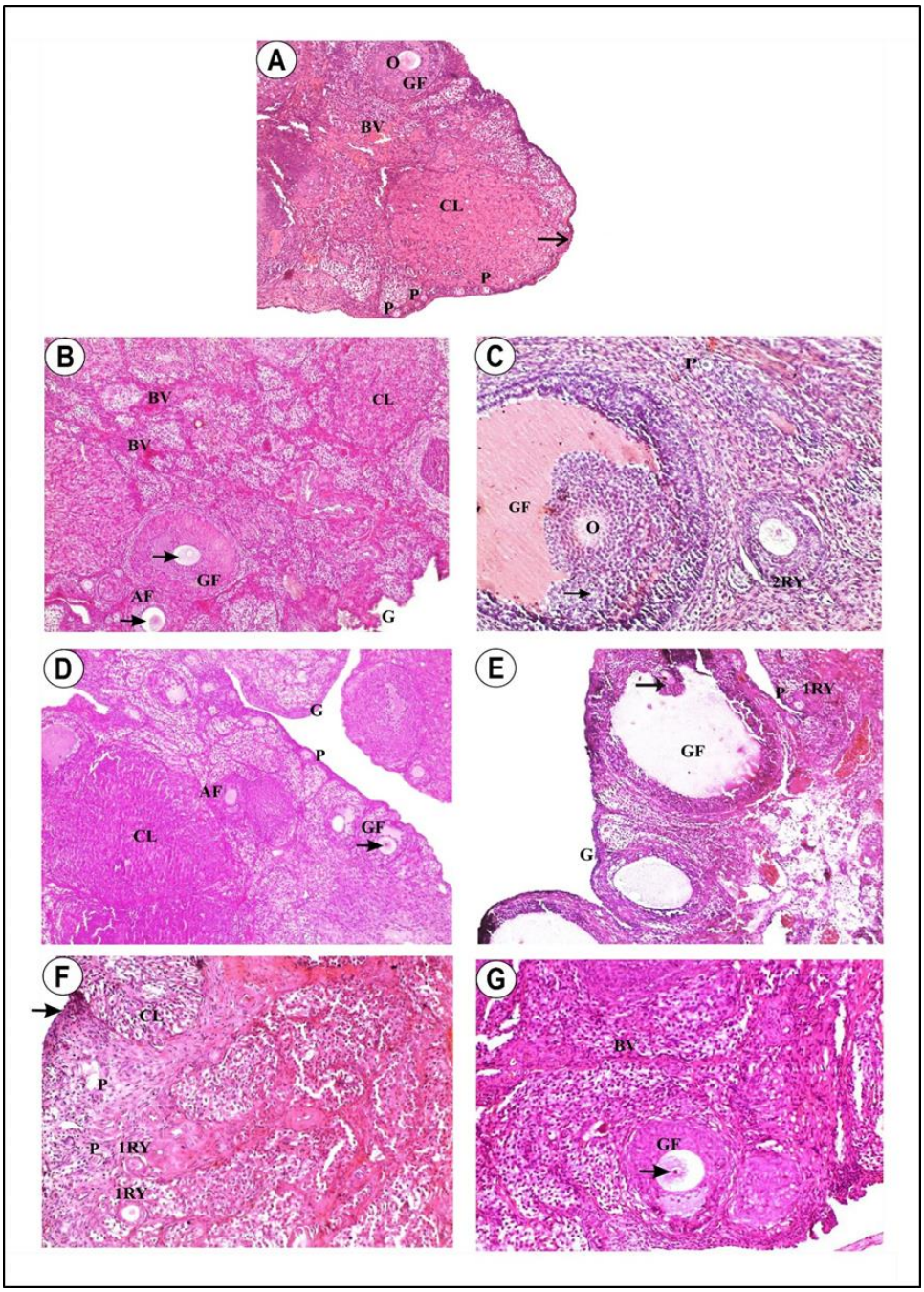

Figure 3. Photomicrographs of the ovarian tissue showing effects of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/day) on MTX -induced ovarian dysfunction in female rats.

