The mutagenic effects of ivermectin in germinal cells and serum protein of the mouse

Karima Mohammad Sweify, Iman Abd El Moneim Darwish* and Dalia Demerdash Abd El Monem Hafez
Women's College for Arts, Science and Education, Ain Shams University, Cairo, Egypt

Abstract: Ivermectin (IVM) is a broad-spectrum anti-parasite agent. It is extremely toxic to fish and aquatic life. Some animals showed reduction in the fertility, the number of variable fetuses and sperm count following treatment with (IVM). Therefore, the objective of the current work was to investigate the mutagenicity of IVM on meiotic chromosomes of mice. The variations in protein fractions of blood serum were also studied using sodium Dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).Animals received single injections only of 200ug/kg b.wt. for meiotic chromosome study. Whereas single and double treatment for serum protein examinations. Analysis of the treated samples revealed significant increase in meiotic aberrations, 33.83% vs 5.8% for the control ($P < 0.001$). Single injection induced much variation in the percentage area of the separated protein than that produced by double treatment. These findings supports the mutagenicity of IVM, accordingly cautious use of IVM is advisable.

Keywords: Ivermectin (IVM), meiotic preparations; quadrivalent chain (CIV), quadrivalent ring (RIV), Autosome univalent (A-U), hexavalent chain (CVI), sex univalent (X-Y U), Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), percentage area (% area).

INTRODUCTION

Ivermectin is an antiparasitic drug, a derivate of avermectins, and a product of fermentation of an actinomycete, *Streptomyces avermitilis.* Ivermectin is presently used in mass treatment of onchocerciasis, other filariasis, some intestinal nematode infections, but also in scabies, and more rarely in resistant head lice (Chosidow and Gendrel, 2016). It is used extensively globally for treatment of helminthic and ectoparasitic infections in animals and humans (Derua *et al.,* 2016).

Single treatment, whether topical or oral, is associated with high cure rate in a week post IVM treatment. However, repeating treatment after one week may be required to achieve 100% cure (Ahmad *et al.,* 2016). Ivermectin was effective, safe and well tolerated (Ali *et al.,* 2015). New uses for IVM are identified regularly, including possible antibacterial, antiviral, and anticancer potential (Omura and Crump, 2014).

Ivermectin is found effective when administered through oral and subcutaneous routes (Panigrahi *et al.,* 2016). The synergistic effect of combinations of antibiotics and ivermectin could be used to achieve complete eradication of lice (Sangaré *et al.,* 2016). Two applications of topical ivermectin provided a cure rate of 63.1% at the 2-week follow-up, which increased to 84.2% at the 4-week follow-up after repeating the treatment (Goldust *et al.,* 2013). A single application of ivermectin was more effective than vehicle control in eliminating head-louse infestations at 1, 7, and 14 days after treatment (Pariser *et al.,* 2012). A 200 microg/kg/d ivermectin dose was an adequate therapeutic regimen in the treatment of uncomplicated strongyloidiasis in children (Ordónez and Angulo, 2004).

IVM induced higher mortality, reduced fecundity, feeding difficulty, and incomplete ecdysis (Sheele and Ridge, 2016) and caused population decline and biodiversity loss (Verdú *et al.,* 2015). Dominant clinical symptoms of adverse effects and toxicity of ivermectin in animals are tremor, ataxia, central nervous system (CNS) depression and coma which often results in mortality (Trailovic *et al.,* 2011) and caused renal tubular necrosis (DeMarco *et al.,* 2002). In addition, reports on adverse events are rare although this drug can cause cardiac dysfunction and liver disease (Sparsa *et al.,* 2006), dizziness (Nontasut *et al.,* 2005) and encephalopathy in individuals heavily infected with *Loa loa* microfilariae who were treated with ivermectin against onchocerciasis (Esum *et al.,* 2001).

On the other hand, Dou *et al.* (2016) indicated that ivermectin is a potential option of the treatment of breast cancer. Mangia *et al.* (2016) reported transporter proteins (P-glycoproteins, PgP) expression of an *Ixodes ricinus*-derived tick cell line following treatment of ivermectin. Raza *et al.* (2016) noticed significant increase in transcription of some ATP binding cassette (ABC) transporter genes following 3 h exposure to both ivermectin (IVM) and levamisole (LEV) in the resistant isolate only. Molinari *et al.* (2013) observed a time-dependent increase in ivermectin (IVM) and IVM-

**Corresponding author:* e-mail: imandarwish73@yahoo.com

containing technical formulation Ivomec® (IVO; 1% IVM)-induced DNA damage.

Furthermore, co-administration of both ivermectin and verapamil induced genotoxicity in both dam and embryonic cells indicated by reduced mitotic index, increased number of micro nucleated erythrocytes in both, and increased different types of chromosomal aberrations in dam cells, while ivermectin alone showed some genotoxic effect as mentioned by el-Ashmawy *et al.* (2011). Varó *et al.* (2010) showed significant changes in the expression of 36 proteins in gilthead sea bream liver protein profile following oral administration of IVM by difference Gel Electrophoresis Technology (DIGE). While IVM and ivomec did not modified SCE frequencies, they induced DNA-strand break revealed by single cell gel electrophoresis (SCGE) as indicated by Molinari *et al.* (2010).

The present communication is dealing with the hazardous effect of IVM in germ cells through chromosomal aberration test. Electrophoresis analysis of serum protein was also done by using SDS-PAGE.

MATERIALS AND METHODS

Chemicals and solutions

Ivermectin was used in the form of Bomectin injection (Bomac-Laboratories LTD). The recommended dose is 200ug/kg b.w. (Chouela *et al.*, 2002).

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. They were supplied with standard laboratory chow and tap water. Mice were allowed to acclimate for at least one week prior to the study.

Animals were divided into main groups. The first group, which acted as control, contained 6 animals injected with sterile distilled water. The second group of animals (6 mice) was injected intraperitoneally with single doses of 200ug/kg ivermectin. Meiotic chromosomes were prepared after 12 days to cover preleptotene stage as recommended by Ciranni and Adler (1991).

Preparation of mammalian meiotic chromosomes

Chromosomal preparations were made according to the method described by Evans *et al.* (1964). Male albino mice were injected i.p. with 0.1 ml of colchicine solution, 2 hours before sacrificing them by cervical dislocation.

