

شبكة المعلومات الجامعية

بسم الله الرحمي الرحم



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

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



يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٥٠ مئوية ورطوبة نسبية من ٢٠-٠٤% To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



بعض الوثائق الاصلية تالفة



بالرسالة صفحات لم ترد بالاصل



AIN SHAMS UNIVERSITY UNIVERSITY COLLEGE FOR WOMEN (ARTS, SCIENCE AND EDUCATION) CAIRO, A.R.E.

EVALUATION OF THE MUTAGENIC POTENTIAL OF IVERMECTIN

A Thesis
Submitted

By

Dalia Demerdash Abd EL Monem Hafez

Assistant Lecturer In Zoology Department Ain Shams University (B. Sc., 1998, M. Sc., 2003)

For The Degree of Ph. D. In Zoology (Cytogenetics)

2005

YNYA

N



وقل اعملوا فسيري الله عملوا فسيري الله عملكم ورسوله والمؤمنون







سورة التوبة آية ٥٠٥



AIN SHAMS UNIVERSITY UNIVERSITY COLLEGE FOR WOMEN (ARTS, SCIENCE AND EDUCATION) CAIRO, A.R.E.

Evaluation of the mutagenic potential of Ivermectin

Under Supervision of

Prof. Dr. KARIMA MOHAMMAD SWEIFY

Prof. of Cytogenetics
Zoology Department
UNIVERSITY COLLEGE FOR WOMEN
(ARTS, SCIENCE AND EDUCATION)
Ain Shams University

Dr. IMAN ABD EL MONEIUM DARWISH

Lecturer of Cytogenetics
Zoology Department
UNIVERSITY COLLEGE FOR WOMEN
(ARTS, SCIENCE AND EDUCATION)
Ain Shams University

2005

Approval Sheet

Name

: Dalia Demerdash Abd El Monem Hafez

Title

: Evaluation of the mutagenic potential of

Ivermectin.

Scientific Degree

: Ph. D.

Supervisors

Karima

Prof. Dr. KARIMA MOHAMMAD SWEIFY

Prof. Of Cytogenetics

Zoology Department UNIVERSITY COLLEGE FOR WOMEN

(ARTS, SCIENCE AND EDUCATION)
Ain Shams University

Dr. IMAN ABD EL MONEIUM DARWISH iman

Lecturer of Cytogenetics

Zoology Department
UNIVERSITY COLLEGE FOR WOMEN
(ARTS, SCIENCE AND EDUCATION)
Ain Shams University

Head of Zoology Department

Qualification

Name : Dalia Demerdash Abd El Monem Hafez

Scientific Degree : M. Sc. (Cytogenetics)

Department : Zoology

College : women College for Arts, Science and

Education

University : Ain Shams University

B.Sc. Graduation Year: May 1998

M. Sc. Graduation Year : 2003

ABSTRACT

Ivermectin (IVM) is a broad-spectrum anti-parasitic agent. It shows an excellent anthelmintic effect in veterinary and human medicine. The objective of this study was the evaluation of potential mutagenicity of IVM on *Mus musculus* in vivo. This was achieved through chromosomal aberration assay (CAA) and micronucleus test (MNT) in bone marrow cells. CAA was also studied in spermatocytes. Variation in constitution of serum proteins on the molecular level was also investigated.

For CAA & MNT, animals received single and/or double doses of $200\mu g/kg$ b.w. IVM. The sampling times were 1, 2, 3, 7 and 14 days after the last injection. While in CAA in spermatocytes, male mice treated with single injection of $200\mu g/kg$ b.w. IVM. Meiotic chromosomes were prepared after 6h, 2, 5, 10 and 12 days to cover the different phases of meiotic division.

In molecular study, animals treated in a similar manner. Blood samples were collected after 1, 7 and 14 days of the last injection. Total protein content was determined. The electrophoretic patterns of serum proteins were studied by using SDS-PAGE.

After single and double injection of IVM the damage cell percent (DC%) and the chromosome aberration percent (CA%) were significantly elevated through the first 3 days, and reduced after 7 and 14 days. The peaking time was different according to exclusion or inclusion of gaps. Single dose of IVM gave high score of MN than that obtained by double doses. In both groups MNPCEs were decreased by increasing time. The percentage of abnormal metaphases in germ cells was 33.83 versus 5.83 for the control.

