IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 2 Ver. III (Feb 2015), PP 17-29
www.iosrjournals.org

The cytogenetic potential of ivermectin on bone marrow cells of mice in vivo

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Abstract:*Ivermectin (IVM) is a broad-spectrum anti spectrum agent. It shows an excellent anthelmintic effect in veterinary and human medicine. The objective of this study was the evaluation of potential cytogenicity of IVM on Mus musculus in vivo.This was achieved through chromosomal aberration assay (CAA) and micronucleus test (MNT) in bone marrow cells.For CAA & MNT, animals received single and/or double doses of 200ug/kg b.w. IVM. The sampling times were 1, 2, 3, 7, and 14 days after the last injection.The maximum values of the chromosomal aberrations percent (excluding gap) attained after 3 days, when including gap, the readings obtained after 1, 2, 3 and 7 days of single treatment were approached to each other, then decreased after 14 days. The chromosomal aberrations percent obtained after repeated injection showed elevation through the first 3 days and reduction after 7 and 14 days. After repeated dosing the chromosomal aberrations percent (CA%) reached its highest value after 2 and 3 days with including and excluding gaps, respectively. Single dose of IVM gave high score of MN than that obtained by double doses. In both groups MNPCEs were decreased by increasing time. On the other hand, IVM treatment induced highly significant decrease in the ratio of PCEs/NCEs at all the experimental periods, with the exception of data obtained after 14 days.IVM induced high level of chromosome aberrations in somatic cells, as it is ascertained by chromosome aberration assay and micronuclei production in bone marrow cells. This study revealed high clastogenic and genotoxic potential of IVM on mice*

Keywords:*Ivermectin (IVM), chromosomal aberration assay (CAA), micronucleus test (MNT), (polychromatic erythrocytes), NCEs (normochromatic erythrocytes)*

I. Introduction

Ivermectin is a macrocyclic lactone (avermectins) proved by the actinomycete Streptomyces avermitilis [1].Cooperia oncophora was the predominant species after deworming in larvae treated with IVM [2]. However, oral ivermectin, although not licensed in many countries, might be useful, particularly for patients who cannot tolerate or comply with topical therapy and in institutional scabies epidermics [3].

The most favourable dosage for red-and roe deer was 2 x 0.4 mg ivermectin/kg body weight administered at an interval of one week, although equally good results could be obtained with 1 x 0.3 - 0.4 mg ivermectin/kg body weight [4]. There are some literature relating the therapeutic efficacy of IVM. Accordingly, it might be possible that ivermectin could help eradicate, suppress, or prevent a bedbug infestation [5]; might constituted an additional potential tool for the control of Glossina palpalis gambiensis [6]; induced adult mortality and decreases the hatch rate of eggs in Aedes aegypti [7]; improves the therapeutic outcomes of both albendazole and mebendazole against Trichuris trichiura [8]and was effective as rodent systemic insecticides against blood feeding sand flies [9].Ivermectin demonstrated high efficacy and tolerability in the treatment of Pediculosis capitis [10]; it could be rapidly advanced into clinical trial for leukemia [11]; resolved retinal edema and electroretinographic changes [12]; is active against numerousspecies of helminths and arthropods [13]; is effective against microfilariae [14]; and is recommended as the treatment of choice for onchocerciasis, a filariasis that produces river blindness [15].On the other hand, ivermectin interfered with the immune system [16]; disturbed the kinetic behavior [17]; inhibited lipopolysaccharide-induced production of inflammatory cytokines [18]; induced immunopotentiating effect [19]; caused salivation, coma, mydriasis, slight fever, lacrimation, mydriasis, protrusion of third eye-lid, tachycardia and ataxia [20]; and induced neuronal damage and impaired neuronal-glial metabolism [21] in animalsMoreover, ivermectin caused severe complications, including lethargy, confusion, coma , fever, myalgia, and postural hypotension [22]; severe neurologic effects [23] ; in patients who were heavily infected with Loa Loa. On the cytogenetic levels, ivermectin caused DNA lesions [24]; reduced mitotic index, increased number of micronucleated erythrocytes, and increased different types of chromosomal aberrations [25] ; induced single DNA-strand breaks in vitro and inhibited cell growth either in vitro or in in vivo bioassays, were scarce [26]; caused genetic selection associated with a lower reproductive rate in the female parasites Onchocerca volvulus [27] and induced non significantly elevated in the sperm head abnormalities [28]. However, ivermectin (IVM) and its commercial formulation (IVM 1.0%)

did not modified sister chromatid exchange frequencies, while they induced DNA-strand breaks revealed by single cell gel electrophoresis SCGE in Chinese hamster ovary (CHO(KI)) cells [29].

