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FUMAGILLIN-INDUCED CHROMOSOME ABERRATIONS IN MOUSE BONE-MARROW CELLS

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Abstract – The genotoxicity of fumagillin was evaluated assessing the mitotic index and chromosome aberrations in mouse bone-marrow cells. Mice were given fumagillin orally in doses 5, 10 and 20 mg/kg b.w. All doses significantly ($p<0.001$) reduced the mitotic index. The medium and maximum doses led to significant ($p<0.01$ and $p<0.001$, respectively) increases in numeric aberrations. The highest dose induced both structural and numeric aberrations ($p<0.001$), and insertions on the first pair of autosomes that were amplified in the 1C and 1F regions. These results pointed to the genotoxic potential of fumagillin in the range of medium and maximum doses applied.

Key words: Fumagillin, genotoxicity, mitotic index, chromosome aberrations, insertions, BALB/c mice.

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INTRODUCTION

Fumagillin is an antibiotic derived from the fungus *Aspergillus fumigatus*. It has proved most effective in suppressing cryptosporidiosis and microsporidiosis caused by *Enterocytozoon bieneusi* which can be fatal in HIV-infected persons (Molina et al., 2000; Conteas et al., 2000). Due to its antiparasitic efficacy, fumagillin has also been widely applied in veterinary medicine against microsporidiosis of bees and fish (Katznelson and Jamieson, 1952; Bailey, 1953; El-Matbouli and Hoffman, 1991; Morris et al., 2003).

Fumagillin is very stable in honey (Furgala, 1962) where it was detectable after having been kept at 80°C for 35 days (Assil and Sporns, 1991). Mixed with syrup it is very effective in suppressing *Nosema* in hibernating honeybee colonies. However, it has not been effective against dormant spores of *Nosema apis*, or against *Nosema ceranae*.

To date some adverse effects of fumagillin have been discovered. After the treatment of *Nosema apis*-infected bees with fumagillin the electron-density of the mitochondrial matrix in corpora allata increased and the dimensions of the mitochondria diminished in comparison with untreated infected bees (Liu, 1990a). Fumagillin produced certain effects on the secretion granules of the hypopharyngeal glands of bees which increased and had a homogeneous structure, which is explained by the changes in the secretory activities of glands (Liu, 1990b). It was confirmed that fumagillin largely increased mortality in bees as well as the number of fungi (Rada et al., 1997).

Fumagillin has also been used in the treatment of microsporidiosis in fish including those caused by *Myxobolus cerebralis* (El-Matbouli and Hoffman, 1991; Karagouni et al., 2005) and *Tetracapsuloides bryosalmona* (Hedrick et al., 1988; Kent and Dawe, 1994; Le Gouvelo et al., 1999; Morris et al., 2003).

Fumagillin is effective in curing microsporidial keratoconjunctivitis (Roseger et al., 1993, Wilkins et al., 1994). It has appeared highly effective against chronic *Enterocytozoon bieneusi* infections in patients with immunodeficiencies (Molina et al., 2002). For further administration, however, appropriate therapeutical models should be significantly improved in order to avoid side effects (Conteas et al., 2000).

Data on the genotoxic effects of fumagillin obtained from *in vitro* studies showed discrepancy, being either positive (Stoltz et al., 1970; Stanimirović et al., 1999; Stevanović et al., 2000 and 2008; Kulić 2006) or negative (Purchase et al., 1978; Mortelmans et al., 1986; Heil, 1996). In addition, there are insufficient data on the genotoxic effects of fumagillin *in vivo*.

Having considered the aforementioned, there are plausible reasons for testing the genotoxic effects of fumagillin *in vivo* and *in vitro*; firstly, because the effects were different in various tests; secondly, they depended largely on the doses of fumagillin and, thirdly, there interactions of fumagillin with endogenous and exogenous factors have been reported (Ames, 1989; Albertini et al., 2000; Norppa, 2003; Stanimirović et al., 2005, 2007; Stevanović et al., 2008).

The aim of this work was to evaluate the possible *in vivo* genotoxic effects of fumagillin at doses lower than those used in bee-keeping.

MATERIAL AND METHODS

The genotoxic effects of fumagillin (dicyclohexylamine, CAS No. 101-83-7, Fumagillin–Evrotom, Serbia) were observed in the following three doses: 5, 10 and 20 mg/kg bw.

