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Effect of Royal jelly in genotoxic and cytotoxic effect of Fumagillin in white mice

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ABSTRACT

This study was conducted in Laboratories of college of science, Tikrit University, and Faculty of science, Dohok University, Iraq between 2014 and 2016. The aim of study was to evaluate the potential of royal jelly to ameliorates genotoxic and cytotoxic effects of Fumagillin in white mice *Mus musculus*. Fumagillin which produced by *Aspergillus fumigates* is a natural antibiotic used in veterinary against *Microsporidia* parasites in bees and fish. It also used in human medicine for AIDS patients and other immune dysfunction. In this current study we evaluate genotoxicity of Fumagillin by micronucleus test in bone marrow cells, chromosome aberration in primary spermatocytes and cytotoxicity by mitotic index in bone marrow cells of white mice, and study the activity of royal jelly in removing genotoxic and cytotoxic effects of Fumagillin. The fumagillin was given as repeated oral gavages for 7 successive days after preparing the doses 10, 15, and 20 mg/kg.b.wt. in 50% of sugar syrup. The positive control group was given single I.p. dose of 20 mg/kg.b.wt. of methotrexate. 100 mg of royal jelly alone and with each dose of Fumagillin was given to evaluate the effect of royal jelly in toxic effect of Fumagillin. The results showed that the two dosages 15 and 20 mg/kg.b.wt of Fumagillin induced significant increase in the frequency of micronuclei, the means was (24.60 ± 2.37, 53.00 ± 4.59) respectively compared with negative control (8.20±1.39), and significant increase in the means of total chromosome aberration in bone marrow cells of the mice in the these groups (9.00±0.92,17.20±1.24) respectively compared with (6.00±0.83) for negative control. In primary spermatocytes, the dose 20mg/kg.b.wt. of fumagillin induced significant increase in the mean of chromosome aberration (14.00 ± 2.07) compared with (3.90 ± 0.50) for negative control. The three dosages 10, 15, and 20 mg/kg.b.wt of Fumagillin induced significant decrease in mitotic index (3.90 ± 0.29, 3.60 ± 1.80, and 2.90 ± 0.50) respectively compared with (7.10 ± 0.29) for negative control. Royal jelly showed activity to ameliorates genotoxic and cytotoic effects of Fumagillin. The results of the current study suggest that the tested dosages of Fumagillin have genotoxic effect represented as increase in the frequency of micronuclei, means of chromosome aberration, and cytotoxicity represented as activity against bone-marrow cell proliferation of white mice. The royal jelly has the activity to ameliorates genotoxic and cytotoxic effect of Fumagillin.

Introduction

Most of organisms exposed to many environmental pollutant, such as chemicals, radiation, gaseous, and others, which have high biological effects leads to harmful lesions [1]. Honey bee *Apeis melliferae* as many other organisms may exposed to many types of pollutants and infected with many diseases, some of which attack larval stages, such as American foulbrood, and some other which attack adults.

Adults of honey bee insects infected by bacteria, fungi, viruses, or protozoa, like amoeba and Nosema.
Many methods used to control these diseases, by some chemicals and antibiotics such as Sulphaquinoxalin, Gramicidin, and Fumagillin, by fumigation or spray or mixing with food [2, 3]. Some of these antibiotics may increase genetic damage in mammalian especially human.
The Fumagillin antibiotic which used to control honey bee infection by the protozoan *Nosema apis* considered as one of these pollutants [4]. This disease cause high cost in honey bee colonies and the best method for its control is chemical treatment by Fumagillin, which produced by the fungus *Aspergillus fumigatus* , and has the trade name fumidil-B. Fumagillin attacks the parasite productive stages in honey bee intestine, but it not affect spores. So the disease may take 2-3 year for healing. When this antibiotic used at the season of honey production, the honey may be contaminated with fumagillin, and must not considered for human consume [5]. Fumagillin is stable in honey [6].
The studies of Stanimirovic *et al* [7] showed that exposure of humane lymphocytes in vitro to low concentrations of Fumagillin caused high decrease in proliferative potential. When the team evaluates genetic effects in human lymphocyte cultures with cytogenetic tests, the results showed high frequency of sister chromatid exchange (SCEs), which considered as marker for genotoxicity and cytotoxicity of Fumagillin. This mean that the fumagillin exerts risk against honey workers and consumers.
There were enough data about in vivo genotxoicity of Fumagillin in dose related manner, The Fumagillin may has interactions with body inside and outside factors [8,9].
Multiple negative effects of many drugs and medications lead to using of natural compounds, which produced by animal and plant organisms in treatment of diseases such as cancer. Among these important natural compounds which used against many types of diseases, are the honey bee products [4]. Honey bee was considered human friend for several thousand years. It provides honey, bee wax, pollen grains, propolis, bee venom and royal jelly. Royal jelly has high feeding value and many health benefits, so it described as (Natures Own Supertonic) and (Nature Miracle Food), due to its wide healing potential. Royal jelly was used in cancer management, and to ameliorate effects of toxins since it has folic acid, which considered as important factor in toxic defense [10].
Due to the absence of studies about activity of royal jelly against genotoxic effects of the antibiotic Fumagillin, this study was designed to reveal the potential of locally produced Royal jelly in removing cytotoxic and genotoxic effects of Fumagillin in white laboratory mice *Mus musculus*, using the following cytogenetic tests and markers:

1- Micronucleus (mn) test in bone- marrow of male white mice.
2- Chromosome aberration in bone- marrow cells of male white mice.
3- Chromosome aberration in white mice primary spermatocytes.
4- Mitotic index MI in bone- marrow cells of white mice.

Materials and methods

The current study was conducted between may 22, 2011 and June22, 2012 in the laboratories of the College of Science – Tikrit University and Faculty of Science- Duhok University, Iraq the aim of study was to detect genotxoic and cytotoxic effects by induced chromosomal changes which resulted from treatment of white mice with 10,15, and 20 mg/kg.bwt. of Fumagillin and the ameliorating effect of the treatment with 100 mg/ kg. bwt. of Royal jelly. Adult male Swiss albino mice (*Musmus culus*) BALB/c with 8-10 weeks age and 26-28 grams in average weight were grouped into nine groups with five males for each. Each group of mice was put in a separate cage and treated for 7 consecutive days. Fumagillin were orally administrated with doses of 10, 1, and 20 mg/kg.b.wt according to Stanimirovic *et al*. [7]. Because of weak solubility in water, Fumagillin does not dissolve readily in water. So it is recommended to mix fumagillin in small amounts of warm water (not more than 32–34^{o}C), stirring to make a paste, and then the water-honey syrup was added gradually with shaking occasionally, to prepare medicated water-honey syrup. Negative control was given 50% sugar solution. Methotrexate (MT) 20 mg/kg.bwt. was given intraperitoneally and considered positive control due to its known clastogenicity and mutagenicity [11]. Experimental doses were obtained by dissolving in 1:1 sugar-water syrup, as in the formulation usually used for application in beekeeping, and administrated to the mice by oral gavage. Scince tested doses of fumagillin were 10, 15, and 220 mg/kg.bwt., each mouse received 0.25, 0.375, and 0.5 mg respectively, i.e. 1.75, 2.625, and 3.5 mg/kg.bwt. in a 7 day treatment. Royal jelly 100 mg/kg.bwt. was given alone and in mixing with each dose of fumagilin. Then the animals were stayed under stable environment with 12/12 hr of dark and light, 21^{o} c and free feeding and drinking water. After 7 days the mice were scarified and sampled as recommended for each experiment. Micronucleus test was conducted by the method which described by Scmidt, [12], mitotic chromosome of bone marrow cells were prepared with the method which described by Adler, [13] with modifications of Zimonjic *et al.,* [14]. The method of Berwen and Preston, [15] was applied in preparing diakinases chromosome of primary spermatocytes. Mitotic index was determined in 1000 bone-marrow cell for each treatment.
Statistical analysis: Statistical package of social sciences (SPSS) was used for data analysis by t-test.

Result and discussion

Evaluation of chromosome breakage and chromosome loose in bone- marrow after mice treatment with Fumagillin and Royal jelly using micronucleus (mn) test

Table (1) shows dose dependent increased numbers of polychromatic erythrocytes(PCEs), The Fumagillin caused dose related significant increase in the mean of bone - marrow PCEs, (206.6±1.32), (232.4±1.43), and (345.2 ± 0.86) with (10, 1, and 20) mg/kg.bwt.) of Fumagillin respectively compared with (197.80 ± 1.74) in negative control. Also the results showed that the dosages (15, 20) mg/kg.bwt) of Fumagillin caused significant increase in the frequency of MNiPCEs (17.80 ± 1.28), (33.60 ± 1.53) respectively compared with ((10.22±0.60) for negative control. This may suggest a role of fumagillin in the induction of micronuclei formation in PCEs, which may due to genotoxic and / or cytotoxic effect of the used dosages of fumagillin (Table1). Previous results showed that there were dose related increase in PCEs and MNiPCEs which refer to cytotoxic effect in addition to genotoxic effect of fumagillin [16].