Therefore, the goal of the current work was to determine the chromosomal aberrations induced in bone marrow cells after IVM treatment. The aberration was scored in metaphase chromosomes and through micronucleus test as well.

II. Materials And Methods

Experimental animals:

The Swiss albino male mice (Mus musculus) aged 9-12 weeks were used in all experiments. They were supplied by Abbasia Farm of the Egyptian Organization for Vaccine and Biological Preparations. Mice were divided into different experimental groups of 6 animals in different cages. They were supplied with standard laboratory chaw and tap water. Mice were allowed to acclimate for at least one week prior to the study.

Chemicals: Ivermectin was used in the form of Bomectin injection (Bomac-Laboratories LTD). It was purchased from Sigma Chemical Company. The recommended dose is 200ug/kg b.w. [30].

Treatment for bone marrow chromosomes and micronucleus test:

For these assays 66 Swiss albino mice (body weight 25-35g) were used. Animals were divided into three main groups. The first group, which acted as control, contained 6 animals injected with sterile distilled water. The group of animals (30 mice) was injected intraperitoneally with single dose of 200 ug/kg ivermectin. The third group of animals contained 30 mice, was injected intraperitoneally with single dose of ivermectin 200ug/kg, and the dose was repeated 2 weeks later. Each of the second and the third group was divided into 5 equal subgroups were killed after 5 consecutive periods of 1, 2, 3, 7 and 14 days

Chromosomal preparation from bone-marrow cells:

Chromosomal preparations were made according to the method proposed by some authors [31]. Colchicine (0.04%) was injected intraperitoneally at dosage of 0.1 ml/20g body weight. Animals were sacrificed by cervical dislocation 2 hr after colchicine injections. The bone marrow cells were flushed out with buffer solution from one femur into a centrifuge tube. The content were aspirated well using Pasteur pipette, then centrifuged at 1000 r.p.m. for 10 minutes. After centrifugation, the supernatant fluid was discarded and the sedimented cells were resuspended in prewarmed hypotonic KCl solution, incubated at 37^0C for 25 minutes and centrifuged at 1000 r.p.m. for 10 minutes. Cells were fixed in freshly prepared 3: 1 methanol-glacial acetic acid for 30 minutes. Cell suspension was washed twice by centrifugation and resuspended in fixative. The final suspension was prepared in a 0.5 ml of fixative. Few drops of this suspension were dropped on a clean ice-cold slide. During dropping the slides were placed in warm plate, air-dried and stained. For each animals, 30 metaphases were analyzed for chromosomal aberrations in light research microscope with 1000 X magnification.

Micronucleus test (MNT):

Bone marrow smears were made according to the method previously described [32].Bone marrow was flushed out from the femur by syringe, contained 1 ml of foetal calf serum into a clean centrifuge tube. Cells were aspirated well with Pasteur pipette to get homogenous cell suspension. After centrifugation for 10 minutes at 1500 r.p.m. the supernatant fluid was removed and the sedimented cells were resuspended in one new drop of foetal calf serum. After resuspension, bone marrow suspension was smeared on a clean, dry and grease slides. Slides were aged overnight and fixed in absolute methanol for 15 minutes. After that, the slides stained for 10 minutes in Giemsa, rinsed thoroughly in distilled water, blotted with filter paper and examined. For micronucleus assay, 1000 polychromatic erythrocytes (PCEs) were examined by a light research microscope at 1000 X magnification. The micronucleated PCEs were registered.

PCEs/NCEs ratio:

In addition, a total of about 1000 erythrocytes of both types PCEs and NCEs were counted for each animal to determine the PCEs/NCEs ratio.

Statistical analysis: Incidence of abnormal metaphases and micronucleated cells were analyzed for significance by Student′s t-test.

III. Results

Chromosomal aberrations assay:The results after therapy with single dose:

As it is well known, the diploid number of mouse chromosomes is 40 (2N=40). Table (I) shows the frequency of chromosomal aberrations induced in mouse bone marrow cells 1, 2, 3, 7 and 14 days post i.p. injection with single dose of 200ug/kg ivermectin.

The chromosomal aberration data were evaluated as the percentage of aberrant metaphase cells (including andexcluding gaps). The Table contains also the different types of chromosomal aberrationsrecorded in the examined cells. A single i.p. injection of ivermectin resulted in a significant ($P \leq 0.001$) increase in percentage of

aberrant cells. The percentage were found to be (42.78 & 37.22), (43.3 & 37.78) and (44.4 & 42.78) after 1, 2and 3 days with and without gap, respectively. The aberrant metaphases were decreased after 7 and 14 days of the treatment, the mean percentage reached (42.78 & 32.2) and (32.78 & 27.78).

