

**EVALUATING THE PROTECTIVE ROLE OF SOME
ANTIOXIDANTS AGAINST THE INDUCED FREE
RADICALS RESULTING FROM DIFFERENT
ENVIRONMENTAL POLLUTANTS**

Submitted By

Eman Gomaa Mohamed Kelany

B.Sc. of Agricultural Science(General), Faculty of Agriculture, Cairo University, 1985

Diploma of Environmental Sciences, Institute of Environmental Studies & Research,

Ain Shams University, 1998

M. Sc. of Environmental Science, Institute of Environmental Studies & Research,

Ain Shams University, 2007

A thesis submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
In
Environmental Science

Department of Environmental Agricultural Science
Institute of Environmental Studies and Research
Ain Shams University

2014

APPROVAL SHEET

EVALUATING THE PROTECTIVE ROLE OF SOME ANTIOXIDANTS AGAINST THE INDUCED FREE RADICALS RESULTING FROM DIFFERENT ENVIRONMENTAL POLLUTANTS

Submitted By

Eman Gomaa Mohamed Kelany

B.Sc. of Agricultural Science(General), Faculty of Agriculture, Cairo University, 1985

Diploma of Environmental Sciences, Institute of Environmental Studies & Research,

Ain Shams University, 1998

M. Sc. of Environmental Science, Institute of Environmental Studies & Research,

Ain Shams University, 2007

This thesis Towards a Doctor of Philosophy Degree in
Environmental Science Has been Approved by:

Name

Signature

1-Prof. Dr. Farouk Mohamed El Tellawy

Emeritus Prof. of Food Technology - Department of Environmental
Agricultural Science – Institute of Environmental Studies and Research
Ain Shams University

2-Prof. Dr. Helmy Taha Omran

Prof. of Food Technology - Department of Food Technology
Faculty of Agriculture
Suez Canal University (Ismailia)

3-Prof. Dr. Farouk Guindi Moawad

Prof. of Biochemistry - Department of Biochemistry
Faculty of Agriculture
Ain Shams University

4-Prof. Dr. Kamal Mamoun El Deib

Prof. of Biochemistry
National Organization for Drug Control and Research
2014

**EVALUATING THE PROTECTIVE ROLE OF SOME
ANTIOXIDANTS AGAINST THE INDUCED FREE
RADICALS RESULTING FROM DIFFERENT
ENVIRONMENTAL POLLUTANTS**

Submitted By

Eman Gomaa Mohamed Kelany

B.Sc. of Agricultural Science(General), Faculty of Agriculture, Cairo University, 1985

Diploma of Environmental Sciences, Institute of Environmental Studies & Research,

Ain Shams University, 1998

M. Sc. of Environmental Science, Institute of Environmental Studies & Research,

Ain Shams University, 2007

**A thesis submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
In
Environmental Science
Department of Environmental Agricultural Science**

Under The Supervision of:

1-Prof. Dr. Usama Mohamed Mohamed Radwan

Prof. of Food Technology and Head of Department of Environmental
Agricultural Science - Institute of Environmental Studies and Research
Ain Shams University

2-Dr. Kamal Mamoun El Deib

Associate Prof. of Biochemistry – Molecular Drug Evaluation Dep.
National Organization for Drug Control and Research

2014

Acknowledgement

First I am deeply thankful to Allah to the grass of whom the present work was realized.

*I wish to express my deepest gratitude, everlasting appreciation and sincere appreciation to Prof. Dr. **Usama Mohamed Radawan** Professor of Food Technology and head of the Environmental agricultural Science Dep., Institute of Environmental Studies and Research, Ain Shams University for valuable assistance, useful advice, guidance and his unlimited helps in writing thesis.*

*I am also greatly indebted to Prof. Dr. **Kamal Mamoun El Deib**, Professor of Biochemistry and head of Molecular Drug Evaluation Dep., National Organization for Drug Control and Research for his supervision, help and advice in writing the thesis and encouragement.*

*I wish to express my deepest gratitude and everlasting appreciation to Prof. Dr. **Mohamed Abdel Razeq El-Nawawy**, Professor of Microbiology, Food Science Dep., Faculty of Agriculture, Ain Shams University for valuable assistance, useful advice*

*Deepest gratitude and many thanks to Dr. **Mahgoub Mohammed Ahmed**, Associate Professor of Biochemistry, Molecular Drug Evaluation Dep., National Organization for Drug Control and Research his help in the analysis part of this study.*

Last but not least, my thanks are dedicated to all staff members of Molecular Drug Evaluation Dep., National Organization for Drug Control and Research for their help.