A: Ovarian tissue of control group of female rats showing intact germinal epithelium (→), ovarian follicles in different stages of development: primordial follicles (P), Graffian follicles (GF) containing oocyte (O) and corpus luteum (CL), blood vessels (BV) (H&E× 200).

B: Ovarian tissue of the MTX group showing detached germinal epithelium (G), shrunken oocyte (→) in the antral follicles (AF) and Graffian follicles (GF), corpus luteum (CL), medulla contains dilated blood vessels (BV) (H&E× 200).

C: Ovarian tissue of MTX group rats showing primordial follicles (P), secondary follicles (2RY), Graffian follicles (GF) containing shrunken oocyte (O), follicular cell vaculation (→) (H&E× 400).

D: Ovarian tissue of MTX+L-Car50 group showing intact germinal epithelium (G), primordial follicles (P), Graffian follicles (GF) containing regular oocyte (→), antral follicles (AF) and corpus luteum (CL) (H&E× 200).

E: Ovarian tissue of MTX+L-Car100 group showing intact germinal epithelium (G), primordial follicles (P), primary follicles (1RY) and Graffian follicles (GF) containing regular oocyte (→) (H&E× 400).

F: Ovarian tissue of MTX+FA group showing intact germinal epithelium (→), primordial follicles (P), primary follicles (1RY) and corpus luteum (CL) (H&E× 200).

G: Ovarian tissue of MTX+FA group showing Graffian follicles (GF) containing regular oocyte (→), blood vessels (BV) less congested (H&E× 200).

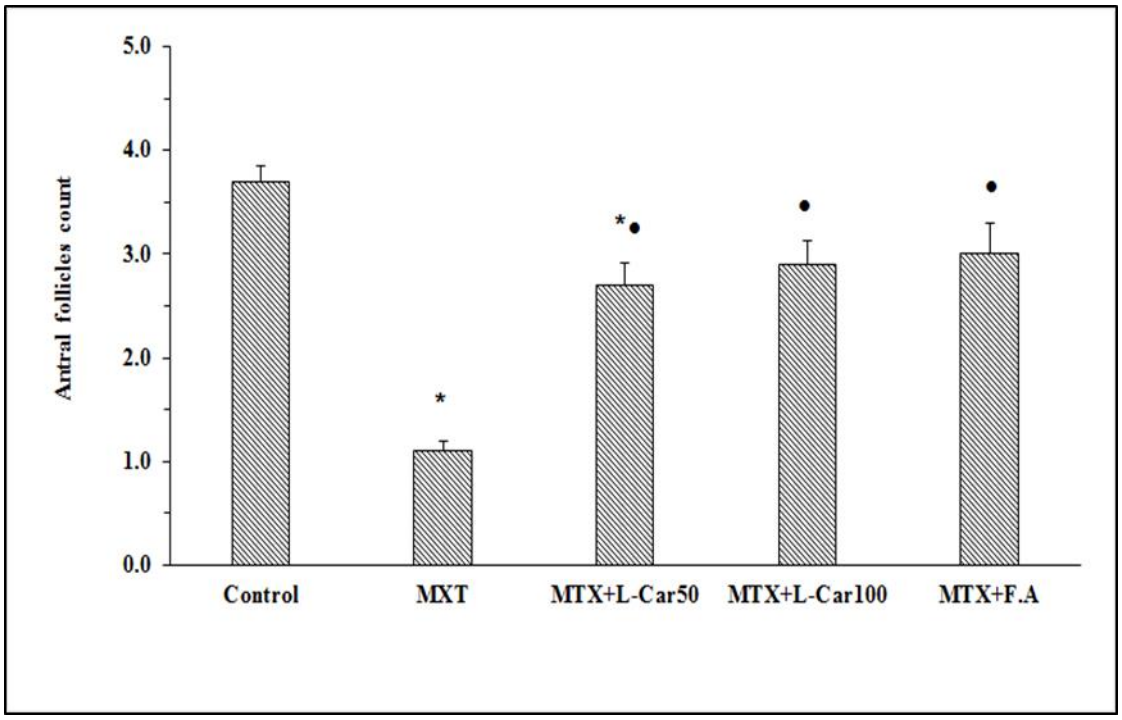

Figure 4. Effect of oral L-car (50, 100 mg/kg/ day) and folic acid (250 µg/kg/ day) on total antral follicles count in MTX -induced ovarian dysfunction in female rats. Data represent mean ± SE of 10 observations. MXT= Methotrexate, L-car= L-carnitine.
* Significant change at ($p < 0.05$) vs. control group.
• Significant change at ($p < 0.05$) vs. MTX group.

