

**MOLECULAR AND GENOTOXIC STUDIES OF SOME
NATURAL AND SYNTHETIC COMPOUNDS**

By

ASMAA MAHER METWALY MOHAMED
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2013

THESIS

**Submitted in partial Fulfillment of the
Requirements for the Degree of**

MASTER OF SCIENCES
In
Agriculture Sciences
(Agricultural Biochemistry)

Department of Agricultural Biochemistry
Faculty of Agriculture
Cairo University
EGYPT
2019

Format Reviewer

Vice Dean of Graduate Studies

APPROVAL SHEET

**MOLECULAR AND GENOTOXIC STUDIES OF SOME
NATURAL AND SYNTHETIC COMPOUNDS**

**M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

By

**ASMAA MAHER METWALY MOHAMED
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2013**

APPROVAL COMMITTEE

DR. OMAIMA AHMED KHAMISS.....
Professor of Cell culture and Molecular Virology, GEBRI, Sadat City University

DR. FATEN MOHAMED ABOUL ELALA.....
Professor of Biochemistry, Fac. Agric., Cairo University

DR. AHMED MAHMOUD ABOUL- ENEIN.....
Professor of Biochemistry, Fac. Agric., Cairo University

DR. ADEL RASHAD AHMED ABD- ALLAH.....
Professor of Pharmacology and Toxicology, Fac. Pharm., Al-Azhar University

Date: 21/2 / 2019

SUPERVISION SHEET

**MOLECULAR AND GENOTOXIC STUDIES OF SOME
NATURAL AND SYNTHETIC COMPOUNDS**

**M.Sc. Thesis
In
Agric. Sci. (Agricultural Biochemistry)**

By

ASMAA MAHER METWALY MOHAMED
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2013

SUPERVISION COMMITTEE

Dr. AHMED MAHMOUD ABOUL- ENEIN
Professor of Biochemistry, Fac. Agric., Cairo University

DR. EMAN AHMED HANAFY
Lecturer of Biochemistry, Fac. Agric., Cairo University

DR. ADEL RASHAD AHMED ABD- ALLAH
Professor of Pharmacology and Toxicology, Fac. Pharm., Al-Azhar University

Name of Candidate: Asmaa Maher Metwaly Mohamed

Degree: Master

Title of Thesis: Molecular and Genotoxic Studies of Some Natural and Synthetic Compounds

Supervisors: Dr. Ahmed Mahmoud Aboul Enein

Dr. Eman Ahmed Hanafy

Dr. Adel Rashad Abd-Allah

Department: Agricultural Biochemistry

Approval: 21/02/2019

ABSTRACT

Many health risks occur as a result of the use of synthetic food additives in industry, on a large scale, that was illustrated by many health and cancer organizations. So that, the present study investigated the genotoxicity effects of nine food additives that includes different classes of synthetic and natural additives (Colors, Preservatives, Antioxidants, Flavors and Sweeteners) on lymphocytes from rats or human volunteer.

The genotoxicity was estimated using the alkaline comet assay, while the cytotoxicity was determined by Trypan blue exclusion method and the IC_{50} was calculated for each compound. Significant genotoxic effect obtained in the comet assay presented as tail percentages and tail moment for DNA strand breaks. Hence, the migration of its fragmentations from the head (chromosomes in nucleus) by electrophoretic mobility was determined on low melting agarose gel.

Furthermore, the investigation of the genotoxic effects of tested food colorants and sweeteners including; Erythrosine, Carminic acid, Aspartame and Sorbitol on another cell type (human lymphocyte cells) were proceeded using the alkaline comet assay. Their ability to induce apoptosis, which is required to be sure that the tested compounds are safe on human health, was also studied.

The results showed that most of the investigated natural food additives have no detected genotoxicity effects at the tested concentrations, while most of the corresponding synthetic food additives gave significant genotoxic results at the same concentrations. However, Carminic acid as a natural coloring agent showed genotoxic result similar to its corresponding synthetic food coloring agent (Erythrosine). Furthermore, Isoamyl acetate and Butylated hydroxyl anisole (BHA) as synthetic additives, showed no genotoxic effect. Aspartame at 6 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$ revealed a significant DNA damage at p . value <0.05 .

