



**CYTOGENTICAL STUDIES OF
MITOMYCIN-C DRUG ON THE BONE
MARROW CHROMOSOMES OF MALE
ALBINO MICE *Mus musculus***

A THESIS SUBMITTED FOR
THE AWARD OF THE M.SC. DEGREE
OF SCIENCE TEACHER PREPARATION
(ZOOLOGY)

BY

Ayman Mohammad Abdullah Husain

(Ed.-B.Sc.)

General Diploma in Science Teacher Preparation – Zoology (2010)

Special Diploma in Science Teacher Preparation – Zoology (2011)

Supervised By

Prof. Dr. Nagla Zaky Ibrahim El-Alfy

Professor of Cytogenetics - Biological and Geological Sciences Department

Faculty of Education - Ain Shams University

Dr. Mahmoud Fathy Mahmoud

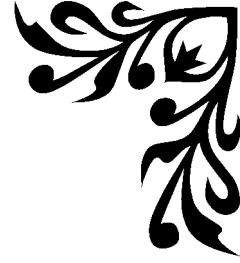
Lecturer of Zoology - Biological and Geological Sciences Department -

Faculty of Education - Ain Shams University

TO

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

2014



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَاللَّهُ خَلَقَ كُلَّ دَابَّةٍ مِنْ مَّاءٍ ۖ فَمِنْهُمْ مَنْ يَمْشِي
عَلَى بَطْنِهِ وَمِنْهُمْ مَنْ يَمْشِي عَلَى رِجْلَيْنِ وَمِنْهُمْ
مَنْ يَمْشِي عَلَى أَرْبَعٍ ۚ يَخْلُقُ اللَّهُ مَا يَشَاءُ ۚ إِنَّ
اللَّهَ عَلَى كُلِّ شَيْءٍ قَدِيرٌ

﴿النور : ٤٥﴾



APPROVAL SHEET

Name: Ayman Mohammad Abdullah Husain

Title: CYTOGENTICAL STUDIES OF
MITOMYCIN-C DRUG ON THE BONE
MARROW CHROMOSOMES OF MALE
ALBINO MICE *Mus musculus*

Supervisors Approved

Prof. Dr. Nagla Zaky Ibrahim El - Alfy

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



Dr. Mahmoud Fathy Mahmoud

Lecturer of Zoology,
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 deepest gratitude and my heartfelt thanks to Prof. Dr. Nagla Zaky EL-Alfy Professor of Cytogenetic, Zoology 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. I am grateful to her for her excellent direction in the completion of this work.

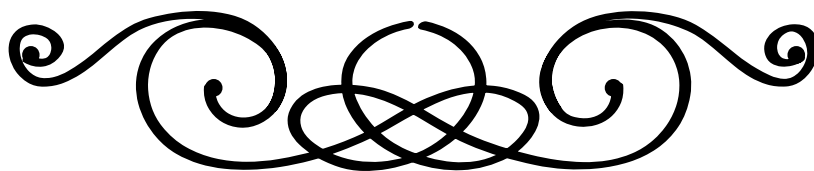
It pleases me to offer special thanks to Dr. Mahmoud Fathy Mahmoud lecturer of Zoology, Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, for

their continuous encouragement and advice during the stages of this work. Sincere thanks are also due to them for their guidance and constructive critical reading of this manuscript.

I am greatly indebted to Prof. Dr. Mohammad Abdul-Aziz Fouad, head of Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, for his valuable facilities.

Real thanks are also due to the staff members of the Biological and Geological Sciences Department, Faculty of Education, Ain Shams University for their help and co-operation.

Finally, I am indebted to thank my mother, my father, my wife, my daughter and my siblings for their continuous encouragement.



