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A mixture of honey bee products ameliorates the genotoxic side effects of cyclophosphamide

Maha Aly Fahmy[1*], Nagwa Hassan Aly Hassan[2], Samia Ahmed El-Fiky[3], Heba Gamal Elalfy[3]

[1]*Department of Genetics and Cytology, National Research Centre, 33 El-Bohouth st. Dokki, Giza, P.O. 12622, Egypt*

[2]*Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt*

[3]*Department of Cell Biology, National Research Centre, 33 El-Bohouth st. Dokki, Giza, P.O. 12622, Egypt*

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ABSTRACT

Objective: To evaluate the protective role of a mixture of honey bee products (honey, royal jelly and pollen grains) against the genotoxicity induced by the anticancer drug cyclophosphamide (CP).
Methods: The study included chromosomal aberration analysis in mice bone marrow cells, induction of morphological sperm abnormalities, DNA fragmentation and histopathological changes induced in liver cells of mice. CP was injected intraperitoneally at the dose of 20 mg/kg body weight. The mixture of honey bee products was administrated orally for different periods of time 5, 10 and 15 days with a dose exactly equivalent to the daily intake of human beings.
Results: The results revealed that honey mixture ameliorated the genotoxic side effects of CP. For chromosomal aberrations the percentage reached 25.20 ± 1.30 for CP treated group, while it reached half of that value 12.30 ± 0.54 in CP-group pretreated with honey mixture for 15 days. Breaks, fragments and multiple aberrations were the most pronounced types of aberrations induced after CP treatment and honey mixture reduced these types of abnormalities. CP induced significant percentage of sperm abnormalities 8.52 ± 0.17 compared to control 3.10 ± 0.10. The percentage of sperm abnormalities reached nearly to the control value in CP- mice treated with honey mixture for 15 days. Honey also reduced the incidence of liver DNA damage induced by CP. The results also indicated that CP had a marked damaging effect on liver tissue including severe dilatation, congestion of main blood vessels and massive infiltration of inflammatory cells with irregular general pattern of the tissue. These effects were greatly ameliorated by using oral administration of honey mixture for different periods of time.
Conclusions: The results concluded that honey bee mixture can be used as chemopreventive agent for minimizing the genotoxic side effects of the anticancer drug CP and open the field for its use in many applications.

1. Introduction

Cancer is a leading cause of death worldwide. Several protocols using chemotherapic and radiotherapic approaches have been developed as anticancer treatments[1]. For over 40 years, cyclophosphamide (CP) has been in clinical use for treatment of various forms of cancer and autoimmune diseases and also used as immunosuppressant after organ transplantations (*e.g.* bone marrow transplantations)[2-4].

CP is a cytotoxic bifunctional alkylating agent belongs to the class of nitrogen mustard. It undergoes bioactivation by hepatic microsomal cytochrome P450 mixed function oxidase system to active metabolites that enter the circulatory system. Phosphoramide mustard and acrolein are the two active metabolites of CP[5]. In spite of the therapeutic importance of CP, a wide range of adverse side effects including genetic and reproductive toxicity as well as histological alterations have been demonstrated following its use in human and experimental animals[6-8].

The generation of free radicals and other reactive oxygen species as well as lipid peroxidation have been reported to be the major mechanisms in CP–toxicity[7,9]. Such oxidative stress generates biochemical and physiological disturbances[10]. Single and double strand scissor of DNA are also produced[11,12] leading to chromosome aberrations and depression of mitotic index[13]. With respect to the effect on reproductive organs, it was found that, male cancer patients treated with CP have diminished sperm counts

*Corresponding author: Maha Aly Fahmy, Professor of Cytogenetics–Genetics and Cytology Department, Division of Genetic Engineering and Biotechnology, National Research Centre, Dokki, Cario, Egypt.
Tel: 00201069937580
E-mail: maha_sadky@yahoo.com
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and absence of spermatogenic cycles in their testicular tissue[14]. Increase in the incidence of oligozoospermia and azoospermia was also detected[15,16].

Antioxidants play a vital role against the deteriorating action of free radicals in the organisms. Honey a sweet natural product produced by honey bees from nectar and other plant juice is also a good source of antioxidants. Several studies evidenced that different types of honeys and honey products from various countries have antioxidant capacity depending on the concentration of different bioactive compounds like phenolics, proline, vitamins, catalase, glucose oxidase, organic acids and amino acids[17-19].

The present study was designed to assess the protective role of a mixture of honey bee products (honey, royal jelly and pollen grains) against the genotoxicity induced by the anticancer drug CP and correlated this activity with the chemical composition of these natural products.

2. Materials and methods

2.1. Experimental animals

Male white Swiss mice aged 8-12 weeks and weighting 20-25 g were used in all experiments. Animals were obtained from a closed random bred colony at the National Research Centre. The mice used for any one experiment were selected from mice of similar age (±1 week) and weight (± 2 g). Animals were maintained under controlled conditions of temperature and humidity and received food and water *ad libitum*.

2.2. Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and performed for being sure that the animals do not suffer at any stage of the experiment.

2.3. Chemicals

CP powder (vial) is manufactured by Allmirall Prodesfarma S.L. For Baxter Oncology GmbH–Kantstrasse 2, D–33790 Halle, Germany.

