CYTOGENETIC EFFECTS OF HONEY CONTAMINATED WITH FUMAGILLIN (DICYCLOHEXYLAMINE) ON MALE MICE *MUS MUSCULUS* BALB/C *IN VIVO*

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(Accepted for publication: June 9, 2013)

Abstract

Cytogenetic effects of honey contaminated with fumagillin that collected from different sources in Duhok province was investigated in mouse bone-marrow cells using damaged cells%, (chromatids and chromosomal aberrations and mitotic index (M.I). A group of mice was orally administrated with honey that gave a positive result with qualitative chemical test. Fumagillin was administered to another group of mice by gavage, at doses of 25, 50, 75 mg/kg body weight (b.w) prepared with honey that give a negative result by biochemical test as artificially contaminant honey. All mice were treated for two different periods, 7 and 35 days at 24-hrs intervals .The treated groups were compared with negative control and Cyclophosphamide (40 mg/kg bw) as a positive control. The biochemical test for all honey samples shows that 16.67% of honey samples were contaminated with fumagillin. The honey sample that give a positive result in the presence of fumagillin in biochemical test revealed its ability to increase the damage cell% and chromosomal aberrations in bone marrow cells after 7 and 35 days of treatment .The ability of this sample equivalent to that of honey experimentally contaminated with fumagillin (25 mg/kg b.w).The result of the present study shows that the contaminated honey sample revealed its ability to reduce the M.I after 35 days of treatment in bone marrow cells as compared to negative controls.

Introduction

Honey is a food used since the most remote times and which was appreciated for its characteristic flavor, considerable nutritional value and medicinal properties (Geni *et al*., 2007).The carbohydrates are the main constituents, comprising about 95% of the honey dry weight. Honey also contains organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds (Heitkamp*et al*., 1986), choline, and acetylcholine (Heitkamp, 1984).Most of these compounds known to have antioxidant properties. In most ancient cultures honey has been used for both nutritional and medical purposes (Allsop and Miller, 1996). The relation of the human with the bees goes back to the stone age (Crane, 1983), and the first written reference to honey, was found with a Sumerian tablet writing, that mentions honey used as a drug and an ointment (Crane, 1975). There are several types of contaminants in our countries which can found their ways to honey .These contaminants come from several sources .These sources can be environmental(indirect contamination) or from beekeepers (direct contamination) (Emmanouel *et al* .,2008). The direct contamination, such as: residues of drugs that used in the treatment of bee disease (Louveaux, 1985). The main problem is the contamination by antibiotic, used against the bee brood diseases (Emmanouel *et al*.,2008). Fumagillin **(Dicyclohexylamine)** has acquired importance in veterinary medicine against microsporidiosis of bees (Morris *et al*., 2003).According to the European Agency for the Evaluation of Medicinal Products EMEA (2000) , treatment of Nosema infections in honey bees, fumagillin is the only chemical registered. In the world the utilization of fumagillin is limited because of its toxic side effects (Didier, 2005).Study of Stevanovic *et al*., (2008) indicated that fumagillin may possess genotoxic effects *in vitro*, or may not (Heil *et al*., 1996).Till year 2000 there were no references regarding the genotoxic effects of fumagillin *in vivo*(Toxicological Evalution, 2000). Stanimirovic *et al*., (2007) reported that fumagillin could cause teratogenesis and have genotoxic effects. Stanimirovic *et al*., (2007) investigated the presence of sister chromatid exchange (SCE) in human culture lymphocytes treated with fumagillin. Significant increase of numerical and structural chromosomal aberrations (CA)were observed in cells of mice bone marrow (Stanimorvic *et al* .,2006), whereas Stanimorvic *et al*.,(2010)

reported that the fumagillin has ability to reduce the M.I in mice *in vivo*.

Generally, the contamination levels with antibiotic in Europe do not present a health hazard, and this problem seems to be under control. In the European Union antibiotics are not allowed for that purpose, and thus honey containing antibiotics is also not permitted to be traded on the market (Bogdanov *et al*., 2008). The presence of antibiotic residues in honey and other hive products is not accepted in Europe. In case a product is found contaminated with antibiotics then it should be destroyed and the producer should be penalized. In our countries the honey containing antibiotics such as fumagillin in contamination levels may be found in the markets due to lack of laws and regulation in this regard. There are no references regarding cytogenetic and mutagenic effects of honey contaminated with fumagillin *in vivo* or *in vitro* . Therefore the present study aims to; detect the presence of fumagillin as residues in local honey ,study the cytogenetic effects of local honey that revealed a positive result to the presence of fumagillin by a biochemical test on bone marrow cells, and study the cytogenetic effects of local honey experimentally contaminated with different doses 25, 50, and 75mg/kg b.w of fumagillin*in vivo*.