ABSTRACT

Mitomycin-C (MC) is an anti-cancer drug has a genotoxic effect on the bone marrow cells as this influence extends to the testicular tissue. The aim of this work is to study the genotoxic effect on bone marrow chromosomes, DNA content and testicular tissue of male albino mice after mitomycin-C treatment. Sixty male albino mice (16-17 weeks old and 28 ± 2 g. weight) were used in the present study and divided into five groups each group consists of 12 mice. The first group served as control was injected intrapretonially with (physiological saline solution 1ml/kg b.wt.) while the second group was treated with mitomycin-C (3 mg/kg b.wt.) for one week, group (3) was treated with mitomycin-C (3 mg/kg b.wt.) for two weeks, group (4) was treated with mitomycin-C (6 mg/kg b.wt.) for one week and group (5) was treated with mitomycin-C (6 mg/kg b.wt.) for two weeks. Each mitomycin-C treated animal was intraperitoneally injected single time at the first day of the experiment.

Results indicated that the treating male mouse with mitomycin-C showed chromosomal aberrations in bone marrow cells whether structural aberrations and numerical aberration. Structural aberrations were deletion, fragmentation, chromatid gap, centric fusion, centromeric attenuation, ring and chromosomal gapping. While numerical aberration was polyploidy. Results of chromosomal aberrations indicated that the rates of total aberrations were increased by time and dose.

Abstract

Micronucleus assay illustrated that mitomycin-C treatment induced genotoxicity in bone marrow cells, and the rate of polychromatic erythrocytes with micronucleus was increased by dose. Also, cytotoxicity test showed that the polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) ratio was increased by dose.

In the current study, genetic changes among control and all treated groups have been studied according to RAPD-PCR analysis. Results indicated that mitomycin-C treatment induced genetic changes among control and all treated groups. Comet assay showed that mitomycin-C treatment induced DNA damage in mice lymphocytes and the mean of total comet score was increased by dose and time among all treated groups.

The damage caused in the testis of mice after mitomycin-C treatment displayed variable changes in both the seminiferous tubules and the interstitial tissue. Changes in seminiferous tubules were represented by hypoplasia of the germinal epithelium and spermatogenic arrest at various stages of spermatogenesis. The most prominent changes reported in the intertubular tissue were represented by the presence of a homogeneous and intensely eosinophilic ground substance in the interstitial areas, congestion of blood vessels as well as haemorrhage in the interstitial tissue. The histological changes were also significantly increased by time and dose.

Key words: Mitomycin-C, Chromosomes, Micronucleus, DNA, RAPD-PCR, Comet assay, Histopathology, Testis, Mice.

Contents

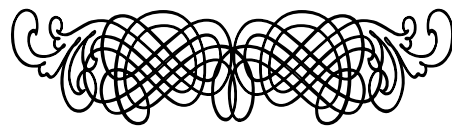
Titles	Pages
LIST OF TABLES	I
LIST OF FIGURES	IV
LIST OF ABBREVIATIONS	XVII
1.INTRODUCTION	1
AIM OF THE PRESENT WORK	4
2.REVIEW OF LITERATURE	5-74
2.1. The chromosomes	5
2.2. DNA Content	32
2.3. Histology of the testis	55
3.MATERIALS AND METHODS	75-103
3.1. Animals:	75
3.2. Drug:	76
3.2.1. Stock solution of drug:	77
3.2.2. Experimental doses of drugs:	77
3.3. Experimental Design:	77
3.4. Preparation of bone marrow chromosomes:	79
3.5. Micronucleus test:	82
3.6. Molecular genetic studies:	84
3.6.1. Random amplified Polymorphic DNA (RAPD-PCR).	84
3.6.1.1. Stock solutions:	84
3.6.1.1.1. DNA extraction buffer (DNA TES):	84
3.6.1.1.2. Tris EDTA buffer (TE):	84
3.6.1.1.3. Tris-Borate EDTA (TBE) 5x:	85
3.6.1.1.4. Potassium 3M, Acetate 5M, pH 4.8:	85
3.6.1.1.5. Sodium Chlorid (NaCl) 6M:	85
3.6.1.1.6. Ethidium Bromide (EtBr):	86
3.6.1.1.7. Sample loading dye:	86
3.6.1.2. DNA Extraction:	86
3.6.1.3. Polymerase Chain Reaction (PCR) Conditions:	88
3.6.1.3.1. PCR mixture:	89
3.6.1.3.2. DNA amplification:	90
3.6.1.4. Sample preparation:	90
3.6.1.5. Gel preparation:	91
3.6.2. Comet assay:	91
3.6.2.1. Requirements	91