In addition, it is clear from the table that cells with gap, deletion, ring and end to end association, break and acentric fragment were highly recorded after 7 days of the treatment. Whereas metaphases which have Robertsonion translocations and dicentric were elevated after 1 and 2 days, respectively. It is important to mention that, through microscopical examination, many metaphases with more than one type of chromosomal aberration were spotted, the high score was detected after 3 days (Fig. 1).

Table (I): Number and mean percentage of metaphases with chromosomal aberrations in mouse bone-marrow cells after single injection with (200 ug/kg) ivermectin

Treatment	Time after treatment (in days)	Structural chromosomal aberrations/300 cells							No of abnormal metaphases					
									With gaps			Without gaps		
		c g	d	R t	e to e a and/or r	a f and/or b	dic	More than one type of aberrations	No.	Mean %	±S.E.	No.	Mean %	±S.E.
control	1	12	4	5	1	2	7	10	41	22.77	0.601	29	16.11	0.307
Single injection with (200ug/kg) ivermectin	1	10	4	27	3	8	5	20	77	42.78	1.013****	67	37.22	0.792****
	2	10	8	13	10	2	15	20	78	43.3	0.966****	68	37.78	1.085****
	3	3	9	16	9	3	13	27	80	44.4	0.614****	77	42.78	0.703****
	7	19	10	6	14	10	8	10	77	42.78	1.108****	58	32.2	0.666****
	14	1	1	-	3	2	2	1	59	32.78	0.872**	50	27.78	0.557****

C g=chromatid and/or chromosomal gap d=deletion R T=Robertsonian translocation e to e a= end to end association r=ring a f=acentric fragment b=break dic=dicentric **p≤0.02=highly significant ****p≤0.001= very highly significant

The results after therapy with two doses:

It is clear from the Table (II) that injection with ivermectin induced high significant increase in the frequency of the damaged cells allover the examined periods (P≤0.001). Peaking was observed at 2 days when gap was taken into consideration and after 3 days by canceling gap. The elevation of the damage was recorded in the first 3 days and the values decreased within samples of 7 and 14 days. In addition, there was an increased occurrence of metaphase cells containing more than one chromosomal aberration after 1 and 14 days in particular.

Comparison between data obtained after single and repeated doses:

a-The chromosomal aberrations percentage (CA%) including gap:

In general, CA% including gap showed its highest value with repeated injection (Table III).
The readings obtained after 1, 2, 3 and 7 days of single injection were relatively approached to each other, and then decreased after 14 days. CA% recorded after repeated dosing reached its highest point 2 days post-treatment, then diminished gradually through the remaining periods.

b-The chromosomal aberrations percentage (CA%) (excluding gap)

Comparing results obtained after single and repeated injection revealed insignificant differences within data of the first and that of the second days (P≥0.05). Data of the third day showed significant differences