ABSTRACT

Eman Goma Mohammed Kelany

**Evaluating the Protective Role of some Antioxidants against the Induced Free Radicals
Resulting From Different Environmental Pollutants**

PhD Thesis, Agric. Sci. Dept.,

**Institute of Environmental Studies and Research,
Ain Shams University (2014).**

This study was designed to investigate the antioxidant activity of 39 substances namely synthetic and natural raw materials used in pharmaceutical preparations and as a food, using different model systems in order to find out new potential sources of natural antioxidants, also comparison study between synthetic and natural compound under investigation. The results showed that all compounds dose-dependently scavenge DPPH free radical. Catechin has the highest free radical by 98.8%, whereas p-cresol has the lowest scavenging activity on DPPH radical by 10.7% at 160 µg/ml. Also, all samples under investigation have inhibitory effect against hydroxyl radical mediated DNA damage and this inhibitory effect is dose-dependent manner. Catechin (97.5%) had the highest scavenging value on hydroxyl radical mediated DNA-damage, whereas 4-hydroxybenzaldehyde had the lowest scavenging effect (9.15%). Also, quercetin exerted the highest inhibition effect by 97.3% at 160 µg/ml against lipid peroxidation in mitochondria induced by ferrous/ascorbate model system. Moreover quercetin and rutin had the highest inhibitory effect against lipid peroxidation in lysosomes induced by ferrous/ascorbate model system. Furthermore, rutin and catechin had the highest inhibitory effect against microsomes lipid peroxidation-induced by ferrous/ascorbate model system, whereas resorcinol exerted the lowest inhibitory effect against microsomes lipid peroxidation. The best five compounds (ascorbic acid, quercetin, caffeic acid, catechin and rutin) by four concentrations; 60, 120, 240 and 480 µg/ml appeared to protect the lysosomal membrane against cisplatin. Ascorbic acid, catechin, caffeic acid and quercetin showed the highest inhibitory effect against cisplatin. The results of this study stated that the five compounds could be taken with cisplatin to reduce its nephrotoxicity.

Key words: antioxidant activity, antioxidant compounds, DPPH free radical, lipid peroxidation, lysosomal membrane, cisplatin.

CONTENTS

Content	Page
INTRODUCTION	1
REVIEW OF LITERATURE	3
1- Acid Compounds	5
2-Flavonoid Compounds	6
3-Amine Compounds	7
4-PHENOL COMPOUNDS	8
5- Other Compounds	9
1-Ascorbic acid	10
2-Caffeic acid	13
3- Quercetin	16
4- Catechin	19
5-RUTIN	22
MATERIALS AND METHODS	26
1-MATERIALS	28
2-METHODS	
2.1. Preparation of mitochondria and microsomes from rat liver for lipid peroxidation test	30
2.2. DNA-sugar damage assay	34
2.3. Free radical scavenging assay (DPPH assay)	35
2.4. Evaluation of the hydroxyl radical scavenging activity	37
2.5. Lysosomal membrane protection	37
RESULTS AND DISCUSSION	
1-FREE RADICAL SCAVENGING ACTIVITY	41
1.1 Free radical scavenging activity of some acids compounds on DPPH radical	41

1.2-Free radical scavenging activity of some flavonoids on DPPH radical	43
1.3-Free radical scavenging activity of some amines on DPPH radical	44
1.4-Free radical scavenging activity of some phenols on DPPH radical	45
1.5-Free radical scavenging activity of p-hydroxy benzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on DPPH radical	46
2-DNA damage system and-Deoxyribose sugar degradation system	48
2.1-DNA damage system	48
2.1.1- Effect of some acids on free radical mediated DNA-damage	48
2.1.2-Effect of some flavonoids on free mediated DNA-damage	49
2.1.3-Effect of some amines on free radical mediated DNA-damage	51
2.1.4-Effect of some phenols on free radical mediated DNA-damage	51
2.1.5-Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on free radical mediated DNA-damage	52
2.2-Deoxyribose sugar degradation system	54
2.2.1-Inhibitory effect of some acids against hydroxyl radical mediated deoxyribose degradation	54
2.2.2-Inhibitory effect of some flavonoids against hydroxyl radical mediated deoxyribose degradation	55