Unexpectedly, Sorbitol as a natural sweetener showed a high genotoxic effect and it induced apoptosis in human lymphocytes. Meanwhile, Erythrosine, Carminic acid and Aspartame gave no detectable genotoxic effects, but they induced apoptosis in the tested cells.

From the obtained results, it can be concluded that the synthetic food additives (Erythrosine, Potassium sorbate and Aspartame) and Carminic acid, as natural food coloring agents, caused DNA strand breaks in rat lymphocyte cells; which in turn leads to dangerous health effects. However, Sorbitol did not depict harmful effects on DNA, when tested on rat lymphocyte cells, but it caused a life cycle disturbances, induces the apoptotic pathway and DNA damage, which revealed its harmful effects at cellular and genetic levels in human lymphocyte cells, at the tested concentration. In contrary, Erythrosine, Carminic acid and Aspartame did not affect DNA, however these additives have bad effects on cell cycle when they were tested on human lymphocyte cells. Likewise, these additives conferred apoptosis insult.

Keywords: Synthetic and natural food additives, cytotoxicity, genotoxicity, alkaline comet assay, Sorbitol, apoptosis

DEDICATION

I wish to thank my family for providing a loving environment for me. My parents, Maher, M. and Rda, A. and my brothers Eslam Maher and Youssef Maher. They raised me, supported me and loved me. To them I dedicate this thesis. I wish to thank my doctors and my colleagues in Biochemistry department for their kind feelings and their love.

ACKNOWLEDGEMENT

Thanks ALLAH for all things in my life (thanks for helping me and encouraging me).

*It is a pleasure to thank many people who made this thesis possible. It is difficult to express my gratitude to my M.Sc. supervisors **Dr. Ahmed Aboul-Enein, Dr. Adel Rashad and Dr. Eman Ahmed** With their enthusiasm, their inspiration, and their great efforts to provided encouragement, sound advice, good teaching, good company, and lots of good ideas.*

*I would like to thank many people who taught me statistical analysis (my doctors and my friend in Agronomy department) and my doctors in research institutes (**Prof. Dr. Oaima Khamis and Dr. Eman Mohamed**) for kind help in comet assay technique.*

CONTENTS

INTRODUCTION.....	Page 1
REVIEW OF LITERATURE.....	3
1. Cytotoxicity.....	3
2. Genotoxicity.....	4
3. Food additives.....	11
a. Erythrosine as synthetic food colorant.....	12
b. Carminic acid as natural food colorant.....	13
c. Potassium sorbate as synthetic preservative.....	14
d. Isoamyle acetate (Banana oil) as synthetic flavor.....	15
e. Clove oil as natural preservative and flavor.....	16
f. Butylated hydroxyanisol (BHA) as synthetic antioxidant...	19
g. Vitamin C as natural antioxidant.....	20
h. Aspartame as synthetic sweetener.....	22
i. Sorbitol as natural sweetener.....	23
MATERIALS AND METHODS.....	25
1. Chemicals.....	25
2. Preparation of tested food additives.....	26
3. Preparation of required chemicals and buffers.....	27
4. Isolation of lymphocyte cells from blood samples.....	28
5. Treatment of lymphocyte cells.....	28
6. Trypan blue exclusion method.....	28
7. <i>In vitro</i> alkaline comet assay.....	29
8. Cell cycle evaluation and apoptotic and necrotic cells detection.....	30 31
RESULTS AND DISCUSSION.....	31
1. The effect of nine food additives on rat lymphocyte cells.....	31
a. Cells viability (cytotoxicity evaluation).....	31
b. Alkaline comet assay.....	35
1. Genotoxicity of (erythrosine and Carminic acid) food colors.....	35

2. Genotoxicity of food preservatives and flavors potassium sorbate, isoamyl acetate and clove oil.....	38
3. Genotoxicity of antioxidants (BHA and vitamin C)	42
4. Genotoxicity of aspartame as synthetic sweetener and sorbitol as natural sweetener.....	45
2. The effect of some food additives normal human lymphocyte cells.....	48
a. Cytotoxicity and IC ₅₀ assessment.....	50
b. Analysis of DNA damage.....	52
c. Analysis of Cell life cycle and apoptosis.....	52
CONCLUSION.....	61
SUMMARY.....	62
REFERENCES.....	67
APPENDIX A.....	82
ARABIC SUMMARY.....	