serum TOS and increased serum TAC, together with decreasing OSI. Ovarian tissue MDA and NO levels were also significantly decreased. Present results are in accordance with the findings of other researches **(Yurut-Caloglu et al., 2015; Eid, 2016; Kelek et al., 2019; Koohpeyma et al., 2019)**. **Surai (2015)** described the antioxidant effect of L-car to be mediated through a different mechanism; it inhibits the enzymes responsible for free radical production and so behaves like a free radical scavenger. In stress conditions, L-car maintains mitochondrial integrity and prevents ROS formation. Also, it Inhibits ROS-generating enzymes, such as xanthine oxidase and NAPDH oxidases. Moreover, L-car maintains optimal redox status of the cell by activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor alpha (PPARα) and inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) with additional synthesis of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), GR, glutathione S-transferase (GST), catalase (CAT) and GSH synthesis. It chelates catalytic metals-promoters of ROS such as Fe and Cu. L-Car regulates vitagenes and synthesis of heat shock proteins (HSPs), sirtuins, thioredoxins and other antioxidant molecules. Furthermore, L-car reduces the accessibility of lipids for peroxidation by transferring fatty acids into the mitochondria for β-oxidation and so reduce the production and accumulation of lipid peroxidation products **(Izgut-Uysal et al., 2003; Dokmeci et al., 2005; Derin et al., 2006, Aboubakr et al., 2020)**. The antioxidant effect of folic acid is due to reduction in plasma homocysteine concentrations **(Hoffman, 2011)**, which may decrease ROS formation and elevate TAC. Folic acid increases GSH regeneration **(Ojeda et al., 2009)** and regulates the transcription of NADPH oxidase **(Sarna et al., 2012)**.

Treatment of rats with MTX produced a significant increase in ovarian tissue caspase-3 level. MTX leads to increased oxidative stress, which has an important role in the induction of apoptosis. Moreover, it binds to DHFR enzyme with greater affinity than folic acid, and inhibits the formation of tetrahydrofolate (necessary for the synthesis of DNA) from folic acid **(Tsurusawa et al., 1997)**. MTX suppress the synthesis of purine and pyrimidine thymidylate resulting in the formation of incorrect DNA and apoptosis **(Heenen et al.,**

1998). Treatment of the rats with L-car (100 mg/kg) produced a significant decrease in the caspase-3 level compared to MTX treated group. This result agrees with **Şener et al. (2006)**, who mentioned that MTX-induced organ toxicity is linked to increasing leukocyte apoptosis and L-car treatment protects against leukocyte apoptosis and oxidative injury. Besides, **Famularo et al. (2004)** illustrated that L-car deficiency contributes to the progression of the infection with human immunodeficiency virus, and patients' treatment with L-car counteracted the unregulated process of lymphocyte apoptosis. Furthermore, **Andrieu-Abadie et al. (1999)** showed that L-car was able to prevent cardiac myocytes apoptosis induced with doxorubicin, implying beneficial effects of L-car against chemotherapeutic drug-induced toxicity. The stabilizing effect of L-car on the outer membrane of mitochondria prevents the efflux of cytochrome C into the cytosol, where cytochrome C released from mitochondria has a pivotal role in the apoptotic cascade and activation of downstream caspases as caspase-3 **(Di Cesare et al., 2007; Shalini et al., 2015)**. Moreover, caspase-3 could be activated by ROS, so the antioxidant property of L-car has a role in its inhibitory effect on caspase-3 level **(Kelek et al., 2019)**.