On the other hand, the results of our current study, showed that treatment with 100 mg/ kg.bwt. of Royal jelly (RJ) with the dosages (10, 1, and 20) mg/kg.bwt. of Fumagillin caused dose related decrease in the means of PCEs (202.40±1.28), (2018.20 ± 0.86), and (243.80±1.31) respectively, compared with (206.60 ± 1.32), (232. 40 ± 1.43), and (345.20 ± 0.86) (table 1). For MNiPCEs, the results showed that treatment with RJ have no significant effect when compared with negative control, and this revel that royal jelly ameliorate genotoxic and cytotoxxic effects of Fumagillin which caused the formation of mn in PCEs.

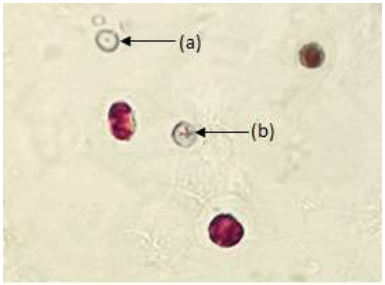

Figure (1) PCEs in the bone-marrow of mice treated with Fumagillin and mn in PCEs. a. one mn., (b) 3 MNi. 100 , May-Grunewald + Gemsa.

Table (1) mn frequency in PCEs of the mice treated with different dosages of Fumagillin and 100mg/kg.bwt. of RJ.

T mg/kg.bwt.	PCEs M ± S.E.	MNPCEs M ± S.E.	MNi M ± S.E.
Sugar solution 1:1	197.80 ± 1.74	10.22 ± 0.60	8.20 ± 1.39 9
MTX 20	530.60 ± 1.91**	110.40 ± 9.03	147.00 ± 6.80**
Fumagillin 10	206.60 ± 1,32*	8.20 ± 1.46	11.80 ± 2.35
15	232.40 ± 1.43**	17.80 ± 1.28**	24.60 ±2.37**
20	345.20 ± 0.86**	33.60 ± 1.53**	53.00 ± 4.59**
Royal Jelly. 100	165.00 ± 1.22**	4.60 ± 0.67	5.80 ± 0.86
Fumagillin + R.J. 10 + 100	202.40 ± 1.28	7.00 ± 0.94	9.80 ± 1.31
15 + 100	218.20 ± 0.86**	12.20 ± 1.24*	16.40 ± 2.06
20 + 100	243.80 ± 1.31**	20.60 ± 1.46**	26.80 ± ± 2.17**

PCEs: Polychromatic erythrocytes; MNPCEs: Micro nucleated PCEs; MTX: Methotrexat; RJ: Royal Jelly; * Sig. at p≤ 0.05; * sig. at p≤ 0.01, t-test for paired samples.

Evaluation of chromosome breakage and chromosome loose in bone- marrow after mice treatment with Fumagillin and Royal jelly using mitotic chromosomes

The results showed significant differences in the means of total structural chromosome aberrations {(Cbs), i.e. breaks, fragments, deletions, translocations). The data in (table 2) shows that the dosage 20mg/kg.bwt. of fumagillin have the largest effect. The mean value of total Cs reached (22.6±1.86) when gaps included and (17.2±1.24) when gaps excluded. Using of Royal jelly 100 mg /kg.bwt. caused decreased mean value of Cbs which reached nearly to the value of negative control. The larger effects are accompanied by the dosages 10, and 15 mg/kg.bwt. The total mean value of Cbs reached 7.8 ± 1.35 with gaps and 5.8 ± 1.06 without gaps, for the dosage 10mg Fum. +100mg RJ., 9.8 ±1.01 with gaps and 7.4 ±1.02 without gaps for 15 mg Fum + 100 mg RJ respectively. While it reached 14.3 ±1.15 with gaps and 10.6±1.16 without gaps for the dosage 20mg Fum + 100mg RJ compared to 22.6 ± 1.86 with gaps and 17.2 ±1.42 without gaps respectively for the negative control, which represent the mean value of total structural chromosome aberration accompanied by treatment with fumagillin only without royal jelly. Although the means did not return to its levels of negative control, there were large decrease, which may lead to the suggestion that using royal jelly may limit the clastogenic effects of Fumagillin in white mice, and the limitation be lager with the lowest tested dosages, i.e 10, and 15 mg/kg.bwt.