Fumagillin does not dissolve readily in water. For that reason, it was stirred in a small quantity of water (≥32-35°C) until it turned into a paste and a water-sugar syrup was gradually added. The solution was administered over seven consecutive days.

Five groups of mice were tested: three groups treated with fumagillin, the positive and negative control. Each group was comprised of six six-month-old BALB/c male mice weighing approximately 20 g. The animals were kept in unchanged conditions under 12/12-h light-dark periods at a constant temperature (21°C) with free access to food and water.

Table 1. Mitotic index in bone marrow cells treated with fumagillin. SE, standard error; SD, standard deviation; ***p<0.001, significant difference (LSD test).

Treatment	Doses	Mitotic index (%)		
		Min - Max		
Water-sugar syrup (negative control)	Conc. 1:1	5.89 – 6.01	Water-sugar syrup (negative control)	Conc. 1:1
	mg/kg b.w.	3.27 – 3.60	Fumagillin	5 mg/kg b.w.
Fumagillin	mg/kg b.w.	3.07 – 3.13		10 mg/kg b.w.
	20 mg/kg b.w.	2.13 – 2.28		20 mg/kg b.w.
Cyclophosphamide(positive control)	40 mg/kg b.w.	14.22 – 14.96	Cyclophosphamide(positive control)	40 mg/kg b.w.

The animals in the negative control group were treated with water-sugar syrup (1:1). Cyclophosphamide, a well-known clastogene and mutagene (Anderson et al., 1995), was used as the positive control and administered i.p. at a dose of 15 mg/kg body weight for seven consecutive days.

Cytogenetic analyses were carried out on the bone-marrow cells obtained from the femur and tibia according to Hsu and Patton (1969), modified by Zimonjić et al. (1990). Preparations were flame-dried and stained with Giemsa solution (Sigma Chemical Co., St. Louis, MO). G-banding was carried out by the tripsin method of Seabright (1971) and Ronne (1991). Chromosomes and sets of chromosomes were identified on the basis of criteria established by the Committee on Standardized Genetic Nomenclature for Mice (1979) and Cowell's photoatlas of mice chromosomes (Cowell, 1984).

Table 2. Cytogenetic parametres in the cells of bone marrow in BALB/c mice in control and experimental groups of animals treated by fumagillin.

Chromosome aberrations	No. of analyzed metaphases	Wather-sugar syrup 1:1 (negative control)		Fumagillin 5 mg/kg b.w.		Fumagillin 10 mg/kg b.w.			Fumagillin 20 mg/kg b.w.		Cyclophosphamide 40 mg/kg b.w. (positive control)
		Mean±SE	(%)	Mean±SE	(%)	Mean±SE			Mean±SE	(%)	Mean±SE
Aneuploidies	600	5.00±0.76	0.83	5.25±0.71	0.87	6.37±0.92	Aneuploidies	600	5.00±0.76	0.83	5.25±0.71
Poliploidies	600	0.25±0.46	0.04	0.50±0.53	0.08	0.75±0.89	Poliploidies	600	0.25±0.46	0.04	0.50±0.53
Gaps	600	2.00±0.93	0.33	2.37±0.52	0.39	2.62±0.74	Gaps	600	2.00±0.93	0.33	2.37±0.52
Acentrics	600	0.75±0.71	0.13	0.87±0.83	0.16	1.25±0.71	Acentrics	600	0.75±0.71	0.13	0.87±0.83
Insertions	600	0.00±0.00	0.00	0.00±0.00	0.00	0.25±0.46	Insertions	600	0.00±0.00	0.00	0.00±0.00

*** Statistically significant difference in comparison to negative control p<0.001,
** Statistically significant difference in comparison to negative control p<0.01

The mitotic index was determined in 1000 cells per treatment. Six-hundred well spread metaphases were analyzed for the presence of chromosome aberrations per each treated group. Statistical analysis was performed by Statistica 6.0 software programme, ANOVA, Student's t-test and LSD-test.

RESULTS

The genotoxic effects of fumagillin in the mouse bone-marrow cells were analyzed assessing the mitotic index (MI) and numeric (CNA) and structural chromosome aberrations (CSA). The doses tested were 5.0, 10.0 and 20.0 mg/kg bw.

All doses of fumagillin (Table 1) induced a significant decrease ($p<0.001$) in the mitotic index (MI=3.41±0.04, 3.11±0.01, and 2.21±0.02%, respectively) in comparison with the negative control (MI=5.94±0.01%). In addition, it was shown that in certain doses fumagillin is capable of provoking both numeric and structural chromosome aberrations.