Multiple assessments can be used to evaluate the ovarian reserve after gonadotoxic therapy (chemotherapy and radiotherapy); among them is the measurement of serum AMH level. AMH is excreted by granulosa cells of pre-antral and antral follicles, and it is the gold standard marker for evaluating the ovarian reserve compared to the other tests, as its level is not fluctuating during the menstrual cycle like other gonadotropins. Moreover, its plasma level is related to the size of the follicle pool in the ovary **(Benian et al., 2013)**. In addition to measurement of serum AMH level, counting the number of ovarian follicles with histological examination (AFC) is also considered an important parameter in assessing the ovarian reserve **(Oner et al., 2011)**. Nevertheless, this method is not practical in human and so animal models were used to evaluate the effects of drugs on ovarian reserve **(Ozcelik et al., 2010)**. In the present study, MTX treatment decreased both AMH level and AFC compared to the control group. In addition, there was a detachment of germinal epithelium, with degenerative changes in the nucleus of Graafian and antral follicles with multiple dilated blood vessels. These results are in agreement with other works **(Karri, 2011; Ulug and Oner, 2014; Soylu Karapinar et al., 2017; Hortu et al., 2020; Gunyeli et al., 2021)**. On the other hand, **Oriol et al. (2008)** reported no significant change in the AMH level after single-dose MTX administration. **Benian et al. (2013)** reported no statistically significant change in the AMH level after multiple dose MTX administration. L-car and folic acid treatment in the present study were able to counteract MTX-induced reduction in AMH level and AFC, and restore the histopathological changes compared to MXT group. Our results are in harmony with the results of **Kalhori et al. (2019)**, who studied the effect of L-car on folliculogenesis in mice following induction of polycystic ovary syndrome. They showed a significant increase in AFC and a significant decrease in the mean number of primary and preantral follicles with the presence of corpora lutea indicating the occurrence of the ovulation with L-car treatment compared to the polycystic ovary syndrome group. Furthermore, Yurut-**Caloglu et al. (2015)** studied the effect of L-car and amifostine against radiation-induced acute ovarian damage in rats and reported a significant increase in the AFC with L-car treatment compared to the group that received radiation only. In addition, **Shohda et al. (2017)** evaluated the protective effect of folic acid on the ovary of female rats affected by MTX and reported a reduction of the histological abnormalities in the ovary of folic acid-treated rats in comparison to MTX-treated rats. These data indicated that L-car may decrease the risk of MTX-induced ovarian toxicity and its use may improve ovarian dysfunction outcomes.

5. Conclusion

Depending on the current biochemical findings, which are supported by the histopathologic evidence, the results of the present study confirm that MXT induced ovarian dysfunction in female rats. Treatment with L-car could ameliorate and protect against this effect through its antioxidant and anti-apoptotic effects. In addition, it improved the ovarian reserve. Bearing in mind that L-car is available in the diet to a large degree, it is beneficial to use L-car supplementation to counteract the toxic side effects of the chemotherapeutic agent.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

References:

Aarsaether, N., Berge, R.K., Aarsland, A., Svardal, A., Ueland, P.M., **1988**. Effect of methotrexate on long-chain fatty acid metabolism in liver of rats fed a standard or a defined, choline-deficient diet. Biochim Biophys Acta Mol Cell Biol Lipids, 958(1), 70-80.

Aboubakr, M., Elsayd, F., Soliman, A., Fadl, S. E., El-Shafey, A., Abdelhiee, E.Y., **2020**. L-Carnitine and vitamin E ameliorate cardiotoxicity induced by tilmicosin in rats. Environ Sci Pollut Res Int, 27,23026-23034.

Aebi, H., **1984**. Catalase in vitro. Meth enzymol, 105, 121-126.

Ali, N., Rashid, S., Nafees, S., Hasan, S.K. and Sultana, S., **2014**. Beneficial effects of Chrysin against Methotrexate-induced hepatotoxicity via attenuation of oxidative stress and apoptosis. Mol Cell Biochem, 385(1-2), 215- 223.

Andrieu-Abadie, N., Jaffrezou, J.P., Hatem, S., Laurent, G., Levade, T., Mercadier, J.J., **1999**. L-Carnitine prevents doxorubicin-induced apoptosis of cardiac myocytes: role of inhibition of ceramide generation. FASEB J, 13,1501-1510.

