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Genotoxic and Cytotoxic Effects of Fumagillin in White Mice

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Key words:
Fumagillin, Genotoxicity, cytotoxicity, micronucleus, chromosome aberration, *Nosema apis*, and PCEs.

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ABSTRACT

Fumagillin (dicyclohexylamine) which secreted by Aspergillus fumigates, is a natural antibiotic, used in veterinary medicine against microsporidiosis in bees and fish. in human medicine it used for the treatment of intestinal amebiasis, microsporidial keratoconjunctivitis and intestinal microsporidiosis due to Enterocytozoon bieneusi in patients with AIDS and other types of immunodeficiency. In our current study, the genotoxicity of fumagillin was evaluated in mouse bone marrow cells using the micronucleus (mn) test, chromosome aberrations in bone-marrow cells and primary-spermatocytes. Mitotic index (MI) wase used to evaluate cytotoxic effects of Fumagillin. Fumagillin was administered to whit mice by gavage in doses of 10, 15, and 20 mg/kg.bwt prepared in 50% sugar solution, repeated for 7days at 24h intervals, with water-sugar syrup 50% as the negative control and Methotrexat as the positive control (20 mg/kg. bwt.) intrapretonial injection. The results of current study shows that Two of experimental doses of fumagillin 15 and 20 mg/kg. bwt. induced a significant increase $p \leq 0.05$ in the frequency of MN (24.60 ± 2.37 and 53.00 ± 4.59 respectively) compared with the negative control (8.20 ± 1.39). Significant increase $p \leq 0.05$ in means of total structural chromosome aberrations (9.00 ± 0.92 and 17.20 ± 1.24 compared with 6.00 ± 0.83 of negative control in bone marrow. In primary spermatocytes, the dose 20 mg/kg. bwt of fumagillin induced significant increase $p \leq 0.05$ in mean of aberration (14.00 ± 2.07 compared with 3.40 ± 0.50 of negative control. All experimental doses induced significant decrease $p \leq 0.05$ in MI (3.90 ± 0.29, 3.60 ± 1.80 and 2.90 ± 1.80 respectively) compared with (7.10 ± 0.29) in negative control. These results suggest that fumagillin (dicyclohexilamine) has an anti proliferative and genotoxic potential in mammal *in vivo* tests.

التأثير السمي الوراثي والسمي الخلوي للفيوماجيللين في الفئران البيض

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الخلاصــة

يعد الفيوماجيللين الذي ينتجه الفطر *Aspergillus fumigates*، مضادا حياتيا طبيعيا يستخدم في الطب البيطري ضد الطفيليات من نوع Microsporidia في النحل والاسماك. كما يستخدم في الطب البشري لمعالجة الاصابة بالاميبا المعوية والاصابات الناتجة عن الطفيلي *Enterocytozoon bienusei* في مرضى الايدز وامراض العوز المناعي الاخرى. في الدراسة الحالية تم تقييم السمية الوراثية للفيوماجيللين في خلايا نقي العظم للفئران البيض باستخدام اختبار النواة الدقيقة mn، والشذوذ الكروموسومي في خلايا نقي العظم والخلايا الابتدائية المولدة للنطف. كما تم استخدام دالة الانقسام لتقدير التأثيرات السمية الخلوية للفيوماجيللين. تم إعطاء الفيوماجيللين بطريقة التجريع الفموي بشكل متكرر كل 24 ساعة ولمدة سبعة ايام متتالية بعد تحضير الجرعات 10، 15، 20 ملغم\كم وزن جسم في محلول سكري 50 %، كما تم إعطاء مجموعة السيطرة السالبة محلول سكري 50 % وبالطريقة نفسها. اما مجموعة السيطرة الموجبة فقد تم إعطاءها جرعة واحدة 20 ملغم\كغم. وزن جسم من الميثوتركسيت MTX بطريقة الحقن داخل الخلب. أظهرت نتائج الدراسة ان بالجرعتين التجريبيتين 15 و 20 ملغم\كغم. وزن جسم من الفيوماجيللين تسببت في حث زيادة معنوية $p \leq 0.05$ في تكرار النوى الدقيقة (24.60 ± 2.37 و 53.00 ± 4.59) على التوالي مقارنة مع السيطرة السالبة (8.20 ± 1.39). كما تبين ظهور زيادة معنوية $p \leq 0.05$ في متوسطات الشذوذ