At the lowest dose of 5 mg/kg bw, fumagillin did not seem to influence either structural or numeric chromosome aberrations in mouse bone-marrow cells, since the mean number of gaps, acentrics and insertions remained similar to the number in the negative control; the same was true for aneuploidy and poliploidy (Table 2).

Significantly increased frequencies ($p<0.01$ or $p<0.001$) of numerical chromosome aberrations (aneuploidies and poliploidies) were observed both in the medium (10 mg/kg bw) and the highest (20 mg/kg bw) dose of fumagillin.

Twice the minimum dose of fumagillin (10 mg/kg bw) resulted in aneuploidies only. Their average number rose to 6.37±0.92, which was significantly more ($p<0.01$) than in the negative control (5.00±0.76). Poliploidy was not more frequent than expected.

Fumagillin administered to BALB/c mice in the dose of 20 mg/kg for seven consecutive days had major consequences on the frequency of both numerical and structural chromosome aberrations. The mean number of numerical chromosome aberrations, both aneuploidies and poliploidies rose considerably ($p<0.001$) to 31.75±1.28 and 5.37±0.74, respectively.

Structural chromosome aberrations (gaps, acentrics and insertions) were noticeably more

frequent in comparison to the negative control only in the highest experimental dose of fumagilline (Table 2).

The average number of gaps more than doubled, increasing from baseline 2.00±0.74 to 5.75±0.89 ($p<0.001$). The frequency of acentric chromosomes rocketed from a negligible (0.75±0.71) in the negative control to 4.37±0.74 ($p<0.001$). The increase in insertions was dramatic ($p<0.001$) having reached 3.25±0.71 on average. After completing the G-band analysis and chromosomal identification, the presence of one large chromosome, unusual for the mouse karyotype, was observed (Figure 1). It was clear that the aberrant chromosome had a surplus of two insertions when compared to the normal autosome from this pair (Figure 2). These insertions were located in the region 1C5 (one segment) and 1E (three segments, between 1E3 and 1E4 bands) (Figure 3).

When taking into consideration total cytogenetic changes, a significant increase ($p<0.01$) in comparison to the negative control (8.00±2.30) was noticed in the medium (11.25±1.83) and in the highest (50.50±1.93) dose group ($p<0.001$).

DISCUSSION

Fumagillin has been frequently used for the suppressing of *Nosema apis* infections in honey bees (Katznelson and Jamieson, 1952; Bailey, 1953). In addition, in humans it has been recommended for curing microsporidial eye infections (Roseger et al., 1993, Willkins et al., 1994) and in the treatment of chronic *Enterocytozoon bieneusi* infections common in HIV patients (Molina et al., 2000, 2002; Conteas et al., 2000). Due to high stability in bees' food (Assil and Sporns, 1991) its residua in honey or other bee products can easily reach final users (Stanimirović et al., 1999, 2005, 2006, 2007; Stevanović et al., 2000, 2006, 2008; Kulić 2006). Furthermore, the data on its genotoxic effects *in vitro* are contradictory: while the results of one group of authors are positive (Stoltz et al., 1970; Stanimirović et al., 1999; Stevanović et al., 2000; Kulić 2006; Stevanović et al., 2008), others have presented negative findings (Purchase et al., 1978; Mortelmans et al., 1986; Heil, 1996). Moreover,

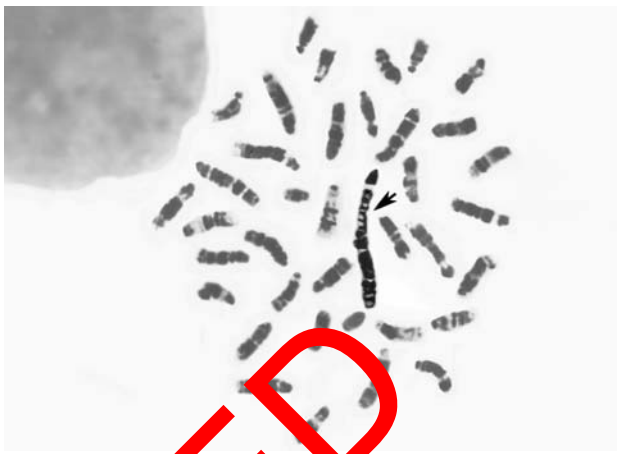

Figure 1. G-band karyotype of a BALB/c mouse treated with fumagillin (20 mg/kg bw) with one large chromosome