Materials and Methods

Thirty samples of honey were collected from different regions Zaxo, Batefa, Zaweta, Sheladiz, Deralok, Akra, Denarta and college of Agriculture apiary in Duhok Governorate during January, February and March 2010. The alcohol moiety of fumagillin (alcohol-I) has been obtained as crystalline and has been shown to contain epoxide grouping. Estimation of epoxide contents compound can reflect the presence of fumagillin as mentioned by (John *et al*., 1956). Each sample revealed positive result (In the presence of fumagillin) was isolated and identified by Thin Layer Chromatography (TLC). Identification of fumagillin in honey samples using TLC is depended on a modified procedure of Richard *et al*., (1989).

Adult male Swiss albino mice (*Mus musculus*) BALB/c with age 8-10 weeks and an average weight of 26-28 grams were grouped into two groups, each group was sub divided in to eight sub groups of mice and treated for two different periods, 7 and 35 days. Each group of mice was put in a separate cage. All groups had equivalent numbers of animals per experiment. Thus, for the cytogenetic test five male mice were used per dose group. Mice were orally administrated with Fumagillin. Fumagillin doses were choosing according to Stanimirovic *et al*. (2007). Fumagillin does not dissolve readily in water. To prepare medicated water-honey syrup, it is recommended to mix fumagillin in small amounts of warm water (not above 32–34°C) and stir into a paste, then the water-honey syrup was added gradually and shake the container occasionally. The Fumagillin mixture was admixed with water–honey syrup shortly before use. The chromosomes were prepared using (Evans *et al*., 1964) method with some modifications .Chromosomal aberrations in bone marrow cells were investigated using Olympus light microscope under magnification power of 100 X oil immersion objective lenses, whereas 40 X objective lens was used to investigate the M.I . The data were analyzed statistically using Statistical Analysis System (SAS, 2010) Program. The Chromosomal Aberrations and M.I were analyzed using Factorial experiments arranged in Completely Randomized Design (C.R.D) which was used to study the effect of treatment, periods and their interaction. The least significant difference (LSD) were used to determine the statistical analysis of the result and P-values from ANOVA tables were tested at $p<0.05$, $P<0.01$ and $P<0.001$ (Steel and Terrie, 1980).

Results

Qualitative Determination of Fumagillin:

The result of qualitative biochemical test shows that, five honey samples of the 30 were positive (16.67%) for the presence of the fumagillin. The red color result of the qualitative chemical analysis method was used as an indicator for the presence of fumagillin in the sample. Depending on these results the positive samples were isolated and identified by TLC(figure 1).

Figure (1) shows the result of qualitative determination of Fumagillin in honey samples.**1-** negative control (acetone, sodium thiosulfate, and phenolphthalein) ,2-Positive control(fumagillin , acetone, sodium thiosulfate, and phenolphthalein) ,3- Positive result, red color (sample contaminated with fumagillin),4,5 and 6- negative results (sample non contaminated with fumagillin).

Identification of fumagillin by Thin Layer Chromatography (TLC)

The results of TLC analysis(figure 2) revealed that the rate of flow (Rf) (Rf = 0.94 cm) were similar for both the standard fumagillin sample(No.1) and honey sample that has been contaminated experimentally with fumagillin (No.2).The contaminated honey sample (No.3) also showed same results (Rf 0.94 cm).

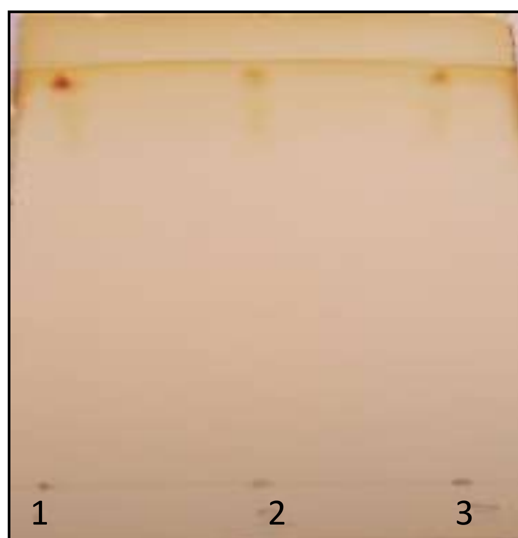

Figure (2): Thin Layer Chromatography of fumagillin after exposure with iodine 1- Standard Fumagillin 2- Honey experimentally contaminated with fumagillin. 3- Sample contaminated with fumagillin .Rate of flow (Rf) for all the three samples were 0.94 cm.

Effects of fumagillin and contaminated honey with fumagillin on chromosomal aberrations in bone marrow cells of Albino male mice

The results revealed a highly significant effect of fumagillin and honey contaminated with fumagillin ($P \leq 0.01$) on the damaged cells and chromosomal aberrations of all eight treatments. The periods and the interaction between treatments and periods also showed highly significant effect ($P \leq 0.01$) on the damaged cells and in all types of chromosomal aberrations except in centromeric break, the differences was non–significant. The effects of honey contaminated with fumagillin increased gradually with increasing of concentrations of fumagillin as it's clear from the value of damaged cells as compared to negative control (Table-1).The present study shows several types of chromatid and chromosomes aberrations; Chromatid break with fragment, Chromatid break without fragment , Ring chromosome, centromeric break, chromatid gaps .By using least significant differences (L.S.D), there were no differences between the damaged cells values in both control negative groups and mice treated orally with honey that revealed negative result by biochemical test in both periods of treatments 7 and 35 days .There were significant differences between the damaged cells values in both periods, the value of damaged cells in the first period was (29.63±3.243) increased to (37.38±4.089) in the second period (Table-1).