Ardeshir, A., Rezaei, M., **2003**. The Effect of Oral Administration of L-Tyrosine, Folic acid and Pyridoxine on Perphenazine Induced Catatonia in Rat. Iran J Pharm Sci, 2(3), 167-171.

Asvadi, I., Hajipour, B., Asvadi, A., Asl, N.A., Roshangar, L. and Khodadadi, A., **2011**. Protective effect of pentoxyfilline in renal toxicity after methotrexate administration. Eur Rev Med Pharmacol Sci, 15,1003-1009.

Babiak, R.M.V., Campello, A.P., Carnieri, E.G.S., Oliveira, M.B.M., **1998**. Methotrexate: pentose cycle and oxidative stress. Cell Biochem Funct, 16, 283-293.

Bancroft, J.D., Gamble, M., **2002**. Theory and practice of histological techniques: Histopathology. Churchill Livingstone, Edinburgh, London, 5th edition.

Barhwal, K., Singh, S. B., Hota, S. K., Jayalakshmi, K., Ilavazhagan, G., **2007**. Acetyl-L-carnitine ameliorates hypobaric hypoxic impairment and spatial memory deficits in rats. Eur J Pharmacol 570, 97-107.

Barnhart, K., Hummel, A. C., Sammel, M. D., Menon, S., Jain, J., Chakhtoura, N., **2007**. Use of "2-dose" regimen of methotrexate to treat ectopic pregnancy. Fertil Steril, 87, 250-256.

Benian, A., Guralp, O., Uzun, D. D., Okyar, A., Sahmay, S., **2013**. The effect of repeated administration of methotrexate (Mtx) on rat ovary: measurement of serum antimullerian hormone (AMH) levels. Gynecol Endocrinol, 29(3), 226-229.

Boonsanit, D., Kanchanapangka, S., Buranakarl, C., **2006**. L-carnitine ameliorates doxorubicin-induced nephrotic syndrome in rats. Nephrology, 11(4), 313-320.

Çakır, T., Özkan, E., Dulundu, E., Topaloğlu, Ü., Şehirli, A.Ö., Ercan, F., Şener, E., Şener, G., **2011**. Caffeic acid phenethyl ester (CAPE) prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pharm Pharmacol, 63(12), 1566-1571.

Cetinkaya, A., Bulbuloglu, E., Kurutas, E. B., Kantarceken, B., **2006**. N-acetylcysteine ameliorates methotrexate-induced oxidative liver damage in rats. Med Sci Monit, 12(8), 274-278.

Daggulli, M., Dede, O., Utangac, M.M., Bodakci, M.N., Hatipoglu, N.K., Penbegul, N., Sancaktutar, A.A., Bozkurt, Y., Türkçü, G. and Yüksel, H., **2014**. Protective effects of carvacrol against methotrexate-induced testicular toxicity in rats. Int J Clin Exp Med, 7(12), 5511-5516.

de Deus, D.M.V., de Lima, E.L.S., Seabra Silva, R.M., Leite, E.P., Cartaxo Muniz, M.T., **2012**. Influence of methylenetetrahydrofolate reductase C677T, A1298C, and G80A polymorphisms on the survival of pediatric patients with acute lymphoblastic leukemia. Leuk Res Treatment, 2012, 292043.

Derin, N., Agac, A., Bayram, Z., Asar, M., Izgut-Uysal, V. N., **2006**. Effects of L-carnitine on neutrophil-mediated ischemia-reperfusion injury in rat stomach. Cell Biochem Funct, 24(5), 437-442.

Di Cesare, M.L., Ghelardini, C., Calvani, M., Nicolai, R., Mosconi, L., Vivoli, E., Pacini, A., Bartolini, A., **2007**. Protective effect of acetyl-L-carnitine on the apoptotic pathway of peripheral neuropathy. Eur J Neurosci, 26, 820-827.

Dokmeci, D., Akpolat, M., Aydogdu, N., Doganay, L., Turan, F.N., **2005**. L-carnitine inhibits ethanol-induced gastric mucosal injury in rats. Pharmacol Rep, 57(4), 481-488.

