ASSESSMENT OF MUTAGENIC AND ANTIMUTAGENIC EFFECTS OF HONEY FORMED BY INNOVATIVE WAY AGAINST CYCLOPHOSPHAMID INDUCED CHROMOSOMAL ABERRATIONS IN BONE MARROW CELLS

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ABSTRACT

The present study has been done to assess the mutagenic and antimutagenic effects of honey formed by innovative way (HW) in comparison with two honey formed by different feed sources ; nectar of flowers (HF) and sugar syrup (HS), against the cytotoxity and genotoxicity induced by cyclophosphamide (CP) in mice bone marrow cells ,which was evaluated using chromosomal aberration (CA). This search was carried out through two stages. In the first stage, mice were orally treated daily with phosphate buffer saline (PBS) as negative control group, and the other three groups were orally treated daily with two doses of the three types of honey (300 and 600 mg/kg) for 7 and 14 days in order to test the clastogenetic effects of the honey,In the second stage, interactions between the ideal dose (300 mg/kg) of each type of honey and the CP were used for 7 and 14 days in order to test the protective effects of honey formed by innovative way as compard to the other types of honey. The results of the first experiment indicated that the three types of honey has no significant clastogenetic effects on chromosomal aberrations of the bone marrow cells of treated mice. The results of the second experiment was showed that (HW) , especially at the ideal dose (300 mg/kg b.w.) exhibited a well protective and high anti-clastogenic efficiency against the genotoxic actions of the cyclophosphamide (CP) on bone marrow cells, by reduce CA frequencies.

Keywords: cytogenetic , supplementary feeding , clastogenetic effects

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تقييم التأثير التطفيرى والمضاد للتتطفر للعسل المنتج بطريقة مبتكرة ضدّ عقار السيكلوفوسفاميد المحفز للتغيرات الكروموسومية في خلايا نخاع العظم للفئران

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المستخلص

أجريت هذه الدراسة لبحث وتقييم التأثير التطفيري والمضاد التطفيري للعسل الذي تم أنتاجه بطريقة مبتكرة (HW) ومقارنتة مع نوعين من العسل المنتج من مصادر تغذية مختلفة وهي رحيق الأزهار (HF) وشراب السكر (HS) ، ضد التاثيرات الخلوية الناجمة عن استخدام السيكلوفوسفاميد (CP) في خلايا نخاع العظم للفئران من خلال دراسة التشوهات الكروموسومية.أُجريت الدراسة الحالية على مرحلتين ؛ شملت الاولى تقسيم الفئران إلى أربع مجاميع(كل مجموعة احتوت على 3 فئران) اعطيت المجموعة الاولى يوميًا وعن طريق الفم محلول دارى الفوسفات الفسيولوجي (PBS) كمجموعة المقارنة السالبة . وأعطيت للمجاميع الاخرى يوميًا تركيزين لكل نوع من انواع العسل قيد الدراسة (300 و600 ملغم ⁄كغم من وزن الجسم) بالتتابع ولمدة 7 و 14 يوما لإختبار التأثيرات الوراثة الخلوية لانواع العسل المختلفة . في حين شملت المرحلة الثانية اجراء التداخل بين الجرعة المثلى لانواع العسل المختلفة (300 ملغم / كغم من وزن الجسم) مع المطفر السيكلوفوسفاميد (CP) لغرض اختبار التاثيرات الوقائية للعسل المنتج بالطريقة المبتكرة .اظهرت نتائج التجربة الأولى انعدام التأثيرات السمية والتطفيرية لأنواع العسل الثلاث (HS، HW، HF) على التغيرات الكروموسومية في خلايا نخاع العظم في الفئران البيضاء المعاملة . بينما أظهرت نتائج التجربة الثانية أن للعسل المنتج بالطريقة المبنكرة (بالأخص في الجرعة المثلى 300 ملغم / كغم من وزن الجسم) تأثيراً وقائياً وكفاءة تثبيطية عالية تجاه التأثيرات السمية الجينية لعقار سيكلوفوسفاميد في خلايا نخاع العظم , من خلال تقليل اعداد التشوهات الكروموسومية .

الكلمات الافتتاحية: الوراثة الخلوية، التغذية التكميلية، التشوهات الكروموسومية.