3.6.2.1.1. Chemicals	91
3.6.2.1.2. Consumables	92
3.6.2.1.3. Instruments	92
3.6.2.2. Preparations of reagents	93
3.6.2.2.1. Phosphate-buffered saline	93
3.6.2.2.2. Normal melting point agarose 0.75% w/v	93
3.6.2.2.3. Low melting point agarose 0.5% w/v	94
3.6.2.2.4. Lysis solutions	94
3.6.2.2.4.1. Stock solution	94
3.6.2.2.4.2. Working solution	94
3.6.2.2.4.5. Electrophoresis buffer	95
3.6.2.2.4.5.1. Stock Solution I	95
3.6.2.2.4.5.2. Stock Solution II	95
3.6.2.2.4.5.3. Working solution	95
3.6.2.2.4.6. Neutralization buffer	95
3.6.2.2.4.7. Reagents for fluorescent staining	96
3.6.2.2.4.7.1. Stock solution	96
3.6.2.2.4.7.2. Working solution	96
3.6.2.3. Protocol for single cell gel electrophoresis (SCGE)	96
3.6.2.3.1. Procedure for collecting blood sample	96
3.6.2.3.2. Procedure for separation of lymphocyte	96
3.6.2.3.3. Procedure for preparation of slides	97
3.6.2.3.3.1. Preparation of agarose	97
3.6.2.3.3.2. Pre-coating of agarose	97
3.6.2.3.3.3. Layering of lymphocyte-LMPA gel mixture	98
3.6.2.4. Procedure for lysis of lymphocyte	99
3.6.2.5. Procedure for alkaline unwinding and electrophoresis of slides	99
3.6.2.6. Procedure for neutralization	100
3.6.2.7. Procedure for staining	100
3.6.2.8. Fluorescent staining method	100
3.6.2.9. Percentage of DNA in the tail	101
3.7. Histological preparation of the testis:	102
3.8. Statistical analysis:	102
4.RESULTS	104-213
4.1. THE CHROMOSOMES	104
4.1.1. Karyotype of the male mouse <i>Mus musculus</i>:	104
4.1.2. Chromosomal aberrations	109
4.1.2.1. Effect of mitomycin-C on chromosomes:	109