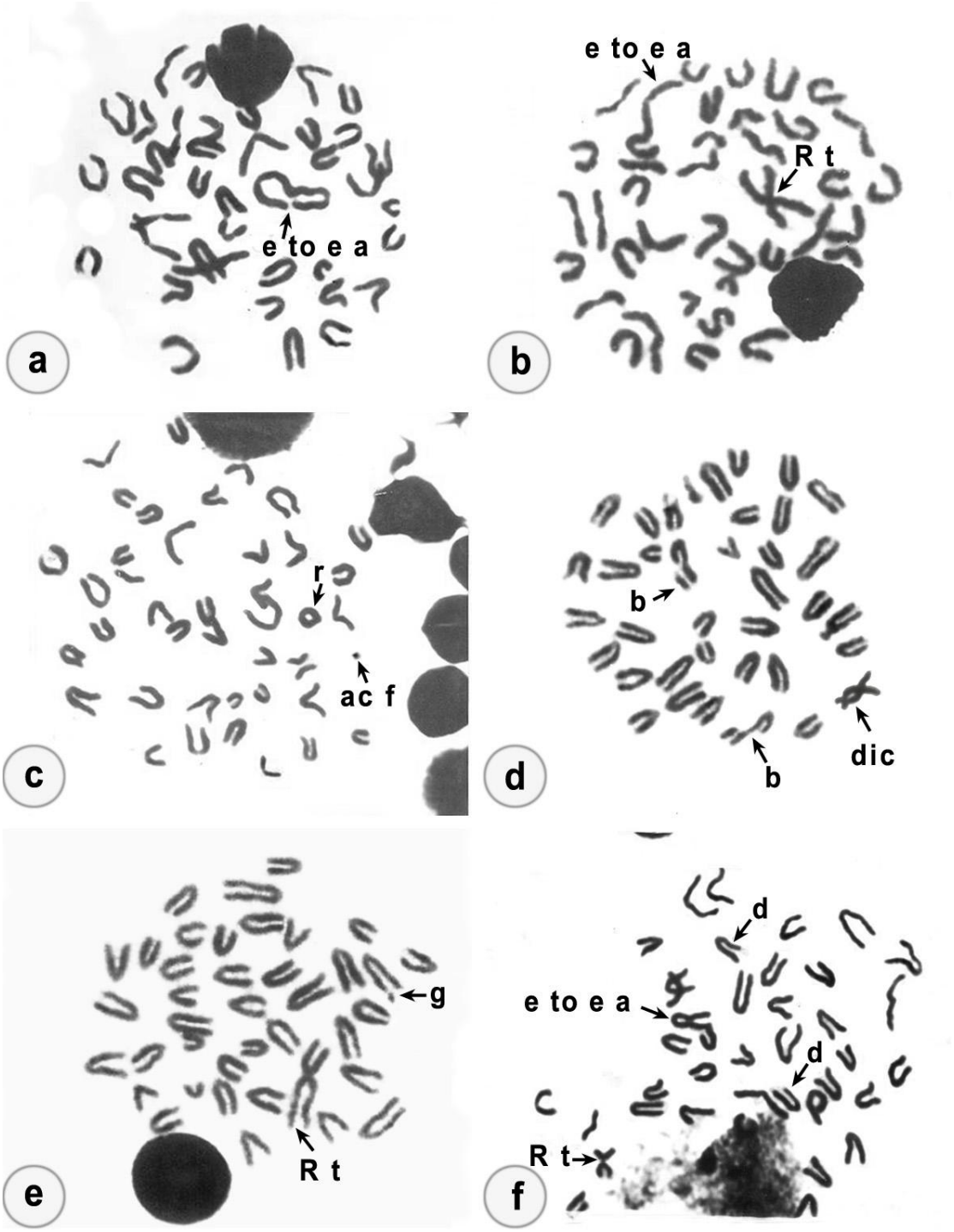

Fig. (1): Structural chromosomal aberrations in metaphase prepared from bone marrow cells after single or double injection with 200ug/kg ivermectin.
e to e a =end to end association R t= Robertsonion translocation
ac f =acentric fragment r=ring B=break dic=dicentric

Table (II): Number and mean percentage of metaphases with chromosomal aberrations in mouse bone-marrow cells after repeated injection with (200 ug/kg) ivermectin

Treatment	Time after treatment (in days)	Structural chromosomal aberrations/300 cells							No of abnormal metaphases					
									With gaps			Without gaps		
		c g	d	R t	e to e a and/or r	a f and/or b	dic	More than one type of aberrations	No.	Mean%	±S.E.	No.	Mean%	±S.E.
control	1	12	4	5	1	2	7	10	41	22.77	0.601	29	16.11	0.307
Repeated injection with (200ug/kg) ivermectin	1	23	6	14	3	10	17	19	92	51.1	0.918****	69	38.3	1.056****
	2	35	7	9	8	6	26	15	106	58.89	0.988****	71	39.4	1.222****
	3	12	14	11	13	15	13	15	93	51.67	0.922****	81	45	0.922****
	7	10	7	6	20	15	4	15	77	42.78	1.579***	67	37.2	1.352***
	14	13	7	5	13	10	2	23	73	40.56	1.447***	60	33.33	1.437***

C g=chromatid and/or chromosomal gap d=deletion R T=Robertsonian translocation e to e a= end to end association r=ring a f=acentric fragment b=break dic=dicentric ***p≤0.01=highly significant ****p≤0.001= very highly significant

Table (III): Percentage of damaged cells in mouse bone marrow after single and repeated injection with 200 ug/kg b.w. of ivermectin

Time in days	DC% including gap		DC% excluding gap	
	Single dose Mean±SE	Repeated dose Mean±SE	Single dose Mean±SE	Repeated dose Mean±S.E.
1	42.78±1.013	51.1±0.918*	37.22±0.792	38.3 ± 1.056^{ns}
2	43.3±0.966	58.89±0.988**	37.78±1.085	39.4 ± 1.222^{ns}
3	44.4±0.614	51.67±0.922*	42.78±0.703	45±0.922*
7	42.78±1.108	42.78 ± 1.579^{ns}	32.2±0.666	37.2±1.352****
14	32.78±0.872	40.56±1.447**	27.78±0.557	33.33±1.437****

SE±=Standard error ns =non significant *p≤0.05=significant
**p≤0.02=highly significant ****p≤0.001=very highly significant

(P≤0.05). Very significant differences were recorded (P≤0.001) among readings of 7 and 14 days.