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INTRODUCTION

Recently,medicines of natural origin had been received a lot of attention due to the believe that these products have an efficient therapeutics as compared to the synthetic drugs. One of the functional foods is honey which has prophylactic and curative properties.Honey is a natural source of antioxidant (30) it might reduce the risk of disease due to the crucial role of oxidative,through reducing the formation of free radicals or neutralizing them and that will produce beneficial effects in human health (19). It has been also documented that honey exhibits several medicinal properties like antitumor, antimutagenic effects (3،10). Honey contains mainly carbohydrates and water, in addition to minerals, proteins, free amino acids, different enzymes, vitamins, organic acids flavonoids , phenolic acids and other phytochemicals (11). The composition of honey might be differant depending on the plant kinds fed by honeybee and external provisions (environment, processing, of honey and storage conditions) (26). Honeybees need several nutrients, like carbohydrates, proteins, lipids, vitamins, and minerals for their growth and development (3). They receive carbohydrates from nectar and proteins from pollen (12). The success of beekeeping depends on the adequate availability of floral sources (28).These can provide abundant supplies of pollen and nectar when in bloom, but limited resources at other times due to a lack of continuity in the flowering phenology of crops during rainy season (dearth period) because of less floral rewards(7) .This is an important concept to be considered in diet preparation for feeding bees in winter (27).During a reliability of nectar and pollen shortages supplement feeding is necessary for maintenance of bee population (12).Sugar syrup is one of the main sources feed to increase food reserves for overwintering and in the spring to stimulate brood rearing (8). Sugar syrup feeding also stimulated honeybees to increase the natural pollen sheding and become capable of producing brood (20).sugar has long been recognized as having a stimulatory effect, such as an increase pollen-gathering and egg-laying activity as well as increased hygienic behavior of honeybee(24). Recently, the interest in magnetic water has been increased water magnetization changes, water properties which become more energized, active, soft and high pH toward slight alkaline and free of germs (2,15).Previous studies with magnetic water, reported that long-term intake of magnetic water (over 8 weeks) may be beneficial in both prevention and treatment of complications in diabetic. Treatment effect of magnetic water not only decreased the blood glucose and glycated hemoglobin levels, but also reduced blood and liver DNA damages in STZ-induced diabetic rats (15). Ma et al. (18) presented the possibility that magnetic water can prevent aging and fatigue by increasing the cell membrane permeability. Also, Buyukuslu et al. (5) indicated that activity of superoxide dismutase was increased in magnetic field. Another study suggested that the administration of magnetized water to animals for at least 6 weeks can suppress the lymphocyte DNA damages induced by DEN (diethyl nitrosamine) (14). The aim of this study is to evaluate the mutagenic and antimutagenic effects of honey formed by innovative way (HW) in comparison with two honey formed by different feed sources ; nectar of flowers (HF) and sugar syrup (HS), against the cytotoxity and genotoxicity induced by cyclophosphamide (CP).

MATERIALS AND METHODS

Cyclophosphamide (CP) drug

Forty mg of CP were dissolved in sterilized distilled water to prepare the required dose and concentration, which is equivalent to (1mg CP/ animal). Dose injected intraperitoneally according to method of Premkumar et al. (22).

Feeding colonies (bees)

Eight colonies were selected as a container of bees belonging local strain of honey bee *Apis mellifera L.* in Iraq . The colonies were fed every three days and from 30 / April to 15/June

1- The first group (three colonies) : colonies were fed on dissolved sugar in magnetic water (water magnetic /,CRYLOMAG MW). The ratio of sugar to water in the groups was (1: 2) (HW).

2- The second group (3colonies): The colonies fed by dissolved sugar in conventional water

(tap water). The sugar to water ratio in the groups was (1: 2) (HS).

3- Third group (3 colonies): feeding the bees naturally on the flowers of sidr trees. They are prescribed for the treatment of many diseases (HF).

Animals and treatments:

Fiften mice at 9- 12 weeks age and 25–30g in weight which were purchased from National Center for Drug Control and Research / Ministry of Health/ Baghdad. They were housed in plastic cages containing hardwood chips, in animal house laboratory in Biotechnology Research Center , Al-Nahrain. The animals were given water and fed with a suitable quantity of water and complete diet.

Experiment design: The animals were divided into 14 groups as follows:

1- Group I: Negative control (3 mice) : Treated with (0.1 ml) phosphate buffer (PBS).

2- Group 2: Positive control (3mice) the animals were intra peritoneally treated with 0.2ml CP 40mg/kg for 24hr

3- Groups 3, 4 and 5: The animals were Treated with two doses (300 and 600 mg/kg) respectively, of each type of honey under study (9 mice)

4- Pre-drug treatment with CP:

The animals group (3 mice) were orally given (0.5ml) honey formed by-floral resources (Group6), sugar with magnetic water (Group7) and sugar syrup (Group8) respectively, per day for 7 and 14 days, before injected CP (0.2 ml).

