STUDY OF THE TOXICOGENOMIC EFFECT OF COMMON FOOD ADDITIVES USING SOME YEAST STRAINS

By

SHIMAA EL-SAYED RASHAD EL-SAYED
B.Sc.Agric. Sci. (Biotechnology), Cairo University, 2004
M.Sc.Agric.Sci.(Genetics), Ain Shamus University, 2012

A Thesis Submitted in Partial Fulfillment
Of
The Requirement for the Degree of

DOCTOR OF PHILOSOPHY
in
Agricultural Sciences
(Genetics)

Department of Genetics
Faculty of Agriculture
AinShams University

2019

Approval Sheet

STUDY OF THE TOXICOGENOMIC EFFECT OF COMMON FOOD ADDITIVES USING SOME YEAST STRAINS

By

SHIMAA EL-SAYED RASHAD EL-SAYED

B.Sc.Agric. Sci. (Biotechnology), Cairo University, 2004
M.Sc.Agric.Sci.(Genetics), Ain Shamus University, 2012

This thesis for Ph.D. degree has been approved by:

Dr. Mohammed Rizk Abu El-Magd El-Ghannam .....
Associate Prof. of Anatomy and Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University.

Dr. Nermin Mahmoud Abdel-Gawad .....
Prof. of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Eman Mahmoud Fahmy .....
Prof. Emeritus of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Fatthy Mohammed Abdel-Tawab .....
Prof. Emeritus of Molecular Genetics, Faculty of Agriculture, Ain Shams University.

Date of Examination:

STUDY OF THE TOXICOGENOMIC EFFECT OF COMMON FOOD ADDITIVES USING SOME YEAST STRAINS

By

SHIMAA EL-SAYED RASHAD EL-SAYED

B.Sc.Agric. Sci. (Biotechnology), Cairo University, 2004
M.Sc.Agric.Sci.(Genetics), Ain Shamus University, 2012

Under the supervision of:

Dr. Fatthy Mohammed Abdel-Tawab

Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (principal supervisor).

Dr. Eman Mahmoud Fahmy

Prof. Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University.

Dr. Ashraf Gamil Attallah

Researcher Prof. of Genetics, Department of Microbial Genetics, National Research Center

ABSTRACT

Shimaa El-Sayed Rashad El-Sayed: Study of the toxicogenomic effect of common food additives using some yeast strains. Unpublished PhD. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2019.

A comet assay was performed to assess DNA damage in yeast deletion strains after treatment by the recommended concentrations of the food additives. yeast knockout haploid strains (YKO) strains exhibited a significant DNA damage ($P < 0.05$) which was observed by an increase in tail length, tail DNA% and tail moment as compared to the normal control strains. The results showed that food additives monosodium glutamate (MSG), sodium benzoate (SB) and saffron induced profound cytotoxicity in cancer cells, i.e., human lung cancer (A549), human breast cancer (MCF7), human colon cancer (Caco-2), respectively. In addition, those food additives generated cytotoxic activities on normal lung cell lines (Wi38), respectively. Flow cytometric analysis demonstrated that treatment of human hepatocellular carcinoma cells (Caco-2) cells with food additives increased G2/M phase cell cycle arrest. The quantitative real time-PCR was used to measure the mRNA levels of *p53*, *Bax*, and *Bcl-2* genes. The data showed that food additives changed transcriptional levels of these related genes. The mRNA expression of *p53* and *Bax* were up-regulated, but, the transcription of *Bcl2* was significantly down-regulated compared to the control in Coca-3 cells. Protein-protein interaction maps provided a valuable framework for a better understanding of the functional organization of the proteome. To detect interacting pairs of human proteins (*RAD51, RECQL, MLH1 and IGF2BP2*) systematically, a protein matrix of human was screened by automated yeast knockout proteins (*RAD51, SGS1, MLH1 and IMP2*) interaction. The results revealed that food additives stimulated cytotoxicity by decreased cell viability in cancer and normal cell lines. Cell cycle blocking at the G2/M phase was evident. Moreover, induction of apoptosis via increased *p53*

and *Bax* mRNA expression levels accompanied with reduced *Bcl2* gene expression which are correlated with apoptosis pathway was observed. Thus, the use of MSG, SB and Saffron could result in negative health biohazards to human beings. This calls for strict control of the threshold levels that should be permitted for the industrial products containing food additives as it could reflect biosafety hazards to human and environment.

Keywords: Yeast knockout, Comet assay, Cell lines, Flow cytometry, Real time-PCR, Neutral red assay.

ACKNOWLEDGEMENT

First, of all my obedience, devotion, deepest thanks and praise are due and fully extended-as always to Allah, the greatest and almighty that has created us and bestowed upon us a lot of blessing, which we cannot enumerate and thank enough.

I would like to express my deepest thanks and sincere gratitude to **Prof. Fatthy M. Abdel-Tawab**, Professor Emeritus of Molecular Genetics, Genetics Dept., Faculty of Agriculture, Ain Shams University for his supervision, suggesting the problem, valuable advices, unfailing help during the course of investigation and in writing the manuscript. This work benefited greatly from his efforts. I would gratefully acknowledge him.

My deep appreciation to **Prof. Eman M. Fahmy**, Professor Emeritus of Genetics, Genetics Dept., Faculty of Agriculture, Ain Shams University, for her supervision, unfailing help during the course of investigation, in writing the manuscript, kind support and motherly guidance .

I'd like to Thank **Prof. Ashraf Gamel**, Prof. of Microbial Genetics, Department of Microbial Genetics, National Research Center, for his advises on sincere guidance throughout the practical work.

Great thanks would be expressed to **Prof. Abd El-Hamid Haggran** Professor of Microbial Genetics, Department of Microbial Genetics, National Research Center, sincere support, effective, valuable help, encouragement and his help in preparation of the manuscript.

My deep appreciation to **Prof. Ekram Salah El-din Ahmed** Professor Emeritus of Genetic, Cell Biology Department, National Research Centre for her excellent advises, and her help in writing and reviewing the manuscript.

It would have been simply impossible to accomplish the tremendous work implied by the thesis goals without the constant support

and friendly attitude of many wonderful people I was honored to know. I would like to offer my sincere gratitude to them.

Finally, thanks are offered to all the staff members and colleagues of Genetics group, Genetic Engineering and Biotechnology Division, Microbial Genetics Department, N.R.C., for help and cooperation.

Great thank are also due to all members of Genetics Department, Faculty of Agriculture, Ain Shams University, for their great help and moral supports during this work.

Acknowledge is extended to my dad and mom, my husband Ahmed, my sons Omar and Mohammed, my sister, Marwa and my brothers, Hossam and Ahmed, I love them all.

CONTENTS

LIST OF TABLES

LIST OF FIGURES