On the other hand, data of both groups (single and repeated injections) showed elevation through the first three days and reduction after 7 and 14 days. Maximum values attained after 3 days of the last injection. Minimum readings achieved after 14 days.

Micronucleated assay:

The induction of MN after single dosing of ivermectin:

The frequency of micronucleated polychromatic erythrocytes (MNPCEs) ranged from 0-5% of the scored cells. The mean is approximately 2.5±1.76. Concerning the spotted micronuclei, most of them had the round-shape (Fig. 2). The incidence of MNPCEs in bone marrow cells of mice after receiving single dose of 200 ug/kg ivermectin is summarized in Table (IV). The results revealed significant increase in the frequency of MN over the corresponding control value.The micronuclei reached its maximum formation after 24h of the treatment. The mean was 11.83. The values tend to decrease throughoutthe remaining experimental periods. The frequencies were 10.5, 7, 6.33 and 5.5 after 2, 3, 7 and 14 days, respectively. The reading for each animal were variable. The lowest and the highest reading were 2/1000 PCEs and 22/1000 PCEs, respectively. Reading the configuration of the observed MN, they occurred in different size and shape. It was found with small size, normal size or abnormal large size. Also, it is important to mention that most of the recognized MNPCEs contain just one MN. However, some cells with two micronuclei or more were spotted in the present samples.

Table (IV): The frequency of micronuclei following treatment with single dose of ivermectin

Serial	Control	MN/1000 after				
		1st day	2nd day	3rd day	7th day	14th day
1	2	15	13	7	2	8
2	5	22	8	5	4	8
3	2	14	6	3	9	6
4	-	5	16	5	5	3
5	2	10	12	8	6	6
6	4	5	8	14	12	2
Total	15	71	63	42	38	33
Mean	2.5	11.83	10.5	7	6.33	5.5
SD±	1.76	6.55	3.78	3.847	3.614	2.509
SE±	0.718	2.676	1.544	1.570	1.475	1.024
T test	-	3.367***	4.698****	2.605*	2.334*	2.397*

SE=Standard deviation SE= Standard error *p≤0.05=significant ***p≤0.01=highly significant
****p≤0.001=very highly significant

The induction of MN after double dosing of ivermectin:

Two injection of 200 ug/kg ivermectin two week apart induced significant increase in the MN level at all the examined periods. The rate of MN production were found to be in the same range at 1, 2 and 3 days post-treatment. Their values were approximately 3-folds that of the control. The number of the positive cells were fluctuated at 7 and 14 days after the last injection. The frequency was dropped to 3.667 and then elevated to 5.333 (Table V). As it is evident from the table, 12 MNPCEs per thousand cells representedthe maximum record in comparison to 2 for the minimum value.

Comparison in the induction of MN after single and double dosing of ivermectin:

The results obtained after single and double treatment with 200 ug/kg ivermectin were arranged in Table (VI). As regards the mentioned Table, the following remarks could be concluded:
Single and double treatment with ivermectin resulted in a marked increase in MNPCEs. This increase was significant comparing to control level. The induction of MN in PCEs was significantly higher in single injection

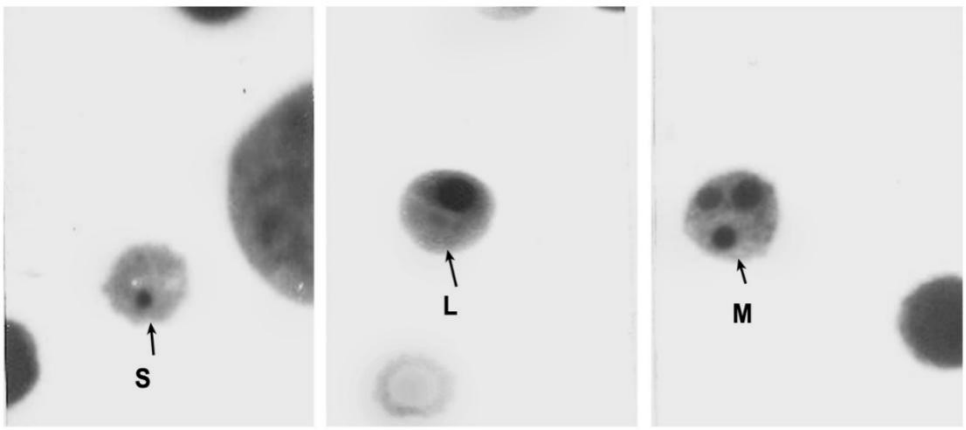

Fig. (2): Micronucleated polychromatic erythrocytes prepared from bone marrow of mice treated with ivermectin. Notice the variability of the size and number:
S= small sized micronuclei L= large sized micronuclei M= More than one micronucleus

