Ain Shams University
Faculty of Women for Arts, Science and Education
Biochemistry and Nutrition Department

The Ameliorative Effect of Propolis Extract Against Methotrexate Induced Oxidative Damage In Rats

M.Sc. Thesis

Submitted to Faculty of Women for Arts, Science and Education, Ain Shams University in partial fulfillment for the requirement of Master Degree in Science " Biochemistry and Nutrition"

By

Nourhan Gamal Mohamed Abd EL-Magid EL-Rahmany

B.Sc. in Biochemistry and Nutrition, Biochemistry and Nutrition Department, Faculty of Women,
Ain Shams University

Under supervision of

Dr. Fatma Abd EL-Hamid Khalil

Professor of Nutrition, Head of Biochemistry and Nutrition Department
Faculty of Women for Arts, Science & Education,
Ain Shams University

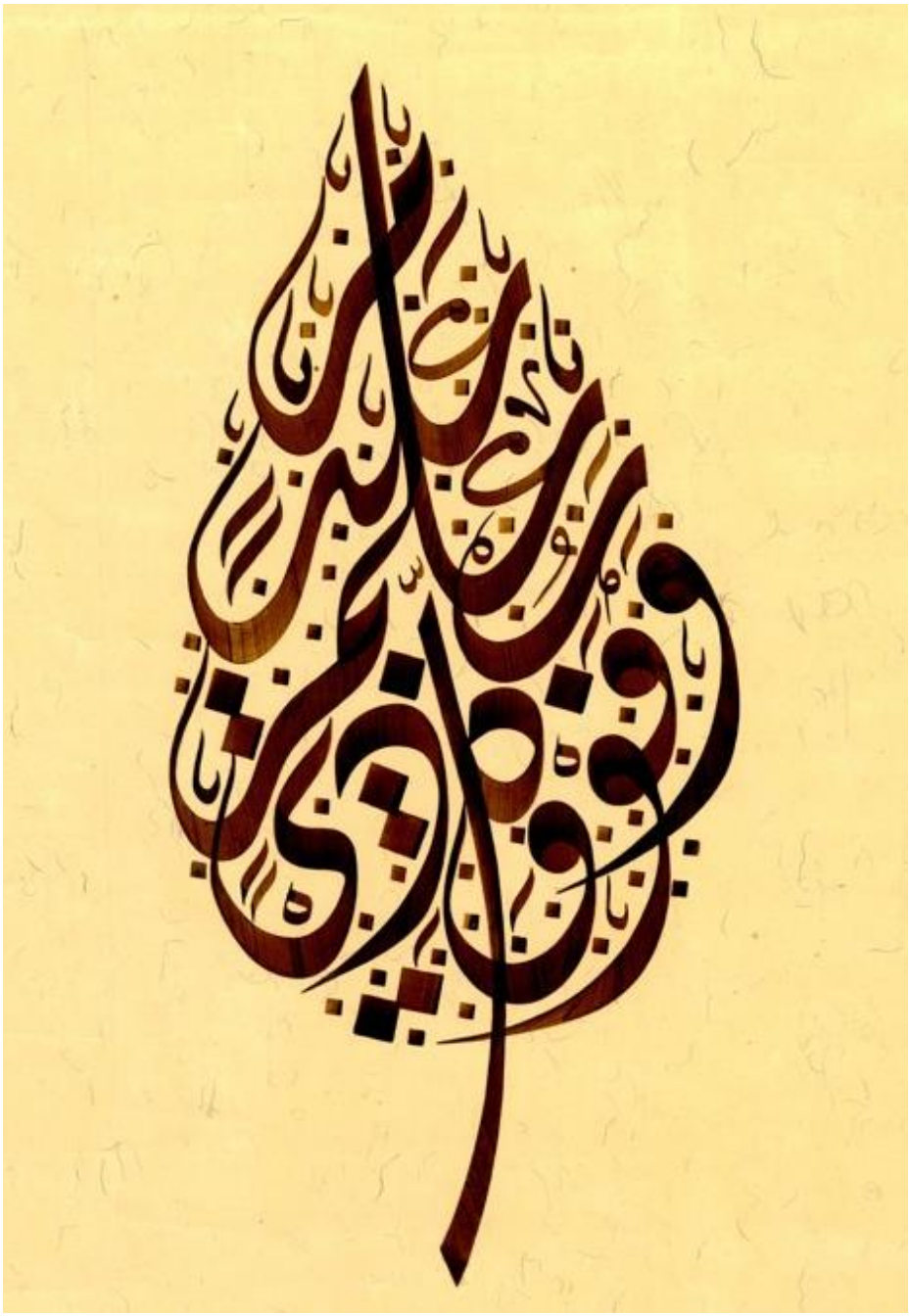
Dr. Amal Ashmawy Ahmed

Lecturer in Biochemistry and Nutrition Department,
Faculty of Women for Arts, Science & Education,
Ain Shams University