الكلمات المفتاحية:
التأثير السمي الوراثي، التأثير السمي الخلوي ، الفيوماجيللين ، الفئران البيض.

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الكروموسومي الكلي (9.00 ± 0.92 و 17.20 ± 1.24) على التوالي مقارنة مع (6.00 ± 0.83) بالنسبة للسيطرة السالبة. اما في الخلايا الابتدائية المولدة للنطف فقد تسببت الجرعة 20 ملغم\كغم. وزن جسم من الفيوماجللين في حث زيادة معنوية $p\leq 0.05$ في متوسط الشذوذ الكروموسومي (14.00 ± 2.07) مقارنة مع (3.90 ± 0.50) في مجموعة السيطرة السالبة. كما تسببت الجرعات التجريبية الثلاثة 10، 15 و 20 ملغم\كم. وزن جسم من الفيوماجيللين بانخفاض معنوي $p\leq 0.05$ في متوسطات دالة الانقسام (3.90 ± 0.29، 3.60 ± 1.80 و 2.90 ± 0.50) على التوالي مقارنة مع (7.10 ± 0.29) بالنسبة الى مجموعة السيطرة السالبة. تفترض النتائج المتحصل عليها من الدراسة الحالية ان الجرعات التي تم اختبارها من الفيوماجيللين لها تأثيرا سميا وراثيا تمثل في زيادة تكرار النوى الدقيقة ومتوسطات الشذوذ الكروموسومي وسمية خلوية تمثلت بالقدرة المضادة لتكثير الخلايا في نقي عظم الفئران البيض.

Introduction:

Fumagillin dicyclohexylamine (fumagillin DCHA) is an antibiotic authorized for use in honey bees for the prevention of infections caused by the *Nosema apis* parasite present in the gut of infected bees. The commercial formulation of fumagillin DCHA is a stabilized water-soluble preparation, Fumidil-B (CEVA Animal Health).

In humans, fumagillin was used in the 1950s for the treatment of intestinal amebiasis (Mc Cowen *et al*., 1951). "More recently, it has been found to be highly effective when used topically in the treatment of microsporidial kerato- conjunctivitis" (Diesenhouse *et al*., 1993; Rosberger *et al*., 1993), and "when used orally in the treatment of chronic Enterocytozoon bieneusi infection in patients with AIDS and other types of immunodeficiency" (Conteas *et al*., 2000; Molina et al., 2000; Molina *et al*. 2002). Watanabe *et al*. (2006) reported that "fumagillin inhibited the infection of human macrophages with HIV-1, and proposed it as a lead compound for the development of a novel type of AIDS therapeutic drug that targets HIV-1 viral protein R (Vpr) activity".

In February 2009 the COM considered studies investigating *in-vitro* chromosomal aberrations, unscheduled DNA synthesis (UDS), *in-vivo* micronucleus and primary DNA damage studies (UDS and Comet), undertaken by Nesslany, and a critical analysis of mutagenicity data of fumagillin DCHA salt, all submitted by the Marketing Authorisation Holder (MAH).

Positive results had been reported for *in vitro* clastogenicity by both the Nesslany, (2006, 2007; Stanimirovic *et al*., 2007a,b; stevanovic *et al*., 2008). However evidence for an *in vivo* clastogenic effect had been reported in (Stanimirovic *et al*., 2006) but not in those undertaken for the MAH by Nesslany, (2004, 2006a,b).