Dokmeci, D., Akpolat, M., Aydogdu, N., Uzal, C., Doganay, L., Turan, F., **2006**. The protective effect of L-carnitine on ionizing radiation-induced free oxygen radicals. Scand J Lab Anim Sci, 33(2), 75-83.

Eid, A., **2016**. Protective effect of L-carnitine against cisplatin-induced testicular toxicity in rats. Azhar J Pharm Sci, 53(1), 123-142.

Famularo, G., De Simone, C., Trinchieri, V., Mosca, L., **2004**. Carnitines and its congeners: a metabolic pathway to the regulation of immune response and inflammation. Ann NY Acad Sci, 1033,132-138.

Fossati, P., Prencipe, L., Berti, G., **1980**. 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, 26(2), 227-231.

Furst, D.E., **1997**. The rational use of methotrexate in rheumatoid arthritis and other rheumatic diseases. Br J Rheumatol, 36,1196-204.

Furuno, T., Kanno, T., Arita, K., Asami, M., Utsumi, T., Doi, Y., Inoue, M., Utsumi, K. **2001**. Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition. Biochem Pharmacol, 62(8), 1037-1046.

Galal, G., Abou Zed, T., Kahilo, K., Shukry, M., **2016**. Effect of L-carnitine on Lead-induced Infertility in female Rats. Int J Sci Res, 5(2), 1492-1499.

Gunyeli, I., Saygin, M., Ozmen, O., **2021**. Methotrexate-induced toxic effects and the ameliorating effects of astaxanthin on genitourinary tissues in a female rat model. Arch Gynecol Obste, 304, 985-997.

Harman, D., **1956**. Aging: a theory based on free radical and radiation chemistry. J Gerontol, 11(3), 298-300.

Heenen, M., Laporte, M., De Graef, C., **1998**. Methotrexate induces apoptotic cell death in human keratinocytes. Arch Dermatol Res, 290,240-245.

Hoffman, M. **2011**. Hypothesis: hyperhomocysteinemia is an indicator of oxidant stress. Med Hypotheses, 77, 1088-1093.

Hortu, I., Ozceltik, G., Ergenoglu, A.M., Yigitturk, G., Atasoy, O., Erbas, O., **2020**. Protective effect of oxytocin on a methotrexate-induced ovarian toxicity model. Arch Gynecol Obste, 301(5), 1317-1324.

Ibrahim, M.A., El-Sheikh, A.A.K., Khalaf, H.M., Abdelrahman, A.M., **2014**. Protective effect of peroxisome proliferator activator receptor (PPAR)-α and -γ Ligands against methotrexate-induced nephrotoxicity. Immunopharmacol Immunotoxicol, 36(2), 130-137.

Izgut-Uysal, V.N., Agac, A., Derin, N., **2003**. Effect of L-carnitine on carrageenan-induced inflammation in aged rats. Gerontology, 49(5), 287-292.

Jahovic, N., Çevik, H., Şehirli, A.Ö., Yeğen, B.Ç., Şener, G., **2003**. Melatonin prevents methotrexate-induced hepatorenal oxidative injury in rats. J Pineal Res, 34, 282-7.

Kalhori, Z., Mehranjani, M.S., Azadbakht, M., Shariatzadeh, M.A., **2019**. L-Carnitine improves endocrine function and folliculogenesis by reducing inflammation, oxidative stress and apoptosis in mice following induction of polycystic ovary syndrome. Reprod Fertil Dev, 31(2), 282-293.

Karri, S., **2011**. Effect of methotrexate and leucovorin on female reproductive tract of albino rats. Cell Biochem Funct, 29(1), 1-21.

Kelek, S.E., Afşar, E., Akçay, G., Danışman, B., Aslan, M., **2019**. Effect of chronic L-carnitine supplementation on carnitine levels, oxidative stress and apoptotic markers in peripheral organs of adult Wistar rats. Food Chem Toxicol, 134, 110851.

Kelly, G.S., **1998**. L-carnitine: therapeutic applications of a conditionally essential amino acid. Altern Med Rev, 3, 345-360.