2.2.3-Inhibitory effect of some amines against hydroxyl radical dextrose mediated deoxyribose degradation	56
2.2.4-Inhibitory effect of some phenols against hydroxyl radical mediated deoxyribose degradation	57
2.2.5-Inhibitory effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin against hydroxyl radical deoxyribose degradation	58
3-Ferrous/ascorbate model system which produced hydroxyl radical and induced lipid peroxidation	60
3.1-Liver mitochondria	60
3.1.1-Effect of some acids on ferrous/ascorbate system induced lipid peroxidation in rat liver mitochondria	61
3.1.2-Effect of some flavonoids on ferrous/ascorbate system induced lipid peroxidation in rat liver mitochondria	62
3.1.3-Effect of some amines on ferrous/ascorbate system induced lipid peroxidation in rat liver mitochondria	64
3.1.4-Effect of some phenols on ferrous/ascorbate model system induced lipid peroxidation in mitochondria	65
3.1.5-Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide biotin, p-hydroxycoumarin and coumarin on ferrous/ascorbate system induced lipid peroxidation in rat liver mitochondria	66
3.2-LIVER LYSOSOMES	67
3.2.1-Effect of some Acids on ferrous/ascorbate system induced lipid peroxidation in lysosomes	68
3.2.2-Effect of some flavonoids on ferrous/ascorbate system induced lipid peroxidation in lysosomes	69

3.2.3-Effect of some amines on ferrous/ascorbate system induced lipid peroxidation in lysosomes	71
3.2.4-Effect of some phenols on ferrous/ascorbate system induced lipid peroxidation in lysosomes	71
3.2.5-Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on ferrous/ascorbate system induced lipid peroxidation in rat liver lysosomes	72
3.3-LIVER MICROSOMES	74
3.3.1-Effect of some acids on ferrous/ascorbate system induced lipid peroxidation in rat liver microsomes	75
3.3.2-Effect of some flavonoids on ferrous/ascorbate system induced lipid peroxidation in rat liver microsomes	76
3.3.3-Effect of some amines on ferrous/ascorbate system induced lipid peroxidation in rat liver microsomes	77
3.3.4-Effect of some phenols on ferrous/ascorbate system induced lipid peroxidation in rat liver microsomes	78
3.3.5-Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on ferrous/ascorbate system induced lipid peroxidation in rat liver microsomes	79
4-Effect of ascorbic acid, quercetin, caffeic acid, catechin and rutin on cisplatin-induced lysosomal membrane damage	80
4.1-Acid phosphatase	80
4.2-β-N- acetyl-glucosaminidase	82
4.3-β-galactosidase	83
Summary	85
REFERENCES	95-122

LIST OF TABLES

TABLE	Page
-Table (1): Free radical scavenging activity of some acid compounds on DPPH radical	42
-Table (2): Free radical scavenging activity of flavonoids compounds on DPPH radical	44
-Table (3): Free radical scavenging activity of some amines compounds on DPPH radical	45
-Table (4): Free radical scavenging activity of some phenolic compounds on DPPH radical	46
-Table (5): Free radical scavenging activity of <i>p</i> -hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide and biotin on DPPH radical	47
-Table (6): Effect of some acids on free radical mediated DNA damage	49
-Table (7): Effect of some flavonoids on Free radical mediated DNA damage	50
-Table (8): Effect of some amines on Free radical mediated DNA damage	51
-Table (9): Effect of some phenols on Free radical mediated DNA damage	52
-Table (10): Effect of <i>p</i> -hydroxybenzaldehyde, riboflavin, selenium dioxide, biotin, <i>p</i> -hydroxycoumarin and coumarin on free radical mediated-DNA damage	53
-Table (11): Inhibitory effect of some acids against hydroxyl radical mediated deoxyribose degradation	55