In Whitwell (2010) Fumagillin DCHA was tested for its ability to induce chromosome aberrations in the bone marrow of male Crl:CD-1(ICR) mice when administered orally by gavage at 25, 50 and 75 mg/kg. bwt./day in a dose volume of 10 ml/kg water-sugar syrup solution over 7 days. The positive control was CPA administered at 40 mg/kg. bwt. in a single dose 16 hours prior to the bone marrow harvest. The bone marrow was sampled 16 hours post the final administration.

The present study was designed to investigate and explain the clastogenicity and aneugenecity of fumagillin in white mice by using micronucleus (mn) test, chromosome aberrations (CA) in bone-marrow and primary spermatocytes, which are "widely used as cytogenetic biomarkers of genotoxic exposure" (Albertini *et al*., 2000). "Both clastogenic and aneugenic effects can be determined using the mn test" (Kirsch-Volders *et al*., 1997; Norppa *et al*., 2003). And cytotoxicity of fumagillin by mitotic index (MI) to detect the cytostatic effects.

Materials and methods:

Genotoxic effects of fumagillin, in the form of fumagillin dicyclohexylamine (Fumagillin-ET®; Evrotom, Ruma, SRJ; Purity: ≥90% by HPLC, CAS No. 23110-15-8) were investigated in bone-marrow cells of BALB/c mice, using the micronucleus (mn) test. chromosomal aberration (CA) in

bone marrow cells and primary spermatocytes. Cytotoxicity of fumagillin were evaluated by mitotic index (MI) to detect the cytostatic effect.

Three experimental doses of fumagillin were tested: 10, 15, and 20 mg/kg. bwt. "since the recommended fumagillin dose for honey bees is 26 mg fumagillin/L" (Webester, 1994), and "there is a lack of data on fumagillin intake levels in humans from the consumption of contaminated honey, or the information is insufficient", as in the reports of (Mladjan and Jovic, 2000 and Kuli´c, 2006) "where fumagillin residue levels range from 8.5 to 12.3 mg/kg in honey harvested from bee colonies irregularly treated with fumagillin in the intensive honey-flow season".

The experimental design for *in vivo* tests included three groups: the negative control, the positive control, and the experimental groups. The experimental groups were divided into three subgroups based on the selected doses of fumagillin. All groups had equivalent numbers of animals per test.

Thus, for the cytogenetic test five animals were used per dose group (male sex). The current study used 6-week-old BALB/c mice with an average weight of 22± 2 gm. Animals were kept under uniform conditions and housed under 12/12-h light–dark period at constant temperature (21 ◦C) with free access to standard laboratory chow and water.

To prepare medicated sugar syrup, it is recommended to mix fumagillin in small amounts of warm water (not above 32–34 ◦C) and stir into a paste, then add prepared water–sugar syrup gradually and shake the container occasionally. The antibiotic mixture should be admixed with water–sugar syrup shortly before use.

Experimental doses were obtained by dissolving fumagillin in 1:1 water–sugar syrup, as in the formulation usually used for application in beekeeping, and administered to the mice by oral gavage. Since tested doses of fumagillin were 10, 15, and 20 mg/kg b.wt., each mouse received 0. 25, 0.375, and 0.5 mg, respectively, i.e. 1.75, 2.625, and 3.5 mg/kg b.wt. in a 7-day treatment. The negative control group was treated with water–sugar syrup. A known mutagen, Methotrexate (MTX) at a dose of 20 mg/kg b.wt. was used for the positive control group "due to its known clastogenic and mutagenic activity" (Anderson *et al*., 1995). MTX was given intraperitoneally (i.p.), and the volume injected was 0.5 ml/animal. All animals of positive control group received one i.p. treatment.

For analysis of micronuclei (MNi) in polychromatic erythrocytes (PCEs) of mouse bone-marrow the method described by Schmid, (1975) was used." At least four slides were made for each animal, allowed to dry overnight and then stained with May–Gruenwald + Giemsa solutions (MOL d.d., Beograd, SCG) according to the standard technique" (Adler, 1984) for conventional assessment of the mn frequency. All slides were coded for microscope analysis at 1000X magnification. Per animal, 1000 polychromatic erythrocytes (PCEs) from each of four randomly selected slides were scored for the presence of MN.