5. Post-drug treatment with CP:

The animals group (3 mice) were orally given CP (40 mg/kg) for one day , then followed by honey (300 mg/kg b.w), formed by floral resources (Group9). Sugar with magnetic water (Group10), and sugar syrup (Group11) respectively, per day for 7 and 14 days.

6. Co-drug treatment with CP :

The animals group (3 mice) were orally given CP (40 mg/kg) with 0.5 ml of honey formed by floral resources (Group12). Sugar with magnetic water (Group 13) and sugar syrup (Group14) respectively, per day for 7 and 14 days . After 24 hrs from last dose, all the groups were sacrificed on days 7 and 14 day and bone marrow samples were taken for cytogenetic analysis (CA).

Chromosome preparation:

Colchicines was injected 2 hrs before sacrificed. Mice were sacrificed by cervical dislocations and bone marrow cells were harvested. Colchicine (4mg/kg b.wt.) was administered intraperitoneally 2 hr before the harvest of the cells. The slides prepared essentially using modified method of Preston, et. al.(23).

Statistical Analysis

The Statistical Analysis System- SAS (24) program was used to evalute effect of different factors. Least significant difference –LSD test was used for significant comparison among means.

RESULTS AND DISCUSSION

Chromosome aberrations test is one of the simplest short term test for biomonitoring of the genotoxicity of chemical carcinogens and the effect of putative chemopreventive agents (27). CA in control samples was 0.86 ± 0.25 % . This value increased after CP treatment to 8.35% (Table 1). These significant ($P<0.05$) increases shows the clastogenic effect of CP. The types of aberrations;found were chromatid breaks, chromatid gaps, deletions, fragment chromatid and ring chromosome. Numerical aberrations included aneoploidy and polyploidy were observed in Fig.1 .The results of the frequencies of total CA in the groups of animals treated with low and high dose of three types of honey, which formed by different feed sources (sugar syrup, sugar with magnetic water and nectar of flowers). The data obtained suggested that the selected doses of honey (300 and 600 mg/kg b.w.), showed no significant differences ($P<0.05$) of CA in mice bone marrow cells as compared with the negative control (Table 1).Whereas, high dose (600 mg\ kg) of HS, caused increased in CA as compared with the negative group with no significant differences in comparison with the positive group .Therefore, the (300 mg/kg.b.w.) as a lowest dose was selected and produced lowest mean value of total CA as compared with the negative control.

Table 1 . Chromosomal aberrations of bone marrow cells in mice treated with different doses of types Honey (HF, HS and HW).

Experimental Groups	Dose (mg/kg)	CA%
Negative control	0	0.86 ± 0.25 a
Positive control (CP)	40	8.35 ± 0.41 b
Honey formed by (floral resources (HF)	300	1.86 ± 0.07 a
	600	2.08 ± 0.09 a
Honey formed by (sugar syrup) (HS)	300	3.86 ± 0.11 c
	600	4.45 ± 0.25 c
Honey formed by (sugar with magnetized water) (HW)	300	1.35 ± 0.21 a
	600	2.20 ± 0.08 a