2.4. Honey bee products

Honey bee, royal jelly and pollen grains from *Apis mellifera* were purchased from the Ministry of Agriculture, Cairo, Egypt.

2.5. Dosing

For CP treatment, the dose of 20mg/kg body weight was intraperitoneally injected.

The dose of each component of honey mixture was exactly calculated by Paget formula[20] to be equivalent to the recommended daily intake of human beings. The honey bee mixture consisted of honey bee (5 g/kg body weight), pollen grains 5% (about 250 mg/kg body weight and royal jelly (100 mg/kg body weight). Honey bee mixture was orally administrated for different periods of time.

2.6. Treatment and experimental design

The experimental design for chromosomal aberration analysis, DNA fragmentation and histological examination was undertaken as follows: One group of mice (six mice) was intraperitoneally injected with CP 24 h before sacrificing. Three groups with six mice in each were treated with CP and honey mixture. In these groups honey bee mixture was orally administrated to mice daily for 5, 10 and 15 days and at the last day CP was intraperitoneally injected to mice at a dose of 20 mg/kg body weight (24 h before sacrificing).

The experimental design for sperm–shape abnormalities followed the recommended protocol of Fahmy *et al*.[21]. Animals were injected *i.p.* with CP for three consecutive days and sacrificed 35 days after the 1st injection. The other three groups of mice received CP (3 successive doses) in addition to honey mixture daily for 5, 10 and 15 days starting from the 1st day of treatment.

A concurrent control group orally administrated with honey bee mixture was taken for each treatment in addition to the non-treated control.

2.7. Cytological preparations

2.7.1. Chromosomal aberrations in mouse bone marrow

For slide preparation and scoring, mice were *i.p.* injected with colchicine 2.5 h before sacrificed. Chromosomal preparations from bone marrow were made according to the technique developed by Doherty *et al*.[22]. A group of six mice was used for each treatment and 100 well spread metaphases were analyzed/animal for scoring different kinds of abnormalities. Scoring was performed under 2500 X magnification with a light microscope (Litz, Germany).

2.7.2. Sperm-shape abnormalities

Sperm were prepared according to the recommended method of Fahmy *et al*.[21] and smears were stained with 1% Eosin Y. A Group of six animals was taken for each treatment and a total of 1000 sperm were counted per animal, scoring different types of sperm abnormalities.Sperm preparations were examined by light microscopy at 1000 × magnification.

2.7.3. DNA-fragmentation

The method of DNA fragmentation was carried out in liver tissue by diphenylamine assay according to Donya *et al*.[23]. Mouse liver cells (25 mg) were mechanically dissociated in hypotonic lysis buffer. The cell lysate was centrifuged at 1 400 r/min for 15 min. then, the supernatant containing small DNA fragments was separated from the pellet containing large pieces of DNA. Both supernatants and pellets were used for the colorimetric determination by diphenylamine assay.

$$\% \text{ of DNA fragmentation} = \frac{O.D\ SN}{O.D\ SN + O.D.pellet} \times 100$$

where *O.D.SN* is optical density of supernatant, and *O.D pellet* is optical density of pellet.

2.7.3. Histopathological examination of liver tissue

Specimens of all animals were dissected immediately after sacrificing and fixed in 10% neutral–buffered formal saline for 48–72 h. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol (70%, 80%, 90% and finally absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55 ℃ and embedded in hard paraffin. Serial sections of 6 μm thick were cut and stained with Haematoxylin and eosin for histopathological investigation[24]. Images were captured and processed using Adobe Photoshop version 8.0.

2.8. Statistical analysis

Statistical analysis was performed with SPSS 15 software. Data were analyzed using One way analysis of variance (ANOVA) followed by Duncan's *post hoc* test for multiple comparisons between pairs.

3. Results

The results showed that the used honey mixture was safe in all tests and its effect tended to be normal compared to the control (non-treated).

The frequency of chromosomal aberrations induced in bone-marrow cells after treatment with CP and honey bee mixture was summarized in Table 1. The results showed that CP induced a significant increase in the percentage of chromosomal aberrations in bone marrow cells 24 h after *i.p.* injection at the dose 20 mg/kg body weight. Such percentage reached 25.20 ± 1.30 compared with 3.00 ± 0.34 for the negative control. The CP–induced aberrations were greatly reduced in animals pretreated orally with honey mixture for different periods of time 5, 10 and 15 days. The percentage of aberrations reached (12.3 ± 0.54) in CP–group treated with honey for 15 days which represent approximately one-half of that of CP–group.

Concerning the types of chromosomal aberrations induced in bone marrow cells, both structural and numerical aberrations were recorded. Break and/or fragments and multiple aberrations were the most types of aberrations after treatment with CP. It was found that treatment with honey mixture reduced these types of aberrations.

Table 2 showed that *i.p.* injection with CP 20 mg/kg body weight for 3 successive days induced significant percentage of sperm abnormalities 35 days after 1st injection. Such percentage reached 8.52 ± 0.17 compared with 3.10 ± 0.10 for the control. The results indicated that oral administration of honey mixture for 5, 10 and 15 days starting from the 1st day of treatment significantly minimized the CP–induced sperm abnormalities and the percentage reached approximately the value of control (untreated) in CP–mice treated with honey for 15 days 3.30 ± 0.15. Coiled tail was the most pronounced morphological sperm abnormalities induced after CP treatment. The percentage of coiled tail abnormality reached 4.950% of the total counted sperm compared with 0.550% for the control. Honey mixture significantly reduced such effect.