Table (V): The frequency of micronuclei following treatment twice with ivermectin

Serial	Control	MN/1000 after				
		1st day	2nd day	3rd day	7th day	14th day
1	2	5	6	4	3	6
2	5	8	10	3	2	3
3	2	8	7	10	3	5
4	-	8	10	12	6	8
5	2	8	8	8	6	4
6	4	6	4	5	2	6
Total	15	43	45	42	22	32
Mean	2.5	7.167	7.5	7	3.667	5.333
SD±	1.76	1.329	2.345	3.577	1.861	1.751
SE±	0.718	0.543	0.957	1.460	0.760	0.715
T test	-	5.184****	5.554****	2.765**	1.030^{ns}	2.795**

SE=Standard deviation SE= Standard error ns=Non-significant
**p≤0.02=highly Significant ****p≤0.001=very highly significant

experiment than in that of the double treatment. There was a detectable variation in MNPCEs at different sacrifice time. The mean of MN dropped from 11.83 after 24h in group 1 to 7.167 in the second group. In both

groups (1 & 2) the frequency of MNPCEs was exponentially decreased with increasing time. The results revealed significant decrease in differences between the values obtained after 1, 2, and 7 days in each of thestudied groups. Meanwhile, the difference was non-significant after 3 and 14 days.

Table (VI): Means of micronuclei induced in mouse bone marrow cells after single and double dosing of 200ug/kg b.w. ivermectin

Serial	MN/1000 after				
	1st day Mean±SD	**2nd day** Mean±SD	**3rd day** Mean±SD	**7th day** Mean±SD	**14th day** Mean±SD
Single dose	11.83±6.55	10.50±3.78	7.00±3.85	6.33±3.614	5.50±2.509
Double dose	* 7.167±1.32	* 7.5±2.345	ns 7.00±3.58	* 3.67±1.86	ns 5.33±1.75

N.B. The mean of MN in control samples was 2.5±1.76
SD=Standard deviation ns=non-significant *p≤0.05=significant

Finally, when the MNPCEs average of each group taken into consideration, comparing to sacrifice time and dosing number, significant negative-relationship was concluded. Table (VII) shows the ratio of PCEs to mature R.B.Cs obtained after single and double dosing of 200 ug/kg ivermectin. PCEs/NCEs ratio in control sample was found to be 1.04±0.024. Ivermectin treatment was found to induce highly significant decrease in the ratio of PCEs /NCEs at all the experimental periods, with the exception of data obtained after 14 days.

Table (VII): PCEs/NCEs ratio recorded in male mice following treatment with single and/or double doses of 200 ug/kg b.w. of ivermectin

Interval	Control		Treated samples			
			Single dose		Double dose	
	Mean	±SE	Mean	±SE	Mean	±SE
1	1.04	0.0242	0.509****	0.0492	0.670****	0.039
2			0.580****	0.083	0.564****	0.0248
3			0.6212****	0.0874	0.599****	0.0928
7			0.515****	0.058	0.571***	0.114
14			1.298-	0.151	1.111-	0.031

SE±=Standard error ****p≤0.001= Very highly significant ***p≤0.01= Highly significant
-P≥0.05=Non significant

At 24h of the treatment, the ratio was greater in samples of double dose than of the single dose (P≤ 0.001). At 2, 3 and 7 days the readings of both group were approximately closed to each others. PCEs/NCEs ratio was elevated at 14 days post-treatment, as it matched that of control value.

IV. Discussion

Ivermectin is a semisynthetic macrocyclic lactone antibiotic agent that is administered orally. It disrupts the function of a class of ligand-gated chloride ion channels, causing persistent opening of the channels [33]. Treatment with ivermectin rapidly reconstituted health in patients with scabies which was associated with considerable morbidity in resource-poor setting [34]. Ivermectin, administered orally at a dose of 200ug per kilogram of body weight, is an effective alternative treatment. Since ingestion of food increases the bioavailability of ivermectin by a factor of two, taking the drug with food will enhance the penetration of the drug into the epidermis [35]. The parasites endemic to certain area (Southern Cameroon) might form a distinct population that exhibited a phenotype of eliciting severe adverse reactions in Loa-infected individual upon ivermectin treatment [36]. In the present investigation, chromosomal aberration assay was used for the detection of genetic damage induced by ivermectin in somatic cells of the mouse in vivo.