Besides that, the results showed that treatment with 100 mg of royal jelly did not cause any significant differences and all values are less or near the values of structural chromosome aberrations in negative control group (Table 2).

Figure (2) shows structural chromosome aberrations in bone-marrow cells of mice after treatment with fumagillin.

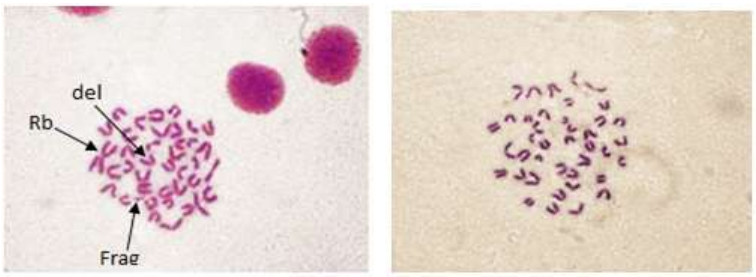

Figure (2) structural chromosome aberrations in bone-marrow cells of mice after treatment with fumagillin

Table (2) Structural and numerical chromosome changes in bone-marrow cells of male whit mice after treatment with fumagillin and royal jelly.

T Mg/kg.bwt.	Structural aberrations						Total aberration M ± S.E.		numerical aberrations M ± S.E.	
	g %	b %	td % .	ace %.	Rob %.	Cen .atten %.	w.g.	wo.g.	ap	pp
S.S.										
1:1 MTX	25.0	22.5	15.0	37.5	-	-	8.0 ± 1.09	6.0 ± 0.83	1.0 ± 0,31	1.4 ± 0.50
20 Fum.	28.3	19.7	12.8	31.5	5.3	2.1	37.4 ± 2.6**	26.8 ± 2.03	4.4 ± 0.50**	6.6 ± 0.67**
10	24.4	18.3	18.5	34.4	4.0	–	9.8 ± 1.09	7.4 ± 0.92		1.8 ± 0.37
15	26.5	20.3	15.6	32.8	4.6	-	12.8 ± 1.06**	9.0 ± 0.92**	1.2 ± 0.20	2.6 ± 0.50
20	23.8	24.7	14.1	29.2	6.1	1.7	22.6 ± 1.86**	17.2 ± 1.42**	1.8 ± 0.37	4.6 ± 0.74
R. J.									3.0 ± 0.31*	
100 Fum. + R.J.	25.8	19.3	16.1	38.7	-	-	6.2 ± 0.86	4.6 ± 067	0.60 ± 0.24	0.80 ± 0.37
10 + 100	25.6	20.5	17.9	35.8	-	-	7.8 ± 1.35	5.8 ± 1.06		
15 + 100	26.5	20.4	16.3	34.6	2.0	-	9.8 ± 1.01	7.4 ± 1.02	0.60 ± 0.24	0.60 ± 0.24
20 + 100	25.3	22.5	1.4	30.9	4.2	1.4	14.3 ± 1.15**	10.6 ± 1.06**	1.0 ± 0.31	1.4 ± 0.50
									1.80 ± 0.20	2.6 ± 0.40*

T: Treatment; S.S:Sugar solution; MTX: Methotrexat; Fum: Fumagillin; td: chromatid deletion; ace: a centric fragment; Rob: Robertsonian translocation; Cen. Atten: centromeric attenution; w.g.: with gaps; wo.g.: without gaps; ap: aneuploidy; pp: polyploidy.

The results of the current study showed that the most effective dose of fumagillin in numerical chromosome changes (i.e. aneuoploidy and euoploidy) was 20 mg/kg.bwt. as the mean value of aneuoploidy was (3.0 ± 0.31) and (4.6 ±0.74) for euoploidy compared with (1.0 ±0.31) and 1.4±0.5) respectively in negative control. More over there were no significant differences in numerical chromosome changes between treatment and negative control groups (Table 2).