Dr. Enas Ali Kamel

Lecturer in Biochemistry and Nutrition Department,
Faculty of Women for Arts, Science & Education,
Ain Shams University

2015



ACKNOWLEDGEMENT

First , I would like to thank ALLAH for his great mercy, generous blesses and for allowing me to achieve this work.

I would like to express my sincere gratitude to ***Prof. Dr. Fatma Abd EL-Hamid Khalil*** *, Head department and Professor of Nutrition, Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University for her supervision, valuable advice, continued assistance, encouragement, following the progress of the work with great interest and guidance throughout the work of the study and I am grateful for everything she has done for me.*

I am grateful to ***Dr. Nagwa Ibrahim Yehia****, Professor of Nutrition, Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University for enlightening me the first glance of research and for her sincere efforts.*

I'd like to express my deepest and sincere gratitude to ***Dr. Amal Ashmway Ahmed****, Lecturer in Biochemistry and Nutrition Department, Faculty of Women, Ain Shams University for her valuable advice and sincere efforts .*

Great appreciation for ***Dr. Enas Ali Kamel,*** *Lecturer in Biochemistry and Nutrition Department,Faculty of Women, Ain Shams University for her continuous assistance and encouragement.*

I'd like to thank all my colleagues of Biochemistry and Nutrition Department,Faculty of Women, Ain Shams University.

I'd like to thank ***Dr.Wesam M.Osman,*** *Assistant Professor of Pathology, Faculty of Medicine - Ain Shams University, for her precious help and for carrying out the histopathological investigations.*

Also, special thanks to ***Dr.Shereen EL-Sayed Mohamed EL-Nahas****, Researcher at Analytical and Measurement laboratory, Botechnology unit – Agrcriculture Research Center for carrying out phytochemical analysis.*

Dedication

I feel deeply grateful to my family for their continuous love, help, advice and support .

I dedicate this work to
My great Mother
and My Sisters
and especially dedicated for
the soul of My Father.

Abstract

Methotrexate (MTX) is an anti-folate drug used to treat cancer and some inflammatory diseases (such as rheumatoid arthritis and psoriasis). The efficacy of MTX is often limited by its severe toxicity. Propolis (bee glue) is one of the most significant bee products that has an important role in balancing antioxidant systems and has an anti-peroxidant effect on several tissues. The present study was conducted to investigate the ameliorative effect of propolis (PP) extract against oxidative damage of MTX on blood and liver, kidney and brain tissues in rats. One hundred and twenty male Wistar albino rats with mean body weights 90 g ± 5 g were divided into 5 groups. Control group (G_1), Saline + DMSO group (G_2), propolis extract group (G_3), MTX group (G_4) and MTX plus propolis extract co-administered group (G_5). Rats were administered their respective doses of propolis extract and/or MTX for 3, 6 and 9 weeks intervals. The results showed that the MTX significantly reduced hemoglobin concentration (Hb), hematocrit % (Hct), mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBCs), white blood cell count (WBCs) and platelets count. While significantly increased mean corpuscular volume (MCV) and lymphocytes %. Moreover, MTX caused significant increase of malondialdehyde (MDA) level and significant decrease in reduced glutathione (GSH) concentration and antioxidant enzyme activities (superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR)) in liver and brain tissues as compared to control group (G_2) in a time dependent manner. MTX administration also caused significant increase in serum amino transferase AST, ALT and alkaline phosphatase (ALP) activities in a time dependent manner, but a significant increase in total bilirubin only in 9 weeks as compared to control group (G_2). On the other hand, MTX impaired kidney function as reflected by a significant increase in serum urea and creatinine levels and decrease in serum uric acid level as compared to control group (G_2). Results suggested that co-administration of propolis with MTX (G_5) normalized all these altered parameters as compared to MTX-treated group in the three different time intervals. Propolis extract administration also recovered the structural and functional integrity of the hepatic cells.

