

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

# بسم الله الرحمن الرحيم





HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرونيله



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



# THE POSSIBLE PROTECTIVE ROLE OF PLANT POLYPHENOLS AGAINST THE GENOTOXICITY OF ACROLEIN ON BONE MARROW CHROMOSOMES AND DNA IN MALE ALBINO MICE

A THESIS SUBMITTED FOR
THE AWARD OF THE P.H.D. DEGREE
OF SCIENCE TEACHER PREPARATION
(ZOOLOGY)

#### BY

#### Sally Ramadan Gabr Eid El-Ashry

General Diploma in Science Teacher Preparation – Zoology (2010) Special Diploma in Science Teacher Preparation – Zoology (2011) Master degree in Science Teacher Preparation – Zoology (2015)

#### Supervised By

#### Prof. Dr. Nagla Zaky Ibrahim El-Alfy

Professor of Cytogenetics - Biological and Geological Sciences Department–Faculty of Education - Ain Shams University

#### Prof. Dr. Mahmoud Fathy Mahmoud

Professor of Cell Biology and Histology–Biological and Geological Sciences Department-Faculty of Education–Ain Shams University

TO

BIOLOGICAL AND GEOLOGICAL SCIENCES DEPARTMENT-FACULTY OF EDUCATION - AIN SHAMS UNIVERSITY

2021



## **APPROVAL SHEET**

Name: Sally Ramadan Gabr Eid El-Ashry

Title: THE POSSIBLE PROTECTIVE ROLE OF PLANT POLYPHENOLS AGAINST THE GENOTOXICITY OF ACROLEIN ON BONE MARROW CHROMOSOMES AND DNA IN MALE ALBINO MICE

**Supervisors** Approved

#### Prof. Dr. Nagla Zaky Ibrahim El-Alfy

Professor of Cytogenetics, Biological and Geological Sciences Department, Faculty of Education, Ain Shams University.

#### Prof. Dr. Mahmoud Fathy Mahmoud

Professor of Cell Biology and Histology, Biological and Geological Sciences Department, Faculty of Education, Ain Shams University.

## **ACKNOWLEDGMENT**

First of all, I wish to offer my deep thanks to ALLAH for the support in every step which enabled me to overcome all the problems that faced me throughout the work.

I would like to express my special appreciation and thanks to Prof. Dr. Nagla Zaky Ibrahim EL-Alfy Professor of Cytogenetic, Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, for suggesting the point and supervising the whole work. Sincere thanks are also for her continuous guidance and critical reviewing of this manuscript. Her advice on both research as well as on my career have been priceless. I am grateful to her for her excellent direction in the completion of this work.

It pleases me to offer special thanks to prof. Dr. Mahmoud Fathy

Mahmoud professor of Cell Biology and Histology, Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, for his continuous encouragement and advice during the stages of this work. Sincere thanks are also due to him for his guidance and constructive critical reading of this manuscript.

I would also like to thank head of Biological and Geological Sciences Department, Faculty of Education, Ain Shams University and the staff members for their co-operation.

Special thanks to my family. Words cannot express how grateful I am to my mother and my father for all of the sacrifices that you've made on my behalf. I am greatly indebted to thank my siblings, my husband and my beloved children Sila & Younis for their continuous encouragement.

### **ABSTRACT**

Acrolein, a highly reactive unsaturated aldehyde, is considered as a mutagenic environmental pollutant which can cause oxidative stress by generation of reactive oxygen species. Quercetin and resveratrol are two naturally occurring plant polyphenols with high antioxidant properties, present in fruits, vegetables and numerous dietary compounds.

The present work is mainly concerned with the study of the protective role of oral pretreatment with quercetin 50 mg/kg b. wt. alone, resveratrol 12.5 mg/kg b. wt. alone and the mixture of both quercetin/resveratrol against the cytotoxicity and genotoxicity of acrolein 10 mg/kg b. wt. on bone marrow chromosomes and DNA content of male albino mice (*Mus musculus*) by using chromosomal aberration assay, mitotic index, chromosomal C-banding analysis, chromosomal G-banding analysis, sister chromatid exchange analysis, micronucleus test, comet assay and quantitative real time-polymerase chain reaction (qRT-PCR) analysis.

In this study, acrolein was administrated orally to mice for four consecutive days, while quercetin and/or resveratrol were given orally to mice for eight days (four days prior to acrolein treatment followed by other four days along with acrolein treatment).

The results obtained showed that oral administration of acrolein to mice for four consecutive days significantly increased (P < 0.001) the incidence of aberrant metaphases, structural and numerical chromosomal

aberrations, the frequency of sister chromatid exchanges, micronuclei formation and cytotoxicity in bone marrow cells in comparison to the control group.

Also, the current results of comet assay showed a significant increase (p < 0.05) in the mean of tail length, tail DNA% and olive tail moment which indicated the induction of DNA damage in the liver cells of mice after oral administration of acrolein 10 mg/kg b. wt. for four consecutive days when compared to those of the control group.