***Values are means (+ standard deviation)**

Interaction between three types of honey (HF ,HS, HW) and (CP) on bone marrow cells in mice : The results of the present investigation confirmed that administration of CP induced asignificant increases in CA as compared to those found in negative control (Tables 2,3,4 and Fig.1.) The development of CA in bone marrow cells of mice induced by CP, may be due to inducing free radicals which have the ability to cause damage to DNA and RNA and inhibit some enzymes by reacting with amino acids (1). This results are consistent with those reported in other studies (28). On the other hand, it has been revealed that oxidative or mutagenic damages of CP could be inhibited by intake of antioxidants and/or free radical scavengers Therefore, it is important to find complementary antioxidant compound that block genotoxicity of CP-induced (9) . At this context, honey-bee have been found to have antioxidant and antimutagenic factors (10). This is a novel study, as used magnetic water in supplementary feeding of bee, in addition it is the first study evaluate the cytogenetic effects of honey (HW) against genotoxicity induced by CP in mouse bone marrow.The present results showed that the mice group treated with honey, before CP (G4) had high significant decreases of total structural aberrations and numerical aberrations ($P<0.05$), as compared to mice group injected with CP alone. Post treatment (G6) showed different protection effect as shown in Table 3. Furthermore, treatment with a mixture of both honey (HW, HF). has ability to reduce CA in similar to the reduction ability of pre-treatment. It was clear that post treatment with honey may activate the suppressing agent or the promoters of DNA repair mechanism and it may increase the error free repair fidelity in the cell (4). The results obtained could reflect the effects of the both honey on the prevention of DNA damage by affecting metabolic pathways being antioxidant or acting on DNA replication (13). as results observed using honey HS in three treatments (before, after and mixture) at two period (7 and 14 day). The experimental results showed significant differences ($P\leq0.05$) between treatments in their effect on all types of CA as well between the two periods. The present data showed in pre treatment (G5) (Table 4), a significant decrease ($P<0.005$) of CA as compared with positive controls. Whereas post treatment (G7) showed no reduction in the frequency of CA in comparison with the positive control. Meanwhile, simultaneous administration (Group10) showed higher effect to reduce the CA comparing with positive controls. It was also observed that there was increased in total CA with increasing treatment period, recording the highest frequency of CA especially, after 14 day on post treatment (Table 3) . Treatment with both honey, before the drug and as a mixture provided protection ratios for CAs more than these ratios when given after drug. So, both honey could be classified as desmutagen in the first order, and bio antimutagenic in the second order (21) .The results showed that HW achieved the best effectiveness to protection against damage induced by CP as compared with HS.Similar results of were found HF, which means that they have similar mechanism of HF to reduce genotoxicity of CP. This result is similar to that observed in the previous study of our laboratory which suggested that using magnetic water in supplementary feeding of bee can firming the protective effect of honey against CP by reduction frequencies of micronucleus in mice bone marrow cells (9) . The mechanism of the effect of HW to modulate cytogenetic effect of CP are not clear. it may be due to use magnetized water in honey bee feeding. Magnetic water has higher pH and electric conductivity as compared to general drinking water (31). Water

magnetization changes water properties which becomes more energized, active, the lowes and highest pH toward slight alkaline and free of germs (6). Mentioned that, water solution increases the fluidity. Physics shows that water change its weight under the influence of magnetic fields. More hydroxyl (OH-) ions are created to form alkaline molecules and reduce acidity.For this reason cancer cells do not survive well in an alkaline environment (9). It is possible that magnetized water activates antioxidant enzymes in the body and reduces DNA damages (17), due to its ability to act as a free radical scavenger and increase the concentrations of endogenous antioxidants such as glutathione (13). The mechanism for protection may be attributed to used magnetic water in formed the honey could influence effectively on the oxidant antioxidant balance (16). Magnetic water can also prevent aging and fatigue by increasing the cell membrane permeability (18).