Cytogenetic analysis was performed according to Hsu and Patton, (1969) as modified by (Zimonjic *et al*., 1990). Rinsing marrow of long bones (femur), Slides were made by the flame-dried technique and later stained with Giemsa (Sigma Chemical Co., St. Louis, MO). G-banding of chromosomes was done by the trypsin method of Seabright, (1971) and Ronne, (1991). Chromosomes and chromosomal bands were identified on the basis of criteria established by the Committee on Standardized Genetic Nomenclature for mice (COM , 1979) and Cowell's photo atlas of mouse chromosomes (Cowel, 1984), in order to identify chromosomes that take part in the formation of Robertsonian (Rb) metacentric chromosomes.

500 well-spread metaphases Were analyzed for each treatment to detect the presence of chromosomal aberrations (CA); whereas the mitotic index (MI) was determined on 1000 or more cells. The slides were randomly selected prior to scoring.

Primary spermatocyte chromosomes analysis was performed according to (Berwen and Preston,1978). Animals were sacrified,Tunica were removed and testis teased in 2.2% trisodium citrate solution. Cell suspension transferred to 3ml test tube and centrifuged for 10 minutes at 800 c/min. The supernatant discarded and pellets suspended in 1.1% trisodium citrate solution for 20 minutes. Test tubes were centrifuged at 800 cycle/min. for 10 minutes, supernatant discarded and

cold fixative 1:3 GAA/ methanol was added drop by drop with continuous shaking. Fixation repeated three times. Slides were made by the flame-dried technique and later stained with Giemsa (Sigma Chemical Co., St. Louis, MO).

100 well-spread metaphases Were analyzed for each treatment to detect the presence of chromosomal aberrations (CA).

Statistical analyses were carried out with the software program SPSS version 17.5 using the Analysis of Variance (ANOVA), the Student's *t*-test, and the LSD-test.

Results and discussion:

Table 1 and Figure 1 show PCEs, MNiPCEs, and MNi in bone-marrow cells from mice after treating with 20 mg/kg.b.wt of MTX, 10, 15, and 20 mg/kg.b.wt of fumagillin. significant differences $p \leq 0.05$ were noticed in PCEs of fumagillin treated groups compared with negative control group which given 1:1 sugar solution. Significant differences were noticed in micronucleated polychromatic erythrocytes (MNiPCEs) and micronuclei (MNi) in treated groups with 15 and 20 mg/kg.b.wt of fumagillin. Micronucleated polychromatic erythrocytes and micronuclei increased in liner manner with increased dosage of fumagillin which may refer to genotoxic effects of the antibiotic.

Table 1: Means ± S.E. of PCEs, MNiPCEs, and MNi in bone marrow cells of white mice in negative control, positive control and test groups which treated with three different dosages of fumagillin

T/ D	No. of animals	No. of screened cells	PCEs M ± S.E	MNiPCEs M ± S.E	MNi M ± S.E
Sugar solution 1:1	5	1000	197.80 ± 1.74	10.22 ± 0.60	8.20 ± 1.39
Methotrexat 20mg/kg.b.wt	5	1000	530.60 ± 1.91**	110.40 ± 1.03	147.00 ± 6.80**
Fumagillin 10mg/kg.b.wt.	5	1000	206.60 ± 1.32*	8.20 ± 1.46	11.80 ± 2.35
Fumagillin 15mg/kg.b.wt.	5	1000	232.40 ± 1.43**	17.80 ± 1.28**	24.60 ± 2.37**
Fumagillin 20mg/kg.b.wt.	5	1000	345.20 ± 0.86**	33.60 ± 1.53**	53.00 ± 4.59**

