Faculty of Women for
Arts, Science and Education

# Hepatotoxicity of Methotrexate and Possible Ameliorative Effect of L-Carnitine: Histological and Ultrastructural Study

**A Thesis Submitted for (M.Sc.) Degree in Zoology**

**By**

**Najwa Abdalgader Ahmad Algaw**
**(B.Sc. of Zoology)**

**Board of Scientific Supervisors**

**Professor Dr. Madiha Abdel Moneim Ashry**
**Prof. of Histochemistry Faculty of Women For Arts, Science and Education, Ain Shams University**

**Professor Dr. Shadia Mohamed Kadry**
**Prof .Of Histology and Histochemistry**
**Faculty of Women for Arts, Science and Education**
**Ain Shams University**

**Dr. Nadia Ragheb Ali Abuzeid**
**Assistant Consultant, Electron Microscopy Laboratory,**
**Ain Shams Specialized Hospital.**

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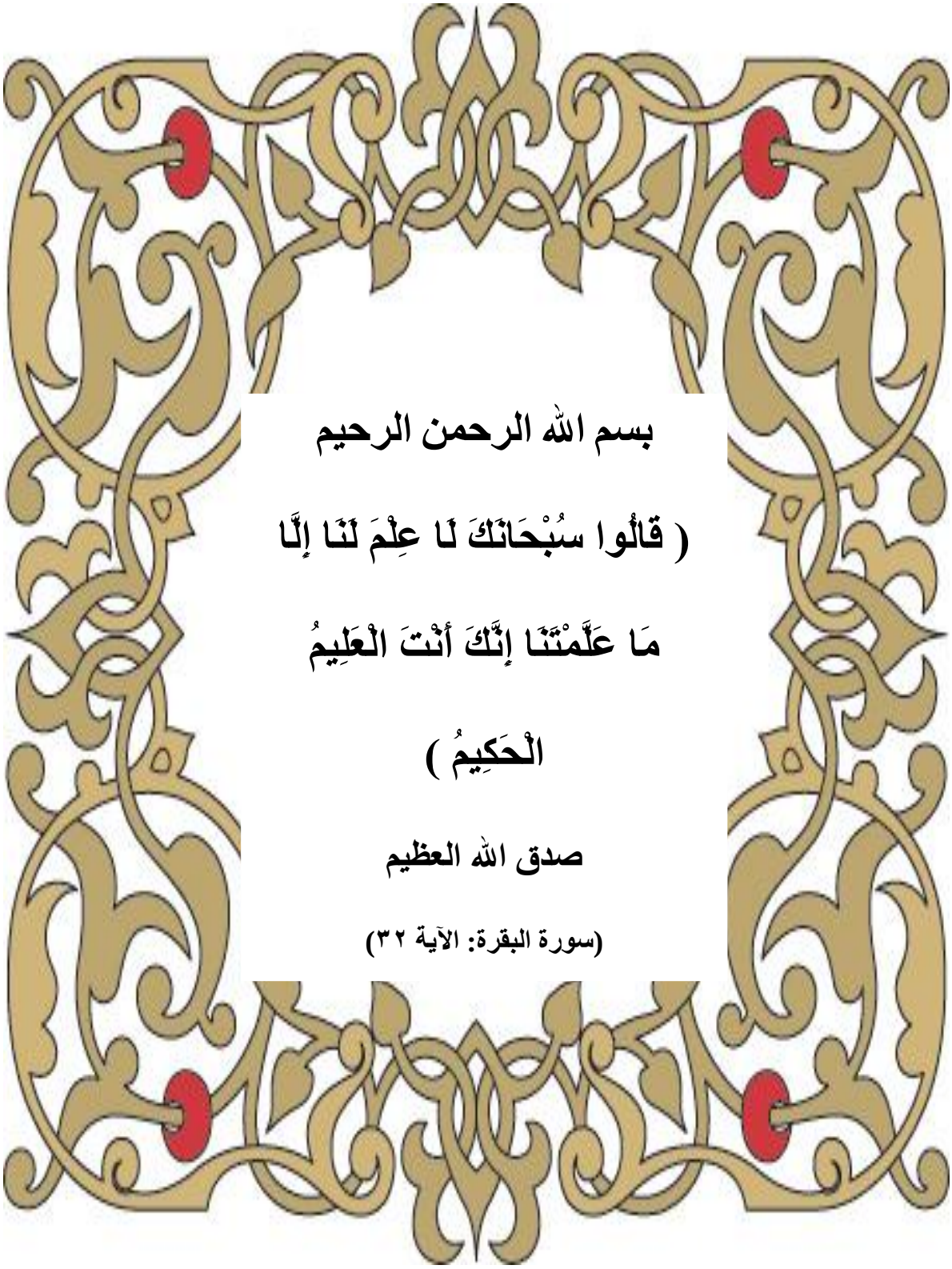