4.1.2.1.1. Chromosome type aberrations:	110
4.1.2.1.1.1. Structural chromosomal aberrations:	110
4.1.2.1.1.1.1. Chromatid aberrations:	110
4.1.2.1.1.1.1.1. Deletions (D):	110
4.1.2.1.1.1.1.2. Fragments (F):	111
4.1.2.1.1.1.1.3. Chromatid gap (C.g):	112
4.1.2.1.1.1.2. Chromosomal aberrations:	113
4.1.2.1.1.1.2.1. Centric fusion (Cf):	113
4.1.2.1.1.1.2.2. Centromeric attenuations (Ca):	114
4.1.2.1.1.1.2.3. Ring form (R):	115
4.1.2.1.1.1.2.4. Chromosomal gap (Ch.g):	116
4.1.2.1.1.2. Numerical chromosomal aberration:	130
4.1.3. Results of Micronucleus assay and cytotoxicity test:	140
4.1.3.1. The Micronucleus assay:	140
4.1.3.2. Cytotoxicity assay (PCE/NCE ratio):	155
4.2. RESULTS OF DNA CONTENT	159
4.2.1. Results of The randomly amplified polymorphic DNA (RAPD) analysis:	159
4.2.1.1. Primer A-14:	159
4.2.1.2. Primer B-19:	166
4.2.2. Results of Comet assay:	173
4.3. HISTOLOGICAL OBSERVATIONS	184
4.3.1. Testis of control mice:	184
4.3.2. Testis of mitomycin-C treated mice:	191
4.3.2.1. Mice treated with 3 mg/kg b.wt. Of mitomycin-C after one week (group 2):	191
4.3.2.2. Mice treated with 3 mg/kg b.wt. Of mitomycin-C after two weeks (group 3):	193
4.3.2.3. Mice treated with 6 mg/kg b.wt. of mitomycin-C after one week (group 4):	195
4.3.2.4. Mice treated with 6 mg/kg b.wt. of mitomycin-C after two weeks (group 5):	196
5. DISCUSSION	214-259
5.1. THE CHROMOSOMES	214
5.1.1. Chromosomal aberrations	215
5.1.2. Micronucleus assay	225
5.2. THE DNA CONTENT	234
5.2.1 The randomly amplified polymorphic DNA (RAPD) analysis	234

5.2.2. Comet assay	242
5.3. THE TESTIS	249
6. SUMMARY AND CONCLUSION	260-265
7. REFERENCES	266-355
8. ARABIC SUMMARY	1-9

LIST OF TABLES AND LIST OF FIGURES



List of Tables

Table No.		Page
Table (1)	The primers code, sequences and the G+C percentage.	94
Table (2)	The mean and standard deviation of chromosomal aberrations in metaphases cells of male albino mice <i>Mus musculus</i> of control group, (group 2) treated with mitomycin-C 3 mg/kg b.wt. for one week and (group 3) treated with mitomycin-C 3 mg/kg b.wt. for two weeks.	126
Table (3)	The mean and standard deviation of chromosomal aberrations in metaphases cells of male albino mice <i>Mus musculus</i> of control group, (group 4) treated with mitomycin-C 6 mg/kg b.wt. for one week and (group 5) treated with mitomycin-C 6 mg/kg b.wt. for two weeks.	132
Table (4)	The relationship between the mean of chromosomal aberrations of male albino mice in control group and all treated groups with mitomycin-C (group 2, 3, 4 and 5).	139

List of Tables

Table (5)	The relationship between the mean of total of chromosomal aberrations of control group and all treated groups with mitomycin-C (group 2, 3, 4 and 5) after one and two weeks (W1, W2) of treatment.	141
Table (6)	The relationship between the mean of total of aberrations for all types of chromosomal aberrations in all treated groups with mitomycin-C (group 2, 3, 4 and 5).	141
Table (7)	The mean and standard deviation of Polychromic erythrocytes (PCEs), Polychromic erythrocytes (MNPCEs) with Micronucleus, Normochromic erythrocytes (NCEs), and genotoxicity in bone marrow of male albino mice <i>Mus musculus</i> of control group and treated groups.	157
Table (8)	DNA characterization for control and treated samples with mitomycin-C of male albino mice <i>Mus musculus</i> using primer A-14.	167
Table (9)	RAPD profiles of different groups using primer A-14.	168

List of Tables

Table (10)	Similarity index and genetic distances between control and treated samples with mitomycin-C of male albino mice <i>Mus musculus</i> .	169
Table (11)	DNA characterization for control and treated samples with mitomycin-C of male albino mice <i>Mus musculus</i> using primer B-19.	177
Table (12)	RAPD profiles of different groups using primer B-19.	175
Table (13)	Similarity index and genetic distances between control and treated samples with mitomycin-C of male albino mice <i>Mus musculus</i> .	176
Table (14)	The mean and standard deviation of the comet score of peripheral blood leukocytes of male albino mice <i>Mus musculus</i> of control group and treated groups.	186