Data showed that long term administration of MTX for 9 weeks produce maximum damage over 6 or 3 weeks, whereas, propolis administration in combination with MTX for 9 weeks offers better alleviation over 6 or 3 weeks.

Key words: Methotrexate, Propolis extract, Oxidative damage, Liver function, Kidney function Antioxidant system, Rats.

List of abbreviations

4-AAP	4-aminoantipyrine
AChE	Acetyl choline esterase
ADA	Adenosine deaminase
ALP	Alkaline phosphatase
ALT	Aspartate aminotransferase
AOAC	Association of official analytical chemists
AST	Alanine aminotransferase
b.wt.	Body weight
CAPE	Caffeic acid phenethyl ester
CAT	Catalase
CE	Catchein
CBC	Complete blood picture
CCl4	Carbon tetrachloride
CFUs	Colony forming units
COX	Cyclooxygenase
$cPLA_2$	Cytosolic phospholipase A_2
CNS	Central nervous system
dAMPA	4-amino-4-deoxy-N_{10} -methyl pteroic acid
DHBS	3, 5-dichloro-2-hydroxybenzene sulfonic acid
DHFR	Dihydrofolate reductase
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
2,4-DNPH-ine	2, 4-dinitrophemyl-hydrazine
DTNB	5, 5` dithiobis-2 - nitrobenzoic acid
dTMP	deoxythymidylate
EAC	Ehrlich ascites carcinoma cells
EDTA	Ethylenediaminetetraacetic acid
EDRF	Endothelium-derived relaxing factor
EPO	Erythropoeitin
fl	Femtoliters
GAE	Gallic acid equivalent
GAR	Glycinamide ribonucleotide
GFR	glomerular filtration rate

GGT	γ -glutamyl traspeptidase
G6PDH	Glucose-6-phosphate dehydrogenase
GC-MS	Gas chromatography-mass spectrometry.
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-s-transferase
Hb	Hemoglobin
Hct	Hematocrit
HDAC	Histone deacetylase
HDL-C	high-density lipoprotein cholesterol
H_2O_2	Hydrogen peroxide
H & E	Hematoxylin eosin
HMP	Hexose monophosphate shunt
IFN-γ	Interferon -gamma
IL-6	Interleukin-6
IL-10	Interleukin-10
IL	Interleukin
iNOS	Inducible nitric oxide synthase
i.p.	Interperitoneally
LDL-C	Low lipoprotein cholesterol
LOX	Lipoxygenase
LPO	Lipid peroxidation
L.S.D	Least significanct difference
MAT	Methionine S-adenosyl transferase
MTX	Methotrexate
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDA	Malondialdehyde
NADP	Nicotinamide adenine dinucleotide phosphate
NAG	N-acetyl-beta-D-glucosaminidase
NBT	Nitroblue Tetrazolium
NMDA	N-methyl-D-aspartate
NK cells	Natural killer cells
NF-κB	Nuclear factor kappa

NF-κB-RE	Nuclear factor-κB response element
NO	Nitric oxide
NOS	Nitric oxide synthase
NRC	National Research Council
mRNA	Messanger Ribonucleic acid
5'NT	5' nucleotidase
4-tert-OP	Octylphenol
7-OH-MTX	7- Hydroxy-Methotrexate
$ONOO^-$	Peroxynitirite
PFF	Protein free filtrates
p.o	Per os
PP	Propolis
PMS	Phenazine methosulphate
RA	Rheumatoid arthritis
PCO	Protein carbonyls
RBCs	Red blood cell count
ROO-	Peroxyl radical
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SAM	S-adenosyl-methionine
SAH	S- adenosyl homocysteine
$sPLA_2$	Secretory phospholipase A_2
S.D	Standard deviation
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
STZ	Streptozotacin
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
THF	Tetrahydrofolate
TNF-α	Tumor necrosis factor-alpha
TS	Thymidylate synthase
T-SH	Total thiol
VLDL-C	Very low-density lipoprotein cholesterol
XO	Xanthine oxidase
WBCs	White blood cell count

List of content

List of tables

List of figures