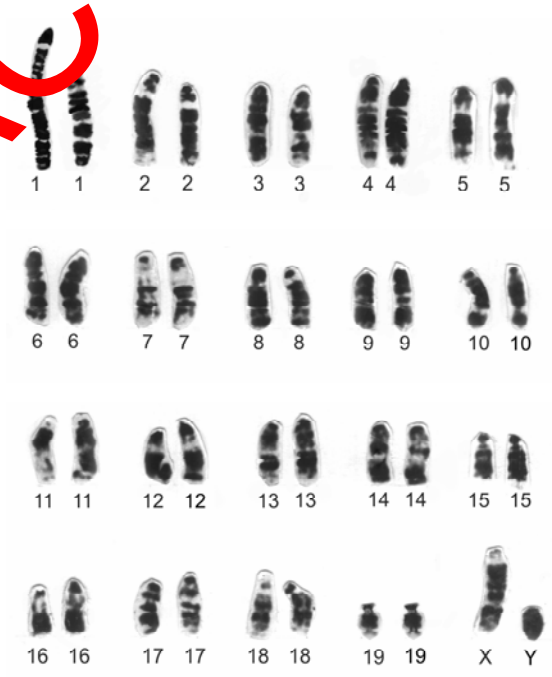

Figure 2. G-band karyogram of a BALB/c mouse treated with fumagillin (20 mg/kg bw) with heterozygous insertions in 1C5 and 1E regions of the first pair of autosomes

there is very little information on the genotoxic effects of fumagillin *in vivo,* which was obtained with much higher doses (Stevanović et al., 2006; Stanimirović et al., 2007). Having analyzed the aforementioned it is clear that further evaluation of the genotoxic effects of fumagillin is necessary.

In our *in vivo* study the MI and CA were monitored in bone-marrow cells of BALB/c mice treated with selected doses of fumagillin (5, 10, and 20 mg/kg bw). The results showed that all experimental doses induced a significant decrease ($p < 0.001$) in MI in comparison with the negative control. As expected, treatment with cyclophosphamide resulted in an increase in the MI. These results are in accordance with the findings of many other authors who examined the antiproliferative effects (anti-angiogenic effects) of fumagillin (Ingber et al., 1990; Wang et al., 2000; Mazzanti et al., 2004). The molecular target of fumagillin and its analogue TNP-470 is methionine aminopeptidase-2 (MetAP-2) (Griffith et al., 199[illegible]; Sin *et al.,* 1997; Liu et al., 1998). Fumagillin binds MetAP-2 on His-231, inactivating the enzyme. MetAP-2 removes the N-terminal methionine from most proteins involved in cell-cycle regulation as part of the translocation process, so its inhibition results in cell-cycle arrest and apoptosis (Fardis et al., 2003). These findings provided a starting point for the rational design of novel fumagillin analogues with fewer side effects (Fardis et al., 2003).

The results of the present study point to the capability of fumagillin at certain doses to cause numerical and structural chromosome aberrations *in vivo*. The highest tested dose (20 mg/kg bw) resulted in the increase in the frequencies of aneuploidies, poliploidies, gaps, acentrics and insertions ($p<0.001$). These results are in accordance with the previous *in vivo* findings of Stanimirović et al. (2007), although, the doses of fumagillin investigated by him were much higher (25, 50 and 75 mg/kg bw) than those in the current work (5, 10 and 20 mg/kg bw). Our *in vivo* findings of chromosome aberrations agree with the previous *in vitro* results of Stanimirović et al., (1999),

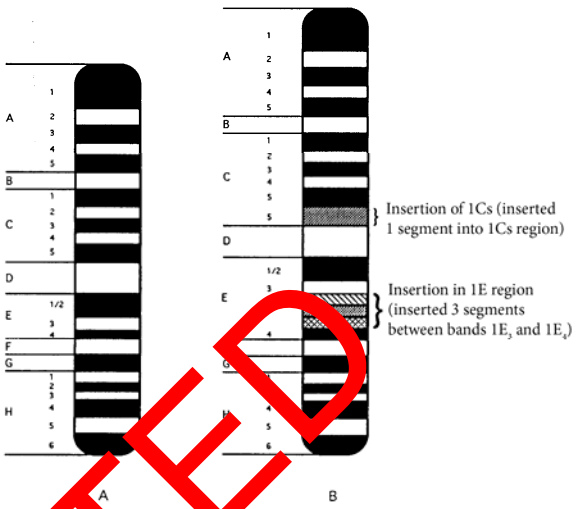

Fig. 3. Idiogram of the first pair of autosomes of a BALB/c mouse (A-normal chromosome, B-chromosome with insertions in 1C5 and 1E region)

Stevanović et al. (2000 and 2008) and Kulić (2006), which claimed that fumagillin significantly increased the frequencies of structural chromosome aberrations.