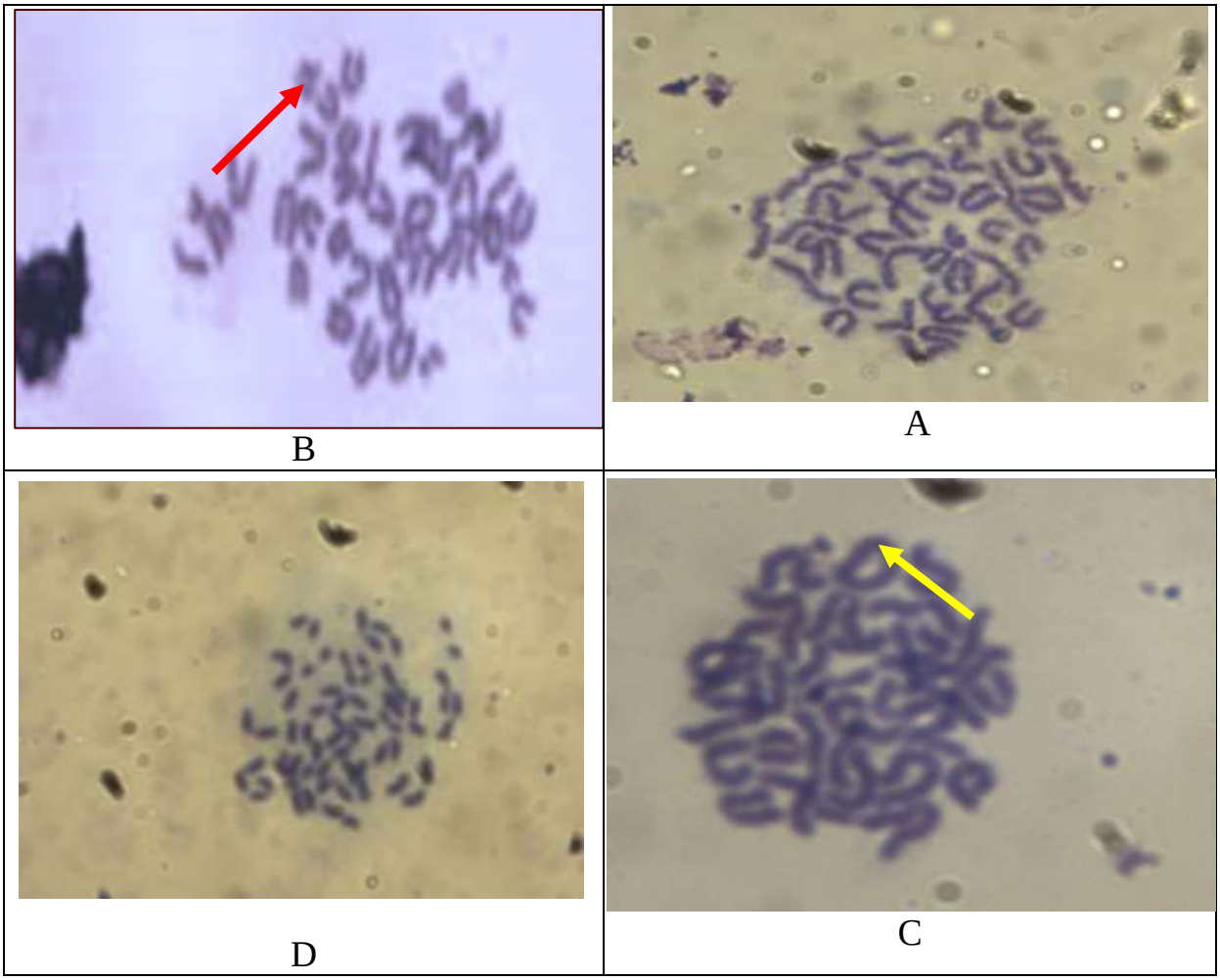

Figure 1.Cells in metaphase stage taken from mice treated with CP the positive control (40mg/ kg), showing: Normal chromosome (A), chromosome break (B), ring chromosome (C), and fragment chromatid (D) (100 x).

Table 2. Protective effects of Honey formed by (floral resources) against cyclophosphamide induced structural and numerical chromosomal aberrations in mice bone marrow cells.

Experimental Groups		Dose mg/kg	Chromosomal aberrations				Chromatid aberrations			Total
			Acentric Fragment	Ring	Poly	Dele	*Gap	Break	Fragment	
G1		0	0.67 ± 0.002	0.0 ± 0.00	0.00 ± 0.00	0.0 ± 0.00	0.054 ± 0.001	0.12 ± 0.003	0.97 ± 0.05	1.814 ± 0.07
G2		40	1.17 ± 0.007	1.80 ± 0.0004	0.0135 ± 0.0002	0.086±0.002	0.452 ± 0.07	1.02 ± 0.07	3.86 ± 0.11	8.4015 ± 0.52
7 day	G3	300	0.677 ± 0.007	0.0 ± 0.00	0.0023 ± 0.00002	0.0 ± 0.00	0.07 ± 0.003	0.023 ± 0.0004	1.16 ± 0.06	1.9323 ± 0.06
	G6		0.56 ± 0.01	0.848 ± 0.002	0.005 ± 0.00001	0.00 ± 0.00	0.076 ± 0.0006	0.56 ± 0.002	2.12 ± 0.09	4.169 ± 0.15
	G10		0.85 ± 0.008	1.022 ± 0.003	0.0098 ± 0.00033	0.050 ± 0.001	0.25 ± 0.004	1.00 ± 0.0005	2.051 ± 0.07	5.2328 ± 0.26
	G12		0.78 ± 0.004	0.56 ± 0.002	0.00 ± 0.00	0.030 ± 0.0004	0.18 ± 0.002	0.69 ± 0.0002	1.89± 0.04	4.13 ± 0.13
14	G3	300	0.0 ± 0.0	0.0 ± 0.00	0.00 ± 0.00	0.0 ± 0.00	0.86 ± 0.005	0.05 ± 0.002	0.83 ± 0.05	0.94 ± 0.08
	G6		0. 32 ± 0.0002	0.43 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.015 ± 0.0002	0.23 ± 0.0005	1.85 ± 0.05	2.845± 0.07
day	G9		0.45 ± 0.001	0.73 ± 0.004	0.0012 ± 0.00004	0.013 ± 0.0002	0.15 ± 0.0004	0.88 ± 0.002	1.97 ± 0.03	4.1942± 0.11
	G12		0.55 ± 0.003	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.55 ± 0.03	1.035 ± 0.07	2.135 ± 0.04
LSD value		---	0.459 *	0.573 *	0.0061 *	0.0369 *	0.366 *	0.593 *	0.731 *	2.166 *