CP also induced liver DNA fragmentation (damage) which was ameliorated by using honey mixture (Table 3).

Table 1

Percentage of metaphases with different types of chromosomal aberrations induced by treatment with CP and honey bee mixture. %.

Treatment and dose	Chromatid and/or chromosome gap	Break and/or frag	Break and/or frag + gap	Del	Del + frag/break or gap	Ring	MA	Endo	Poly	Abnormal metaphase mean% ± SE
Control (untreated)	1.33	1.00	–	–	–	–	–	0.67	–	3.00 ± 0.34^{a}
CP (20 mg/kg body weight)	6.00	10.00	1.00	2.00	0.33	0.17	4.67	1.00	–	25.20 ± 1.30^{d}
Honey (5 days)	1.00	0.67	–	–	–	–	–	–	1.00	2.67 ± 0.62^{a}
Honey (5 days) + CP	2.67	5.67	0.67	2.00	0.33	0.33	4.67	–	0.33	16.67 ± 0.75^{c}
Honey (10 days)	1.67	0.33	–	–	–	–	–	–	1.17	3.20 ± 0.35^{a}
Honey (10 days) + CP	3.00	5.00	–	1.33	0.33	0.33	2.33	0.67	0.67	13.67 ± 1.20^{bc}
Honey (15 days)	0.83	0.67	–	–	–	–	–	0.33	1.00	2.80 ± 0.28^{a}
Honey (15 days) + CP	2.00	4.67	1.00	0.67	0.33	0.33	2.67	0.33	0.33	12.30 ± 0.54^{b}

No. of mice in each group was six. No. of examined metaphases was 600 (100/mice). Frag: Fragment: Del: Deletion; MA: More than five aberrations in the same metaphase (with the same or different types of aberrations); Endo: Endomitosis; Poly: Polyploidy. Values with different superscript letters within a column represent significant statistical differences ($P < 0.05$).

Table 2

Percentage of different types of sperm abnormalities induced after treatment with CP and honey bee mixture.

Treatment and dose	Head abnormalities (%)							Tail abnormalities (%)	Abnormal sperm mean% ± SE
	Without hook	Amorphous	Triangle	Banana	Big	Small	Forked	Coiled tail	
Control (untreated)	0.62	1.02	0.45	0.10	0.25	0.07	0.05	0.55	3.10 ± 0.10^{b}
CP (20 mg/kg body weight)	1.42	0.78	0.33	0.40	0.10	0.32	0.22	4.95	8.52 ± 0.17^{e}
Honey (5 days)	0.62	0.27	0.48	0.25	0.17	0.03	–	0.75	2.57 ± 0.13^{a}
Honey (5 days) + CP	1.02	0.98	0.40	0.22	0.10	0.12	0.03	2.68	5.55 ± 0.14^{d}
Honey (10 days)	0.55	0.23	0.38	0.25	0.23	0.08	0.07	0.60	2.40 ± 0.10^{a}
Honey (10 days) + CP	0.72	0.88	0.38	0.18	0.05	0.12	0.15	1.82	4.30 ± 0.13^{c}
Honey (15 days)	0.23	0.33	0.30	0.32	0.37	0.07	0.07	0.53	2.20 ± 0.10^{a}
Honey (15 days) + CP	0.78	0.58	0.23	0.38	0.02	0.20	0.12	1.02	3.30 ± 0.15^{b}

No. of mice in each group was six. The number of examined sperm was 6000 (1000/mice). Values with different superscript letters within a column represent significant statistical differences ($P < 0.05$).

The results of the present study revealed the normal structure of the liver tissue in control group (Figure 1), and also there's normal liver tissue in 5, 10 and 15 days of oral administration of honey mixture (control honey), where the administration of honey for 15 days showed vesicular nuclei of hepatocytes (Figure 2).

CP 20 mg/kg body weight had a marked damaging effect on liver tissue including severe dilatation, congestion of main blood vessels and massive infiltration of inflammatory cells with irregular general pattern of the tissue (Figure 3). These effects were greatly ameliorated by using pre-oral administration of honey mixture for different periods of time. A positive effect was obtained by using honey mixture for 5 days included marked reduction of inflammatory cells infiltration, although the main blood vessels were still dilated and congested. The results in 10 days showed only a few inflammatory cells beside blood vessels that showed mild dilatation with or without congestion and the administration of honey for 15 days showed great amelioration of liver tissue, and the general architecture of tissue was approximately like the normal (Figure 4).

Table 3

Percentage of DNA fragmentation induced in liver cells after treatment with CP and honey bee mixture. mean ± SE.

Treatment and dose	% DNA fragmentation
Control (untreated)	61.47 ± 0.58^{b}
CP (20 mg/kg body weight)	70.67 ± 0.34^{e}
Honey (5 days)	61.17 ± 0.45^{ab}
Honey (5 days) + CP	70.23 ± 0.18^{de}
Honey (10 days)	59.62 ± 0.23^{ab}
Honey (10 days) + CP	68.50 ± 0.21^{d}
Honey (15 days)	57.92 ± 0.34^{a}
Honey (15 days) + CP	65.77 ± 0.85^{c}

The number of mice was six in each group. Values with different superscript letters within a column represent significant statistical differences ($P < 0.05$).