Testes were removed and placed in isotonic sodium citrate solution (2.2% wt/vol) at room temperature. Tunica was pierced and testes were swirled in the solution to remove adherent fat. Then testes were transferred into fresh 2.2% sodium citrate solution and the tubules were gently pulled out. The mass of tubules was held with fine, straight forceps and their contents were teased out with similarly fine, but curved forceps. When the tubules appeared flat and opaque they have been allowed to settle, then the supernatant fluid was transferred into centrifuge tubes.

After centrifugation at 500 r.p.m. for 5 minutes, the supernatant fluid was discarded and the sedimented cells were resuspended in approximately 3 ml of 1% sodium citrate solution (hypotonic), and left for 12 minutes at room temperature.

After centrifugation as much as possible, the supernatant fluid was removed. Cells were resuspended in the remainder by flicking the tube with a thumb till a thin film of suspension was formed. Cells were fixed by adding about 0.25 ml of fixative (3 methanol: 1 acetic acid) rapidly, and directly on to the suspended cells. More fixative was added, until the tube was about one-third full. After 5 minutes, cells were sedimented again by centrifugation and were resuspended in fresh fixative. The change of fixative was repeated after further 10 minutes. The final suspension was prepared in a 0.5 ml of fixative. Few drops of this suspension were allowed to fall on a grease free slide at room temperature. After drying, slides were stained for 5 minutes in Giemsa.

Scoring criteria

One hundred MI spermatocytes were scored per animal. Metaphase spreads were selected at low magnification (20 x) on the basis of morphological criteria. Numerical or structural abnormalities were verified at high magnification (100x).

For structural aberration analysis (MI): Only MI with 20 bivalents was scored. Reciprocal translocations as a chain or ring multivalents were classified. The presence of univalents (autosomal univalents and/or sex univalents) or chromosome breaks and fragment were recorded.

Serum protein electrophoresis

Eighteen male mice were injected with single dose of 200ug/kg IVM. After two weeks, nine of the animals received another dose of IVM. Blood were collected from each group after 1, 7, and 14 days of the last injection.

Blood collection for protein electrophoresis

Blood serum of the treated animals and accompanied control (three for each duration) were prepared. The electrophoretic patterns of serum protein were studied by using SDS-PAGE which proposed by Laemmli (1970). Blood samples were collected from eye plexus by nonheparinized haematocrit tubes into eppendorf tubes. The eppendorf tubes were put in the refrigerator for 1 hour of clotting then centrifuged at about 3000 r.p.m. for 10 minutes. The supernatant serum was transferred into a

new clean eppendorf tube using Pasteur pipette. The tubes were kept in the deep freezer at -20^{o}C till analysis

Polyacrylamide gel electrophoresis (SDS-PAGE) for serum protein analysis

Serum protein analysis of control and treated mice was done using SDS-PAGE under reducing conditions by using of B-mercaptoethanol according to the method of Laemmli (1970) with some modifications.

Sample preparation and the separation process

Serum was diluted with distilled water (1:3 v/v, respectively), i.e. 120ul distilled water was added to 40 ul serum. Then, the diluted serum was combined with an equal volume of treatment buffer (160ul) in eppendorf tubes. Eppendorf tubes were put in boiling water for (5-10) min, the samples were cooled at room temperature and 10ul from each sample was applied to the gel wells. Samples were stored frozen for more runs.

Once samples had been placed in the wells, the apparatus was connected to a power supply and a current was applied. The run was carried out with constant voltage of 100 volts at the stacking gel and 150v at the separating gel. When the tracking dye band was within 2-3 mm of the bottom of the gel, migration was stopped by turning of the electrical field.

Processing the gel (staining, destaining and preserving): Gel was removed carefully from the glass sandwich. It was necessary to loosen the gel by spraying water around the edges of the gel with a plastic pipette. Gel was covered with staining solution, sealed in plastic box and left in stain for 30 minutes at 60^{o}C.

Destaining

After staining gel was transferred into destaining solution, the solution was changed several times till the background appeared clear. Then it placed in final destaining solution.

Preserving

After destaining, gel was transferred into plastic box contained preserving solution and left for 15 minutes at 50^{o}C. This solution helps to keep the gels flexible and resistant to cracking.

Analysis of gel lanes

Analysis of gel lanes was carried out using computer software program (Gel-Pro Analyzer version 3.1).

Ethical approval

All experiments were conducted according to the protocol approved by Faculty of Medicine Ethics Committee (MFEC), Ain Shams University, Egypt.

STATISTICAL ANALYSIS

Chromosome aberration frequencies and polyploidy frequencies in treated and control animals were compared by Student-t-test.

RESULTS

Chromosomal studies in germ cells

Control samples

Most of the counted dividing spermatocytes showed 20 bivalents constituent (19 II + XY) (fig.1). This fig. represents the normal structure of diakinesis metaphase I cell of the mouse spermatocytes. Such structure could be speculated in about 94.17% of the examined cells (table 1).

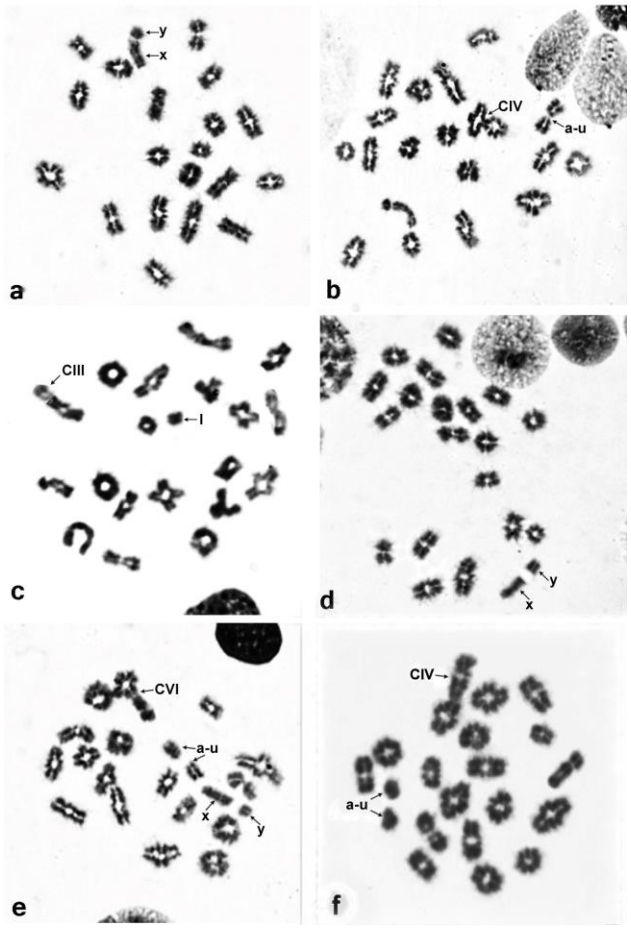


Fig. 1: 1ry spermatocytes at diakinesis-metaphase I showing

a-The twenty bivalents of control samples arrows showing the attached sex chromosomes