As recommended by many investigators [37], the number of aberrant metaphase cells (excluding and including gaps) were scored. Ivermectin induced significantly high frequencies of chromosome aberration as compared with control. The incidence of damage was gradually elevated through 1, 2, 3 & 7 days of experimentation. After which the frequencies of chromosomal aberration percent (CA%) was reduced after 14 days. Such reduction may be due to the fact that cells with severe chromosomal damage might have been deleted in cell cycle following the treatment [38]. To the author′s opinion, the drug causes chromosome aberration which seems to be reversible as the drug eliminated or diminished from the bone marrow through the metabolic process.

Considering the concentration of the drug and the sampling time, no clear-cut relationship could be concluded, as the data were fluctuated. Single dose injections mostly revealed high level of damage than double dose. On the other hand, peaking times were also differed. As for chromosomal aberrations percent CA% (excluding gap) peaking was achieved after three days of the treatment with single and/or double doses.
Meanwhile, when including gap, the peak was observed after three days of single injection and two days after double treatment. The fluctuation in the percentage of damage can be attributed to the fact that the various lymphocyte subpopulations apparently show different sensitivities to mutagen [39]. They could also be due to the type of DNA effect produced by a drug [40].

The alteration in the peak time when considering the gap, attract the attention to the genetic significance of gap (whether chromatid or chromosomal). As it represented a clear indication of the genotoxic potential of the chemicals [41].

In the present work, chromosome gap were frequently observed after treatment of ivermectin. It is always located at a definite region. This figure is firstly reported in the literature [42].

Centromeric attenuations were detected in abundant number (although it is not added to the calculation). Centromeric attenuations resulted from chromosomal break attack the centromeric region and lead to separation of the two chromatidsas explained by some investigators [43].

In the present investigated samples, the most common types of aberrations noted were dicentric, Robertsonion translocation, deletion and end to end association. This means that, the drug induced both chromatid and chromosomal type of aberrations.It is of interest to present briefly the two types of aberrations stable and unstable [44]. Stable aberrations (deletion, duplication, inversion and balanced translocation) can be transmitted through repeated cell divisions, and thus persist in the cell population. The unstable type are, chromosome breaks, give rise to acentric fragment, dicentric chromosomes, ring and various other asymmetrical rearrangements. They usually cause the death of the cell through the loss of vital genetic material or mechanical hindrance of mitosis.

So, according to the previous description, the present results indicate that ivermectin is clastogenic induce both stable and unstable damage. Consequently, this also explains the inconstancy of the data and of the peaking time.

To confirm our findings on metaphase assay, the mutagenicity of ivermectin have further been evaluated by means of micronucleus test (MNT). It has been proved to be a quick, trusty and precise assay for the screening of potential clastogens.

Generally micronucleus is derived from chromosome losing centromere or chromosome fragment after the chromosome was damaged by certain physical and chemical factors. Micronuclei incidence may reflect the extent of how chromosomes are damaged [45].

In this study, the frequency of micronucleated polychromatic erythrocytes (MNPCEs) in control samples was found to be 2.5±1.76%. This score come to that of some investigators [46].

After 1, 2, 7 days of the treatment with 200ug/kg ivermectin, the micronucleus induction was more in animals received single dose than that treated with double doses. Meanwhile, after 3 and 14 days, the data were found to be identical in both treatments.

High level of micronucleus formation was observed after 24h. This results is in a good agreement with that of many investigators [47], they close the sampling time only at 24h because the highest frequency of MNPCEs were obtained at that time.

Such observation indicates the rapid metabolism and elimination of the drug [48].

Although the value was decreased gradually through the time intervals of experimentation, yet the frequency of MN remained higher than that of the control. Such a dropping down of dose-effect relationships is a typical phenomenon of the MNT [49].

To the author′s opinion, the decrease in the MN level at later time for the single dose and the lower and constant score after double therapy may be due to the removal of the metabolites from the animal body with time. Also, the cells may attain a sort of resistance or adaptation to the repeating of ivermectin injection.

Accordingly, IVM may affect the multi-drug-resistance gene (mdrl). This gene encode P-glycoprotein that transports a variety of drugs from the brain back into the blood [50].

