Age Dependent Immunomodulation, Genotoxicity and Oxidative Stress due to Pesticide Mixture Exposure and Protective Potential of Antioxidants in Albino Rats

Thesis

Submitted in partial fulfillment for the MD degree in forensic medicine and clinical toxicology

By

Mustafa Mohsen Mustafa Afify

Assistant Lecturer of Forensic Medicine
And Clinical Toxicology
Faculty of medicine - Beni Sueif University

Under supervision of

Prof. Dr. Aly Gamal Eldin Abd El-Aal

Professor of Forensic Medicine
And Clinical Toxicology
Faculty of Medicine – Cairo University

Prof. Dr. Dina Ali Shokry

Professor and Head of Forensic Medicine
And Clinical Toxicology Department
Faculty of Medicine – Cairo University

A. Prof. DR. Manal El -Sayed El-Halwagy

Assistant Professor of Biochemistry
Mammalian toxicology Department
Central pesticide laboratory

Dr. Mohamed Adly Mohamed

Lecturer of Forensic Medicine
And Clinical Toxicology
Faculty of Medicine – Cairo University

Faculty of Medicine
Cairo University
2009

Abstract

This thesis was designed to study dose-related and age-related effects of the mixture of four pesticide residues extract (chloropyrifos, profonofos, fenitrothion and dicofol) on Oxidative Stress, Genotoxicity and immunotoxicity, and Protective Potential of natural Antioxidants (garlic 250 mg/kg and alpha lipoic acid 60mg/kg), 120 males of Albino rats will be divided into two main groups according to age; weaning group (2 months age) and Adult group (6 months age). Each age group of rats were divided into 6 subgroups (*10 rats in each)* the 1st group served as control, *the 2nd&3rd* groups were orally treated with high & low level of pesticides residue mixture, respectively, the 4th group served as +ve control (antioxidants only), the 5th &6th groups were orally treated with antioxidants 1h after administration of high & low level of pesticides residue mixture, respectively, All groups were force-fed by gastric intubations 5 days per week for 3 months. The oxidative stress status of treated animals has been evaluated by assessment of reduced glutathione (GSH), Glutathione-S-Transferase (GST), malondialdehyde (MDA). In addition, the acetylcholinesterase (AChE) activity was measured as a biomarker of toxicity. The mean *comet tail length* and *Comet DNA %* were used to measure DNA damage. We used IgG, IgM, rate of leucocyte phagocytosis and of lymphocyte transformation as immunotoxicological biomarkers to test the immune function as well as Histopathological studies in lymph node. Our result revealed that pesticide mixture induce inhibitory effect on AChE, depletion in GSH content, alteration in GST and elevation in lipid peroxidation (MDA). A significant increase in mean *comet tail length* and Comet DNA *%* indicating DNA damage was observed. The damage was dose related. The results showed that pesticides mixture produced a decrease in Both IgG and IgM, the rate of lymphocyte transformation and the rate of leucocyte phagocytosis also decrease in both age groups. In additions, our result revealed that natural antioxidants (ALA and garlic extracts) have more or less counteracting effect on Oxidative Stress, Genotoxicity and immunotoxicity caused by pesticides.

(Key words: pesticide residues, acetylcholinesterase, Oxidative Stress, lipid peroxidation, Genotoxicity, comet assay, immunotoxicity, lymphocyte transformation, phagocytosis)

ACKNOWLEDGEMENT

First of all greatest thanks and merciful to Allah, the great creator and the source of all man's knowledge.

I would like very much to express my deepest gratitude and sincere thanks to **Prof. Dr. Aly Gamal El-Din** *Professor of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University, for his fatherly attitude, sincere encouragement, valuable guidance, kind supervision and scientific support.*

Wording is not enough to express my sincere gratitude and deep appreciation to **Prof. Dr. Dina Ali Shokry** *Professor and Head of Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Cairo University, for the idea and planning of this work, her efficient and indispensable help, faithful guidance and willing efforts to facilitate the difficulties during this work.*

I offer my great appreciation and thanks to **A. Prof. Dr. Manal El - Sayed El-Halwagy** *Assistant Professor of Biochemistry, Mammalian toxicology Department , Central pesticide laboratory for her meticulous supervisions, sincere guidance, significant input and kind help in every step of this work.*

Thanks to **Dr. Mohamed Adly Mohamed**, *Lecturer of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Cairo University for his supervision and encouragement.*

I wish also to express my sincere thanks to **Prof. Dr. Nevine Darwish** *Professor of pathology, Research institute of ophthalmology, for her support in the Histopathological part of this work, and for her valuable advices*

Thanks to **Dr. Sanaa El Sawi,** *Researcher in Central laboratory for analysis of pesticide residues and heavy metal in food, for her help.*

Last, but not least, my deep thanks and gratitude to all my professors and my colleagues and to my family for their kind support and encouragement.

Mustafa M. Afify

This work is dedicated

TO SOUL OF MY FATHER

TO MY MOTHER

TO MY WIFE

AND

TO MY KID

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ABTS	2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)
AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
AGE	aged garlic extract
ALA	alpha lipoic acid
ALS	alkali-labile sites
AOX	Antioxidants
ARfD	Acute Reference Dose
CA	chromosome aberrations
CAT	catalase
CFU	colony forming units
CTL	cytotoxic T cells
DHLA	dihydrolipoic acid
DSB	double-strand breaks
EMRL	Extraneous Maximum Residues Limits
EPA	Environmental Protection Agency
GGT	gamma-glutamyl transpeptidase
GR	glutathione reductase
GPx	glutathione peroxidase
GSH	Reduced glutathione
GST	glutathione-S-transferase
HDPM	High Dose Pesticides Mixture
H2O2	hydrogen peroxide
LD50	lethal dose in 50% of animals
LDPM	Low Dose Pesticides Mixture

LGL	large granular lymphocyte
MALT	mucosa-associated lymphoid tissues
MDA	malonyl aldehyde
MEM	Minimum Essential Medium (culture medium)
MHC	major histocompatibilty complex
MRL	Maximum Residue Level
MTT	[3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] The MTT assay has been used to test cytotoxicity of reagents and cell viability.
NPD	Nitrogen Phosphorous Detectors
O2-·	superoxide anion
^{1}O2	singlet oxygen
OC	Organochlorines
OH	hydroperoxyl radicals
OFR	Oxygen free radicals
OP	Organophosphate
PBS	Phosphate Buffered Saline
PRC	Pesticide Residues Committee
PSD	Pesticide Safety Directorate
RDA	Recommended Dietary Allowance
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute, Media
SCE	sister chromatid exchanges
SOD	superoxide dismutase
SSB	single -strand breaks
TCR	T cell receptor
WHO	World Health Organization

LIST OF FIGURES

LIST OF PHOTOMICROGRAPH

LIST OF TABLES