Results of quantitative real time-polymerase chain reaction (qRT-PCR) analysis revealed that acrolein administration reduced the expression levels of two genes coding for antioxidant enzymes; glutathione peroxidase 1 (GPx1) and superoxide dismutases 1 (SOD1) mRNA in the mice liver tissues in comparison to that of the control group.

On the contrary, oral pretreatment of mice with quercetin and/or resveratrol significantly reduced the incidence of aberrant metaphases, structural and numerical chromosomal aberrations, the frequency of sister chromatid exchanges, micronuclei formation and cytotoxicity in bone marrow cells in comparison to acrolein-treated group.

Further, results of comet assay revealed that the antigenotoxic potential of quercetin and/or resveratrol caused a significant (P < 0.05) reduction in the comet parameters and the genotoxicity of liver cells when compared to acrolein-treated group.

Furthermore, the current results of quantitative real time-polymerase chain reaction (qRT-PCR) analysis demonstrated that pretreatment of quercetin and/or resverarol before acrolein administration upregulated the expression levels of GPx1 and SOD1 mRNA in comparison to acrolein administered group.

No considerable difference was observed between the protective effects of quercetin alone, resveratrol alone and also the mixture of both quercetin/resveratrol against acrolein-induced clastogenesis, cytotoxicity and genotoxicity. However, oral pretreatment of quercetin alone showed the best protective effect against acrolein-toxicity.

Therefore, natural foods rich in quercetin such as apples, honey, raspberries, onions, red grapes, cherries, citrus fruits and green leafy vegetables and resveratrol such as peanuts, grapes, blueberries, cocoa and dark chocolate should be included in the human daily diet or can be replaced by daily nutritional supplements of quercetin and resveratrol to protect against the deleterious effects of clastogenic agents like acrolein.

Key words: Acrolein, Quercetin, Resveratrol, Mice, Chromosomes, DNA.

# **Contents**

Title	page
LIST OF TABLES	I
LIST OF FIGURES	III
LIST OF ABBREVIATIONS	XXII
1. INTRODUCTION	1-6
1.1. Acrolein	1-3
1.2. Plant polyphenols (quercetin and resveratrol)	3-6
AIM OF THE PRESENT WORK	7
2. REVIEW OF LITERATURE	8-56
2.1. The normal karyotype of the male albino mouse (Mus musculus)	8, 9
2.2. Effect of acrolein on bone marrow chromosomes and DNA of male albino mice (Mus musculus)	10-37

2.3. The protective role of some plant polyphenols against the genotoxicity of acrolein	38-56
2.3.1. Quercetin (Q)	40-47
2.3.2. Resveratrol (RES)	47-56
3. MATERIALS AND METHODS	57-100
3.1. Experimental Animals	57, 58
3.2. Cages and water bottles	58
3.3. Chemicals	58-61
3.3.1. Acrolein	59
3.3.2. Quercetin	60
3.3.3. Reseveratrol	60, 61
3.4. Experimental Design	62-64
3.5. Preparation of bone marrow chromosomes	65-67
3.5.1. Mitotic index (MI%)	67
3.6. Chromosomal banding preparation	68-71
3.6.1. C-banding technique	68, 69

3.6.2. G-banding technique	70, 71
3.7. Sister chromatid exchange (SCE) analysis	72-74
3.8. Micronucleus test	75, 76
3.9. Comet assay	77-87
3.9.1. Requirements	77, 78
3.9.2. Preparations of reagents	79-82
3.9.3. Protocol for single cell gel electrophoresis (SCGE)	82-87
3.9.3.1. Tissue collection and preparation	82
3.9.3.2. Procedure for separation of hepatocytes	82
3.9.3.3. Procedure for preparation of slides	83
3.9.3.4. Preparation of agarose	83
3.9.3.5. Pre-coating of agarose	83
3.9.3.6. Layering of hepatocytesLMPA gel mixture	84
3.9.3.7. Procedure for lysis of hepatocytes	85
3.9.3.8. Procedure for alkaline unwinding and electrophoresis of slides	85, 86
3.9.3.9. Procedure for neutralization	86

3.9.3.10. Visualization and analysis of Comet Slides	86, 87
3.10. Quantitative real time-Polymerase Chain Reaction (qRT-PCR) analysis	88-99
3.10.1. Isolation of total RNA	88-93
3.10.1.1. Components of the kit	88, 89
3.10.1.2. Buffer and solution preparation	89, 90
3.10.1.3. Sample preparation	90
3.10.1.4. RNA isolation	90-93
3.10.2. Synthesis of cDNA from RNA (Reverse Transcription)	93-96
3.10.2.1. Components of the kit	93, 94
3.10.2.2. First strand cDNA synthesis procedure	94, 95
3.10.2.3. PCR amplification of first strand cDNA	95, 96
3.10.3. Polymerase Chain Reaction (PCR)	96-99
3.10.3.1. PCR procedure	97, 99
3.11. Statistical Analysis	100