IVM elevated the total protein content. Single injection induced much variation in the percentage area of the separated proteins than that produced by double treatment. In conclusion, this study revealed high clastogenic and genotoxic potential of IVM on mice.

Contents

		Page
Acknow	vledgment.	
Introduction.		1
Aim of the present work.		6
Chapter 1: Literature review.		7
1-1:	Origin and chemistry of ivermectin.	7
1-2:	Pharmacokinetics and safety of ivermectin	9
1-3:	Mechanism of action.	12
1-4:	Therapeutic efficacy of ivermectin.	14
1-5:	Side effects of ivermectin.	16
1-6:	Chromosomal studies.	19
1-6-1:	Mitotic chromosomes of mouse <i>Mus</i> musculus.	19
1-6-2:	Cytogenetic effects of avermectin group and other anthelmintic drugs.	19
1-7:	Micronucleus test (MNT).	22
1-7-1:	Anthelmintic drugs and MNT.	25
1-8:	Meiotic chromosomes.	28
1-8-1:	Various therapeutic treatments.	30
1-9:	The protein binding of ivermectin.	32
1-10:	SDS-PAGE electrophoresis.	34

		page
1-10-1:	Normal serum proteins.	34
1-10-2:	Electrophoresis of serum proteins.	36
	DNA electrophoresis.	40
Chapter -2- Materials and methods.		42
A-	Cytogenetic analysis.	42
A-1-	Experimental animals.	42
A-2-	Chemicals and Solutions.	43
A-2-1-	Chemicals and solutions used for mitotic chromosomes and micronucleus test.	43
A-2-2-	Chemicals and solutions used in meiotic chromosomes.	43
A-2-3-	Giemsa Stain.	44
	Plan of the work.	44
A-3-	Treatment for bone marrow chromosome aberrations and micronucleus test.	46
A-3-1	Chromosome preparation from bone- marrow cells.	46
A-3-1-1	Analysis of the slides.	47
	Statistical analysis.	48
	5- Micronucleus test (MNT).	48
	Scoring of the micronuclei.	49
	PCEs/NCEs ratio.	49
	Statistical analysis.	50
A-4-	Treatment for meiotic chromosomes.	50

		page
A-4-1	MI spermatocytes.	50
A-4-2	MII spermatocytes.	51
A-4-3-	7-Preparation of mammalian meiotic chromosomes.	51
A-4-4-	Scoring criteria.	53
A-4-5-	Statistical analysis.	53
B-	Molecular genetics analysis.	54
B-1-	Blood collection for protein analysis.	54
B-2-	Determination of serum total proteins.	54
B-2-1-	Principle	55
B-2-2-	Reagents	55
В-2-3-	Procedure	56
B-2-4-	Calculation	56
B-3-	Polyacrylamide gel electrophoresis (SDS-PAGE) for serum protein analysis.	56
В-3-1-	Principle of the method.	57
B-3-2-	Reagents (stock solutions).	57
B-3-2-1	Formulation of SDS-polyacrylamide separating and stacking gels.	62
B-3-2-2	Preparation of gel.	63

		page
	Sample preparation and the separation process.	
	d- Processing the gel (staining, destaining and preserving).	64
B-3-2-5	Analysis of gel lanes.	65
Chapter -3- Results.		67
A-	chromosome study in somatic cells.	67
A-1-	The results after therapy with single dose.	67
A-1-1-	The structural chromosomal aberrations.	67
1-a-	The aberrant metaphases percent.	67
1-b-	Chromosomal aberrations percentage CAs%.	77
A-1-2-	The numerical chromosomal aberrations.	80
A-2-	The results after therapy with two single doses.	84
A-2-1-	The structural chromosomal aberration.	84
1-a-	The percent of the damaged cells DC%.	84
	Chromosomal aberration percentage CA%.	86
A-2-2-	The numerical chromosomal aberrations.	88
A-3-	Comparison between data obtained after single and repeated doses.	90
A-3-1-	Structural chromosomal aberration.	90