The interactions between treatments and periods indicated that the highest value of damaged cells is (89±0.836) in treatment with cyclophosphamide (+ve control) after 35 days, and by using least significant differences (L.S.D), there were differences between the damaged cells values of cyclophosphamide (+ve control) and value of damaged cells in mice treated with fumagillin alone (75mg/kg b.w) 89±0.836 and 57±0.316, respectively. The result of present study in Table(1) shows that the value of damaged cells in mice treated with honey contaminated with fumagillin after 7 days treatment was (15.8±0.0200) increased after 35 days of treatment in to(20.0±0.774). Statistically the effect of 25 mg/kg b.w revealed the same effect of honey

contaminated with fumagillin .The most common affected type of chromosomal aberrations was ring chromosome with value of 22.80±0.374 after treatment with cyclophosphamide (+ve control) after 35 days, followed by chromatid gap with value of 18.20 ±0.374 in the same treatment, whereas the least value of 1.20±0.200 for chromatid break without fragments was found in mice treated with honey that give negative result in biochemical test.

The results of present study was revealed highly significant differences (P≤0.01)between two periods of treatment 7 and 35 days in M.I of bone marrow cells. After 7 days of treatment there were no differences between M.I values in both control negative groups and mice those treated orally with honey that revealed negative result by biochemical test; 13.28±0.324 and 13.26± 0.287,respectively (Table -2) .The results indicated that the low value (6.60 ± 0.539) for M.I analyses of bone marrow cells was found in mice those treated with fumagillin alone with 75mg/kg b.w . Table (2) shows that all experimental doses of fumagillin prepared by water-honey syrup 25, 50, and 75mg/kg b.w induced decrease of M.I 8.48±0.837, 8.06±0.472, and 6.64±0.429 as compared with the value of M.I in negative control (13.28±0.324).The sample of honey that showed positive result to the presence of fumagillin by biochemical test with M.I. value (12.0± 0.695) that is equivalent to the M.I values in both control negative groups and mice those treated orally with honey that revealed negative result by biochemical test; 13.28±0.324 and 13.26± 0.287, respectively. However, there were no significant differences between M.I values in both experimental doses of fumagillin prepared by water-honey syrup 25and 50mg/kg b.w 8.48±0.837, 8.06±0.472,respectively, and between the value of M.I of mice treated with honey contaminated with fumagillin and the M.I of mice treated with 25 mg/kg b.w(12.00±0.695,8.48±0.837, respectively .

After 7 days of treatment there were no significant differences between both experimental doses of fumagillin those prepared by water-honey syrup 25and 50mg/kg b.w 8.48±0.837and 8.06±0.472, respectively .Table(2) shows no difference between the M.I of bone marrow cells in both control negative groups and mice treated orally with honey that revealed negative result by biochemical test after 35 days treatment; 14.30±0.374 and 14.10± 0.266,respectively.However, after 35 days of treatment there were no significant differences between M.I values in both experimental doses of fumagillin prepared by water-honey syrup 25and 50mg/kg b.w 7.0±0.273, 5.80±0.374,respectively .

Statistical analysis indicated that the effect of fumagillin alone75mg/kg b.w on M.I. of bone marrow cells after 35 days of treatment 3.70 ±0.431 was equivalent to that of fumagillin at the same concentration that was prepared by water –honey syrup 3.60±0.664 (Table-2).

The value of M.I. of cyclophosphamide as a positive control in both periods of treatment (Table-2) shows that there were no significant differences with experimental doses of fumagillin prepared by water-honey syrup 25mg/kg b.w. The present results in Table (2) shows that all experimental doses of fumagillin prepared by water-honey syrup 25, 50, and 75mg/kg b.w after 35 days resulted decrease in M.I 7.00±0.273, 5.80±0.374, and 3.60±0.664, respectively as compared with the value of M.I in negative control (14.30±0.374).There were significant differences between the M.I values in both periods, the value of M.I in the first period was 9.69±0.449 decreased to8.36±0.652 in the second period.