-Table (12): Inhibitory effect of some flavonoids against hydroxyl radical mediated deoxyribose degradation.	56
-Table (13): Inhibitory effect of some amines against hydroxyl radical mediated deoxyribose degradation.	57
-Table (14): Inhibitory effect of some phenols against hydroxyl radical mediated deoxyribose degradation	58
-Table (15): Inhibitory effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin against hydroxyl radical mediated deoxyribose degradation.	59
-Table (16): Effect of some acids on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver mitochondria	62
-Table (17): Effect of some flavonoids on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver mitochondria.	63
-Table (18): Effect of some amines on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver mitochondria	65
-Table (19): Effect of some phenols on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver mitochondria	66
-Table (20): Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, Biotin, p-hydroxycoumarin and coumarin on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver mitochondria	67
-Table (21): Effect of some acids on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver lysosomes	69
-Table (22): Effect of some flavonoidson Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver lysosomes	70
-Table (23): Effect of some amines on Fe ²⁺ /ascorbate system induced lipid peroxidation in rat liver lysosomes	71

-Table (24): Effect of some phenols on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver lysosomes.	72
-Table (25): Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver lysosomes.	74
-Table (26): Effect of some acids on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver microsomes.	75
-Table (27): Effect of some flavonoids on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver microsomes	77
-Table (28): Effect of some on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver microsomes	77
-Table (29): Effect of some phenols on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver micosomes	78
-Table (30): Effect of p-hydroxybenzaldehyde, tocopherol, riboflavin, selenium dioxide, biotin, p-hydroxycoumarin and coumarin on Fe^{2+} /ascorbate system induced lipid peroxidation in rat liver microsomes	80
-Table (31): Comparative effects of ascorbic acid, quercetin, caffeic acid, catechin and rutin on cisplatin-induced release of acid phosphatase	81
-Table (32): Comparative effects of ascorbic acid, quercetin, caffeic acid, catechin and rutin on cisplatin-induced release of β -N- acetyl glucosaminidase	82
-Table (33): Comparative effects of ascorbic acid, quercetin, caffeic acid, catechin and rutin on cisplatin-induced release of β -glactosidase.	83

LIST OF FIGURES

FIGURES	Page
-Fig. 1. Preparation of mitochondria and microsomes from rat liver.	30
- Fig.2. Preparation of lysosomal fraction from rat liver.	31
- Fig. 3. Reaction between MDA and TBA.	34
- Fig.4. Scavenging of free DPPH [•] by antiradical species.	36

1-INTRODUCTION

Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil (**Stavric, 1994**). Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of bio-membranes and dysfunction of cells and tissues (**Cho et al., 2003**). Synthetic or natural antioxidants play a significant role in protecting living organisms from the toxic effect of various chemicals by preventing free radical formation (**Sheweita et al., 2001**).

On the other hand, reports revealing that synthetic antioxidants could be toxic, with regard to food additives or pharmaceutical products safety, identifying alternative natural and probably safer source of food antioxidant is needed.

Free radicals originating from cellular metabolism primarily in the mitochondria can act directly on liver tissues, In addition attack critical target molecules or attack polyunsaturated fatty acid in membranes and initiate lipid peroxidation and liver cirrhosis (**Irmak et al, 2002**).

Free radicals are defined as molecules having an unpaired electron in the outer orbit. They are generally unstable and very reactive. There is a lot of evidence revealing the role of reactive oxygen species (ROS) in several diseases. ROS are generated as byproducts of cellular metabolism, primarily in the mitochondria (**Szocs, 2004**). These ROS are scavenged by antioxidant enzymes namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). Under some circumstances, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction of oxygen radicals, inactivation of detoxification

systems, and failure to replenish antioxidants in tissues adequately (**Irmak et al., 2002**).

This study was designed to investigate the antioxidant activity of some compounds using different model systems in order to find out new potential sources of natural antioxidants also, comparison study between synthetic and natural compound under investigation

Recently special attention is focused on the substitution of synthetic antioxidants used in foods and pharmaceutical preparations by natural ones. Therefore, the present study was designed to compare the antioxidant activity of synthetic sources used as food additives and in the pharmaceutical preparations with the natural ones. To achieve this aim, the following topics were considered;

PART 1

-1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) scavenging activity system

PART 2

-Deoxyribonucleic acid (DNA) damage system and-deoxyribose sugar degradation system

PART 3

-Ferrous/ascorbate model system which produced hydroxyl radical and induced lipid peroxidation in liver mitochondria, microsomes and lysosomes.

PART 4

-Effect of ascorbic acid, quercetin, caffeic acid, catechin and rutin on cisplatin-induced lysosomal membrane damage.