Koohpeyma, H., Goudarzi, I., M, Lashkarbolouki, T., Elahdadi Salmani, Shabani M., **2019**. Postnatal administration of homocysteine induces cerebellar damage in rats: protective effect of folic acid. Neurotox Res, 35, 724-738

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., Cosic, V., **2001**. Method for the measurement of antioxidant activity in human fluids. J Clin Path, 54(5), 356-361.

Kose, E., Sapmaz, H.I., Sarihan, E., Vardi, N., Turkoz, Y., Ekinci,N., **2012**. Beneficial effects of montelukast against methotrexate-induced liver toxicity: a biochemical and histological study. ScientificWorldJournal, 2012, 987508.

Leitão, R.F., Brito, G.A., Oriá, R.B., Braga-Neto, M.B., Bellaguarda, E.A., Silva, J.V., Gomes, A.S., Lima-Júnior, R.C., Siqueira, F.J., Freire, R.S., Vale, M.L., **2011**. Role of inducible nitric oxide synthase pathway on methotrexate-induced intestinal mucositis in rodents. BMC Gastroenterol, 11, 90.

Montgomery, H.A., Dymock, J.F., **1961**. The determination of nitrite in water. Analyst, 86, 414-416.

Morsy, M.A., Ibrahim, S.A., Amin, E.F., Kamel, M.Y., Rifaai, R.A., Hassan, M.K., **2013**. Curcumin ameliorates methotrexate-induced nephrotoxicity in rats. Adv Pharmacol Sci, 2013, 387071.

Mukherjee, S., Ghosh, S., Choudhury, S., Adhikary, A., Manna, K., Dey, S., Sa, G., Das, T., Chattopadhyay, S., **2013**. Pomegranate reverses methotrexate-induced oxidative stress and apoptosis in hepatocytes by modulating Nrf2-NF-κB pathways. J Nutr Biochem, 24(12), 2040-2050.

Mutavdzin, S., Gopcevic, K., Stankovic, S., Jakovljevic Uzelac, J., Labudovic Borovic, M., Djuric, D., **2019**. The effects of folic acid administration on cardiac oxidative stress and cardiovascular biomarkers in diabetic rats. Oxid Med Cell Longev, 2019, 1342549.

Ohkawa, H., Ohishi, N., Yagi, K., **1979**. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95(2), 351-358.

Ojeda, M.L., Nogales, F., Jotty, K., Barrero, M.J., Murillo, M.L. and Carreras, O., **2009**. Dietary selenium plus folic acid as an antioxidant therapy for ethanol-exposed pups. Birth Defects Res B Dev Reprod Toxicol, 86(6), 490-495.

Oner, G., Ozcelik, B., Ozgun, M. T., Ozturk, F., **2011**. The effects of metformin and letrozole on endometrium and ovary in a rat model. Gynecol Endocrinol, 27(12),1084-1086.

Oriol, B., Barrio, A., Pacheco, A., Serna, J., Zuzuarregui, J. L., Garcia-Velasco, J. A., **2008**. Systemic methotrexate to treat ectopic pregnancy does not affect ovarian reserve. Fertil Steril, 90(5), 1579-1582.

Ozcelik, B., Turkyilmaz, C., Ozgun, M. T., Serin, I. S., Batukan, C., Ozdamar, S., Ozturk, A., **2010**. Prevention of paclitaxel and cisplatin induced ovarian damage in rats by a gonadotropin-releasing hormone agonist. Fertil Steril, 93,1609-1614.

Pınar, N., Kaplan, M., Özgür, T., Özcan, O., **2018**. Ameliorating effects of tempol on methotrexate-induced liver injury in rats. Biomed Pharmacother, 102,758-764.

Rauchova, H., Koudelova, J., Drahota, Z., Mourek, J., **2002**. Hypoxia-induced lipid peroxidation in rat brain and protective effect of carnitine and phosphocreatine. Neurochem Res, 27,899-904.

Sarna, L.K., Wu, N., Wang, P., Hwang, S., Siow, Y.L., Karmin, O., **2012**. Folic acid supplementation attenuates high fat diet induced hepatic oxidative stress via regulation of NADPH oxidase. Can J Physiol Pharmacol, 90, 155-165.