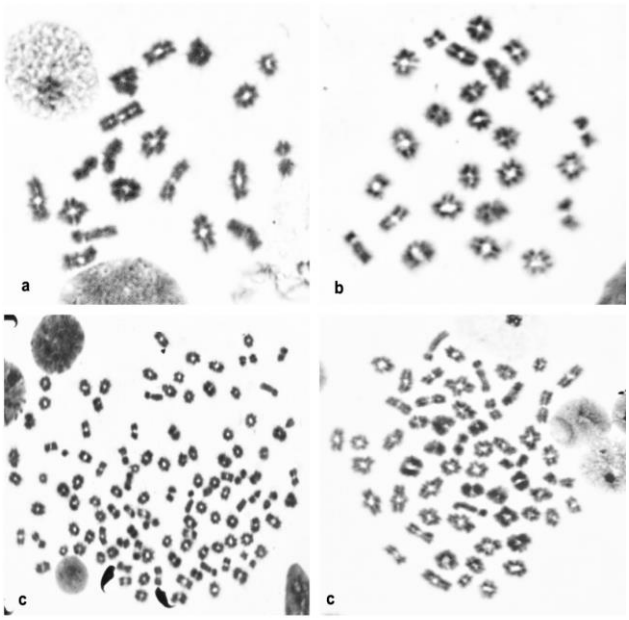
b-17 bivalents with univalent formation of auto somal chromosomes and the quadrivalents tanslocation.

c-18 bivalents with CIII + I of auto somes

d-19 bivalents with univalent formation of sex chromosomes (x-y). e-Multivalent configuration (15 bivalents + CVI + A-U + x-y u) configuration

f- 17 bivalents with univalent formation of auto somes and CIV

This union results by the homologous joined to each other largely at their terminal ends. Each metaphase spread consists of 20 pairs of chromosomes. Each pair of homologous chromosomes known as bivalents. The autosomes pairs regularly forming 19 identical bivalents; the sex chromosomes associated together with the noncentromeric ends. The auto some bivalents acquire a more rounded shape of termination of chiasmats as the chromosomes shorten.



A= Hypoploid metaphase with 19 chromosomes
B= Hyperploid metaphase with 25 chromosomes
C= polyploidy.

Fig. 2: a-c Primary spermatocytes at metaphase I illustrating

On the other hand, the abnormal metaphases in the control samples constitute 5.83% of the examined cells. The observed aberrations are almost translocations in addition to low frequency of autosome and sex univalent.

The different types of chromosomes damage which may find in diakinesis metaphase I could be mentioned according the description of Leonard (1975): First of all, the term of reciprocal translocation creates exchanges the terminal segments between non-homologous chromosomes. At meiotic synapsis heterozygosity for a reciprocal translocation results from production of A quadrivalent configuration in form of a ring (RIV). This occurs when the noncentromeric ends of the chromosomes maintain association with terminalyzing chiasmata.

A chain of four fig. (CIV) by failure of association with chromosomes in one arm. A chain of three plus a univalent fig. (CIII + 1), by failure of association of two adjacent arms. In addition, more complex configurations can be observed. It was found that sex univalent and the chain of four figs. (CIV) were frequently observed in the control samples. Autosome univalent and chain of hexavalents (CVI) or a quadrivalents ring (RIV) were rather rare.

Effects of ivermectin on spermatocyte chromosomes: Structural aberrations: Cytological analysis of diakinesis-metaphase I spermatocytes of the treated samples revealed significant increase in chromosome aberrations over the control values. Single i.p. injections of 200ug/kg b.w. ivermectin caused 33.83% of abnormal metaphases. The detected types of aberrations can be arranged in a descending manner as, sex univalents, CIV, and autosome univalents. Sex univalents observed in 66 metaphases out of 600, with a percentage of11, fig. (1). Chain of four (CIV) occurred in 56 of 11 cells that is equal 9.3%. Autosome univalents found in 6% of the scored cells. Other types of aberrations, autosome plus sex univalents, ring of four (RIV), chain of six (CVI) and fragments were rather rare (table 1).

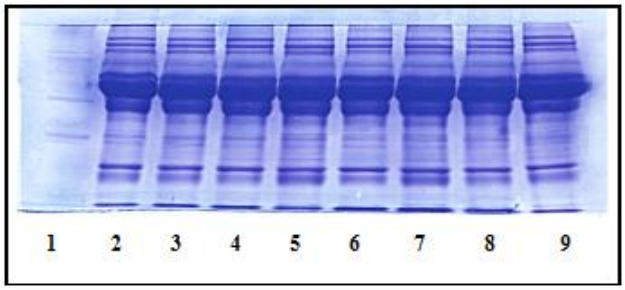


Fig. 3: Gel represent protein pattern of *Mus musculus* 1day after treatment with single dose of ivermectin. Lanes from left to right respectively: Lane 1: High molecular weight marker. Lane 2: Control. Lanes (3-8): Treated animals. Lane 9: Control.

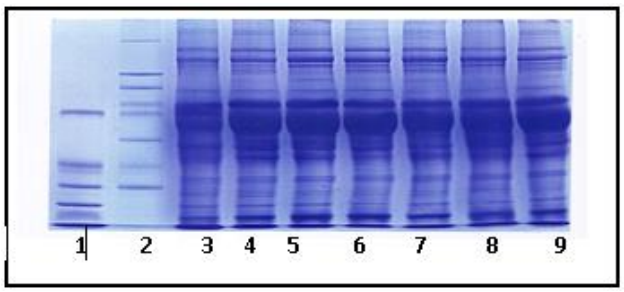


Fig. 4: Gel represent protein pattern of *Mus musculus* 7 days after treatment with single dose of ivermectin. Lanes from left to right respectively: Lane 1: Low molecular weight marker. Lane 2: High molecular weight marker. Lane 3: Control Lanes (4-9): Treated animals.

It is of interest to mention that, some of the examined metaphases were found to have multiple reciprocal translocations (fig. 1). In addition, polyploidy was also observed in 3% of the counted cells (fig. 2).