Table (1): Mean ± SE for the effect of fumagillin and honey contamminated with fumagillin (Treatments, Periods, and their Interaction) on chromosomal aberrations in bone marrow cells of Albino Male Mice.

periods	Treatments	Normal cells	Damaged cells	Chromatid break with fragment	Chromatid break without fragment	Ring chromosome	Centromeric break	Chromatid Gap
7 days	(-ve control)	88.80±0.489	11.20±0.489	1.40±0.244	1.40±0.244	3.60±0.244	2.40±0.489	2.40±0.244
	(-ve Honey)	88.40±0.400	11.60±0.400	1.40±0.244	1.20±0.200	4.40±0.400	2.20±0.400	2.40±0.244
	(+ve Honey)	84.20±0.200	15.80±0.200	1.60±0.244	2.40±0.244	4.80±0.200	2.60±0.200	4.40±0.244
	(25 mg/kg bw)	83.20±0.583	16.80±0.583	1.60±0.244	2.40±0.244	4.60±0.244	2.20±0.583	6.00±0.547
	(50 mg/kg bw)	75.60±0.244	24.40±0.244	2.60±0.244	4.60±0.400	5.40±±0.400	6.40±0.244	6.00±0.447
	(75 mg/kg bw)	58.40±0.244	41.60±0.244	4.80±0.200	8.40±0.244	11.60±0.244	8.20±0.244	8.80±0.200
	(75 mg/kg bw) Fumagillin alone	58.00±0.447	42.00±0.447	4.60±0.244	7.00±0.447	13.20±0.374	8.80±0.447	8.40±0.244
	(+ve control) CP (40 mg/kg bw)	26.40±0.509	73.60±0.509	13.00±0.316	14.20±0.374	17.40±0.244	15.00±0.509	14.20±0.374
L.S.D		**1.229**	**1.229**	**0.659**	**0.931**	**0.901**	**0.829**	**0.979**
35 days	(-ve control)	87.20±0.200	12.80±0.200	1.60±0.244	1.60±0.244	4.20±0.374	2.40±0.200	3.60±0.244
	(-ve Honey)	88.20±0.200	11.80±0.200	1.40±0.244	1.40±0.244	3.40±0.244	2.20±0.200	3.40±0.244
	(+ve Honey)	80.00±0.774	20.00±0.774	4.00±0.316	2.80±0.200	5.60±0.509	2.80±0.774	4.80±0.374
	(25 mg/kg bw)	78.60±o.509	21.40±0.509	3.60±0.244	2.80±0.200	5.60±0.244	3.40±0.509	6.00±0.316
	(50 mg/kg bw)	68.00±0.707	32.00±0.707	4.20±0.374	4.00±0.316	6.00±0.316	7.00±0.707	10.80±0.583
	(75 mg/kg bw)	45.00±0.836	55.00±0.836	7.80±0.489	9.20±0.374	15.20±0.374	8.20±0.836	14.60±0.244
	(75 mg/kg bw) Fumagillin alone	43.00±0.316	57.00±0.316	8.60±0.244	8.60±0.244	16.40±0.244	8.40±0.316	15.00±0.316
	(+ve control) CP (40 mg/kg bw)	11.00±0.836	89.00±0.836	15.80±0.374	16.80±0/374	22.80±0.374	15.40±0.836	18.20±0.374
L.S.D		**1.783**	**1.783**	**0.962**	**0.846**	**1.020**	**0.9745**	**1.020**
Period								
7 days		70.38±3.243	29.63±3.243	3.88±0.596	5.20±0.679	8.13±0.784	5.98±0.692	6.58±0.594
35 days		62.63±4.089	37.38±4.089	5.88±0.722	5.90±0.802	9.90±1.085	6.23±0.687	9.55±0.884
L.S.D		**0.5178**	**0.5178**	**0.2912**	**0.2976**	**0.3256**	**0.3058**	**0.346**

SE= standard error. Any cell containing one or more aberrations is counted as one damaged cell (Preston *et al*., 1987).

Continuous

periods	Treatments	Normal cells (100)	Damaged cells	Chromatid break with fragment	Chromatid break without fragment	Ring chromosome	Centromeric break	Chromatid Gap
Interaction Treatment / Period — 7 days	(-ve control)	88.80±0.489	11.20±0.489	1.40±0.244	1.40±0.244	3.60±0.244	2.40±0.489	2.40±0.244
	(-ve Honey)	88.40±0.400	11.60±0.400	1.40±0.244	1.20±0.200	4.40±0.400	2.20±0.400	2.40±0.244
	(+ve Honey)	84.20±0.200	15.80±0.200	1.60±0.244	2.40±0.244	4.80±0.200	2.60±0.200	4.40±0.244
	(25 mg/kg bw)	83.20±0.583	16.80±0.583	1.60±0.244	2.40±0.244	4.60±0.244	2.20±0.583	6.00±0.547
	(50 mg/kg bw)	75.60±0.244	24.40±0.244	2.60±0.244	4.60±0.400	5.40±±0.400	6.40±0.244	6.00±0.447
	(75 mg/kg bw)	58.40±0.244	41.60±0.244	4.80±0.200	8.40±0.244	11.60±0.244	8.20±0.244	8.80±0.200
	(75 mg/kg bw) fumagillin alone	58.00±0.447	42.00±0.447	4.60±0.244	7.00±0.447	13.20±0.374	8.80±0.447	8.40±0.244
	(+ve control) CP (40 mg/kg bw)	26.40±0.509	73.60±0.509	13.00±0.316	14.20±0.374	17.40±0.244	15.00±0.509	14.20±0.374
35 days	(-ve control)	87.20±0.200	12.80±0.200	1.60±0.244	1.60±0.244	4.20±0.374	2.40±0.200	3.60±0.244
	(-ve Honey)	88.20±0.200	11.80±0.200	1.40±0.244	1.40±0.244	3.40±0.244	2.20±0.200	3.40±0.244
	(+ve Honey)	80.00±0.774	20.00±0.774	4.00±0.316	2.80±0.200	5.60±0.509	2.80±0.774	4.80±0.374
	(25 mg/kg bw)	78.60±o.509	21.40±0.509	3.60±0.244	2.80±0.200	5.60±0.244	3.40±0.509	6.00±0.316
	(50 mg/kg bw)	68.00±0.707	32.00±0.707	4.20±0.374	4.00±0.316	6.00±0.316	7.00±0.707	10.80±0.583
	(75 mg/kg bw)	45.00±0.836	55.00±0.836	7.80±0.489	9.20±0.374	15.20±0.374	8.20±0.836	14.60±0.244
	(75 mg/kg bw) Fumagillin alone	43.00±0.316	57.00±0.316	8.60±0.244	8.60±0.244	16.40±0.244	8.40±0.316	15.00±0.316
	(+ve control) CP (40 mg/kg bw)	11.00±0.836	89.00±0.836	15.80±0.374	16.80±0/374	22.80±0.374	15.40±0.836	18.20±0.374
L.S.D		**1.465**	**1.465**	**0.824**	**0.842**	**0.921**	**0.865**	**0.979**