Indeed, *in vitro* research on fumagillin has not revealed indicators of its genotoxic potential in the Salmonella test using *Salmonella typhimurium* types TA 98, TA 100, TA 1535 and TA 1538 (Purchase et al., 1978; Mortelmans et al., 1986). On the other hand, in the research carried out by Stoltz et al., (1970) human lymphocytes incubated in the presence of dicyclohexylamine sulfate for 5 and 24 hours showed a marked dose-dependent increase in aberrations.

The National Toxicology Program (2006a, b) has reported positive genotoxic effects of dicyclohexylamine nitrate in the Salmonella test (National Toxicology Program 2006a) while dicyclohexylamine did not exhibit genotoxicity (National Toxicology Program 2006b). Also, there are certain data on the genotoxic effects of secondary metabolites (gliotoxin and verruculogen) of *Aspergillus fumigatus* which fumagillin is derived from. Gliotoxin causes changes in the DNA

(Golden et al., 1998) and it appeared to be genotoxic in *in vitro* test systems (Niemien et al., 2002); meanwhile, verruculogen produced effects in Salmonella/microsomal mutagenicity assays (Sabater-Vilar et al., 2003).

The results of our research clearly showed an increase in the frequency of chromosome aberrations provoked by fumagillin, especially at the dose of 20 mg/kg bw, which significantly influenced the amplification of certain chromosome regions on one of the chromosomes of the first pair of autosomes in a certain number of the animals tested. By G-band analyses we showed that these insertions were located in the region 1C5 (one segment) and 1E (three segments, between 1E3 and 1E4 bands). These results are in accordance with the findings of Kulić (2006), who investigated a synthetic analogue of fumagillin (dicyclohexylamine-ET).

Also, the same type of insertion was found when investigating some other substances, e.g. levamisole hydrochloride *in vivo* in rats (Stanimirović et al., 1998). These authors claim that the insertions provoked by levamisole hydrochloride were observed on the q arm of the first pair of autosomes between q21 and q22, where the q21a derived from endogenous duplication or amplification of DNA region was inserted. These findings point to the instability of the first pair of autosomes in mice and rats, which was already described by Agulnik et al. (1988, 1990) and Stanimirović et al. (1995, 1998). For a more certain explanation of the reason for the insertion further investigations are necessary.

Finally, although there is no reliable information regarding fumagillin residue levels in food except those of Mladjan and Jović (2000) and Kulić (2006), our results concerning the increased frequencies of chromosome aberrations (poliploidy, aneuploidy, gaps, acentrics and insertions) induced by fumagillin lead to the conclusion that fumagillin residues in food may have genotoxic effects that could increase the risk for chromosome aberrations and cancer (Stanimirović et al., 2006, 2007; Stevanović et al., 2006, 2008). Moreover, beekeepers, who are occupationally exposed to fumagillin, may also be at genotoxic risk. There is an urgent necessity for their compulsory education concerning consumers' safety. Similar caution should be taken with patients treated with fumagillin against microsporidia.

Additional studies of the adverse effects of fumagillin should be undertaken in order to provide all the necessary data to define a minimal residual level for this substance, our results should not be disregarded in any case.

REFERENCES

Агулник С.И., Агулник А.И., А.О. Рувинский (1990). Мейотический драйв абберантной 1-j хромозоми у домовой мыши. Генетика, **6**, 664-669.

Агулник С.И., Орлов И.П., А.И. Агулник (1988). Новий вариант 1-j хромозоми у домовой мыши. Цитология, **30**, 773-778.

Albertini, R.J., Anderson, D., Douglas, G.R., Hagmar, L., K. Hemminki (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. *Mutat. Res.* 463, 111-172.

Ames, B.N. (1989). Mutagenesis and carcinogenesis: Endogenous and exogenous factors. *Environ. Molec. Mut.* 14, Suppl. **16**, 66-77.