Electrophoretic patterns of serum protein in Mus musculus

In the present work, the effect of ivermectin was observed as changes in the number of bands of various serum proteins.

Scoring of SDS-PAGE gel of mice sera proteins in control series revealed the presence of 26 to 32 bands while they were 28: 33 in samples obtained from the treated mice. The majority of the control bands matched that of the treated. However, some newly formed bands were observed in the experimental samples (table 2). From the table, it is clear that the number of the newly formed bands are 1, 6, 1 in mice received single dose of ivermectin; they are 3, 1, 2 in samples obtained after two injections.

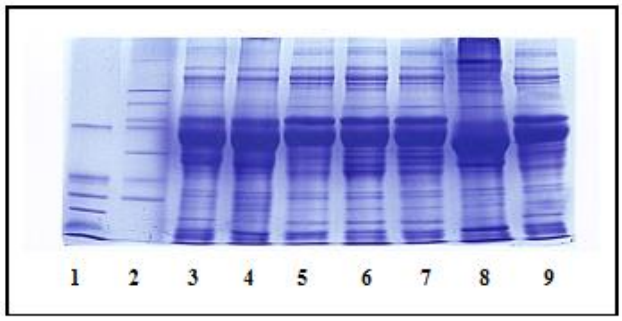


Fig. 5: Gel represent protein pattern of *Mus musculus* 14 days after treatment with single dose of ivermectin. Lanes from left to right respectively: Lane 1: Low molecular weight marker. Lane 2: High molecular weight marker. Lane 3: Control. Lanes (4-9): Treated animals.

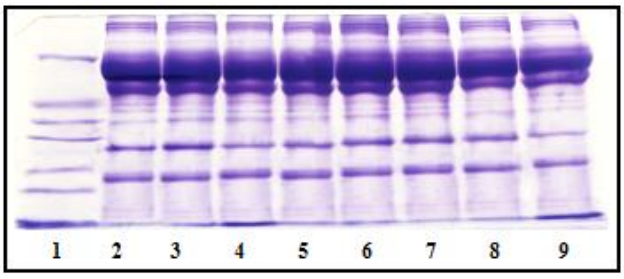


Fig. 6: Gel represent protein pattern of *Mus musculus* 1 day after treatment with repeated dose of ivermectin. Lanes from left to right respectively: Lane 1: Low molecular weight marker. Lane 2: Control. Lanes (3-9): Treated animals.

Concerning the relative mobility (RF), no significant alterations

were recorded in almost all of the detected fractions comparing to the control values (figs. 3-8). The % area showed significant increase and / or decrease due IVM therapy while most of the protein showed non-significant alteration (tables 3), eg: after one day of single IVM dosing, significant increase was observed in the % area of 5 bands (peak number (PK)) 16, 18, 19, 22 and 29 (data not shown). Whereas 6 bands showed significant decrease in their % areas, PK numbers, 7, 8, 9, 10, 14 and 25. Non-significant variations were observed in a number of 18 bands. Generally, single injection of IVM induced much variation in % areas of the separated proteins than that produced by the double treatment. In samples prepared after 7 days of single dosing, significant decrease in the area percentage was appeared in 7 bands. The average area percentage of serum belonging to animals received single dose of IVM was statistically increased from one bands after 14 days comparing to that of the accompanied control ($P<0.01$). Remarkable reduction in the average area percentage was occurred after 1 day of double injections (7 bands). Significant increase in area percentages was recorded in three bands after 14 days of repeated injections. In samples studied after 1, 7, & 14 days of repeated dosing, 20, 23 & 25 bands were found. Their area percentages were not statistically differing comparing to the control values.

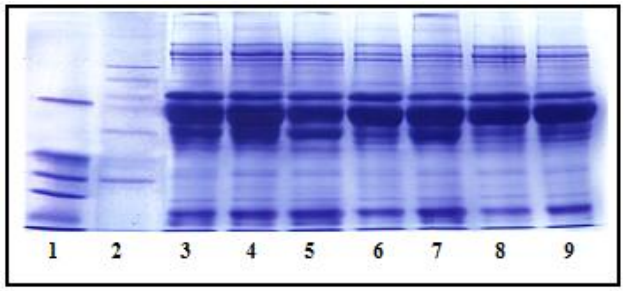


Fig. 7: Gel represent protein pattern of *Mus musculus* 7 days after treatment with repeated dose of ivermectin. Lanes from left to right respectively: Lane 1: Low molecular weight marker. Lane 2: High molecular weight marker. Lane 3: Control. Lanes (4-9): Treated animals.

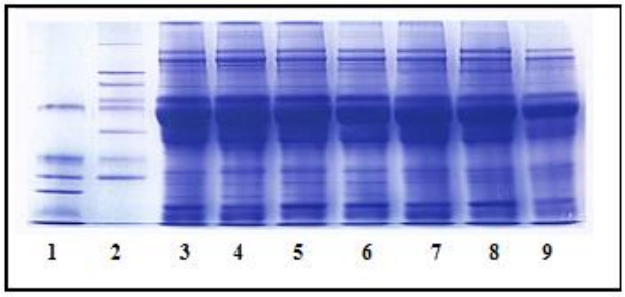


Fig. 8: Gel represent protein pattern of *Mus musculus* 14 days after treatment with repeated dose of ivermectin. Lanes from left to right respectively: Lane 1: Low molecular weight marker. Lane 2: High molecular weight marker. Lane 3: Control. Lanes (4-9): Treated animals.

DISCUSSION

Ivermectin is a semi-synthetic lactone drug that exhibits broad anti-helminthic specificity (Goa et al., 1991). Over the past several years, severe adverse reactions to ivermectin treatment have been reported on individuals residing in onchocerciasis endemic areas that are also endemic for *Loa loa* (Boussinesq *et al.*, 2003).

In this study, the aberrant metaphases in control sample represented in 5.83% of the examined cells. Sex univalent and chain of four (CIV) were frequently observed. Auto some univalent and (CVI) or (RIV) were rather rare. These observations are in a good agreement with that of Das and Roy (1990), Benova (1992) and Ahmed and Othman (2004). They found the aberrant metaphases in the untreated mice were ranging between 5.5: 10%. In the current study, chromosomes spreads were prepared for 12 days post-treatment with single dose of 200ug/kg IVM, to cover preleptotene stage.