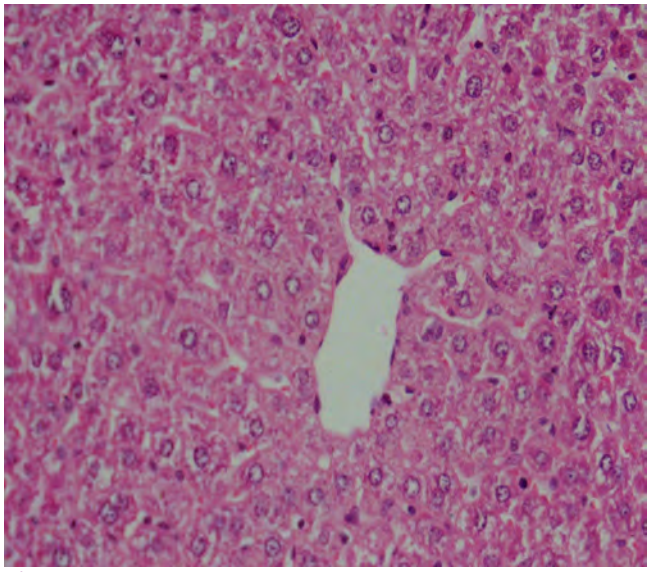

Figure 1. Photomicrograph of a section of liver tissue from a control mouse.

The structure of the liver is normal. Notice the central vein and the cords of hepatocytes radiating from it.

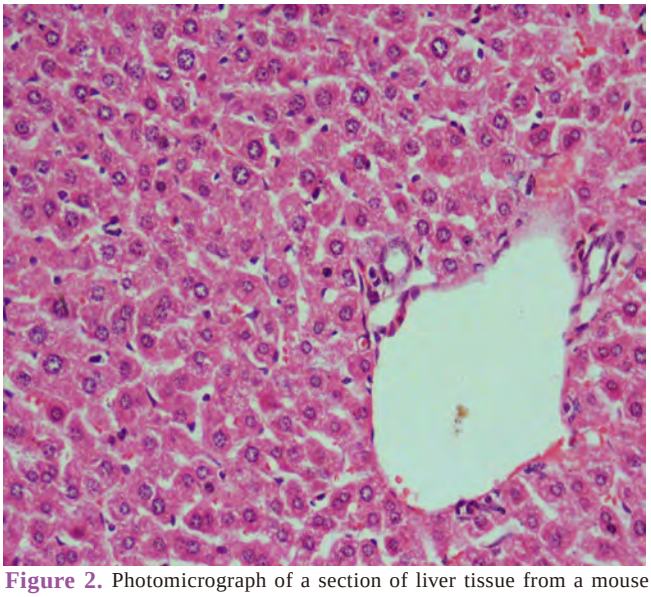

Figure 2. Photomicrograph of a section of liver tissue from a mouse received honey for 15 days.

The liver tissue is absolutely normal. Notice the vesicular nuclei of hepatocytes denoting increased activity of these cells.

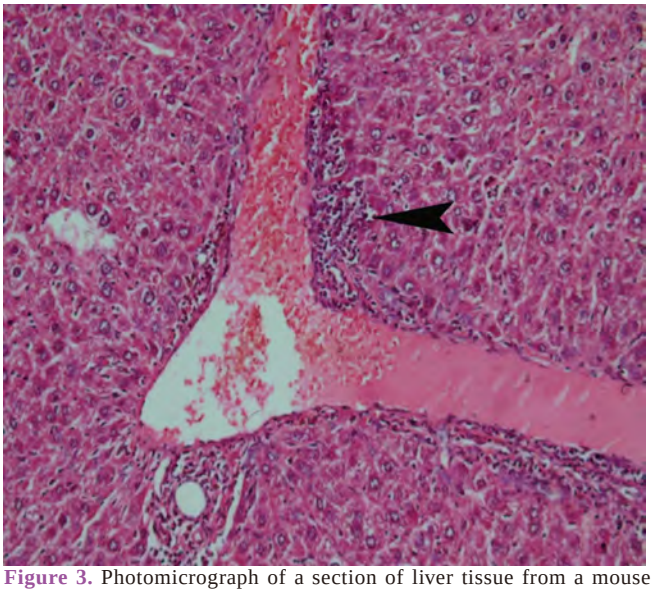

Figure 3. Photomicrograph of a section of liver tissue from a mouse received CP.

Severe dilatation and congestion of main blood vessels and massive infiltration of inflammatory cells with distortion of general architecture of the tissue can be seen.