*** Statistical significance (P< 0.05)**

G1: Negative control, G2: Positive control (CP),G3: Honey formed by (floral resources), G6: Pre - CP, G9: Post-CP, G12: co-treatment(7and 14 days)

Table 3 . Protective effects of Honey formed by (sugar with magnetized water) against cyclophosphamide induced structural and numerical chromosomal aberrations in mice bone marrow cells

Experimental Groups		Dose mg/kg	Chromosomal aberrations				Chromatid aberrations			Total
			Acentric Fragment	Ring	Poly	Dele	Gap	Break	Fragment	
G1		0	0.20 ± 0.008	0.043 ± 0.002	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.005	0.23 ± 0.02	0.56 ± 0.03	1.053 ± 0.06
G2		40	1.17 ± 0.005	1.80 ± 0.05	0.0135 ± 0.00094	0.086 ± 0.0004	0.67 ± 0.02	1.02 ± 0.06	3.86 ± 0.07	8.6195 ± 0.46
G4		300	0.012 ± 0.007	0.051 ± 0.001	0.00 ± 0.00	0.00 ± 0.00	0.052 ± 0.007	0.62 ± 0.02	0.87 ± 0.03	1.605 ± 0.09
7 day	G7		0.73 ± 0.004	0.65 ± 0.05	0.00 ± 0.00	0.021 ± 0.0007	0.24 ± 0.03	0.88 ± 0.04	0.90 ± 0.05	3.421 ± 0.15
	G10		0.87 ± 0.02	1.27 ± 0.02	0.001 ± 0.0004	0.00± 0.00	0.452 ± 0.03	0.96 ± 0.07	1.15 ± 0.08	4.703 ± 0.15
	G13		0.34 ± 0.02	0.54 ± 0.003	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.004	1.33 ± 0.05	1.03 ± 0.06	3.69 ± 0.09
`14 day	G4	300	0.010 ± 0.0004	0.026 ± 0.0009	0.00 ± 0.00	0.00 ± 0.00	0.035 ± 0.0006	0.20 ± 0.006	0.62 ± 0.02	0.891 ± 0.07
	G7		0.42 ± 0.005	0. 00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.55 ± 0.009	0.98 ± 0.05	1.95 ± 0.08
	G10		0.68 ± 0.02	0.72 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.26 ± 0.005	0.95 ± 0.023	1.00 ± 0.06	3.61 ± 0.11
	G13		0. 25 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.62 ± 0.08	0.87 ± 0.04	1.74 ± 0.04
	LSD value	---	0.287 *	0.475 *	0.0135 NS	0.0359 *	0.296 *	0.573 *	0.577 *	`0.892 *

*** Statistical significance (P< 0.05),**

G1: Negative control, G2: Positive control (CP),G4: Honey formed by (sugar with magnetized water), G7: Pre - CP, G10: Post-CP, G13: co-treatment(7and 14 days)

Table 4 . Protective effects of Honey formed by (sugar syrup) against cyclophosphamide induced structural and numerical chromosomal aberrations in mice bone marrow cells