Anderson, D., Bishop, J.B., Garner, R.C. and P.B. Selby (1995). Cyclophosphamide: review of its mutagenicity for an assessment of potential germ cell risk, *Mutat. Res.* 330, 115–118.

Assil, H.I. and *P. Sporns*, (1991). ELISA and HPLC methods for analysis of fumagillin and its decomposition products in honey. *J. Agric. Food Chem.* **39**, 2206-2213.

Bailey, L. (1953). Effect of fumagillin upon Nosema apis (Zander). *Nature* **171**, 112-213.

Committee on Standardized Genetic Nomenclature for Mice (1979). New rules for nomenclature of genes, chromosome anomalies and inbred strain of mous. *Mouse News Letter* **61**, 4-11.

Conteas, C.N., Berlin O.G., Ash, L.R., J.S. Pruthi (2000). Therapy for human gastrointestinal microsporidiosis. *Am. J. Trip. Med. Hyg.* **63**, 121-127.

Cowel, J.K. (1984). A photographic representation of the variability of the G-banded structure of the chromosomes of the mouse karyotype. *Chromosoma* **89**, 294-320.

El-Matbouli, M., R.W. Hoffmann (1991). Prevention of experimentally induced whirling disease in rainbow trout Oncorhynchus mykss by Fumagillin. *Dis. Aquat. Organ.* **10**, 109-113.

Fardis M., Pyun, H.J., Tario, J., Jin, H., Kim, C.U., Ruckman, J., Lin, Y., Green, L. and B. Hicke (2003). Design, synthesis and evaluation of a series of novel fumagillin analogues. *Bioorg. Med. Chem.* **11**, 5051–5058.

Furgala, B. (1962). Residual fumagillin activity in sugar syrup stored by wintering honeybee colonies. *J. Apic. Res.* **1**, 35-37.

Golden, M.C., Hahm, S.J., Elessar, R.E., Saksonov, S. and *J.J. Steinberg* (1998). DNA damage by gliotoxin from Aspergillus fumigatus. *Mycoses*, **41**, 97-104.

Griffith E.C., Su, Z., Niwayama, S., Ramsay, C.A., Chang, Y.H. and *J.O. Liu* (1998). Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2, *Proc. Natl. Acad. Sci. USA*. **95**, 15183–15188.

Hedrick, R.P., Groff, J.M. and *T. McDowell* (1988). Oral administration of Fumagillin DCH protects Chinook salmon Oncorhynchus tshawytscha from experimentally-induced proliferative kidney dise*. *Dis. Aquat. Organ.* **4**, 165-168.

Heil, J., Reifferscheid, G., Waldmann, P., Leyhausen, G. and *W. Geurtsen* (1996). Genotoxicity of dental material*. *Mutat. Res.* **368**, 181-194.

Hsu, T.C. and *G.L. Patton* (1969). Bone marrow p*parations for chromosome studies. In: Comparative mammalian cytogenetics. (Ed. Benirschke), 1-395. Springer-Verlag, Berlin.

Ingber D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H. and *J. Folkman* (1990). Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature*, **348**, 555-557.

Karagouni, E., Athanassopoulou, F., Lytra, A., Komis, C. and *E. Dotsika* (2005). Antiparasitic and immunomodulatory effects of innovative treatments against *Myxobolus sp.* infection in *Diplodus puntazzo*. *Vet. Parasitol.* **134,** 215-228.

Katznelson, H. and *C.A. Jamieson* (1952). Control of Nosema disease of honey-bees with fumagillin. *Science* **115**, 70-71.

Kent, M.L. and *S.C. Dawe* (1994). Efficacy of fumagillin DCH against experimentally-induced Loma salmonee (Microsporea) infections in Chinook salmon Oncorhynchus tshawytscha. *Dis. Aquat. Organ.* **20**, 231-133.

Kulić, M. (2006). Research on genotoxic potential of dicyclohexylamine in vivo and in vitro. PhD Thesis, Faculty of Biology, University of Banjaluka.

Le Gouvello, R., Pobel, T., Richards, R.H. and *C. Gould* (1999). Field efficacy of a 10-day treatment of fumagillin against proliferative kidney disease in rainbow trout Oncorhynchus mykiss. *Aquaculture*, **171**, 27-20.

Liu S., Widom, J., Kemp, C.W., Crews, C.M. and *J. Clardy* (1998). Structure of human methionine aminopeptidase-2 complexed with fumagillin. *Science*, **282**, 1324–1327.