The latter is the most sensitive stage before meiotic divisions, when cytogenetic analysis is carried out in MI spermatocytes (Ciranni and Adler, 1991). Further, the majority of chemicals act as S-dependent agents, and premeiotic S-phase occurs in the mouse 12 days before meiotic divisions (Adler, 1996).

IVM treatment elevated the abnormality to 33.83%, i.e. 6-fold increase was occurred. Sex univalents, CIV, autosome univalents represented in 11%, 9.3% and 6%, respectively. Autosomes plus sex univalents, RIV, CVI, fragments were rarely picked out.

Literature on cytogenetic effects of IVM is very meager. However, many chemicals and drugs have mentioned to inducing such univalents formation of mice (Kar and Das, 1987). Abd-EL-Baset *et al.* (2000) observed various signs of chromosomal aberrations in germ cells of mice due to exposure to sunset yellow and / or ponceu 4R as a synthetic colors. Also, chromosomal aberrations were significantly recorded in germ cells of mice after exposure to pepon and prostaplex (used in treatment of prostatic disorders (Ahmed and Othman, 2004).

Concerning the effects of IVM on pairing behaviour, both autosomes and sex chromosomes showed susceptibility to univalent formation. X-Y bivalents showed a higher sensitivity than the auto somal bivalents. That is mean, following IVM treatment, X and Y separation was highly detected than autosomes. This observation is in accordance with that of Hu and Zhu (1990) following the effect of uranyl fluoride containing uranium; Imai *et al.* (1981) in hybrids between wild mice and inbred laboratory mice; Amer *et al.* (2002) as the effect of malathion. According to Das and Roy (1990) such behaviour may result either from early breakdown of association or from a complete lack of it.

In the present study, a multivalent configurations were occasionally observed such as (18 II + III + I), (18II + CIV), (17II + CVI), (15II + CXI). Such phenomenon may explaine as proposed by Lyon and Meredith (1966) who believed that the type of aberration may be correlated with the relative length of the chromosome segments that are exchanges. They added, in very small segments chiasmata will be rare, resulting in a multivalent figs. of

Table 1: Number and percentage of the different types of chromosomal aberrations in primary spermatocytes of male mice after single i.p.injection with 200ug/kg ivermectin

Experimental group	Time after treatment in days	No. of mice	Examined metaphases	No. of abnormal metaphases	Abnormal metaphases		No. of metaphases with different types of chromosomal aberrations							
					Mean %	±S.E.M.	A.U.		X-Y U.	A.U.+ X-Y U.	RIV	CIV	CVI	F	Polyploidy
Control	12	6	600	35	5.83	0.94	No.	3	10	2	1	13	1	-	5
							%	0.5	1.6	0.3	0.16	2.16	0.16	-	0.83
Single i.p.injection with 200ug/kg ivermectin	12	6	600	203	33.83	3.3 ****	No.	36	66	6	5	56.4[a]	11	1	18
							%	6*	11 ***	1 n.s.	0.83 n.s.	9.3 ****	1.83 *	0.16 n.s.	3*
							t-test								

A.U= Autosomal univalent X-Y U= X-Y univalent A.U. + X-Y U= Autosomal univalent + X-Y univalent RIV=ring IV CIV= Quadrivalent chain CVI= Hexavalent chain F=Fragment a= chain III+I S.E.M.=Standard error mean n.s.=Non significant *= P<0.05 significant **= highly significant ***=p≤ 0.01 ****= p≤ 0.001 using simple T-test

Table 2: Summary of the electrophoretic changes in protein profile pattern o f *Mus musculus* following treatment with ivermectin

Time intervals	Control groups	Treatment	Treated groups		
	Total bands		Total bands	Shared bands	New bands
1day	28	Single injection with (200ug/kg) ivermectin	29	28	1
7 days	27		33	27	6
14 days	32		33	32	1
1 day	26	Repeated injection with (200 ug/kg) ivermectin two weeks apart	29	26	3
7 days	27		28	27	1
14 days	29		31	29	2

Table 3: Effect of ivermectin on % areas of serum protein bands of the treated mice

Treatment	Time intervals	The type of changes and the number of bands		
		Increase	Decrease	Non significant variations
Single injections	1day	5	6	18
	7 days	5	7	21
	14 days	1	5	27
Repeated injections	1day	2	7	20
	7 days	1	4	23
	14 days	3	3	25

the chain of IV or chain III + I type. The presence of several configurations also indicates a serial induction of translocations. For example, fig. of CIV suggests breaks in 2 chromosomes, the presence of two quadrivalents indicates breaks in 4 chromosomes (Cacheiro *et al.*, 1974). This explanation is in accordance with that of Matter and Generoso (1974) who proposed that the types of genetic damage referred to chromosomal breakage and all classes of aberrations that may arise from initial breaks. This view is further confirmed by that of Cacheiro *et al.* (1974); Pacchierotti *et al.* (1983).

The present observations pointed to the mutagenic effects of IVM. The frequency of translocation is significantly higher than that found in the control samples.

The effect of mutagens in germ cell in both mans and mouse has been discussed in many occasions but rare for IVM. In a number of studies in mice carrying male-sterilizing auto somal translocations, spermatocytes loss has been associated with the apposition of unpaired auto somal and sex chromosome elements during pachytene and this in turn, might interfere with the genetic activity of sex chromosomes (Chandley *et al.*, 1986).

In addition, the genotoxic effects of ivermectin were reviewed by several investigators. Accordingly, Li *et al.* (2004) suggested that the increasing proportion of Gpi-AA genotype and perhaps Gpi-A allele in a population might be useful as a potential resistant biomarker of *Oxya chinensis* to pesticide avermectin. Osei-Atweneboana *et al.* (2012) showed that IVM resistance had been selected and the genotype (1183GG/1188CC/1308TT, 1545GG) was strongly associated with the resistance phenotype. Also, the genotoxicity of IVM was examined by El Makawy and Mahrous (2008) through the cytokinesis block micronucleus assay and chromosomal aberrations in buffalo lymphocytes *in vitro* and Sweify *et al.* (2015) on bone marrow cells of mice *in vivo*.

