دراسة مقارنة على مؤشرات وراثية وخلوية وجزيئية للتسمم بالمعادن الثقيلة فى فئران المعمل (*مس ماسكيولس*)

رسالة مقدمة الى كلية العلوم-جامعة القاهرةلاستيفاء الدراسة المقررة
لدرجة الماجستير فى العلوم
علم الحيوان
(وراثة)

رسالة مقدمة من
هيدان مصطفى سالم عبد الهادى
بكالوريوس علوم – جامعة القاهرة
قسم علم الحيوان - كلية العلوم
جامعة القاهرة

٢٠٠٩

Comparative study on cytogenetic and molecular biomarkers of heavy metal toxicity in the laboratory mice (*Mus Musculus)*

A Thesis

Submitted to Faculty of Science,Cairo University

In Partial Fulfillment of the Requirements for the degree of

M.Sc. In Zoology

(Genetics)

By

Haidan Mostafa Salem El Shorbagy

B.Sc. Cairo University

Zoology Department

Faculty of Science

Cairo University

٢٠٠٩

APPROVAL SHEET

Title of the M.Sc. Thesis

"Comparative study on Cytogenetic and Molecular biomarkers of Heavy metal toxicity in the laboratory mice (*Mus musculus*)"

Name of Candidate

Haidan Mostafa Salem ElShorbagy

Submitted to

Zoology Department
Faculty of Science, Cairo University

Supervisor committee:

١-Prof. Dr. Mohamed Akmal A.El-Ghor …….
Professor of Genetics,
Zoology Department,
Faculty of Science, Cairo University

٢-Dr. Magda M.Noshy ……..
Associate Professor of Genetics
Zoology Department,
Faculty of Science, Cairo University

٣-Dr. Mohamed Hussein Awwd ……
Associate Professor of Molecular Biology,
Zoology Department,
Faculty of Science, Benha University

Head of Zoolog Department
Faculty of Science Cairo University
Prof. Dr.Kawther S. Abou El-Ala

TO

My Family

My Husband

And

My daughters

Hala and Jana

Acknowledgment

I would like to express my most sincere gratitude to Prof. Dr Akmal A.El Ghor prof. of Genetics, Zoology Department, Faculty of Science ,Cairo University, for his useful guidance, supervision and careful reading and criticizing the manuscript. His effort in putting this thesis in its final form is greatly appreciated.

I also wish to express my deep appreciation to Dr. Magda M.Noshy, Associate professor of Genetics, Zoology Department, Cairo University for her keen supervision, as well as the revision of the manuscript and unlimited help.

Sincere thanks to Dr. Mohammed Awwad, Associate professor of molecular biology, Zoology Department, Faculty of Science, Banha University, for his helpful and guidance during the investigation as well as for careful reading of manuscript.

My profound gratitude to my family, my aunt Zozo and my husband.

Finally, I wish to thank all members of Zoology Department, Faculty of Science, Cairo University, for their cooperation.

Contents

LIST OF TABLES

List of Figures

ABSTRACT

The induction of micronuclei in mice bone marrow cells and point mutation at the restriction sites of ٨ different restriction enzymes, five cutters(BalI , BclI, BglII, HindIII and PstI) and three non cutters (BamHI, EcoRI and NcoI) of mice MT-I gene were investigated.

Cadmium chloride was administered to adult male mice as single i.p. dose of ٢,٥ and ١,٢٥ mg/kg b.w. and as repeated i.p doses of ١,٢٥ mg/kg b.w. for ٤ week at ٧٢h intervals. $CdCl_2$ induced a significant and dose-dependent increase in the percentage of MNPCEs reached ٢,٨٦ % with the highest tested dose compared with negative control.

On the other hand administration of lead acetate doses of ٣٢٣ and ١٦١,٥ mg/kg b.w. single i.p injection and ٨ mg/kg b.w. as repeated i.p. doses for ٤ week at ٧٢h intervals had induced a significantly higher MN frequency than negative control.

Null point mutation induction was detected using PCR/RFLP method after different treatments with the two tested doses of cadmium chloride or lead acetate.

In conclusion, results of the present work indicated that cadmium chloride and lead acetate induced significant cytogenetic and cytotoxic effects in mice bone marrow cells with no point mutation in MT-I gene at the restriction sites of the restriction enzymes used. Thus it may be concluded that MN assay can serve as a better preliminary biomarker that reflects cadmium and lead exposure.

However, further investigation is needed to evaluate other molecular assays to detect mutations that may be produced by heavy metals.

Chapter I

Introduction and aim of the work

Heavy metals are found in increasingly hazardous concentrations in air, food, and water. Agency for toxic substances and disease registry **(ATSDR, ١٩٩٩)** lists cadmium and lead among the top of the ٢٧٥ most hazardous substances in the environment which have been identified as the most probable causes of heavy metal-related disease observed in primary care medicine.

As a class heavy metals can either be mutagenic by themselves or can enhance the mutagenic effect of other agents **(Minissi and Lombi, ١٩٩٧).**

Cadmium is a toxic heavy metal of continuing occupational and environmental concern. Cadmium exposure mainly occurs in industrial settings, however, cigarette smokes and Cd-contaminated water, air and food are other non-occupational sources of human exposure to cadmium. It has wide variety of adverse effects and it has been classified as a human carcinogen by the International Agency for Research on Cancer and the US National Toxicology Program **(Rossman et al., ١٩٩٢ and Who and Lyon, ١٩٩٣)**. Moreover, it's clearly a potent multi-tissue animal carcinogen **(Schimada et al., ١٩٧٦; Vikina et al., ١٩٧٨; Who and Lyon, ١٩٩٣).** The mechanisms of the carcinogenic activity of cadmium are not clearly defined but may involve non-genotoxic or indirect genotoxic events since cadmium is, in general, a weak mutagen **(Dzhokhadze and Lezhava, ١٩٩٤).**

Several *in vitro* studies have shown that cadmium produces DNA strand breaks, DNA–protein cross-links and oxidative DNA damage mostly at high cytotoxic concentrations **(Deknudt and Gerber, ١٩٧٩; Forni, ١٩٩٢).**

However, reports regarding the *in vitro* induction of chromosomal aberrations by cadmium compounds or the data obtained from *in vivo* assays were contradictory.