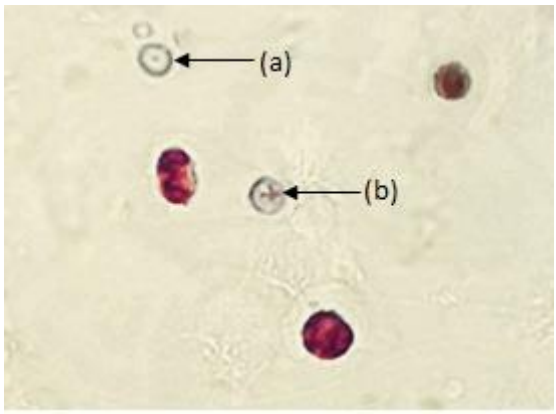

Figure 1: micronucleated polychromatic erythrocytes. May- Grunewald + Gimesa, 100 X. (a) single mn. (b) bi MNi.

The micronucleus test in erythrocytes of mouse bone marrow was proposed as a screening test by Boller and Schmid, (1970), and by Heddle, (1973). The frequency of micronuclei can be most easily evaluated in young erythrocytes shortly after the main nucleus is expelled. These young erythrocytes are termed polychromatic erythrocytes (PCE) and are distinguished from mature normochromatic (NCE) ones by their different staining properties. With a combination of Giemsa and May-Gruenwald staining the PCEs stain bluish to purple due to their high content of RNA in the cytoplasm. In contrast NCEs stain reddish to yellow and are also slightly smaller than PCEs. Treatment-induced micronuclei derived from chromosomal fragments produced during S-phase of the preceding cell cycle may not appear in PCEs earlier than 10 h after injection of the animal with the test chemical.

In this study, treatment with 10, 15, and 20 mg/kg. b. wt. of fumagilline induced significant increase $p \leq 0.05$ of PCES, MNPCEs, and MNi in dose related manner (table 1) which points to genotoxic properties of this antibiotic, this result in accordance with Stevanovic *et al*., (2006).

The current study showed significant increase $p \leq 0.05$ in PCEs which may refer to genotoxic effect that interfere with cell cycle mechanism. "Depression of bone marrow proliferation, evidenced in a drastic reduction of PCEs, adversely affects the micronucleus yields" (Venitt and Parry, 1984). On other hand increasing MNi PCEs may be related to increased frequency of PCEs.

Table 2 and figure 2 reveal noticed structural chromosome aberration in bone-marrow cells of white mice in each of negative control, positive control and test groups which treated with 10, 15, and 20 mg/kg.b.wt. of fumagillin. Results of the current study showed increased percentages of Rb translocation. Figure 3 shows banded metaphase chromosomes and other types of structural chromosome aberrations which were also noticed, so this result are in accordance with the previous *in vivo* findings of Natekar, (2007).

Stanimirovic *et al*., (2007b) reported the same findings with other fumagillin concentrations (25, 50, and 75 mg/kg b.w.)

"Gaps should not be counted as significant aberrations unless they present in a much higher than usual frequency" (Brusik, 1980). So results were analyzed with and without gaps. Open breaks should be considered as indicators of genetic damage, as should configurations resulting from repair of breaks. The later includes translocations, multiradials, rings, multicentrics, *etc*. Breaks are the most effect of genotoxic substance. It may be chromosomal when genotoxic effect occurred at G1 or it may be chromatid type if genotoxic effect occurred at G2 of mitotic cell cycle.