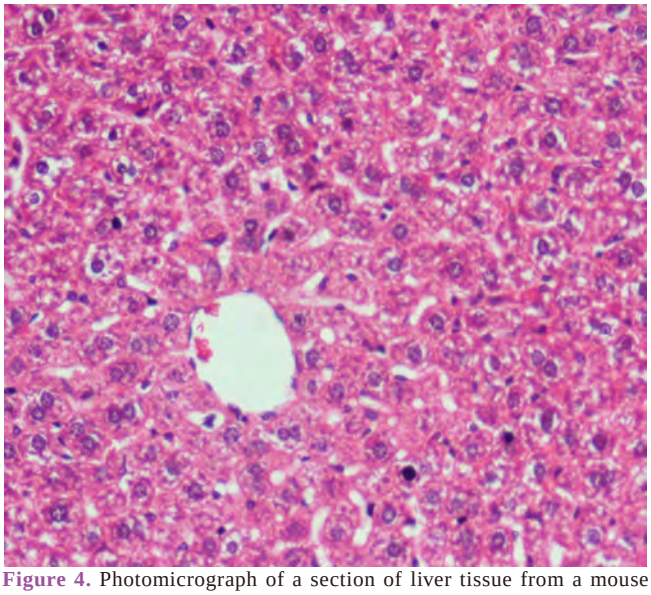

Figure 4. Photomicrograph of a section of liver tissue from a mouse received CP and honey bee mixture for 15 days.

Great amelioration of liver tissue can be seen, and the general architecture of tissue is near the normal.

4. Discussion

The antineoplastic drug CP is a commonly used chemotherapeutic agent for treatment of a wide range of neoplastic diseases and some autoimmune disorders[3,4]. CP was reported to increase the frequency of secondary treatment–related tumors in human cancer survivors due to its genotoxicity[25]. The present work aimed to ameliorate the genotoxic side effects induced by CP treatment using a mixture of honey bee products (honey bee, royal jelly and pollen grains).

The genotoxicity of CP in the present work was evidenced by inducing significant percentage of chromosomal aberrations in mice bone marrow cells. Such results are coincide well with the findings of other authors who proved that CP causes gene mutation,

chromosome breakage, rearrangement, micronuclei and sister chromatid exchange in somatic cells[26,27].

Significant percentage of sperm abnormalities was recorded after CP treatment. Both head and tail abnormalities and sharp decline in sperm count were demonstrated. The head abnormalities most probably reflect a change in DNA content while the coiling of sperm tail involves its orientation, which give an impression of a reduced sperm movement and affect fertility[21]. Germinal cell degeneration, abnormality in sperm head morphology and genotoxicity of different doses of cyclophosphamide in mice were also detected by Tripathi and Jena[28]. CP was also demonstrated to induce an epididymis specific effect on sperm count[29] which can be explained by increase in apoptosis at specific stages of germinal cell cycle and reflect the effect of CP for inducing spermatogenic cell death. In addition, adult male patients treated with CP have demonstrated diminished sperm counts and an absence of spermatogenic cycle in their testicular tissue[14,15].

The results also demonstrated that CP at the tested dose induced significant increase in liver DNA fragmentation as compared to control. The active metabolites of CP named "phosphoramide mustard" and "acrolein" were reported to be bi-functional alkylating in nature and can potentially produce DNA-DNA or DNA-protein cross-links and induce single strand breaks by alkylating DNA at N7 position of guanine[12,30]. Several research studies showed that CP induced DNA damage and apoptosis by a free radical mediated mechanism. It was also reported to induce DNA damage and apoptosis in non-tumor cells which eventually leads to genetic mutation and secondary tumors in these cells[8,30].

CP was reported to be cytotoxic to normal cells. Its metabolites can interact with the big molecules such as proteins, membrane lipids and nucleic acids[31]. In the present work histological examination of liver cells revealed that CP induced severe damaging effect on liver tissue. These findings are coincide well with the results obtained by Ince *et al*.[7] who demonstrated severe damage in liver tissue of rats exposed to *i.p.* injection with CP 75 mg/kg body weight.

It was reported that the toxic side effect of CP is linked with its metabolite acrolein[32]. The damage may occur through the generation of oxidative stress which result in decrease in the activities of the main antioxidant enzymes and increase in free radicals and lipid peroxidation that attack these tissues[31].

The fact that antioxidants have several preventive effects against different diseases, such as coronary diseases, inflammatory disorders, neurologic degeneration, aging and cancer has led to the search for food rich in antioxidants. Honey has been used as a traditional food and medicinal source since ancient times. However, recently many scientists have been concerning with the antioxidant property of honey products[17]. The study of the present work investigated the combined effect of honey mixture and CP. The results revealed that the used honey mixture was safe in all the tests and its effect was normal compared to control. In addition, honey mixture ameliorated all the side effects induced by CP.

With respect to chromosomal aberrations in bone marrow cells, honey was found to significantly reduced the aberrations by CP by about 34%, 46%, and 51% in 5, 10 and 15 days treatment respectively. Honey also reduced the percentage of the different types of aberrations. In sperm, the percentage of abnormalities induced after treatment with CP 8.52 ± 0.17 decreased and reached to normal value 3.30 ± 0.15 in 15 days of treatment compared to the negative control 3.10 ± 0.10. Also the results demonstrated a reduction in the percentage of the induced coiled tail sperm. Such percentage reached 1.020% in CP-treated mice administrated honey for 15 days. This value represents about 79% reduction below the frequencies induced by CP (4.95%). Liver DNA damage was also improved by honey treatment. Honey also ameliorated the histopathological changes induced by CP in liver cells and the liver tissue was recovered to near the normal architecture in 15 days treatment.