بسم الله الرحمن الرحيم

( قالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا

مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ

الْحَكِيمُ )

صدق الله العظيم

(سورة البقرة: الآية ٣٢)

# Qualifications

**Name: Najwa Abdualgader Algaw**

**Scientific Degree: B.Sc.**

**Department: Zoology**

**College: Faculty of Science**

**University: El-Margeb University, Libya.**

**B.Sc. Graduation year: 2010**

# *Approval Sheet*

**Name: Najwa Abdualgader Algaw**

**Title: Hepatotoxicity of Methotrexate and Possible Ameliorative Effect of L-Carnitine: Histological and Ultrastructural Study.**

**Scientific Degree: B.Sc.**

## Supervisors

**Professor Dr. Madiha Abdel Moneim Ashry**
**Prof. of Histochemistry Faculty of Women For Arts, Science and Education, Ain Shams University**

**Professor Dr. Shadia Mohamed Kadry**
**Prof .Of Histology and Histochemistry**
**Faculty of Women for Arts, Science and Education**
**Ain Shams University**

**Dr. Nadia Ragheb Ali Abuzeid**
**Assistant Consultant, Electron Microscopy Laboratory,**
**Ain shams Specialized Hospital.**

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## ABSTRACT

Methotrexate (MTX), a folic acid antagonist, is a chemotherapeutic agent widely used in the treatment of some types of cancers and various inflammatory diseases such as psoriasis and rheumatoid arthritis.

The efficacy of this agent in high doses has been associated with hepatotoxicity. 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.

The present study aimed to investigate the protective effect of L-Carnitine against Methotrexate induced hepatotoxicity on the rat liver . Fifty male albino rats weighing 120-130g.were used to study the histological and ultrastructural alterations as well as histochemical changes-including total proteins and total polysaccharides in liver tissues.The experimental animals were equally divided into five groups , 10 rats each, and treated as follows : (1) rats did not receive any treatment (control group ); (2) rats given 300mg L-Carnitine /100g b.w. for 4 weeks ; (3) rats given 0.045mg Methotrexate /100g b.w. for 4 weeks ; (4) rats given 0.045mg Methotrexate /100g b.w. with 300mg L-Carnitine /100g b.w. for 4 weeks ; (5) rats given 0.045mg Methotrexate /100g b.w. for 4 weeks then given 300mg L-Carnitine /100g b.w. for another 4 weeks . Rats were injected intraperitoneally twice per week.

The results of the present study in methotrexate group showed dilatation and congestion of blood vessels with collection of chronic inflammatory cells, severe ballooning and vacuolation of hepatocytes. Some nuclei of the degenerated cells showed pyknosis while other showed karyolysis .Enlarged von kupffer cells within dilated sinusoids were also detected, in addition to haemorrhagic and vacuolar necrosis

.Histochemical studies showed that methotrexate decreased total proteins and polysaccharides in the liver tissues. Ultrastructure studies revealed Dilatation and congestion of the hepatic sinusoids. pyknotic nucleus with abnormally electron dense chromatin as well as irregular nuclear membrane , electron dense mitochondria , fragmented rough endoplasmic reticulum , increased lipid droplets and increased collagen fibers.

Methotrexate + L-Carnitine group, showed significant improvement in the histological, histochemical and ultrastructural alterations.