Other types of structural aberration may be resulted from breaks. Fragments may be resulted from one break or it may be from two interstitial breaks in one chromatid or it may be in the whole chromosome. When more than one break occurred in the chromosome, fragment may be centric which have centromere or a centric when it have no centromere. Centric fragment may be named as minute chromosome. A centric fragments may be loosed when sampling delayed, so deletion may be noticed without fragment. If time of sampling is accurate we may notice breaks, fragments, and/or deletions. Translocation may be resulted from two breaks occurred in two chromosomes at the same time. Two broken chromosomes may be rejoined and translocation occurred. When breaks affect centromeric region, centric fusion may be occurred and this type of aberration called Robertsonian translocation if it include acrocentric chromosomes. Ring chromosome may be resulted from two breaks in one chromosome at two regions about centromere. When considering probabilities one event more likely than two events may be happened at the same time in the same chromosome. Chemicals less likely than Radiation in causing more than one break in the same chromosome at one time, so watching ring chromosomes may be more difficult in a such study.

Table 3 shows numerical chromosome aberrations in bone-marrow cells of white mice in each of negative control, positive control and test groups which treated with 10, 15, and 20 mg/kg.b.wt. of fumagillin. Results indicate that means of aneuploidy and euoploidy less than that of negative control, so it may be refer that fumagillin treatment decreased this type of chromosome aberration in this study. So this results discordant with Kulic *et al* (2009) which reported that "the mean

number of numerical chromosomal abberrations, both aneuploidies and polyploidy rose considerably ($p<0.001$) to 31.75±1.28 and 5.37±0.7440, respectively".

In the current study significant increase $p\leq 0.05$ were noticed in structural aberrations with 15, and 20 mg/kg.b.wt. of fumagillin. Percentage of translocation increased with 10, 15, and 20 of fumagillin in positive manner when compared with negative control (table 2). Stanimirovic *et al*., (2006) reported "that 50 mg/kg.b.wt of fumagillin significantly increased the frequency of Rb translocations". They noticed after G-band analysis that chromosome 4 and 19 participated in the formation of unusual Rb metacentric. Rb translocation considered one of balanced aberrations, so it may be survived. "When this type of aberration occurred in sexual cells it may cause disorders in animals and humans" (Down syndrome, cancer, infertility, etc.).

Table 2: Structural chromosome aberration in bone-marrow cells of white mice treated with three different dosages of fumagillin

T/ D Mg/ kg.b.wt	gaps %	w. gaps M ± S.E.	w.o. gaps M ± S.E.	Cell with structural aberration				
				br%	frag%	del%	t%	r%
S. S 1:1	25	8.0 ± 1.09	6.0 ± 0.83	22.5	37.5	15.0	-	-
MTX 20	28.3	37. 40 ± 2.65**	26. 80 ± 2.03**	19.7	31.5	12.8	5.3	2.0
F 10	24.4	9. 80 ± 1.06	7. 40 ± 0.92	18.3	34.6	18.3	4.0	-
F 15	26.5	12. 80 ± 1.06**	9. 0 ± 0.92**	20.3	32.8	15.6	4.6	-
F 20	23.8	22. 60 ± 1.86**	17. 20 ± 1.42**	24.7	29.2	14.1	6.1	1.7

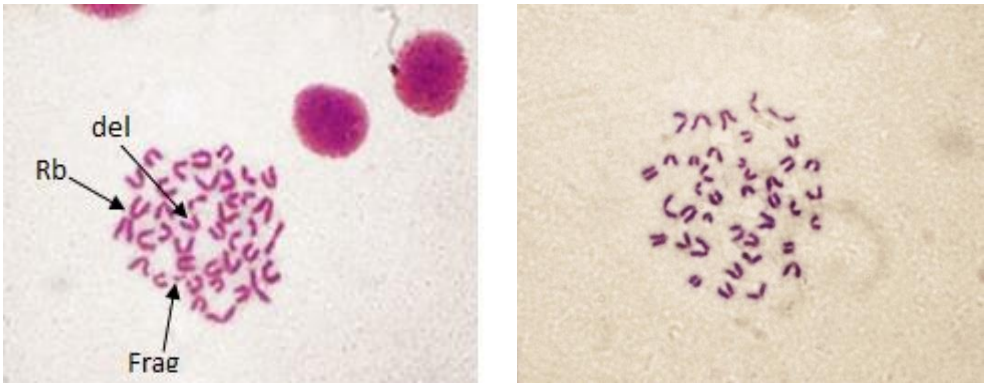

Figure 2: structural chromosome aberrations in treated and untreated mice. n= 40 Giemsa 100X. del: deletion. Rb: Robertsonian translocation. Frag: fragment.