The results showed that no concurrent induction of numerical chromosome changes with 100mg of royal jelly treatment when compared with negative control. Table 2 showed that the mean value of total numerical chromosome changesaneuoploidy (1.0±0.20) in mice treated with 20 mg/kg.bwt. of fumagillin + 100 mg of royal jelly which is very close to mean vale of the same type of aberration in negative control group which reached (1.0 ± 0.31).

Figure (3) shows banded metaphase chromosomes. N=40 G. band. Giemsa. 100X.

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

These results showed that the used doses of fumagillin in our current study induced structural and numerical chromosome aberration in bone-marrow cells of white mice, and this concord with the results

of (Milan *et al* [17] which shoed dose related increase of aberration and that was ascertained by Stanimorvic, [18].

Besides that, our results showed that the royal jelly have the potency to decrease the mean value of structural and numerical chromosome aberrations, although these mean value did not return to the mean values of structural and numerical chromosome aberration in negative control (Table 2). This ability may relate to the content of proteins, vitamins, and amino acids, in royal jelly, especially folic acid which resist toxins [10].

Chromosome aberrations in primary spermatocytes of male white mice treated with fumagillin and royal jelly.

The results of our current study showed significant increase in the mean value of chromosome aberration in primary spermatocytes of the treated group with 20 mg/kg.bwt. of fumagillin (14.0 ±2.07) compared with (3.4 ± 0.) in negative control (Table 3).

One other hand these results showed no significant differences between negative control and treatment with 100 mg of royal jelly (Table 3).

Figure 4. showed chromosomes of primary spermatocytes in Diakinesis from female mice treated with 20mg/kg.bwt. of fumagillin, we can see the autosomal bivalents, sexual X-Y bivalents, autosomal univalentes (A. uni. val).

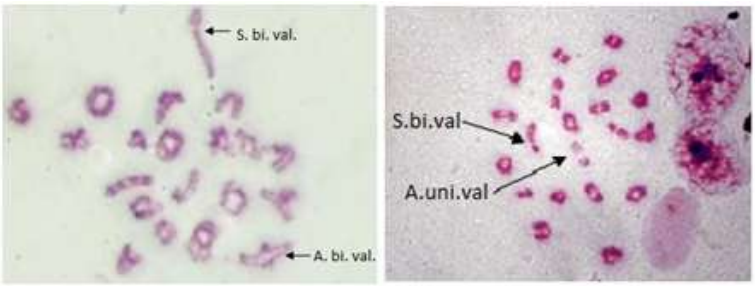

Figure 4. Primary spermatocytes Chromosomes in diakinesis. Notice the A. uni. Val.

Table (3) Chromosomal changes in primary spermatocyytes of male white mice treated with fumagillin and royal jelly

T Mg/kg.bwt.	ace %.	A.uval %.	S.uval %	Chain-4 %.	Total aberration M ± S.E.
Sugar solution 1:1	-	11.70	64.70	23.50	3.40 ± 0.50
MTX 20	13.60	20.0	40.0	26.30	19.60 ± 1.48**
Fumagillin 10	13.60	13.60	45.45	27.20	4.40 ± 0.81
15	16.20	18.60	37.20	27.90	1.46 ± 8.60
20	14.20	21.40	35.70	28.50	14.00 ± 2.07**
Royal Jelly. 100	8.30	17.0	50.0	25.0	2.40 ± 0.74
Fumagillin + R.J. 10 + 100	8.30	-	58.30	33.30	2.40 ± 0.50
15 + 100	13.60	9.0	45.40	31.80	4.40 ± 0.92
20 + 100	12.80	17.90	38.40	30.70	7.8 ± 1.39**

T: treatment; ace: a centric fragment; A.uval: autosomal univalent; S.uval: seual univalent. M: mean; S.E.: standard error mean.

The results in Table (3) also showed that treatment with 10 and 15 mg/kg.bwt. of fumagillin with 100mg of royal jelly have no significant differences when compared with negative control. The mean value of total aberration were largely less than that of treatment with fumagillin only. Besides that, the mean value of total chromosome aberration coincides with the dosage 20mg/kg.bwt. of fumagillin and 100mg of royal jelly (7.8 ± 1.39) showed significant decrease when compared with treatment with fumagillin only (14.0 ± 2.07), although its value did not return to the level of negative control (7.8 ± 1.39) Table (3).