Moreover, El-Nahas and El-Ashmawy (2008) concluded that ivermectin had slight effects on male fertility. Molinari *et al.* (2009) highlighted that IVM and ivomec exert both genotoxicity and cytotoxicity in mammalian cells *in vitro*, at least in CHO(K1) cells. Although both abamectin and ivermectin do not induce *in vitro* and *in vivo* gene mutations in either bacterial or mammalian cells, there is no concrete evidence of a clear clastogenic effect exerted both *in vitro* and *in vivo* in mammalian cells (Molinari *et al.*, 2010). el-Ashmawy *et al.* (2011) concluded that combined treatment of ivermectin and verapamil severely affect fetal genetic material and development and induced genotoxic effect in somatic cells of the dams. Molinari *et al.* (2013) concluded that the decrease in DNA lesions was mostly related to IVM-induced cytotoxicity rather than attributable to a repair process.

These studies revealed high clastogenic and genotoxic potential of IVM

The variation in the protein fractions due to IVM treatment was examined through SDS-PAGE. It is flexible and powerful technique widely used for protein separation based on their M. wt. (Laemmli *et al.*, 1993 and Oswald, 2016). Comparing to the control samples, some newly bands were developed in the treated samples. The appearance of these bands may due to break down of a high M.wt protein (Dutta *et al.*, 1992); or may be attributed to the formation of adducts between IVM and DNA which in turn influenced the transcription rate of

genes encoding plasma protein (Mackiewicz *et al.,* 1992). Also El-Deeb *et al.*(1996) suggested that exposure to pollutants may play a role in increasing polymorphism ratio among treated samples by causing disappearance or appearance of certain bands. So, our results may be explained through the reports of Stromberg and Guillot (1987) and Mycek *et al.* (1997). They postulated that some pollutants or drugs cause misreading of the genetic code and therefore cause abnormal protein synthesis.

On the other hand, the % areas of the protein fraction was elevated to some treated samples, meanwhile it was reduced in others. Increasing and / or decrease in the relative area percentage were observed which indicate the effect of IVM on the electrophoretic patterns of the serum protein fraction, with this respect Abd El-Latif (2000) found that fluoxetine (antidepressant) induce various abnormal pictures of protein areas during the different developmental stage of mice embryos referring to the binding of the drug with certain specific proteins which induced many signs of teratogenicity and genotoxicity. The present findings are in harmony with the results reported by Ibrahim *et al.* (1995) who proposed that the formation of new bands or fluctuation of the % area of some bands are most probably cause from irreversible protein break down which appear in the presence of the old bands after drug exposure. Finally, although the remarkable side effect, of IVM, yet parenteral use of IVM may be a safe and effective treatment for severe parasitic infections (Turner *et al.,* 2005 and Barrett *et al.,* 2016). It is of interest to mention the conclusions of Omura (2008) that IVM has improved the lives and productivity of billions of humans, livestock and pets around the globe, and promises to help consign to the history books two devastating and disfiguring diseases that have plaqued people throughout the tropics for generations, while new uses for it are continually being found.

The effect of avermectin on serum protein was mentioned in the literature. Impair protein and energy metabolism, immune system function, and performance resultant from clinical psoroptic mange, improved substantially within 8 weeks of successful treatment for injectable ivermectin as stated by Rehbein *et al.* (2016). Merola *et al.* (2009) showed p-glycoprotein defects for increased susceptibility to ivermectin toxicosis in dogs. Ivermectin interferes with the binding of retinol to the 19.7 kDa Onchocerca protein as indicated by Lal and James (1996). Moreover, Interleukin-6 (IL-6) and C-reactive protein (CRP) were elevated in 25.7% and 50.7% of onchocerciasis patients, respectively, after ivermectin treatment (Njoo *et al.,*1994). In the present study, the mobility of certain protein fractions was found to be affected even with the lowest dose of ivermectin. Based on the above findings, it is clear that the formation of new bands as well as the increase in band relative percentage is most probably caused by irreversible protein breakdown which appears in the presence of these odd bands after the exposure to IVM. These results indicate the effect of IVM on the electrophoretic patterns of the serum protein fractions.

CONCLUSION

In the present work, IVM induced significant increase in the reciprocal translocation figs. of primary spermatocytes. The changes in the serum protein fractions add another warning for the mutagenicity of IVM. It is concluded from the present work that the mutagenic effects fortunately diminished with time. According to the obtained results cautious use of IVM is advisable.

ACKNOWLEDGEMENTS

The helps and cooperation of College of Women, Ain Shams University, Egypt, is gratefully acknowledged.

REFERENCES

Abd El-Baset SE, Ali MO, Hanafy Z M and Ahmed MA (2000). The mutagenic effects of sunset yellow or Ponceu4R as synthetic colours on adult male albino mice. *Al-Azhar. Med. J.*, **29**(3): 235-250.

Abd El-Latif SB (2000). Various effects of an antidepressant drug (fluoxetine) on the embryonic development of mice. MSc. Thesis Faculty of Science. Al-Azhar University p.173.

Adler ID (1996). Comparison of the duration of spermatogenesis between male rodents and humans. *Mutat. Res.*, **352**(1-2): 169-172.

Ahmad HM, Abdel-Azim ES and Abdel-Aziz RT (2016). Clinical efficacy and safety of topical versus oral ivermectin in treatment of uncomplicated scabies. *Dermatol. Ther*., **29**(1): 58-63.

Ahmed SS and Othman EO (2004). Cytogenetic studies on two drugs for natural relief of prostatic disorders. *Cytologia.*, **69**(3): 275-284.

Ali ST, Alinia H and Feldman SR (2015). The treatment of rosacea with topical ivermectin. *Drugs Today (Barc)*, **51**(4): 243-250.

Amer SM, Fahmy MA, Aly FA and Farghaly AA (2002). Cytogenetic studies on the effect of feeding mice with stored wheat grains treated with malathion. *Mutat. Res.*, **513**(1-2): 1-10.

Barrett J, Broderick C, Soulsby H, Wade P and Newsholme W (2016). Subcutaneous ivermectin use in the treatment of severe strongyloides stercoralis infection: two case reports and a discussion of the literature. *J. Antimicrob. Chemother.*, **71**(1): 220-225.

Benova DK (1992). Anticlastogenic effects of a polyvitamin product, Pharmavit, on gamma-ray induction of somatic and germ cell chromosome aberrations in the mouse. *Mutat. Res.*, **269**(2): 251-258.

Boussinesq M, Gardon J, Gardon-Wendel N and Chippaux JP (2003). Clinical picture, epidemiology and outcome of Loa-associated serious adverse events

related to mass ivermectin treatment of onchocerciasis in Cameroon. *Filaria. J.*, **2**(Suppl 1): S4.