Any cell containing one or more aberrations is counted as one damaged cell

Table (3): Mean ± SE for the effect of fumagillin and honey contaminated with fumagillin on Bone marrow M.Iof Albino Male Mice.

Periods	Treatments	Bone marrow Mitotic Index
7 days	(-ve control)	13.28±0.324
	(-ve Honey)	13.26±0.287
	(+ve Honey)	12.00±0.695
	(25 mg/kg bw)	8.48±0.837
	(50 mg/kg bw)	8.06±0.472
	(75 mg/kg bw)	6.64± 0.429
	(75 mg/kg bw) Fumagillin alone	6.60±0.539
	(+ve control) CP(40 mg/kg bw)	9.20±0.374
	L.S.D	**1.407**
35 days	(-ve control)	14.30±0.374
	(-ve Honey)	14.10±0.266
	(+ve Honey)	10.58±0.668
	(25 mg/kg bw)	7.00±0.273
	(50 mg/kg bw)	5.80±0.374
	(75 mg/kg bw)	3.60±0.664
	(75 mg/kg bw) Fumagillin alone	3.70±0.431
	(+ve control) CP (40 mg/kg bw)	7.80±0.374
	L.S.D	**1.369**
	Period	
	7 days	9.69± 0.449
	35 days	8.36± 0.652
	L.S.D	**0.490**

Discussion

Qualitative Tests to Detection of Fumagillin in Honey Samples:

In the present study, we found that (16.67%) of samples contain fumagillin residues, this value is considered as a very high percentage as compared with the study by Diserens, (2007). He found that 1.7% of the honey samples of European market which was analyzed for antibiotic residues were non-compliant with the EU standard.

Identification of fumagillin by Thin Layer Chromatography (TLC)

Each honey sample gave a positive result for the presence of fumagillin by Qualitative chemical test was confirmed by the TLC (figure-2) . The TLC is highly reliable and sensitive test for the fumagillin detection in samples expected to be contaminated with fumagillin (Richard *et al*., 1989). High performance liquid chromatography (HPLC) also can be used to detect fumagillin (Hanaa and Peter, 1991). Zuzana *et al*. (2012) used TLC and HPLC analysis for confirmation of fumagillin, whereas study of Colin *et al*. (1997) indicated that the TLC is a highly sensitive method for the detection of fumagillin.

Effects of fumagillin and honey contaminated with fumagillin on chromosomal aberrations in bone marrow cells of Albino male mice.

The experimental results showed significant differences (P≤ 0.01) between treatments in their effect on all types of chromosomal aberrations and damaged cells as well between the two periods.

The damage effect of fumagillin could be due to the direct interference of the fumagillin derivatives with DNA synthesis during cell growth or replication. Since, the fumagillin, has primarily two epoxide structures capable of alkylating proteins involved in the packaging of DNA (Birch and Hussain, 1969) thereby establishing conditions for damaging DNA. The results of the current study are in favor with a study conducted by Stanimorvic *et al* .(2007), when they evaluated the genotoxic effect of fumagillin in sister chromatid exchange (SCE) and chromosome aberration tests in cultured human peripheral blood lymphocytes at three concentrations (1.02, 3.07 and 9.20 µg/mL). Their results revealed that all tested concentrations of fumagillin significantly increased the SCE frequency per cell and decreased the proliferative activity of human cultured lymphocytes which was manifested in the decrease in mitotic and proliferative indices. In another study done by Kulic *et al*. (2009), fumagillin alone was tested for the ability to provoke chromosomal aberrations in mouse bone marrow cells. Mice were administered fumagillin by gastric probe in doses of 5, 10 and 20 mg/kg b.w., Water-sugary syrup as a negative control and cyclophosphamide (15 mg/kg b.w.) as a positive control. Significantly increased frequencies (p≤0.001) of numerical chromosomal aberrations (aneuploidies and polyploidies) was observed both in the medium (10 mg/kg b.w.) and the highest (20 mg/kg b.w.) dose of fumagillin. Structural chromosomal aberrations (gaps, breaks and insertions) were noticeably more frequent in comparison to negative control only in the highest experimental dose of fumagillin. These results clearly showed that fumagillin in concentrations 10 and 20 mg/kg b.w. had a genotoxic potential *in vivo*. In another study mice were given fumagillin orally in doses 5, 10 and 20 mg/kg bw. All doses showed significantly increase in chromosomal aberrations (Stanimorvic *et al*., 2010). Stanimorvic *et al*.,(2010), found that the highest dose 20 mg/kg b.w. induced both structural and numerical chromosomal aberrations and insertions on the first pair of autosomes that were amplified in the 1C and 1E regions. These results pointed to the genotoxic potential of fumagillin in the range of medium and maximum doses applied.