Liu, T.P. (1990a). Ultrastructure of mitochondria in the corpora allata of honeybees infected by Nosema apis before and after treatment with anti-Nosema drugs. *Tissue Cell*, **22**, 511-515.

Liu, T.P. (1990b). Ultrastructural changes in the secretion granules of the hypopharingeal glands of the honeybee infected by Nosema apis and after treatment with fumagillin. *Tissue Cell*, **22**, 523-531.

Mazzanti C.M., Landle, A., Lorang, D., Costouros, N., Roberts, D., Bedlack, G., and *K. Libutti* (2004). Early genetic mechanisms underlying the inhibitory effects of endostatin and fumagillin on human endothelial cells. *Genome Res.* **14**, 1585–1593.

Mladjan, V. and *M. Jović,* (2000) Antibiotics in beekeeping. Proceedings of the 2nd Symposium in Animal Clinical Pathology and Therapy, *Clinica Veterinaria*, Budva, 211-212.

Molina, J.M., Goguel, J., Sarfati, C., Michiels, J.F., Desportes-Livagel, I. and *S. Balkan* (2000). Trial of oral fumagillin for the treatment of intestinal microsporidiosis in patients with HIV infection. *AIDS*, **14**, 1341-1348.

Molina, J.M., Tourneur, M., Sarfati, C., Chevret, S. and *A. de Gouvello* (2002). Fumagillin treatment of intestinal microsporidiosis. *N. Engl. J. Med.* **346**, 1963-1969.

Morris, D.J., Adams, A., Smith, P. and *R.H. Richards* (2003). Effects of oral treatment with TNP-470 on rainbow trout (Oncorhynchus myksis) infected with Tetracapsuloides bryosalmonae (Malacosporea), the causative agent of proliferative kidney disease. *Aquaculture*, **221**, 51-64.

Mortelmans, K., Haworth, S. Lawlor, T., Speck, W., Tainer, B. and *E. Zeiger* (1986). Salmonella mutagenicity test: II. Results from testing of 270 chemicals. Environ. Mutagen. **8** (Supl. 7), 1-119.

National Toxicology Program (2006a). http:/ntp.niehs.nih.gov/index.cfm?objectid=6DE0B88C-F1F6-975E-7A19428A3AD94D85.

National Toxicology Program (2006b). htpp://ntp.niehs.nih.gov/index.cfm?objectd=6DE0E43F1F6-975E-7B0FA8000D056A43

Nieminen, S.M., Maki-Paakkanen, J., Hirvonen, M.R., Roponen, M. and *A. von Wright* (2002). Genotoxicity of gliotoxin, a secondary metabolite of Aspergillus fumigatus, in a battery of short-term test systems. *Mutat. Res.* **520**, 161-170.

Norppa, H. (2003). Cytogenetic biomarkers and genetic polymorphism. *Toxicol. Lett.* **144**, Suppl. 1, 25.

Purchase, I.F.H., Longstaff, E., Ashby, J., Styles, J.A. and *D. Anderson* (1978). An evaluation of six short-term tests for detecting organic chemical carcinogens. *Br. J. Cancer*, **37**, 873-959.

Rada, V., Machova, M., Huk, J., Marounek, M. and *D. Duskova* (1997). Microflora in the honeybee digestive tract - counts, characteristics and sensitivity to veterinary drugs. *Apidologie*, **28**, 357-365.

Ronne, M. (1991). High resolution banding: present aspects. *Gen. Sel. Evol.* **23**, Suppl. 1, 49s-55s. Elsevier/INRA.

Roserger, D.F., Serdaravic O.N., Evlandson, R.A., Bryan, R.T. and *D.A. Schwartz* (1993). Successful treatment of mikrosporidial keratoconjuctivitis with topical fumagillin in a patient with AIDS. Cornea, **12**, 261-265.

Sabater-Vilar, M., Nijmeijer, S. and *J. Fink-Gremmels* (2003). Genotoxicity assessment of five tremorgenic mycotoxins (fumitremorgen B, paxilline, penitrem A, verruculogen, and verrucosid) produced by molds isolated from fermented meats. *J. Food. Prot.* **66**, 2123-2129.

Seabright, M. (1971). A rapid banding technique for human chromosomes. Lancet, **2**, 971-972.