Şener, G., Ekşioğlu-Demiralp, E., Cetiner, M., Ercan, F., Şirvancı, S., Gedik, N., Yeğen, B.C., **2006**. L-Carnitine ameliorates methotrexate-induced oxidative organ injury and inhibits leukocyte death. Cell biol Toxicol, 22(1), 47-60.

Shalini, S., Dorstyn, L., Dawar, S., Kumar, S., **2015**. Old, new and emerging functions of caspases. Cell Death Differ, 22, 526-539.

Shamberger, R.C., Sherins, R.J., Ziegler, J.L., Glatstein, E., Rosenberg, S.A., **1981**. Effects of postoperative adjuvant chemotherapy and radiotherapy on ovarian function in women undergoing treatment for soft tissue sarcoma. J Natl

Canc Inst, 67, 1213-1218.

Shohda, A.A., El-banna, A., Abdel Fattah, F., **2017**. The possible protective effect of folic acid against methotrexate induced ovarian damage in female albino rats. Light and electron microscopic study. Bull Natl Nutr Inst Arab Repub Egypt, 49(1), 1-24.

Soylu Karapinar, O., Pinar, N., Özcan, O., Özgür, T., Dolapçıoğlu, K., **2017**. Protective effect of alpha-lipoic acid in methotrexate-induced ovarian oxidative injury and decreased ovarian reserve in rats. Gynecol Endocrinol, 33(8), 653-659.

Stika, C.S. **2012**. Methotrexate: The pharmacology behind medical treatment for ectopic pregnancy. Clin Obstet Gynecol, 55, 433-439.

Surai, P.F., **2015**. Antioxidant action of carnitine: molecular mechanisms and practical applications. Veter Sci, 2(1), 66-84.

Tsurusawa, M., Saeki, K., Fujimoto, T., **1997**. Differential induction of apoptosis on human lymphoblastic leukemia Nalm-6 and Molt-4 cells by various antitumor drugs. Int J Hematol, 66,79-88.

Ulug, P., Oner, G., **2014**. Evaluation of the effects of single or multiple dose methotrexate administration, salpingectomy on ovarian reserve of rat with the measurement of anti-Mu¨ llerian hormone (AMH) levels and histological analysis. Eur J Obstet Gynecol Reprod Biol, 81, 205-209.

Vardi, N., Parlakpinar, H., Ates, B., Cetin, A. and Otlu, A., **2013**. The protective effects of Prunus armeniaca L (apricot) against methotrexate-induced oxidative damage and apoptosis in rat kidney. J Physiol Biochem, 69(3), 371-381.

Vardi, N., Parlakpinar, H., Cetin, A., Erdogan, A., Cetin O. I., **2010**. Protective effect of beta-carotene on methotrexate-induced oxidative liver damage, Toxicol Pathol 38, 592-597.

Whittle, S.L., Hughes, R.A., **2004**. Folate supplementation and methotrexate treatment in rheumatoid arthritis: A review. Rheumatology, 43, 267-271.

Yucebilgin, M.S., Terek, M.C., Ozsaran, A., Akercan, F., Zekioglu, O., Isik, E., Erhan, Y. **2004**. Effect of chemotherapy on primordial follicular reserve of rat: An animal model of premature ovarian failure and infertility. Aust New Zeal J Obstet Gynaecol 44, 6-9.

Yurut-Caloglu, V., Caloglu, M., Eskiocak, S., Tastekin, E., Ozen, A., Kurkcu, N., Oz-Puyan, F., Kocak, Z., Uzal, C., **2015**. Comparison of the protective roles of L-carnitine and amifostine against radiation-induced acute ovarian damage by histopathological and biochemical methods. J Cancer Res Ther, 11(2), 447-453.

Zhang, Q., Wang, S.M., Yao, P.B., Zhang, L., Zhang, Y.J., Chen, R.X., Fu, Y., Zhang, J.M., **2015**. Effects of L-carnitine on follicular survival and graft function following autotransplantation of cryopreserved- thawed ovarian tissues. Cryobiology, 71, 135-140.