The results of the present study are in agreement with that obtained by Stanimirovc *et al*., (2007) who found that the same concentrations of the fumagillin (25 mg/kg b.w., 50 mg/kg b.w., and 75 mg/kg b.w.) significantly increased the frequencies (p ≤ 0.01 or p ≤ 0.001) of structural chromosomal aberrations (CA)such as gaps, breaks, and centric rings .Data from other studies have shown that there are certain genotoxic effects of secondary metabolites (gliotoxin and verruculogen) of *Aspergillus fumigates* 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 a positive result in Salmonella/microsomal mutagenicity assays (Sabater-Vilar *et al*., 2003). In the present study the statistical analysis indicates that the effect of fumagillin alone75mg/kg b.w on

damaged cells of bone marrow after 35 days of treatment 57.00 ±0.316 was more than that of fumagillin at the same concentration prepared with water –honey syrup 55.00±0.836. This may be due to ability of honey to reduce the effect of fumagillin .The ability of honey to reduce the effect of fumagillin on the chromosomes of bone marrow cells can be attribute to anti-oxidant property of honey that prevent the production of toxic materials (Perez *et al* .,2007).No data in the area are available on the effect of honey contaminated with fumagillin on bone marrow cells.

The results in table (2) show that all experimental doses of fumagillin prepared with water-honey syrup 25, 50, and 75mg/kg b.w fumagillin alone caused decreases of bone marrow M.I 8.48±0.837, 8.06±0.472, 6.64±0.429 and 6.60 ±0.539 as compared with the value of M.I in negative control (13.28±0.324).These results are in agreement with the findings of many authors considering the antiproliferative effects (antiangiogenic effects) of fumagillin (Molina *et al.*, 2002 and Mazzanti *et al.*, 2004). It can be assumed that the decrease in MI in the current study is the consequence of fumagillin binding on methionine aminopeptidase-2 (MetAP-2), the molecular target of fumagillin and its analogue TNP-470 (Sin *et al.*, 1997 and 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 a part of the translocation process, so its inhibition results in cell cycle arrest and apoptosis (Fardis *et al.*, 2003). This mechanism probably underlies the antiproliferative effect of fumagillin which was manifested in the decrease in MI in our study. Moreover, the results of Mazzanti *et al.* (2004) support the notion that genes DOC1, KLF4, and TC1 are specific for the endothelial cells response to endostatin and fumagillin. Nevertheless, these authors suggested that further studies are necessary to clarify these early mechanisms and to better understand the function of these genes (Mazzanti*et al.*, 2004).

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التأثيرات الوراثية الخلوية للفيوماجلين (Dicyclohexylamine) والعسل الملوث به في ذكور الفئران BALB/c *Mus musculus*

الخلاصة

فيوماجيلين (Dicyclohexylamine) هو مضاد حيوي يفرزبشكل طبيعي من فطر *Aspergillus fumigatus*وهو فطر ينتمي لجنس الرشاشيات. فهو يستخدم في الطب البيطري ضد الطفيلي *Nosema* (Microsporidia: Nosematidae)spp. الذي يصيب نحل العسل (*Apismellifera* L).

في هذه الدراسة الحالية، تم جمع عينات العسل من 30مصدر من مختلف المناطق في محافظة دهوك وضواحيها للكشف عن وجود فيوماجيلين في هذه العينات تم تطبيق الاختبار النوعي الكيميائي**Qualitative chemical test** و بواسطة **TLC**.

تمت دراسة التأثيرات الوراثية الخلوية للعسل الملوثة بالفيوماجيلين والذي أظهر نتيجة ايجابية في كل من الاختبارين الكيمياويين على خلايا نخاع العظم باستعمالمعامل الإنقسام (M.I) والتغيرات الكروموسومية.حيث عوملت الفئران BALB/c عن طريق الفم بالفيوماجلين وبالتراكيز 25، 50، 75 ملغ / كغم من وزن الجسم ،وحضر هذه التراكيز من الفوماجلين مع العسل المخفف بالماء الدافيء.

