BIOCHEMICAL STUDIES OF THE ANTIOXIDANT EFFECTS OF POMEGRANATE ON HEPATIC INJURY COMPOUNDS IN MALE ADULT ALBINO RATS

By

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B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Zagazig Univ., 2009

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Title of Thesis: Biochemical Studies of The Antioxidant Effects of Pomegranate on Hepatic Injury Compounds in Male Adult Albino Rats

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ABSTRACT

In ancient times Egyptians regarded pomegranate fruit as a symbol of fertility due to the round shape and abundant seeds .They used it to heal many ailments. Pomegranate fruit mentioned in our holy Quran not less than three times and paid attention for its importance. Most pomegranate (*Punica granatum Linn., Punicaceae*) fruit parts are known to possess enormous antioxidant activity. The present study was carried out to determine the phenolic and flavonoid contents of pomegranate juice and nanoparticles pomegranate juice. It was an attempt to evaluate the toxic effect of chlorpyrifos (CPF) with either pomegranate juice or nanoparticles pomegranate juice treatments and as antioxidant agents. Therefore, the present study also aimed to elucidate the possible ameliorative role of pomegranate juice (PJ) or nanoparticles pomegranate juice (NPJ) in CPF toxicity when given to male rats and compared with ursodeoxycholic acid (UDCA) drug. This was done through studying the effects of CPF on serum and antioxidant enzymes in liver and kidneys as well as histopathological changes in vital organs such as liver and kidney. Results showed that CPF-intoxicated rats caused significant changes in serum enzyme activity in liver and kidneys, liver function and kidneys function, when compared with normal control. Ingestion of CPF along with PJ or NPJ or UDCA drug improved those parameters. Histopathological reports also revealed that there is a regenerative activity in the liver and kidney cells. PJ and NPJ showed to be hepatoprotective and renal protective against CPF -induced hepatic injury. On the basis of the previous results it can be concluded that pomegranate extracts especially NPJ is a promising natural product, which could be useful for the prevention of diseases caused by oxidative stress and this study reveals biological evidence that supports the use of PJ and NPJ in the treatment of chemical-induced hepatotoxicity and renal toxicity.

Key words: Pomegranate juice, Nanoparicles, Ursodeoxycholic acid, Chlorpyrifos toxicity, Lipid peroxidation, Antioxidant activity

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my father and mother for their patience and help, as well as to my brothers and my sister for all the supports they lovely offered along the period of my post graduation and I can't find adequate words to express my feeling towards them. Also, I wish to express my feeling to my grand father; Khalil (late), my friend; Fawziya (late) and other friends for encouragement.

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ABBREVIATIONS, EXPRESSIONS AND SYMBOLS

4-AAP	4-aminoantipyrine
AA	Antioxidant activity or ascorbic acid
AAE	Ascorbic acid equivalent
AAP	4-aminophenazone
ACh	Acetylcholine
AChE	Acetylcholinesterase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
b.w.	Body weight
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BPA	Bisphenol A
BuChE	Butyrylcholinesterase or pseudocholinesterase or plasma cholinesterase
CAT	Catalase
CDNB	1-chloro-2, 4- dinitrobenzene
CE	Catechin equivalent
CEs	Carboxylesterases
CFIDS	Chronic fatigue immunodeficiency syndrome
ChE	Cholinesterase
CPF	Chlorpyrifos
CPFO	Chlorpyrifos-oxon
CYP450	Cytochrome P450
DHBS	3, 5-dichloro-2-hydroxy benzene sulfonic acid
DNA	Deoxyribonucleic Acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl or 1, 1-diphenyl-2-picryl- hydrazyl
DTNB	5, 5` dithiobis (2 - nitrobenzoic acid)
EA	Ellagic acid
EDTA	Ethylene di-amine tetra acetic acid
EGCG	Epigallocatechin gallate
ENMs	Engineered nanoscale materials
ET	Ellagitannins

FRAP Ferric reducing antioxidant power
GAE Gallic acid equivalent
GK Glycerol kinase
GPO Glycerol phosphate oxidase
GPx Glutathione peroxidase
GR Glutathione reductase
GRAS Generally recognized as Safe
GSH Reduced glutathione
GSSG Oxidized glutathione
GST Glutathione-s-transferase
H&E Hematoxylin and eosin
HDL High density lipoproteins
HF Hydrofloric acid
HPLC High performance liquid chromatograph
HTs Hydrolysable tannins
IC Injury control
LD_{50} Lethal dose
LDH Lactate dehydrogenase
LDL Low density lipoproteins
LMWAs Low molecular weight antioxidants
LPL Lipoprotein lipase
LPO Lipid peroxide
MDA Malondialdehyde
MFO Mixed function oxidase
MP Methyl parathion
MPS Myofascial pain syndrome
$NADP^+$ Nicotinamide adenine dinucleotide phosphate
NC Normal control
ND Not detected
NFkB Nuclear factor k B
NO Nitric oxide
NPJ Nanoparticles pomegranate juice
NPs Nanoparticles
NTE Neuropathy target esterase
OFRs Oxygen free radicals
OP Organophosphorus
OPT Organophosphorothionate
PCB's Polychlorinated biphenyls
PJ Pomegranate juice

PON 1	Paraoxonase 1
PSO	Pomegranate seed oil
PUFA	Polyunsaturated fatty acid
RDA	Recommended dietary allowance
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSA	Radical scavenging activity
SD	Standard deviation
SEM	Scanning electron microscope
SOD	Superoxide dismutase
SPSS	Statistical package for social sciences
SSC	Suspended-sediment concentrations
TA	Tetratable acidity
TAC	Total antioxidant capacity
TB	Total bilirubin
TBA	2-Thiobarbituric acid
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Tertiary butyl hydroquinone
TC	Total cholesterol
TCA	Trichloroacetic acid
TCP	3, 5, 6-trichloro-2-pyridinol
TEM	Transmission electron microscopy
TFC	Total flavonoids content
TG	Triglycerides
TL	Total lipids
Tocs	Tocopherols
TP	Total proteins
TPC	Total phenolic compounds
UA	Uric acid
UDCA	Ursodeoxycholic acid
UV	Ultraviolet
VLDL	Very Low density lipoproteins
WHO	World health organization

CONTENTS

LIST OF TABLES

LIST OF FIGURES