Sin N., Meng, L., Wang, M.Q., Wen, J.J., Bornmann, W.G. an *C.M. Crews* (1997). The anti-angiog agent fumagillin covalently binds and inhibits the meth nine aminopeptidase MetAP-2. *Proc. Natl. Acad. S* 94, 6099–6103.

Stanimirović Z, Stevanović, J., Kulić, Ž. a *V. Stojić* 2006). Frequency of chromosomal berrations a evaluation of genotoxic potential of di clohexylamine fumagilline) in vivo. *Acta Vet.* **4**, 35 366,

Stanimirović, Z., Stevano J., B V. a *I. Radović* (2007). Evaluation of geno xic fe s of fumagillin by cytogenetic ts in v *Mutat. Res.* **628**, 1–10.

Stanimirović, Z., Ste ović, J., Jovanović, S., and *M. Andjelković* (2005) Evalution of genotoxic effects of Apitol® (cymiazole hydrochloride) in vitro by measurement of sister chromatide exchange. *Mutat. Res.* **588**, 152-157.

Stanimirović, Z., Stevanović, J. and *D. Pejović* (1999). Analysis of genotoxic effects Fumagilin®-ET. In: Proceedings of 29th Annual Meeting of the European Environmental Mutagen Society EEMS-99 (85), Copenhagen, 44-45.

Stanimirović, Z., Vučinić, M. and B. *Soldatović* (1998). Cytogenetic changes in bone-marrow cells of wistar rats induced by levamisole hydrochloride. *Acta Vet.* **48**, 255-262.

Stanimirović, Z., Vučinić, M., Soldatović, B. and *M. Vučićević* M. (1995). A large acrocentric chromosome in first par of autosomes in natural populations of Mus musculus Linne, 1758, *Acta Ve* **45**, 15 0.

Stevanović, J., Stanimirov Z., Djeli N. and *S. Djurković* (2000). Is th applica n of umagillin in nosemosis treatment ceptab e fro genotoxicological point of view. Pro edi s of the 2nd Symposium in Animal Clin al Pat logy a Therapy Clinical Veterinaria 2 Budva, 22 2 .

Stevanović, J., Stanimirović, Z., Pejin, I. and *M. Lazarević* (2006). Mo itoring of mitotic index and frequency of micronuclei in evaluation of genotoxic potential of fum illin (dicyclohexylamine) in vivo. *Acta Vet.* **56**, 437 48.

Stevanović, J., Stanimirović, Z., Radaković, M., and *V. Stojić* (2008). In vitro evaluation of the clastogenicity of fumagillin. *Environ. Mol. Mutagen.* **49**, 594-601

Stoltz, D.R., Khera, K.S., Bendall, R. and *S.W. Gunner* (1970). Cytogenetic studies with cyclamat and related compounds. *Science* **167**, 1501-1502.

Wang J., Lou, P. and *J. Henkin* (2000). Selective inhibition of endothelial cell proliferation by fumagillin is not due to differential expression of methionine aminopeptidases. *J. Cell. Biochem.* **77**, 465–473.

Wilkins, J.H., Joshi, N., Margolis, T.P., Cevallos, V. and *D.R. Dawson* (1994). Microsporidial keratoconjunctivitis treated successfully with a short courses of fumagillin. *Eye* **8**, 703-704.

Zimonjić, D.B., Savković, N. and M. *Andjelković* (1990). Genotoksični agensi: efekti, principi i metodi detekcije, 1-395, Naučna knjiga, Beograd.

ХРОМОЗОМСКЕ АБЕРАЦИЈЕ ПРОУЗРОКОВАНЕ ФУМАГИЛИНОМ У ЋЕЛИЈАМА КОСТНЕ СРЖИ МИША

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Испитивана је генотоксичност фумагилина праћењем митотског индекса и хромозомских аберација у ћелијама костне сржи миша. Мишеви су третирани п.о. дозама 5, 10 и 20 мг/кг т.м. Све испитиване дозе фумагилина сигнификантно смањују митотски индекс (п<0,001). Средња и највећа доза сигнификантно (п<0,01, односно п<0,001) повећавају учесталост нумеричких аберација. Највиша доза индукује структурне и нумеричке аберације високо сигнификантно (п<0,001). Фумагилин изазива појаву инсерција на првом пару аутозома. Ради се о амплификацији у 1Ц региону сегмента означеног као 1Ц5 траке, односно оба сегмента (1Е3 и 1Е4 траке) 1Е региону. Резултати указују да фумагилин у опсегу средње и највеће дозе испољава генотоксичност.