عوملت مجموعة من الفئران ب 75 ملغ / كغم من وزن الجسم من الفيوماجلين فقط والمحضر بالماء الدافئ وذلك عن طريق الفم ، مجموعة أخرى من الفئران عوملت عن طريق الفم بالعسل الملوث والتي أعطيت نتيجة إيجابية بالأختبارات الكيمياوية. محلول من الماءوالعسل أعطي الى مجموعة اخرى من الحيوانات عن طريق الفم ، وتركت مجموعة اخرى من الفئران بدون أي معاملة وعدت سيطرة سالبة ايضا" .

عوملت كافة الفئران لفترات 7 و 35 يوما مع 24 ساعة فاصلة. تمت مقارنة كافة المعاملات مع السيطرة السالبة و(40 ملغم / كغم من وزن الجسم. Cyclophosphamideالذي عد كسيطرة موجبة .

أظهر الاختبار الكيمياوي لعينات العسل التي جمعت من النحل المصاب سريريا أن 16.67٪ من هذهالعينات كانت ملوثةبالفيوماجيلين.كافة جرع الفيوماجيلين 25، 50، 75 ملغم / كغم من وزن الجسم لها تاثير معنوي ($P \leq 0.01$) على زيادة الخلايا المحطمة والتغيرات الكروموسومية (chromatid break with fragment ,chromatid break without fragment, Ring chromosome, centromeric break and chromatid gaps) في خلايا نقي العظم بعد كل من 7 و 35 يوما من المعاملة. اظهر الدراسة كذلك أن كل جرع الفيوماجيلين لها القابلية على خفظ معامل الانقسام M.Iبشكل معنوي عند المقارنة مع السيطرة السالبة والموجبة .

ان عينة العسل الملوثة بالفوماجلين والتي اظهرت نتيجة موجبة بالاختبار الكمياوي ابدت قابليتها بزيادة التغيرات الكروموسومية في خلايا نقي العظم بعد 7 و35 يوم من المعامله .وكانت قابلية هذه العينه يكافئ لتاثير عينة العسل الذي لوثت بالمختبر بالفيوماجلين بالتركيز (25 ملغم / كغم من وزن الجسم).أظهرت نتائج الدراسة الحالية ان عينه العسل الملوثه بالفيوماجلين لها القابليه على اختزال معامل الانقسام M.I بعد 35 يوم من المعامله في خلايا نقي العضم بعد المقارنه مع السيطرة السالبة.

كارتێكرنا بۆماوه یا خانەیی یا فۆماجیلین (دایسایكلوهێكسیل ئەمین) و هنگڤینی یێن كەفتینە ژێر كاریگەریا وی دناف مشكێن *Musmusculus* BALB\c

كورتی

ئەنتیبایوتیكێ فۆماجیلین Fumagillin (دایسایكلوهێكسیلئەمین Dicyclohexylamine) ب شێوەكێ سروشتی ژلایێ كەروویێ ئەسپەرجێلەس *Aspergillusfumigatus* دهێتە دروست كرن. ئەڤ ئەنتیبایوتیكە دزانستێ ڤیتەرنەریدا دژی مشەخۆرێ زیانبەخش و نەخۆشی پەیداكەر *Nosema*spp. دهێتە ب كارئینان، ژبەركو ئەڤ مشەخۆرە توشی مێشێن هنگڤینی *ApismelliferaL.* دبیت.

د ماوێ ڤێ ڤەكولینێ دا، 30 نموونەیێن هنگڤینی ل سەرانسەری پارێزگەها دهوكێ و دەوروبەرێن وێ هاتینە كومكرن. تێستێن كیمیایی یێن جۆری هاتینە ب كارئینان بۆ دیاركرنا هەبوونا ڤی ئەنتیبایوتیكی دناڤ نموونەیێن وەرگرتی دا. ل دویڤدا ئەڤ ئەنتیبایوتیكە ب هاریكاریا TLC دناڤ هەر هنگڤینی دا هاتە دیاركرن كو ئەنجامێن ئەرێنی هەبوون د تێستا كیمیایی یا جۆریدا. كارتێكرنا بۆماوەییا خانەیی یا كەفتیە ژێر كاریگەری ئەنتیبایوتیكێ فۆماجیلین كو ئەنجامەكێ ئەرێنی د هەردوو تێستێن زیندەكیمیایی دا هەبوو، هاتە پشكنین دناڤ خانەیێن مەژیێ هەستیكێن مشكان ، ئەوژی ب هاریكاریا فاكتەرێن دابەشبونێ (MI)، گهورینێن كرۆموسومی (CA).