In Methotrexate then L-Carnitine group, showed mild to moderate improvement in all these changes caused by methotrexate. These results indicated that co- and post administration of L-Carnitine reduced the histological, histochemical and ultrastructural changes and induce mild protection against methotrexate toxicity.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ALL** | **Acute Lymphoblastic Leukemia** |
| **ALT** | **alanine transaminase** |
| **AST** | **Aspartate aminotransferase** |
| **ATP** | **Adenosine triphosphte** |
| **acyl-CoA** | **acyl-coenzyme A** |
| **CCl4** | **carbon tetrachloride** |
| **CDDP** | **cis-diamine dichloroplatinum** |
| **DFS** | **disease free survival** |
| **GSH** | **Reduced glutathione** |
| **HD-MTX** | **high doses of methotrexate** |
| **IVF** | **in vitro fertilization** |
| **LC** | **L-Carnitine** |
| **LM** | **light microscopy** |
| **MDA** | **Mallon di aldehyde** |
| **MTX** | **Methotrexate** |
| **MUL** | **Mulberry leaf extract** |
| **NADP** | **nicotineamide adenosine diphosphate** |
| **NAFLD** | **nonalcoholic fatty liver disease** |
| **OAS** | **overall disease** |
| **PAS** | **Periodic Acid Schiff** |
| **QE** | **quercetin** |
| **RA** | **rheumatoid arthritis** |
| **RER** | **Rough endoplasmic reticulum** |
| **ROS** | **reactive oxygen species** |
| **SEM** | **Scanning electron microscopy** |
| **SER** | **Smooth endoplasmic reticulum** |
| **TEM** | **transmission electron microscopy** |
| **TNF** | **tumour necrosis factor alpha** |

# LIST OF FIGURES

# INTRODUCTION

Methotrexate (MTX), a folic acid antagonist, is a chemotherapeutic agent widely used in the treatment of some types of cancers and various inflammatory diseases such as psoriasis and rheumatoid arthritis. However, the efficacy of this agent in high doses has been associated with hepatotoxicity **(Jahovic *et al*., 2003 ; Cetinkaya *et al*., 2006).**

MTX is metabolized and stored in hepatocytes in the polyglutamated form **(Chladek *et al*., 1997) .**The presence of higher levels of polyglutamates causes a longer intracellular presence of the drug and this has been suggested as a mechanism for hepatotoxicity **(Kremer *et al*., 1986).**

The mechanisms of MTX toxicity are not yet fully understood but recent studies have shown that a decrease in the levels of cellular glutathione (GSH) and oxygen radicals are linked with the development of MTX induced toxicity**( Jahovic *et al*., 2003 ; Cetinkaya *et al*., 2006 ; Sener *et al*.,2006 ; Cetin *et al*., 2008 ).**It was demonstrated that cytosolic nicotineamide adenosine diphosphate (NADP) dependent dehydrogenases are inhibited by MTX, suggesting that the drug decreases the availability of NADPH in cells**(Caetano *et al*., 1997).** Under normal conditions, NADPH is used by glutathione reductase to maintain the reduced state of GSH, an important cytosolic antioxidant that protects cells against reactive oxygen species (ROS). Thus, the significant reduction in GSH levels induced by MTX therapy leads to reduction in the level of the antioxidant enzyme defense system, sensitizing the cells to ROS. Considering the relationship between GSH and the deleterious effects of MTX, interest has focused on antioxidant compounds that can either

stimulate GSH synthesis or act as direct antioxidants **(Babiak *et al*., 1998).**

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 **(Vanella *et al*., 2000).** L-Carnitine has an antioxidant activity that combines both free radical scavenging and metal-chelating properties **(Srinivas *et al*., 2007; Salama *et al*., 2012 ; Salama *et al*., 2013).** 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 **( Panneerselvam and Kumaran ,2006).** Human embryos generated in vitro fertilization (IVF) exhibit varying degrees of cytoplasmic fragmentation indicative of apoptosis **(Jurisicova *et al*., 1996).**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 **(Flanagan *et al*., 2010).** Carnitine that not obtained from food is synthesized endogenously from two essential amino acids, lysine and methionine. This occurs in kidney, liver and brain **(Cave *et al*., 2008 and Yildirim *et al*., 2013).** 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 **(Agarwal *et al*., 2005).** Recently,L-carnitine has been proposed as a potential treatment to improve anemia, thrombocytopenia, leukopenia and immunological function **(Tousson *et al*., 2014; Weinhandl *et al*., 2003 ; Saluk- Juszck *et al*., 2010).**

# AIM OF THE WORK

The liver is an organ of prime importance and plays a significant role in metabolism and detoxification of exogenous toxins and therapeutic agents. Since the liver is involved in the performance of varied functions, it may be susceptible to injury resulting from toxic substances.

The present study was carried out to investigate the side toxic effects induced by Methotrexate (MTX) in liver of male rats. Moreover, to evaluate the possible role of L-Carnitine in ameliorating the negative effects and toxicity induced by Methotrexate.

The studied parameters included histological, histochemical and ultrastructural changes of the hepatic tissue.

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