Cairo university
Faculty of veterinary medicine
Dept. of biochemistry and
Chemistry of nutrition

Biochemical Effects of cinnamon Extracts and tomato extract lycopene against methotrexate induced oxidative stress in rats

Athesis presented

By

Fatma mohammed mmahmoud mohammed Osman

For

M.V.SC degree

(Biochemistry & chemistry of nutrition)

Under supervision of

Prof. Dr. Hassan A. H. Amer
Prof. of Biochemistry and
chemistry of nutrition
Faculty of Vet. Medicine
Cairo university

Dr. Mohsen Ahmed Wasfy
Assistant prof. of biochemistry
Chemistry of nutrition
Faculty of Vet. Medicine
Cairo university

Abstract:

Oxidative stress changes in serum constituents by methotrexate and the antagonistic effects of cinnamon and tomato extracts treatment for a period of 4 weeks were the aim of the present work. For this purpose, 60 adult male rats were used and divided into 6 equal groups namely G1 (control), G2 (MTX-treated, 1.25mg/0.05ml), G3 (MTX-cinnamon, 1.25 mg MTX and 1ml cinnamon), G4 (MTX-tomato extract 1,25mg MTX and 0.5 ml tomato extract), G5 (cinnamon-treated, 1ml) and G6 (tomato extract treated, 0.5 ml). At the end of 4 weeks, serum samples were used and analyzed biochemically. Methotrexate induced an elevation in the oxidative stress marker MDA and a decline in most other serum constituents. On the other hand, tomato extract and cinnamon administration with MTX decreased MDA and increased total antioxidant capacity level with improvement of other serum constituents which sustained within their normal ranges. This may be due to the short-term period of application (4 weeks) in this study. Histopathological changes in the liver supporting the changes in the serum parameters.

Dedication

This thesis is dedicated to my wonderful family, it is Allah gift.,

My DAD, Mr/Mohammed osman

I am honored to have father as you

My Mam Amal

I am honored to have you as my parents,

Who have always been so close to me that I found them with me whenever I needed. It is their unconditional love that motivates me to set higher targets.

My lovely sisters,

Asma, Aya and my little sister Malak

My lovely brothers,

Mahmoud and Ahmed

Who have provided me with a strong love shield that always surrounds me and never lets any sadness.

Acknowledgement

*First of all and above all, my deepest thanks to **ALLAH,** the most merciful gracious and compassionate who give us everything and showing us the right path.*

*Words are not enough to express my sincere thanks, appreciation and gratitude to **Prof. DR. Hassan Abd El-Halim Hassan Amer,** Prof of Biochemistry and chemistry of Nutrition, Faculty of veterinary medicine, Cairo University for his valuable suggestions, advices, critical comments, generous help, continuous supervision and constant support. Really I owe him a lot, a he helped me during performance of this thesis, revising this work and correcting the manuscripts. Truly, without his keen interest, this work probably never has been conducted, all respect and appreciation.*

*I would like to express my sincere appreciation and deepest thanks to soul of **DR. Mohsen Ahmed Wasfy**, Assistant prof. of biochemistry and chemistry of nutrition, Faculty of veterinary medicine, Cairo university who has given me great advices and assistance in planning the practical part of this work, But he did not complete this work with us because he is gone,,, God have mercy on his great soul.*

I would like also to express many thanks to all members of biochemistry and chemistry of nutrition department, Faculty of Vet.Med., Cairo University for their helping me.

Contents

List of abbreviations:

AICAR	5-aminoimidazole-4-carboxamide ribonucleotide
AC	Antioxidant capacity
ADP	Adenosine diphosphate
ALB	Albumin
ALT	Alanine transaminase
AMP	Adenosine mono phosphate
AMPK	Adenosine mono phosphate-activated protein kinase
ANF	Atrial natriuretic factor
ANOVA	Analysis of variance
AST	Aspartate transaminase
ATP	Adenosine-5-triphosphate
CAN	Cinnamaldehyde
CAT	Catalase
CE	Cinnamon extract
CMOII	Carotenoid monooxygenase II
CPDG2	Carboxy peptidase
CRP	C-reactive protein
CUA	Conc of Uric acid
CVA	Cerebrovascular accident
DAMPA	Deglutamated 4-amino-4-deoxy-N10-methyl pteroic acid
DHFR	Dihydrofolate reductase
DNA	Deoxy Ribonucleic Acid
DNHP	Dinitrophenyl hydrazine
EDTA	Ethylene diamine tetra acetic acid
FDA	Food and drug administration
FFA	Free fatty acid
G-3-P	Glycerol-3-phosphate
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GssG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HBV	Hepatitis B virus
HDL	High-density lipoprotein
HIV	Human immune deficiency virus
HMG-CoA	3-hydroxy-3-Methyl glutaryl-coenzyme A
IL-1	Interleukin-1
IR β	Insulin receptor β
LDL	Low-density lipoproteins

LPP	Lipid peroxidation products
LPS	Lipopolysaccharide
LSD	Least significant difference
MDA	Malondialdehyde
MTX	Methotrexate
MTx PGs	Methotrexate polyglutamates
MTx-DHFR	Methotrexate dihydrofolate reductase
MTX-HD	Methotrexate high dose
NADPH	Nicotinamide adenine dinucleotide phosphate
NSAIDs	Non steroidal anti inflammatory drugs
OD	Optical density
OSL	Observed safe level
PBs	Phosphate buffer saline
PMNs	Polymorphonuclear neutrophils
POD	Peroxidases
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S.E	Standard error
SAM	S-adenosylmethionine
SD	Standard deviation
SEM	Standard error of mean
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvate transaminase
SOD	Superoxide dismutase
SPSS	Statistical package for social science
T.G	Triglyceride
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloro acetic acid
THF	Tetrahydrofolate
TNF- α	Tumor necrotic fator alfa
TS	Thymidylate synthase
UA	Uric acid
ULN	Upper limit of normal
VLDL	Very-low density lipoprotein
ZMP	Zero moment point
α-KG	Alpha keto glutamate