Mitotic Index (MI) of bone-marrow cells in male white mice after treatment with fumagillin and royal jelly

Table 4. shows that treatment with 10, 15, and 20 mg/kg.bwt. of fumagillin caused dose related significant decrease in the value of mitotic index, the mean values were (3.9 ± 0.29), (3.6 ± 1.8), and (2.9 ± 2.9) respectively compared with (7.1±0.29) for negative control.

Table 4 Mitotic index of bone-marrow cells in male white mice after treatment with fumagillin and royal jellyMI

T Mg/kg.bwt.	M ± S.E.
Sugar solution 1:1	7.10 ± 0.29
MTX 20	4.80 ± 0.25**
Fumagillin 10	3.90 ± 0.29**
15	3.60 ± 0.18**
20	2.90 ± 0.18 **
Royal Jelly. 100	6.90 ± 0.24
Fumagillin + R.J. 10 + 100	4.70 ± 0.20**
15 + 100	4.20 ± 0.12**
20 + 100	3.80 ± 0.20**

Number of scored cell for each animal= 1000

Fumagillin interact with the binding of His-231 and Metap-2 and inhibit the enzyme activity of Metap-2 and removing of the N- terminal methionin from most of proteins that engaged in cell cycle regulation as apart of translation process, so its inhibitory effect resulted in blockage of cell cycle and apoptosis [19].. Other studies reported similar results [20, 21, and 22]. On other hand, results of the current study showed no significant differences after treatment with royal jelly 100 mg alone, the mean value of MI (6.90 ± 0.24) which is very close to the value (7.10 ± 0.29) for negative control. Treatment with fumagillin and royal jelly caused changes in the means value of MI compared to treatment with fumagillin only, (4.70 ± 0.20) compared to (3.90 ± 0.29) for the dosage 10 mg/kg.bwt. of fumagillin, (4.20 ± 0.12) compared to (3.60 ± 0.18) for 14 mg/kg.bwt. and (3.80 ± 0.20) compared to (6.90 ± 0.24) for the dosage 20 mg/kg.bwt. of fumagillin.

Our current study suggest that giving royal jelly with the fumagillin didn't ameliorates the bad effects of the studied doses of fumagillin in MI.

References

[1] Urkan, A. and Rena, G. (1999). The inhibition of the genotoxic effect of environmental pollution and the aging processes by plant anti mutagens. Traditional Biology. 23: 135-142.

[2] Abdullatif, Mohammed Abbas (1986) Honey bee, Clloge of agriculture, Al- Exandria University , Eygpt.

[3] Al Bunni, Mohammed Ali (1979) Honey bee and it products. College of agriculture, Ein Shams University, Eygpt.

[4] Al-Berikdar, Z.T.H. (2007) Evaluation some Immunological, anti mutagenic and hepato protective properties of Oregano leaves extracts (*Origanum vulgare*) CCL4- induced acute hepatic injury in albino male mice. An MSC. Thesis. College of Science. Al-Nahrin University.

[5] Al- Binna Jihad (2013) Bee keeper guide, Ministry of agricultur, Lebanon. pp. 26-28.

[6] Furgala, B. (1962) Residual fumagillin activity in sugar syrup stored by wintering honey bee colonies. J. Apic. Res. 1, 35-37.

[7] Stanimiovic, Z. Pejin, I.I. Kuli, Z. and Djiporovic, M. (2007) Evaluation og Genotoxic Effects of Fumagillin by Sister chromatid- Exchange and Chromosomal Aberration tests in Human Cell Cultures. Actaveterinaria (Beograd), 57 (2-3): 257-273.

[8] Ames, B.N. (1989) Mutagenesis and carcinogenesis: Endogenous and exogenous Factors. Environ. Molec. Mut. 14, Suppl. 16, 66-77.

[9] Stevanovic, J. Stanimirovic, Z. Radakovic, M. and Stojic, V. (2008) *In vitro* evaluation of the clastogenicity of fumagillin. Environ. Mol. Mutagen. 49, 549-601.

[10] Sader, Foaad Fillip (1997) Royal Jelly, Magical Food and medicine, Dar Sader, Beirut,edit. (1).

[11] Anderson, D., Bishop, JB., Garner, RC., and Selby, PB. (1995) Cyclophosphamide: review of its mutagenicity for an assessment of potential germ cell risk, Mutat Res, 330, 115-8.

[12] Schmid, W. (1975) The micronucleus test. Mutat. Res. 31: 9-15.