Our findings concerning the significant antimutagenic effect of honey are consistent with the reports of other authors who demonstrated strong antimutagenic effect of honey from different floral sources against the effect of the mutagen Trp-p-1 using Ames test[33], physical (UV, γ) and chemical (ethyl methane sulfonate) mutagens[34]. Antigenotoxic effect was also demonstrated by royal jelly supplementation. This was evidenced by reducing chromosomal aberrations, mitotic index and DNA damage induced by mutagenic chemicals[35-37]. The results are also coincide well with the finding of Pinto *et al*.[38] who reported that bee pollen was able to reduce chromosome damage induced by the anticancer drugs mitomycin C, bleomycin and vincristine.

The obtained results showing the protective effect of honey mixture on sperm morphology and count are supported by the findings of Al-Sanafi *et al*.[39] who demonstrated an improvement in sperm parameters and male infertility in a number of infertile men received different doses of royal jelly for 3 months. Also in experimental animals royal jelly administration improved sperm count, sperm viability, oxidative stress, testosterone concentration, sperm DNA damage induced by chemicals that affect male reproductive organs[40,41].

The protective role of honey on liver tissue are in agreement with the finding of Mahesh *et al*.[42] who demonstrated that honey reduced the incidence of liver lesions, oxidative stress and damage induced by acetaminophen in rats.

Also royal jelly was reported to have strong hepatoprotective effect against chemicals known to cause liver damage[43,44]. Royal jelly contains 57–KDa glucoprotein which plays a vital role in stimulating hepatocyte development and liver regeneration. Moreover, bee pollen was revealed to induce hepatoprotective effect against carbon tetrachloride CCl_4 induced liver damage[45].

The protective effect of honey that recorded in all parameters of the present data may be related to its strong antioxidant properties. The basic mechanisms proposed for the antioxidant activity of honey included free radical scavenging capacity, metal ion (pro-oxidant) chelation and those related to oxi-reduction capacity of phenolic due to the presence of hydroxyl groups bounded in an aromatic ring. Organic acids (gluconic, malic and citric), carotenoids, vitamins, enzymes (glucose oxidase and catalase) are compounds originally present in honey bee products but they act as antioxidants. Other compounds such as furfural compound (Maillard reaction products), not originally present in honey but formed during honey processing or storage, may also contribute to the antioxidant activity[46].

Phenolic compounds are considered the main bioactive molecules in honey products that have pharmacological and therapeutic interest. Among the phenolic compounds, phenolic acids (caffeic acid, coumaric acid, ferrulic acid, ellagic acid, chlorogenic acid, gallic acid and syringic acid) and flavonoids (chrysin, pinocembrin, pinobanksin, quercetin, kaempferol, luteolin, galangin, apigenin, hesperetin and myricetin) are the most important in *Apis* honey[17]. Significant correlation was found between honey antioxidant activity and its phenolic contents[47]. Alvarez-Suarez *et al*.[48] reported that honey flavonoids were responsible for the complete inhibition of cell membrane oxidation, as well as intracellular reactive oxygen species production and for the recovery of intracellular glutathione. The flavonoid taxifolin has been described in honey from *Apis mellifera*. Taxifolin is characterized by the presence of several hydroxyls that confer strong antioxidant activity[49]. In royal jelly, in addition to flavonoids, proteins and cinnamic acid derivatives have strong antioxidant activity[50].

Tamura *et al*.[51] attributed antioxidant properties of royal jelly to its free amino acids content (*e.g.* aspartic acid, cysteine, tyrosine, glucine, lysine, leucine, valine and isoleucine). Ohta *et al*.[52] reported that hydroxycinnamic acid derivatives isolated from Brazilian bee pollen have free radical scavenging activity nearly equal to α-tocopherol. Bee pollen is also referred to be the only perfectly complete food, as it contains all essential compounds and amino acids needed for human health that possess protective potential and antioxidant activity.

The anticarcinogenic activity of honey could be another proof of its antimutagenic effect, as cancer is considered as a DNA impair function disease. Honey has a remarkable potential to inhibit cancer cell lines and some animal models of cancer[1,53,54,]. Some of the widely reported phenolic constituents of honey (*e.g.* caffeic acid, chrysin, apigenin, quercetin and others) have evolved as anticancer agents in some recently concluded studies. The antiproliferative and apoptotic activity of honey in most cases are ascribed to its phenolic constituents[55].

Honey products also might exert its protective role via interfering with the immune system. Immuno-modulatory effects of royal jelly were documented and have been ascribed to its protein components, especially to the major royal jelly protein 3[56] and apalbumin 1[57].

The mixture of the three honey products contains many vitamins such B-plex vitamins, pantothenic acid (B5), pyridoxine (B6), α-tocopherol, vitamins A, C, D and B and carotenoid–like substances. The role of these vitamins as antioxidants, anti-carcinogenic and anti-mutagenic is well established.Vitamins also play a vital role in immune system, DNA repair and in improving tissue damage and spermiogenesis[58,59].

For our knowledge, it is the first time of using a mixture of honey bee products for ameliorating the genotoxic side effect of a chemotherapeutic drug. Honey mixture used in the present work are successful in ameliorating the genotoxic effects induced by the anticancer drug CP and the results contribute vital and new knowledge to our understanding which may be useful to overcome side effects produced after treatment with chemotherapy and may open the door for developing a new drug consisting of honey bee products.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Jaganathan SK, Balaji A, Vellayappan MV, Asokan MK, Subramanian AP, John AA, et al. A review on antiproliferative and apoptotic activities of natural honey. *Anticancer Agents Med Chem* 2014; **15**: 48-56.

