Mechanistic study of potential antifibrotic effect of methyl palmitate in experimentally induced liver fibrosis

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(2012)

Acknowledgements

No words can be ever said expressing my deep thanks to ***ALLAH.***

Most heartfelt thanks are due to ***Dr. Ebtehal El Demerdash****, Professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, who has made this work possible by her great effort, continuous guidance, support and indispensable help in practical work and thesis writing. In fact, she was more than a supervisor, she never stopped supporting and encouraging me. Her precious advices were always pushing me forward.*

I am greatly thankful to ***Dr. Azza Awad****, Assistant professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, for her help and cooperation.*

I wish to express my appreciation and gratitude to ***Dr. Mariane George,*** *Lecturer of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy,*

Ain Shams University, for her guidance, kind cooperation and discussion throughout the work.

I would like to express my appreciation and thanks to ***Dr.Ashraf Bahi El-Deen Abdel Naim*** *Professor of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmac, Ain Shams University, for his help and support.*

Iam deeply indebted to ***Dr. Dina Hassan,*** *Lecturer of Histology. Department of Histology, Faculty of Medicine, Al-Azhar University, for her great effort in accomplishing the part of histopathological technique.*

I would like to thank ***Dr. Amira Badr,*** *lecturer of Pharmacology and Toxicology and* ***MSCC Pharmacist. Ahmed Esmat Amer*** *and* ***MSCC Pharmacist. Amal Kamal,*** *Assistant lecturers of Pharmacology and Toxicology, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for their continuous help in the practical work.*

It is my great pleasure to thank all members of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University and every person in my Faculty who supported me and helped me in my way.

Finally, but of great importance, I wish to express my deep gratefulness and thanks to my family; my father, my mother and my brothers, for their support and continuous prayers and for all what they endured to tolerate and uphold me in finshing this thesis.

Eman Mohamed Mantawy

Abstract

Fibrosis accompanies most chronic liver disorders and is a major factor contributing to hepatic failure. Therefore, the need for an effective treatment is evident. The present study was designed to assess the potential antifibrotic effect of MP and whether MP can attenuate the severity of oxidative stress and inflammatory response in chronic liver injury. Male albino rats were treated with either CCl_4 (1 ml/kg, twice a week) and/or MP (300 mg/kg, three times a week) for six weeks. CCl_4-intoxication significantly increased liver weight, serum aminotransferases, total cholesterol and triglycerides while decreased albumin level and these effects were prevented by co-treatment with MP. As indicators of oxidative stress, CCl_4-intoxication caused significant glutathione depletion and lipid peroxidation while MP co-treatment preserved them within normal values. As markers of fibrosis, hydroxyproline content and α–SMA expression increased markedly in the CCl_4 group and MP prevented these alterations. Histopathological examination by both light and electron microscope further confirmed the protective efficacy of MP. To elucidate the antifibrotic mechanisms of MP, the expression of NF-κB, iNOS and COX-2 and the tissue levels of TNF-α and nitric oxide were assessed; CCl_4 increased the expression of NF-κB and all downstream inflammatory cascade while MP co-treatment inhibited them. Collectively these findings indicate that MP possesses a potent antifibrotic effect which may be partly a consequence of its antioxidant and anti-inflammatory properities.

Keywords: Liver fibrosis; Methyl palmitate; Carbon tetrachloride; NF-κB; Inflammation.

List of Contents

List of Contents (Cont'd)

List of Abbreviations

4-AAP	4- Aminoantipyrine.
ALT	Alanine aminotransferase.
AST	Aspartate aminotransferase.
ATP	Adenosine-5'-triphosphate.
BCG	Bromcresol green.
Bcl2	B-cell lymphoma 2.
BclxL	B-cell lymphoma-extra large.
BSA	Bovine serum albumin.
CCl_3·	Trichloromethyl free radical.
CCl_300·	Trichloromethyl peroxy radical.
CCl_4	Carbon tetrachloride.
CE	Cholesterol esterase.
cIAPs	Cellular inhibitors of apoptosis.
CO	Cholesterol oxidase.
COX-2	Cyclooxygenase 2.
CYP 450	Cytochrome P 450.
DNA	Deoxyribonucleic acid.

DTNB	Ellman's reagent [5,5'-dithio-bis (2-nitrobenzoic acid)].
ECM	Extracellular matrix.
EGF	Epidermal growth factor.
ELISA	Enzyme linked immunosorbent assay.
ET-1	Endothelin-1.
FGF	Fibroblast growth factor.
GFAP	Glial fibrillary acidic protein.
GK	Glycerol kinase.
GPO	Glycerol phosphate oxidase.
GSH	Reduced glutathione.
H & E	Hematoxylin and Eosin.
H_2O_2	Hydrogen peroxide.
HBV	Hepatitis B virus.
HCC	Hepatocellular carcinoma.
HCV	Hepatitis C virus.
HDV	Hepatitis delta virus.
HFE	(High-iron) gene.
HIV	Human immunodeficiency virus.

HSCs	Hepatic stellate cells.
I.P.	Intraperitoneal.
ICAM-1	Intracellular cell adhesion molecule-1.
IFN-γ	Interferon gamma .
IGF	Insulin-like growth factor.
IgG	Immunoglobulin G.
IκB	Inhibitor of κB.
IKK	Inhibitor of κB Kinase.
IKKK	Inhibitor of κB kinase kinase.
IL-1	Interleukin-1.
iNOS	Inducible form of nitric oxide synthase.
KH_2PO_4	Potassium dihydrogen phosphate.
K_2HPO_4	Dipotassium hydrogen phosphate.
LPL	Lipoprotein lipase.
LPS	Lipopolysaccharide.
MCP-1	Monocyte chemotactic protein-1.
MDA	Malonaldehyde.
MMPs	Matrix metalloproteinases.
MP	Methyl palmitate .

NF-κB	Nuclear factor kappa B.
NK	Natural killer
NO	Nitric oxide.
NOx	Total nitrite/nitrate.
NSAIDs	Non sreroidal anti-inflammatory drugs.
ODN	Oligodeoxynucleotide.
$ONOO^-$	Peroxynitrite .
PDGF	Platelet derived growth factor.
POD	Peroxidase.
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SP Conjugate	Streptavidin-Peroxidase Conjugate.
TBA	Thiobarbituric acid.
TBS	Tris buffered saline.
TCA	Trichloroacetic acid.
TGF-β	Transforming growth factor beta.
TBARS	Thiobarbituric acid reactive substances.
TIMPs	Tissue inhibitors of metalloproteinase.
TNF-α	Tumor necrosis factor alpha.

UDCA	Ursodeoxycholic acid.
UV	Ultraviolet.
VCAM-1	Vascular cell adhesion molecule-1.
XIAP	X-linked inhibitor of apoptosis protein.
α-SMA	Alpha smooth muscle actin.

List of Tables

List of Figures