Cacheiro NL, Russell LB and Swartout MS (1974). Translocations, the predominant cause of total sterility in sons of mice treated with mutagens. *Genetics*, **76**(1): 73-91.

Chandley AC, Speed RM, McBeath S and Hargreave TB (1986). A human 9; 20 reciprocal translocation associated with male infertility analyzed at prophase and metaphase I of meiosis. *Cytogenet Cell Genet*, **41**(3): 145-153.

Chosidow A and Gendrel D (2016). [Safety of oral ivermectin in children]. *Arch Pediatr*, **23**(2): 204-209.

Chouela E, Abeldano A, Pellerano G and Hernandez MI (2002). Diagnosis and treatment of scabies: A practical guide. *Am. J. Clin. Dermatol.*, **3**(1): 9-18.

Ciranni R and Adler ID (1991). Clastogenic effects of hydroquinone: Induction of chromosomal aberrations in mouse germ cells. *Mutat. Res.*, **263**(4): 223-229.

Das RK and Roy B (1990). Evaluation of genotoxicity of clofazimine, an antileprosy drug, in mice *in vivo.* I. chromosome analysis in bone marrow and spermatocytes. *Mutat. Res.*, **241**(2): 161-168.

DeMarco JH, Heard DJ, Fleming GJ, Lock BA and Scase TJ (2002). Ivermectin toxicosis after topical administration in dog-faced fruit bats (Cynopterus brachyotis). *J. Zoo Wildl. Med*., **33**(2): 147-150.

Derua YA, Malongo BB and Simonsen PE (2016). Effect of ivermectin on the larvae of *Anopheles gambiae* and *Culex quinquefasciatus*. *Parasit Vectors*, **9**(1): 131-136.

Dou Q, Chen HN, Wang K, Yuan K, Lei Y, Li K, Lan J, Chen Y, Huang Z, Xie N, Zhang L, Xiang R, Nice EC, Wei YQ and Huang C (2016). Ivermectin induces cytostatic autophagy by blocking PAK1/Akt axis in breast cancer. *Cancer Res*., Jun 14. [Epub ahead of print].

Dutta HM, Dogra JV, Singh NK, Roy PK, Nasar ST, Adhikari S, Munshi JS and Richmonds C (1992). Malathion induced changes in the serum proteins and haematological parameters of an indian catfish *Heteropneustes fossilis* (Bloch). *Bull Environ Contam Toxicol*, **49**: 91-97.

el-Ashmawy IM, el-Nahas AF and Bayad AE (2011). Teratogenic and cytogenetic effects of ivermectin and its interaction with P-glycoprotein inhibitor. *Res. Vet. Sci.*, **90**(1): 116-123.

El-Deeb SI, Ezzat AA, Abdel-Aziz SH and Helmy ZA (1996). Electrophoretic protein polymorphism of Clarias lazera affected by two herbicides. *J Agric Sci Mansoura Univ*, **21**(8): 2859-2868.

El-Makawy AI, and Mahrous KF (2008). Antimutagenic activity of some natural supplements on ivermectin genotoxicity in lymphocytes of Buffalo. *Egypt. J. Hospital Medicine*, **30**: 115-125.

El-Nahas AF and El-Ashmawy IM (2008) Effect of ivermectin on male fertility and its interaction with P-glycoprotein inhibitor (verapamil) in rats. *Environ Toxicol. Pharmacol*., **26**(2): 206-211.

Esum M, Wanji S, Tendongfor N and Enyong P (2001). Co-endemicity of loiasis and onchocerciasis in the South West Province of Cameroon: Implications for mass treatment with ivermectin. *Trans. R. Soc. Trop. Med. Hyg*., **95**(6): 673-676.

Evans EP, Breckon G and Ford CE (1964). An-air drying method for meiotic preparations from mammalian testes. *Cytogenetics*, **3**: 289-294.

Goa KL, McTavish D and Clissold SP (1991). Ivermectin. A review of its antifilarial activity, pharmacokinetic properties and clinical efficacy in onchocerciasis. *Drugs*, **42**(4): 640-658.

Goldust M, Rezaee E, Raghifar R and Hemayat S (2013). Treatment of scabies: the topical ivermectin vs. permethrin 2.5% cream. *Ann. Parasitol*., **59**(2): 79-84.

Hu QY and Zhu SP (1990). Induction of chromosomal aberrations in male mouse germ cells by uranyl fluoride containing enriched uranium. *Mutat. Res.*, **244**(3): 209-214.

Ibrahim IG, Abou-Zeid MM, Zaki ZT and Rady MI (1995). Changes induced by paraquat herbicide in muscle proteins and some hematological parameters of two fishes from Egypt. *Al-Azhar Bull. Sci.,* **6**(1): 637-647.

Imai HT, Matsuda Y, Shiraishi T and Moriwaki K (1981). High frequency of X-Y chromosome dissociation in primary spermatocytes of F_1 hybrids between Japanese wild mice (*Mus musculus molossinus*) and inbred laboratory mice. *Cytogenet. Cell Genet*, **29**: 166-175.

Kar RN and Das RK (1987). Dominant lethality and spermatocytic chromosome aberrations in mice treated with chlordiazepoxide. *Caryologia*, **44**: 267-273.

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680-681.

Laemmli EK, Rozanski TA, Morey AF and Rholl V (1993). The effects of exercise activity on serum prostate specific antigen levels. *Urology*, **150**: 893-894.

Lal PG and James ER (1996). Onchocerca retinol- and ivermectin-binding protein activity. *Parasitology*, **112**(Pt 2): 221-225.

Leonard A (1975).Tests for heritable translocations in male mammals. *Mutat Res*,**31**:291-298.

Li CL, Duan YH, Lu FP, Guo YP and Ma EB (2004). Differential acute mortality among the allozyme genotypes of *Oxya chinensis* by pesticide avermectin. *Yi. Chuan. Xue. Bao.*, **31**(11): 1241-1247.

Lyon MF and Meredith R (1966). Autosomal translocations causing male sterility and viable aneuploidy in the mouse. *Cytogenetics*, **5**(5): 335-354.

Mackiewicz A, Schooltink H, Heinrich PC and Rose-John S (1992). Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. *J. Immunol.*, **149**(6): 2021-2027.

Mangia C, Vismarra A, Kramer L, Bell-Sakyi L, Porretta D, Otranto D, Epis S and Grandi G (2016) . Evaluation of the in vitro expression of ATP binding-cassette (ABC) proteins in an *Ixodes ricinus* cell line exposed to ivermectin. *Parasit. Vectors,* **9**(1): 215-219.