[2] Bergmann M, Wendtner CM. [Chronic lymphocytic leukemia]. *Dtsch Med Wochenschr* 2015; **140**(7): 479-82. German.

[3] Dan D, Fischer R, Adler S, Förger F, Villiger PM. Cyclophosphamide: as bad as its reputation? Long-term single centre experience of cyclophosphamide side effects in the treatment of systemic autoimmune diseases. *Swiss Med Wkly* 2014; **144**: w14030.

[4] Friedman DL, Chen L, Wolden S, Buxton A, McCarten K, FitzGerald TJ, et al. Dose-intensive response-based chemotherapy and radiation therapy for children and adolescents with newly diagnosed intermediate-risk Hodgkin lymphoma: a report from the Children's Oncology Group Study AHOD0031. *J Clin Oncol* 2014; **2**(32): 3651-8.

[5] Vredenburg G, den Braver-Sewradj S, van Vugt-Lussenburg BM, Vermeulen NP, Commandeur J, Vos JC. Activation of the anticancer drugs cyclophosphamide and ifosfamide by cytochrome P450 BM3 mutants. *Toxicol Lett* 2014; **232**(1):182-92.

[6] Comish PB, Drumond AL, Kinnell HL, Anderson RA, Matin A, Meistrich ML, et al. Fetal cyclophosphamide exposure induces testicular cancer and reduced spermatogenesis and ovarian follicle numbers in mice. *PLoS One* 2014; **9**(4): e93311.

[7] Ince S, Kucukkurt I, Demirel HH, Acaroz DA, Akbel E, Cigerci IH. Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. *Chemosphere* 2014; **108**: 197-204.

[8] Yadav L, Khan S, Shekh K, Jena GB. Influence of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) polymerase, in the evaluation of the genotoxicity of doxorubicin, cyclophosphamide and zidovudine in female mice. *Mutat Res Genet Toxicol Environ Mutagen* 2014; **770**: 6-15.

[9] Liu F, Li XL, Lin T, He DW, Wei GH, Liu JH, et al. The cyclophosphamide metabolite, acrolein, induces cytoskeletal changes and oxidative stress in Sertoli cells. *Mol Biol Rep* 2012; **39**(1): 493-500.

[10] Chakraborty P, Sk UH, Murmu N, Das JK, Pal S, Bhattacharya S. Modulation of cyclophosphamide-induced cellular toxicity by diphenylmethyl selenocyanate *in vivo*, an enzymatic study. *J Cancer Mol* 2009; **4**(6): 183-9.

[11] Khandelwal N, Abraham SK. Protective effects of common anthocyanidins against genotoxic damage induced by chemotherapeutic drugs in mice. *Planta Med* 2014; **80**(15): 1278-83.

[12] Liu M, Hales BF, Robaire B. Effects of four chemotherapeutic agents, bleomycin, etoposide, cisplatin, and cyclophosphamide, on DNA damage and telomeres in a mouse spermatogonial cell line. *Biol Reprod* 2014; **90**(4): 72.

[13] Melek FR, Aly FA, Kassem IA, Abo-Zeid MA, Farghaly AA, Hassan ZM. Three further triterpenoid saponins from *Gleditsia caspica* fruits and protective effect of the total saponin fraction on cyclophosphamide-induced genotoxicity in mice. *Z Naturforsch C* 2015; **70**(1-2): 31-7.

[14] Harel S, Fermé C, Poirot C. Management of fertility in patients treated for Hodgkin's lymphoma. *Haematologica* 2011; **96**(11): 1692-9.

[15] Green DM, Liu W, Kutteh WH, Ke RW, Shelton KC, Sklar CA, et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. *Lancet Oncol* 2014; **15**(11): 1215-23.

[16] Shen GQ, Lu GC, Pan TJ, Xiao YJ. [The changes of IGF-I in testis and epididymis on a rat model with oligozoospermia/azoospermia induced by cyclophosphamide]. *Zhonghua Nan Ke Xue* 2005; **11**(9): 664-6, 669. Chinese.

[17] Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: a review. *J Am Coll Nutr* 2008; **27**(6): 677-89.

[18] Chantarudee A, Phuwapraisirisan P, Kimura K, Okuyama M, Mori H, Kimura A, et al. Chemical constituents and free radical scavenging activity of corn pollen collected from *Apis mellifera* hives compared to floral corn pollen at Nan, Thailand. *BMC Complement Altern Med* 2012; **12**: 45.

[19] Noor N, Sarfraz RA, Ali S, Shahid M. Antitumor and antioxidant potential of some selected Pakistani honeys. *Food Chem* 2014; **143**: 362-6.

[20] Paget GE, Barnes JM. Toxicity tests. In: Laurence DR, Bachorach AL, editors. *Evaluation of drug activities: pharmacomertrics*. New York: Academic Press; 1964, p. 133-66.

[21] Fahmy MA, Hassan NHA, Melek FR, Hassan ZM, Al-Ashaal HA. Studies on the genotoxic effect of nickel chloride in mice and the possible protective role of soybean seeds extracts. *Global J Pharmacol* 2014; **8**(4): 625-34.