LIST OF TABLES

List of figures

List of photos

1- Introduction

Methotrexate is one of most successful drug in cancer chemotherapy **(Takimoto and Ellegra, 1995)**.It was used at higher doses as a cancer therapy and since 1990 it is used at much lower doses to treat rheumatic diseases (**Benedek, 2010)**.Side effects of MTX high dose (MTX-HD) may be life threatening, however those of various doses of oral MTX are variable because of the interindividual variability of gastrointestinal absorption of this drug. Methotrexate also causes kidney damage, which is a frequent complication of high-dose therapy. It is manifested by elevated serum creatinine and decreased creatinine clearance. Although the biochemical mechanism is still debated, intravenous administration of leucovorin (a racemic mixture of isomers of folinic acid) at a scheduled time after the infusion of high dose MTX therapy was found to be beneficial to healthy cells, and protects them from the cytotoxic action of MTX. When used, MTX can cause a number of organ damage. Hepatotoxicity is a common complication of long term treatment with MTX **(Olsen, 1991)**. Methotrexate is well known to cause serum aminotransferase elevations and long term therapy has been linked to development of fatty liver disease, fibrosis and even cirrhosis.

MTX causes oxidative tissue damage by increasing lipid peroxidation in the liver tissue and decreasing the level of antioxidant enzymes**.** Oxidative stress thus refers to the situation in which there is a significant imbalance between free radicals and the antioxidant defense system. The resulting harm is termed oxidative damage. Cells normally deal with mild oxidative stress by up regulating the synthesis of antioxidant defense mechanisms through changes in gene expression. However, at higher levels of oxidative stress, cell injury occurs when adaptation is not adequate for the elimination of oxidation products. This leads to oxidative damage to all types of biomolecules including DNA, proteins and lipids that have been associated with many diseases. The target of oxidative damage varies depending on the characteristics of the cell and the type and degree of stress imposed. According to **Halliwell, (2007),** "antioxidant is any substances that delays, prevents, or remove oxidative damage to a target molecule".

Four very important antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and catalase and various other low molecular weight antioxidant molecules such as glutathione**.** It is thought that the detrimental effects of MTX are partly due to its direct toxic action by increasing ROS production. It has further been reported that MTX administration induces oxidative stress and significantly reduces antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase in liver, intestinal mucosa and spinal cord tissues of rats **(Uzar et al., 2006)**.

Recently, tomato extract has received particular attention as a result of studies indicating that it has highly efficient antioxidant and free radical scavenging capacity **(Atessahin et al., 2006a; Atessahin et al., 2006b).**Cellular and molecular studies have shown tomato extract to be one of the most potent antioxidants and has been suggested to prevent carcinogenesis and atherogenesis by protecting critical biomolecules such as DNA, proteins, lipids and low density lipoproteins (LDLs) **(Pool-Zobel et al., 1997; Rao and Agarwal, 1998)**.The antioxidant properties of tomato extract constitute the major focus of research with regards to its biological effects. Dietary intake of tomato extract has been shown to increase circulatory and tissue levels of tomato extract. Acting as an antioxidant, it can trap ROS and reduce oxidative stress and damage to cellular components including lipids, proteins, and DNA **(Agarwal and Rao, 2000)**.The individual antioxidant role of tomato extract isomers and their interconversions remain unclear. Interestingly, whereas limited in vitro studies show convincing antioxidant and anticarcinogenic effects of tomato extract, tomato extract has been extensively studied because of its potent ability to decrease oxidative stress, and has been shown to significantly enhance total antioxidant capacity, while simultaneously decreasing oxidation of lipids, proteins and DNA. In light of the vast amount of scientific research demonstrating a potential link between tomato extract and decreased risk of cancer, the FDA performed an evidence-based review and concluded that there was some limited evidence to support an association between tomato consumption and reduced risk of gastric, ovarian, pancreatic and prostate cancers, although at this time, the existing evidence is not

powerful enough for the FDA to make official qualified health claims **(Kavanaugh et al., 2007)**.

Cinnamon In addition to being an antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering compound, cinnamon has also been reported to have activities against neurological disorders, such as Parkinson's and Alzheimer's diseases. The pharmacological prospective of cinnamon and its use in daily life has been well documented. Antioxidants have been considered the most important drivers in the progress and existence of humans, as they respond to free radicals and damage in metabolic diseases and age-related syndromes of humans and other animals **(Halliwell, 2011)**.

Mancini-Filho et al., (1998) reported various extracts of cinnamon, such as ether, aqueous, and methanolic extracts that have shown considerable antioxidant activities. Different flavonoids isolated from cinnamon have free-radical-scavenging activities and antioxidant properties **(Okawa et al., 2001).** In a comparative study among 26 spices, cinnamon showed the highest antioxidant activity, indicating that it can be applied as an antioxidant used in foods (**Shan et al., 2005**). All of the extracts had moderate amounts of phenolic compounds and showed potential activity against hydrogen peroxide, nitric oxide, and lipid peroxide free radicals **(Aravind et al., 2012).**