Matter BE and Generoso WM (1974). Effects of dose on the induction of dominant-lethal mutations with triethylenemelamine in male mice. *Genetics,* **77**(4): 753-763.

Merola V, Khan S and Gwaltney-Brant S (2009). Ivermectin toxicosis in dogs: A retrospective study. *J. Am. Anim. Hosp. Assoc*., **45**(3): 106-11.

Molinari G, Soloneski S, Reigosa MA and Larramendy ML (2009). In vitro genotoxic and cytotoxic effects of ivermectin and its formulation ivomec on Chinese hamster ovary (CHOK1) cells. *J. Hazard Mater*., **165**(1-3): 1074-1082.

Molinari G, Soloneski S and Larramendy ML (2010). New ventures in the genotoxic and cytotoxic effects of macrocyclic lactones, abamectin and ivermectin. *Cytogenet. Genome Res*., **128**(1-3): 37-45.

Molinari G, Kujawski M, Scuto A, Soloneski S and Larramendy ML (2013). DNA damage kinetics and apoptosis in ivermectin-treated Chinese hamster ovary cells. *J. Appl. Toxicol*., **33**(11): 1260-1267.

Mycek MJ, Harvey RA and Champe PC (1997). Lippincott's Illustrated Reviews Pharmacology 2 and ed. Lippincott Raven publishers. Philadelphia New York pp.314-317.

Njoo FL,,Hack CE, Oosting J, Luyendijk L, Stilma JS and Kijlstra A (1994). C-reactive protein and interleukin-6 are elevated in onchocerciasis patients after ivermectin treatment. *J. Infect Dis*., **170**(3): 663-668.

Nontasut P, Claesson BA, Dekumyoy P, Pakdee W and Chullawichit S (2005). Double-dose ivermectin vs albendazole for the treatment of gnathostomiasis. *Southeast Asian J. Trop Med. Public Health,* **36**(3): 650-652.

Omura, S (2008). Ivermectin: 25 years and still going strong. *Int. J. Antimicrob. Agents,* **31**(2): 91- 98.

Omura S and Crump A (2014). Ivermectin: Panacea for resource-poor communities? *Trends Parasitol*., **30**(9): 445-455.

Ordóñez LE and Angulo ES (2004). Efficacy of ivermectin in the treatment of children parasitized by Strongyloides stercoralis. *Biomedica*., **24**(1): 33-41.

Osei-Atweneboana MY, Boakye DA, Awadzi K, Gyapong JO and Prichard RK (2012). Genotypic analysis of B-tubulin in *Onchocerca volvulus* from communities and individuals showing poor parasitological response to ivermectin treatment. *Int. J. Parasitol. Drugs and Drug Resist,* **2**: 20-28.

Oswald N (2016). How SDS=PAGE works. *Bite size Bio*, http://bitesizebio.com/580/how-sds-page-works.

Pacchierotti F, Bellincampi D and Civitareale D (1983). Cytogenetic observations, in mouse secondary spermatocytes on numerical and structural chromosome aberrations induced by cyclophosphamide in various stages of spermatogenesis. *Mutat. Res.,* **119**: 177-183.

Panigrahi PN, Gupta AR, Patra RC, Mohanty BN, Maiti A and Sahoo GR (2016). Comparative anthelmintic efficacy of ivermectin delivered through different routes in gastrointestinal nematode infected dogs. *J Parasit. Dis.,* **40**(1): 46-51.

Pariser DM, Meinking TL, Bell M and Ryan WG (2012). Topical 0.5% ivermectin lotion for treatment of head lice. *N. Engl. J. Med*., **367**(18): 1687-1693.

Raza A, Kopp SR, Bagnall NH, Jabbar A and Kotze AC (2016). Effects of *in vitro* exposure to ivermectin and levamisole on the expression patterns of ABC transporters in *Haemonchus contortus* larvae. *Int. J. Parasitol. Drugs Drug Resist,* **6**(2): 103-115.

Rehbein S, Visser M, Meyer M and Lindner T (2016). Ivermectin treatment of bovine psoroptic mange: Effects on serum chemistry, hematology, organ weights and leather quality. *Parasitol. Res.,* **115**(4):1519-1528.

Sangaré AK, Rolain JM, Gaudart J, Weber P and Raoult D (2016). Synergistic activity of antibiotics combined with ivermectin to kill body lice. *Int. J. Antimicrob. Agents*, **47**(3): 217-223.

Sheele JM and Ridge GE (2016). Toxicity and potential utility of ivermectin and moxidectin as xenointoxicants against the common bed bug, *Cimex lectularius* L. *Parasitol Res*., **115**(8): 3071-3081.

Sparsa A**,** Bonnetblanc JM, Peyrot I, Loustaud-Ratti V, Vidal E and Bédane C (2006). Systemic adverse reactions with ivermectin treatment of scabies. *Ann. Dermatol. Venereol*., **133**(10): 784-787.

Stromberg PC and Guillot FS (1987). Hematology in the regressive phase of bovine psoroptic scabies. *Vet Pathol.*, **24**(5): 371-377.

Sweify KM, Darwish IA and Hafez DDA (2015). The cytogenetic potential of ivermectin on bone marrow cells of mice *in vivo. IOSR-JESTFT,* **9**(2): 17-29. www.iosrjournals.org.

Trailovic SM, Ivanovic SR and Varagic VM (2011). Ivermectin effects on motor coordination and contractions of isolated rat diaphragm. *Res. Vet. Sci*., **91**(3): 426-433.

Turner SA, Maclean JD, Fleckenstein L and Greenaway C (2005). Parenteral administration of ivermectin in a patient with disseminated strongyloidiasis. *Am. J. Trop. Med. Hyg.,* **73**(5): 911-914.

Varó I, Rigos G, Navarro JC, del Ramo J, Calduch-Giner J, Hernández A, Pertusa J and Torreblanca A (2010). Effect of ivermectin on the liver of gilthead sea bream *Sparus aurata*: a proteomic approach. *Chemosphere*, **80**(5): 570-577.

Verdú JR, Cortez V, Ortiz AJ, González-Rodríguez E, Martinez-Pinna J, Lumaret JP, Lobo JM, Numa C and Sánchez-Pinero F (2015). Low doses of ivermectin cause sensory and locomotor disorders in dung beetles. *Sci. Rep*., **5**: 13912.