In the present study, the positive results obtained in chromosomal aberration assay and in MNT revealed the genotoxic potential of ivermectin. This is contradictory to the previous observation that no evidence about the genotoxic potential of ivermectin could be obtained in the MNT [51]. With the exception of this report, no other published literature is available regarding the cytogenetic effect of ivermectin.

The discrepancies between the previous observations and the present results may be attributed to the differences in the mode of administration and strain of mouse and more importantly, the mechanism of action of each drug. The mechanism of MN formation was explained by several investigators. The in vivo rat micronucleus assay measures the number of micronuclei present in polychromatic erythrocytes (PCE) from rat bone marrow [52], and is used in determining potential carcinogenicity of compounds and their ability to cause chromosomal damage in replicating cells [53].

The micronucleus test detects mutagenic substances, thus altering the equitable distribution of chromosomes during cell division [54].

To the author′s opinion, the presence of chromosomal aberration in bone marrow cells of the treated animals suggests that the MN resulted from chromosomal breakage.

Since MN are formed either from acentric chromosome fragments or from lagging chromosomes that fail to migrate to the poles during anaphase, the determination of MN frequencies is a reliable method for evaluating the potential of a chemical to induce structural and/or numerical chromosomal alterations. Of the variety of in vivo assays used to detect genotoxic chemicals, the most common is the in vivo rodent erythrocyte MN assay [55].MN with higher DNA content are more likely to contain a chromosome arising from events producing chromosome loss, whereas MN with lower DNA content are more likely to contain a chromosome fragment arising through breakage events [56].

Certain antineoplastic drugs interfere with topoisomerase by stabilizing the enzyme-DNA cleavable complex. So, small acentric fragments resulting from unresolved stabilization of the cleavable complex and the accompanying chromosome stickiness at anaphase would persist as MN in the cytoplasmic region [57]. They added that, the higher MN frequency may reflect not only interphase damage but also mitotic damage as well. In our study, many micronuclei which had abnormal morphology were occasionally observed. Some PCEs contained more than one micronucleus. The occurrence of multiple and morphologically abnormal MN had been early documented [58]. It may possibly involve a more complex mechanism of micronucleus formation than clastogenicity [59]. The development of abnormally shaped micronucleus indicates that an unusual mechanism of MN production might exist [60].

In addition, MNT can provide useful information on cytotoxicity of the agents being tested. The cytotoxicity is assessed by scoring the ratio of PCEs to NCEs [61].

In the current study, ivermectin proved to be clastogenic a statistically highly significant increase of MN frequency was observed which was accompanied by a decrease in PCEs/NCEs ratio. After 14 days of the treatment PCEs/NCEs ratio was found to be statistically increased.

To the author′s opinion, the decrease in the PCEs/NCEs and the increase by passing the time indicate that the drug has cytotoxic effect in the erythropoietic system of the treated mice. This cytotoxicity disappeared by the withdrawal of the drug. The decrease in PCEs/NCEs ratio may indicate a cell-cycle delaying effect or, a selective killing of the dividing cells [62]. The change in PCEs/NCEs ratio may attribute to the effect of the used agent on the mitotic cycle [63].

IVMinduced cytotoxicity rather than attributable to a repair process [24]; severely affect fetal genetic material and development and induced genotoxic effect in somatic cells of the dams in Wister rats [25]; was able to induce single DNA-strand breaks in vitro and inhibited cell growth either in vitro or in vivo bioassays [26]; exerted both genotoxicity and cytotoxicity in mammalian cells in vitro, at least in CHO (K1) cells [29];

induced a high single nucleotide polymorphism (SNPs) association and a loss of polymorphism [64]; caused genetic selection on Onchocerca volvulus which was associated with a lower reproductive rate in the female parasites [27], and had cytotoxic activity in the cell culture of murine myeloma Ns/o, Erlich carcinoma ascites and human larynx carcinoma Hep-2 [65]. Other literaturesuggested an association between certain genes and ivermectin resistance like Cooperia oncophora GluClalpha3 gene [66] and orfX gene in Streptomyces avermitilis [67].

V. Conclusion

In the present study, high level of chromosomal damage and micronuclei formation were induced in bone marrow cells indicating the genotoxic potential of IVM. These results reflect the latent harmful effects which may be encountered during treatment with IVM.It is concluded from the present work that the mutagenic effects fortunately diminished with time. According to the obtained results cautions use of IVM is advisible.

Acknowledgements

Dr.Iman Darwish and Dr.Dalia Demerdash would like to acknowledge Prof. Dr. Karima Sweify, Women's College, Ain Shams University, Cairo, Egypt forher assistance in the manuscript preparation.

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