ئەنتیبایوتیكێ فۆماجیلین ب رێكا گاڤاج (رێكا دانا ب زۆری یا دەرمانان بۆ ناڤ دەڤێ كەسێ نەخۆش یان زیندەوەرێ ڤەكۆلین ل سەر دهێتە كرن) هاتەدان بۆ مشكێ BALB/c ب ژەمێن (25، 50، 75 میلیگرام\ كیلوگرام) ژكێشا گشتی یا لەشی (B.W)، هەروەسا وەك ماددەیەكێ كاریگەر و پیسكەر بۆ هنگڤینی هاتەدان. ئەڤ ئەنتیبایوتیكە دگەل ئاڤا گەرم بتنێ ب ژەمەیا(75میلیگرام\ كیلوگرام ژكێشا گشتی یا لەشی) هاتە دان بۆ كومەكا مشكان ب رێكا دەڤی، لێ هنگڤینی سروشتی هاتەدان بۆ كومەكا دی یا مشكان ب هەما رێك كو ڤێ كومێ ئەنجامێن ئەرێنی د تێستا كیمیایی یا جۆریدا هەبوون (ئەنتیبایوتیكێ فۆماجیلین دناف هنگڤینی دا هە بوو). هەردیسان شلەیەكێ پێكهاتی ژ هنگڤینی و ئاڤێ ب رێكا دەڤی هاتەدان بۆ كومەكا دی یا مشكان و ئەڤ مشكە هاتنە ب كارئینان وەك كۆنترولێ نێگەتیف. هەردیسان كومەكا دی ژی مشكان وەك كۆنترولێ نیگەتیف هاتنە ب كارئینان دگە ل خارن وفە خارنا ئاڤێ بتنێ بێ كو هیچ تشتەكی پێ بهێتە دان .

هەمی ئەو مشكێن ڤەكولین ل سەر هاتیە كرن د دوو ماوەیێن ژێكجودادا سەرەدەری دگەل هاتەكرن، ئەوژی د ماوێ 7 رۆژاندا و دماوێ 35 رۆژاندا ب بەردەوامی و دماوێ هەر 24 دەمژمێراندا. ئەو كومێن مشكان یێن سەرەدەری دگەل هاتیەكرن دگەل مشكێن كۆنترولێن نێگەتێڤ و ماددێ سایكلوفوسفوئەماید (40میلیگرام\كیلوگرام ژ كێشا لەشی) وەك كۆنترولێ پوزەتیف هاتنە هەڤبەركرن، تێستێن زیندەكیمیایی كو ل سەر هەمی هنڤینی ژ وان جهین ئیش لی هەین هاتنە ئەنجامدان دیاركر ب رێژا 16.67٪ كەفتنە بن كاریگەریا ئەنتیبایوتیكێ فۆماجیلین.

هەمی ژەمێن ئەنتیبایوتیكێ فۆماگیلین (25، 50، 75 میلیگرام\ كیلوگرام ژ كێشا لەشی) كارتێكرنەكا بەرچاڤ هەبوو ل سەر ($P\leq 0.01$) ژ كارئێخستنا خانەیان و تێكچوونا كرۆموسومان (هندەك كرۆماتید دشكێن و دبنە پارچە پارچە، هندەك كرۆماتید ژی دشكێن لێ نابنە پارچە پارچە، گروڤربوونا كرۆموسومان، سێنترومیرێ دناڤبەرا دوو كرۆماتیدا دشكێت، هندەك كەلێن و ڤالاهی دكەڤنە دناڤ كرۆموسوماندا)، ڤێ چەندێ

دناڤ خانەیێن مەژییێ هەستیکان روویدا ئەوژی پشتی 7 رۆژان ژ سەرەدەریکرنێ و پشتی 35 رۆژان ژ سەرەدەریکرنێ.هەردیسان ئەنجامێن ڤێ ڤەکۆلینێ دیارکرن کو ئەنتیبایوتیکێ فۆماجیلین کارتێکرنەکا بەرچاڤ ل سەر کێمکرنا فاکتەرێن دابەشبوونێ هەیە، ب تایبەت دەمێ دهێتە هەڤبەرکرن دگەل هەردوو کۆنترولێن نێگەتیف و پوزەتیف. هنگڤینی یێن ئەنجامەکێ پوزەتیف هەی ل دەمێ هەبوونا ئەنتیبایوتیکێ فۆماجیلین د تێستێن زیندەکیمیایی دا شیانێن خو دیارکرن بۆ زێدەکرنا تێکچوونا کرۆموسومان دناڤ خانەیێن مەژییێ هەستیکان ئەوژی پشتی 7 روژێن سەرەدەریکرنێ و پشتی 35 روژێن سەرەدەریکرنێ. شیانێن ڤان نموونە ین وە رکرتی د هاوتانە دگەل وان نموونە یێن تاقیکرن ل سەر هاتیەکرن و کەفتینە ژێر کاریگەریا فۆماجیلینی (25 میلیگرام\ کیلوگرام کێشا لەشی).

ئەنجامێن ڤێ ڤەکۆلینێ دیاردکەن کو هنگڤینی یێن کەفتینە ژێر کاریگەریا فۆماگینی شیانێن خو دیاردکەن بۆ کێمکرنا فاکتەرێن دابوشبوونێ دناڤ خانەیێن مەژییێ هەستیکان ئەوژی پشتی 35 رۆژان ژ سەرەدەریکرنێ ب تایبەت ژی دەمێ دهێنە هەڤبەرکرن دگەل کۆنترولێن نێگەتیف.