[13] Adler, I.D. (1982) in Cytogenetic Assays of Environmental Mutagens, Hsu, T.C. (ed) Allan-held, Osmun and Co. Publ. New Jersey, p. 249.

[14] Zimonjic, D.B. Savkovic, N. and Andjelkovic, M. (1990) Genotoxic agents: the effects: principles and methodology of detection. Naučna knjiga, Beograd, 1-395.

[15] Brewen, J.G. and Preston, R.J. (1973) Chromosome aberrations as a measure of utagenesis: Comparisons in vitro and in vivo and in somatic and germ cells. Environmental Health Persp. 16: 157-166. Environ. Health Perspect. 6, 157-166.

[16] Simar, S. (2010) Study of Genotoxic Activity in Mouse combining the Bone Marrow Micronucleus test with Comet assay in stomach. Fumagillin DCH. Institut Pasteur, December 2010, Study Number FSR-IPL 090903 (In confidence report**).(**

[17] Milan, K. Nevenka, A. Zoran, S. Siniša, R. and Snežana M. (2009) Examination of genotoxic effects of Fumagillin*in vivo. Original scientific paper*,41, (3), :329-338.

[18] Stanimirovic, Z. Nevenka, A. Kulic M, and Maletic M. (2010) Fumagillin-induced chromosome aberrations in mouse bone –marrow cells. *Arch. Biol. Sci.*, Belgrade, 62 (1): 47-55.

[19] Fardis, M. Pyun, H.J. Tario, J. Jin, H. Kim, C.U. and Ruckman, J. (2003) Design, synthesis and evaluation of a series of novel fumagillin analogues. *Bioorg Med Chem*, 11: 5051-5058.

[20] Ingber, D. Fujita, T. Kishimoto, S. Sudo, K. Kanamaru, T. Brem, H. and Folkman, J. (1990) Synthetic analogues of fumagillin that inhibit

angiogenesis and suppress tumour growth. *Nature*, 348 (6301):555-557.

[21] Wang, J. Lou, P. and Henkin, J. (2000) Selective inhibition of endothelial cell proliferation by fumagillinisnot due to differential expression of methionine aminopeptidases. *J Cell Biochem,* 77: 465-473.

[22] Mazzanti, C.M. Tandle, A. Lorang, D. Costouros, N. Roberts, D. Bevilacqua, G. and Steven K. (2004) Early genetic mechanisms underlying the inhibitory effects of endostatin and fumagillin on human endothelial cells. *Genome Res,* 14, 1585-93.

تأثير الغذاء الملكي في السمية الوراثية والسمية الخلوية للمضاد الحياتي فيوماجيللين في الفئران البيض

وجدي صبيح صادق

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

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، والتعرف على تأثير الغذاء الملكي في إزالة الآثار السمية الوراثية والخلوية للفيوماجيللين. تم اعطاء 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 اعطاء مجموعة السيطرة السالبة محلول سكري 50 % وبالطريقة نفسها. اما مجموعة السيطرة الموجبة فقد تم إعطاؤها جرعة واحدة 20 ملغم\كغم. وزن جسم من العقار ميثوتركسيت MTX بطريقة الحقن داخل الخلب. كما تم تقييم تأثير الغذاء الملكي في الحد من التأثير السمي للفيوماجيللين، اذ تم اعطاء 100 ملغم من الغذاء الملكي مع الجرعات المذكورة من الفيوماجيللين. أظهرت نتائج الدراسة ان بالجرعتين التجريبيتين 15 و 20 ملغم\كغم. وزن جسم من الفيوماجيللين تسببت في حث زيادة معنوية 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 في متوسطات الشذوذ الكروموسومي الكلي في خلايا نقي العظم في فئران المجموعتين المذكورة (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 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 في متوسط 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. واظهر الغذاء الملكي قدرة على الحد من التأثيرات السمية الوراثية والسمية الخلوية للفيوماجيللين.

تفترض النتائج المتحصل عليها من الدراسة الحالية ان للجرعات التي تم اختبارها من الفيوماجيللين تأثيرا سميا وراثيا تمثل في زيادة تكرار النوى الدقيقة ومتوسطات الشذوذ الكروموسومي وسمية خلوية تمثلت بالقدرة المضادة لتكثير الخلايا في نقي عظم الفئران البيض وان للغذاء الملكي القدرة على الحد من التأثير السمي الوراثي والسمي الخلوي للفيوماجيللين.