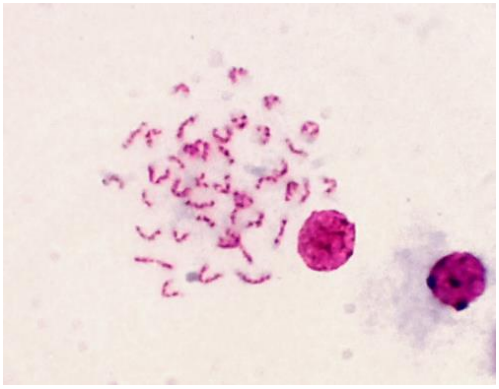

Figure 3: banded metaphase chromosomes. N=40 G. band. Giemsa. 100X.

Table 3: Numerical chromosome aberration in bone-marrow cells of white mice treated with three different dosages of fumagillin

T/ D Mg/ kg.b.wt	Cell with numerical aberrations		Aneu M ± S.E.	Eu M ± S.E.
	aneu%	euo%		
S.s 1:1	10.6	4.2	10.0 ± 0.31	1.4 ± 0.50
MTX 20	10.0	5.0	4.4 ± 0.50**	6.6 ± 0.67**
F 10	10.34	5.17	1.2 ± 0.20	1.8 ± 0.37
F 15	11.68	5.19	1.8 ± 0.37	2.6 ± 0.50
F 20	11.02	5.88	3.0 ± 0.31*	4.6 ± 0.74*

Table 4 shows percentage of dividing cells (MI) in mice bone-marrow of negative , positive and treated groups with 10, 15, and 20 mg/kg.b.wt. of fumagillin. The current study showed that MI decreased with increasing of dosages.

MTX the positive control is an anti-metabolite drug which means it is capable of blocking the metabolism of cells. It acts by inhibiting the metabolism of folic acid. "Methotrexate is cell cycle S-phase selective, and has greater negative effects on rapidly dividing cells (such as malignant and myeloid cells), which are replicating their DNA and thus inhibits the growth and proliferation of these cells" (Natekar, 2007).

This current study found that treatment with fumagillin caused decrease in proliferative cells in reverse relation with increased dosage. This may refer to cytotoxic effects which interfere with cell division if any kind of numerical chromosome aberration occurred with it. Or it may be considered as genotoxic effects if any kind of structural chromosome aberration occurred with it. The results of this study showed a significant $p \leq 0.05$ structural chromosome aberration in treated groups which support the suggestion of genotoxic effects of fumagillin.

Table 4: MI in bone-marrow cells of white mice treated with three different dosages of fumagillin

T/ D	No. of animals	No. of screened cells	Mitotic index(%) M ± S.E.
Sugar solution 1:1	5	1000	7.10 ± 0.29
Methotrexat 20mg/kg.b.wt	5	1000	4.80 ± 0.25**
Fumagillin 10mg/kg.b.wt.	5	1000	3.90 ± 0.29**
Fumagillin 15mg/kg.b.wt.	5	1000	3.60 ± 1.80**
Fumagillin 20mg/kg.b.wt.	5	1000	2.90 ± 1.80**

In table 5 figure 4 the noticed chromosome aberrations in primary spermatocytes of white mice in each of negative control, positive control and test groups which treated with 10, 15, and 20 mg/kg.b.wt. of fumagillin. Results of this study showed significant increase $p \leq 0.05$ in total chromosome aberrations especially with higher dosage of fumagillin 20 mg/kg.b.wt. (table 5). When considering percentages of chromosome aberration types, the current study noticed that percentages of autosomal univalents (A.uni.), chain IV (Ch.IV) and fragments (Frag.) increased in linear manner with the increased dosages of fumagillin.