Experimental Groups		Dose mg/kg	Chromatid aberrations			Chromosomal aberrations			Total
			Fragment	Break	Gap	Deletions	Ring	Acentric Fragment	
7 day	G1	0.00	0.00 ± 0.00	0.66 ± 0.04	0.76 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.008	1.57 ± 0.005
	G2	40	3.86 00 ± 0.08	2.15 ± 0.08	3.32 ± 0.06	1.05 ± 0.01	1.85 ± 0.04	1.32 ± 0.003	13.55 ± 0.72
	G5	300	1.15 00 ± 0.006	0.62 ± 0.03	1.55 ± 0.02	0.00 ± 0.00	0.87 ± 0.04	0.012 ± 0.0006	4.202 ± 0.31
	G8		0.00 00 ± 0.00	0.87 ± 0.08	2.645 ± 0.08	0.09 ± 0.005	1.40 ± 0.04	0.56 ± 0.008	6.315 ± 0.37
	G11		2.89 00 ± 0.06	1.534 ± 0.08	2.26 ± 0.06	0.75 ± 0.002	0.845± 0.06	1.15 ± 0.02	9.429 ± 0.61
	G14		2.81 00 ± 0.07	1.38 ± 0.04	2.44 ± 0.09	0.75 ± 0.008	0.00 ± 0.00	0.15 ± 0.006	7.53 ± 0.35
14 day	G5		2.13 00 ± 0.04	0. 65 ± 0.03	1.90 ± 0.03	0.01 ± 0.002	0.86 ± 0.02	0.58 ± 0.009	6.13 ± 0.25
	G8		1.22 00 ± 0.02	2.03 ± 0.07	2.15 ± 0.05	0.84 ± 0.009	1.10 ± 0.03	0..94 ± 0.04	8.28 ± 0.52
	G11	300	3.25 ± 0.07	1.89 ± 0.04	2.76 ± 0.06	0.84 ± 0.02	1.06 ± 0.03	0.95 ± 0.02	10.75± 0.78
	G14		2.16 ± 0.06	1.13 ± 0.06	2.54 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.02	5.98 ± 0.022
LSD value		---	0.863 *	0.749 *	0.827 *	0.562 *	0.783 *	0.533 *	2.317 *

*** Statistical significance (P< 0.05)**

***G1: Negative control, G2: Positive control (CP),G5: Honey formed by (sugar syrup), G8: Pre - CP, G11: Post-CP, G14: co-treatment(7and 14 days)**

This study was amied to evaluate the importance of using magnetic water in supplementary feeding for the purpose of forming honey. It was found that using of magnetic water instead of general water as an supplementary feeding for bees improved the honey quality which consequently improves antioxidant status, and limit dangerous effect of anticancer drug CP. This strategy is necessary for diminishing the deleterious side effects of anticancer drug with preservation of its chemotherapeutic efficacy. However, further studies in this field are required to confirm these results.

REFERENCES

1. Aditya, M.; G .,Vishhal; N. ,Hemant and S. ,Vishal. 2013. Protective effect of (*Curry Leaf*) leaves extract against genotoxicity induced by cyclophosphamide in mouse bone marrow cells. Glo. Vete. 10(2): 128-133

2. Al-Mufarrej ,S.; H.A. ,Al-Batshan; M.I. ,Shalaby and T.M., Shafey .2005. The effects of magnetically treated water on the performance and immune system of broiler chickens. Inte J Poul Sci 4(2): 96-102.

3. Breeze,T.D. ; A.P., Bailey; K.G.,Balcombe and S.G. Potts 2011. Pollination services in the UK: How importantare honey bees? Agriculture, Ecosystems and Environment.142 (3):137-143

4.Gautam,S.; S.,Saxena and S.,Kumar 2016.Fruits and vegetables as dietary sources of antimutagens. J Food Chem Nanotechnol 2(3): 97-114

5. Buyukuslu, N.O. and C. C., Atak .2006. The effect of magnetic field on the activity of superoxide dismutase. Journal of Cell and Molecular Biology 5(1): 57-62.

6. Cho, Y.I. and S.H., Lee .2005. Reduction in the surface tension of water due to physical water treatment for fouling control in heat exchangers. ICH and Mass Transfar. 32(2): 1-9

7. Decourtye ,A.; E.,Mader and N., Desneux.2010.Landscape enhancement of floral resources for honey bees in agroeco systems. Apidologie. 41(1): 264-277

8. De Grandi Hoffmanh, G. ;G., Wardell;F., Ahumada Segura ;T., Rinderer ;R. , Danka and J., Pettis .2008.Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. J Apic Res and Bee World .47(4):265-270.

9. Ekhlas, M.F. ; A. C., Rukaibaa and Q. A.,Lina. 2017 .Investigation of role magnetized water used in supplementary feeding for honeybees to modulate the genotoxic side effects induced by cyclophosphamide in mice bone marrow cells . J Contemp Med Sci .3 (12) : 313–318

10. Elfiky, S. A; I. M., Farag; K. M., Zoheir; N. H., Hassan, and H. G., Elalfy.2013. The protective role of honey-bee products against the genotoxic effects of cyclophosphamide in male mice .J. Appl. Sci. Res. 9(8): 4745-4758

11. El Sohaimy ,S.A. ; S.H.D.,Masry and M.G., Shehata. 2015 . Physicochemical characteristics of honey from different origins .Annals of Agricultural Science 60(2): 279–287

12. Lee, H.J.; H.R., Jo; E.J., Jeon and M.H.,Kang.2010.Effect of the magnetized water supplementation on lymphocyte DNA damage in mice treated with diethyl nitrosamine. Korean. J. Nutr.43(6):570-577

13. Lee ,H.J.**;**H.R. Jo; E.J., Jeon and M.H. Kang. 2013 .Effect of the magnetized water supplementation on blood glucose, lymphocyte DNA damage, antioxidant status, and lipid profiles in STZ-induced rats. Nutr Res Pract .7(1):34-42

14. Lee ,H.J.;Y.K., Park and M.H., Kang. 2011. The effect of carrot juice, β carotene supplementation on lymphocyte DNA damage, erythrocyte antioxidant enzymes and plasma lipid profiles in Korean smoker .Nutr Res Pract.5(6):540-547

15. Liboff ,A.R; S., Cherng; K.A., Jenrow and A.,Bull. 2003. Calmodulin-dependent cyclic nucleotide phosphodiesterase activity is altered by 20 μ Tmagnetostatic fields. Bio Electro Magnetics. 24(1):32-38

16. Ma, Y.L.;H., Ren; S., Ren; E.K., Zhen and G . Hao. 1992.A study of the effect of magnetic water on enzyme activities by potentiometric enzyme electrode method. J Tongji Medi Univ 12(4): 193-196.

17. Maurya, S.; A. K., Kushwaha; S. Singh and G. Singh. 2014. An overview on antioxidative potential of honey from different flora and geographical origins. IJNPR, 5(1): 9-19

18. Mohebodini, H and G., Tahmasbi. 2013 . Effect of dietary thiamine on growth of the Iranian honey bee colonies (*Apis mellifera meda*) in different seasons .Agriculture and Forestry.59(3):119-126.

19. Oliveira, R.J.; L.R., Ribeiro; A.F., da Silva and R., Matuo. 2006. Evaluation of antimutagenic activity and mechanisms of action of beta-glucan from barley, in CHO-k1 and HTC cell lines using the micronucleus test. Toxicol. In Vitro. 20(7): 1225-1233

20. Premkumar, K.;S., Kavitha; S.T., Santhiya and A.R. , Ramesh 2004. Interactive effects of saffron with garlic and curcumin against Cyclophosphamide induced genotoxicity in mice. Asia. Pac. J Clin Nutr. 13(3): 292-294

21. Preston, R.J.; B.J.; S.,Dean ; H.,Galloway; A.F., M., Holden and M., Shelby. 1987. Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat. Res. 189(2): 157-165

22. Sammataro, D. and W., Milagra .2012. comparison of colonies of honey apis mellifera supplemented with sucrose of high fructose bcorn syrup .Journal of Insect Science. 13(19):1-13

23. SAS. 2012.Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA

24. Saxena, S.; S., Gautam and A.,Sharma.2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem. 118(2): 391–397

25. Sihag, R. C. and M., Gupta .2013. Testing the effects of some pollen substitute diets on colonies build up and economics of beekeeping with *Appis mellifera* L. Journal of Entomology 10 (3):120-135

26. Skubida , P ; P., Semkiw, and K., Pohoreck .2008 . Stimulative feeding of bees as one factor in preparing colonies for early nectar flows. Journal of Apicultural Science .52(1): 65-72

27. Tolera ,K.2014. Testing the effect of dearth period supplementary feeding of honey bee (*Apis mellifera)* on brood development and honey production .International Journal of Advance Research. 2(11):319-324

28. Tripathi, R.; D.S. Pancholi and P., Tripathi. 2011. A 14-Day subchronic genotoxicity study of nimesulide in mouse bone marrow cells in vivo. Pharmacology online. 1: 544-551

29. Vallianou, N .G.; P., Gounari; A., Skourtis; J., Panagos and C., Kazazis .2014 . Honey and its anti-Inflammatory, anti-bacterial and anti-oxidant properties. Gen Med (Los Angel) 2(1): 132.

30. Wang, X. H.; L.,Andrae, and N. J.,Engeseth. 2002.Antimutagenic effect of various honeys and sugars against Trp-p-1.Journal of Agricultural and Food Chemistry.50(.23):6923–6928

31. Xu, Y.B. and S.Y., Sun. 2008. Effect of stable weak magnetic field on Cr(VI) bio-removal in anaerobic SBR system. Biodegradation.19(3): 455-62.