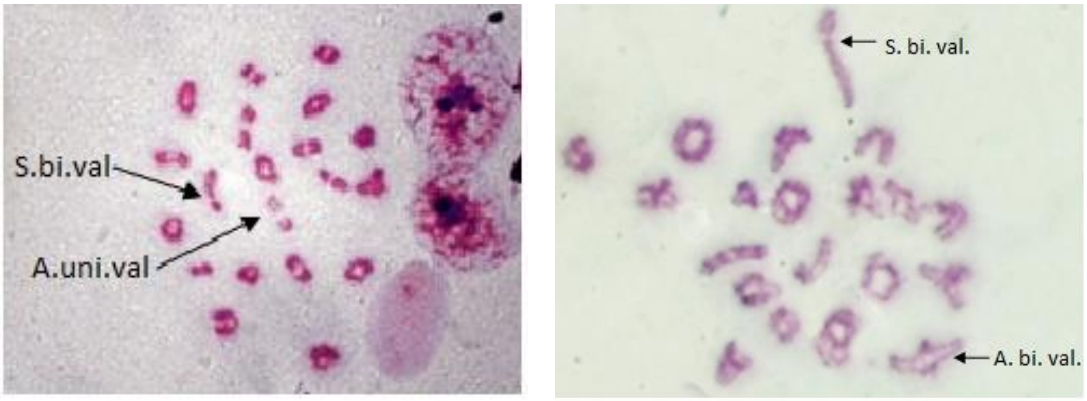

Figure 4: shows 20 bivalents in primary spirmatocytes in non treated, 19 bivalents and 2 univalent in treated mice. S. bi. Val.: sexual bi valents. A.bi. val.: Autosomal bivalents. A. uni. Val.: Autosomal uni valent.

Table 5 chromosome aberration in primary spermatocytes of white mice treated with three different dosages of fumagillin

T/D Mg/ kg.b.wt	A.uni. %	S.uni. %	Ch.IV %	Frag. %	Total M ± S.E.
S.s 1:1	11.7	64.5	23.5	-	3.4 ± 0.50
MTX 20	20	40	26.3	13.6	19.6 ± 1.48**
F 10	13.6	45.45	27.2	13.6	4.4 ± 0.81
F 15	18.6	37.2	27.9	16.2	8.6 ± 1.46
F 20	21.4	35.7	28.5	14.2	14.0 ± 2.07**

Meiotic preparations from seminiferous tubules a technique that introduced by Ford and Evans (1969), Were used for studying chromosomes of mouse primary spermatocytes at diakinesis and metaphase 1. In normal state we must find 19 autosomal and 1 sexual (X-Y) bivalents. Univalents, Qudrivalents, Ch. IV are the most types of chromosome aberrations in this kind of studies. More over we may watch structural aberrations like deletions, fragments, translocations and numerical

aberrations such as aneuploidy and euoploidy. In the current study, A. uni., S. uni., and Ch. IV were noticed (figure 4). Fragments were also noticed (table 5).

"Sterility**,** which can be directly correlated both with spermatogenic breakdown during meiosis and chromosome abnormality, has been reported in several animal species and in particular in certain hybrids, notably the mule" (Benirschke, 1967). "This can very well attributed to failed synapsis and subsequent gross genetic imbalance" (Hamerton, 1971).

Results of the current study concerning the increased frequencies of Micronuclei and structural chromosome aberrations (gaps, and Rb translocation) induced by fumagillin lead to the conclusion that fumagillin may have genotoxic effects that could increase risk for cancer and chromosomal aberrations and this in accordance with other *in vivo* studies (Stanimirovic *et al*.,2006; 2007a; Stevanovic *et al*., 2006; 2008). Beekeepers who are occupationally exposed to fumagillin may also be at genotoxic risk. Moreover, there is necessity for education of beekeepers concerning consumers' safety.

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