[22] Doherty AT, Baumgartner A, Anderson D. Cytogenetic *in vivo* assays in somatic cells. *Methods Mol Biol* 2012; **817**: 271-304.

[23] Donya SM, Farghaly AA, Abo-Zeid MA, Aly HF, Ali SA, et al. Malachite green induces genotoxic effect and biochemical disturbances in mice. *Eur Rev Med Pharmacol Sci* 2012; **16**: 469-82.

[24] Cuce G, Çetinkaya S, Koc T, Esen HH, Limandal C, Balcı T, et al. Chemoprotective effect of vitamin E in cyclophosphamide-induced hepatotoxicity in rats. *Chem Biol Interact* 2015; **232**: 7-11.

[25] Schuurman HJ, Smith HT, Cozzi E. Tolerability of cyclophosphamide and methotrexate induction immunosuppression in nonhuman primates. *Toxicology* 2005; **213**(1-2): 1-12.

[26] Jain R, Jain SK. Effect of *Buchanania lanzan* Spreng. bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice. *Asian Pac J Trop Med* 2012; **5**(3): 187-91.

[27] El-Souda SSE, Mohammed RS, Marzouk MM, Fahmy MA, Hassan ZM, Farghaly AA. Antimutagenicity and phytoconstituents of Egypitan *Plantago albicans* L. *Asian Pac J Trop Dis* 2014; **4**(2): S946-51.

[28] Tripathi DN, Jena GB. Astaxanthin inhibits cytotoxic and genotoxic effects of cyclophosphamide in mice germ cells. *Toxicology* 2008; **248**(2-3): 96-103.

[29] Jalali AS, Hasanzadeh S, Malekinejad H. *Achillea millefolium* inflorescence aqueous extract ameliorates cyclophosphamide -induced toxicity in rat testis: stereological evidences. *Chin J Nat Med* 2012; **10**(4): 247-54.

[30] Zhang QH, Wu CF, Yang JY, Mu YH, Chen XX, Zhao YQ. Reduction of cyclophosphamide-induced DNA damage and apoptosis effects of ginsenoside Rb(1) on mouse bone marrow cells and peripheral blood leukocytes. *Environ Toxicol Pharmacol* 2009; **27**(3): 384-9.

[31] Lata S, Singh S, NathTiwari K, Upadhyay R. Evaluation of the antioxidant and hepatoprotective effect of *Phyllanthus fraternus* against a chemotherapeutic drug cyclophosphamide. *Appl Biochem Biotechnol* 2014; **173**(8): 2163-73.

[32] Kern JC, Kehrer JP. Acrolein-induced cell death: a caspase-influenced decision between apoptosis and oncosis/necrosis. *Chem Biol Interact* 2002; **139**(1): 79-95.

[33] Wang XH, Andrae L, Engeseth NJ. Antimutagenic effect of various honeys and sugars against Trp-p-1. *J Agric Food Chem* 2002; **50**(23): 6923-8.

[34] Saxena S, Gautam S, Maru G, Kawle D, Sharma A. Suppression of error prone pathway is responsible for antimutagenic activity of honey. *Food Chem Toxicol* 2012; **50**(3-4): 625-33.

[35] Cavuşoğlu K, Yapar K, Yalçin E. Royal jelly (honey bee) is a potential antioxidant against cadmium-induced genotoxicity and oxidative stress in albino mice. *J Med Food* 2009; **12**(6): 1286-92.

[36] Abd El-Monem DD. The ameliorative effect of royal jelly against malathion genotoxicity in bone marrow and liver of rat. *J Am Sci* 2011; **7**(12): 1251-6.

[37] Galaly SR, Abdella EM, Mohammed HM, Khadrawy SM. Effects of royal jelly on genotoxicity and nephrotoxicity induced by valporic acid in albino mice. *Beni-Suef Univ J Basic Appl Sci* 2014; **3**: 1-15

[38] Pinto B, Caciagli F, Riccio E, Reali D, Sarić A, Balog T, et al. Antiestrogenic and antigenotoxic activity of bee pollen from *Cystus incanus* and *Salix alba* as evaluated by the yeast estrogen screen and the micronucleus assay in human lymphocytes. *Eur J Med Chem* 2010; **45**(9): 4122-8.

[39] Al-Sanafi AE, Mohssin SA, Abdulla SM. Effect of royal jelly on male infertility. *Thi-Qar Med J* 2007; **1**(1): 1-12.

[40] Silici S, Ekmekcioglu O, Eraslan G, Demirtas A. Antioxidative effect of royal jelly in cisplatin-induced testes damage. *Urology* 2009; **74**(3): 545-51.

[41] Zahmatkesh E, Najafi G, Nejati V, Heidari R. Protective effect of royal jelly on the sperm parameters and testosterone level and lipid peroxidation in adult mice treated with oxymetholone. *Avicenna J Phytomed* 2014; **4**(1): 43-52.

[42] Mahesh A, Shaheetha J, Thangadurai D, Rao DM. Protective effect of Indian honey on acetaminophen induced oxidative stress and liver toxicity in rat. *Biologia* 2009; **64**(6): 1225-31.

[43] Kanbur M, Eraslan G, Beyaz L, Silici S, Liman BC, Altinordulu S, et al. The effects of royal jelly on liver damage induced by paracetamol in mice. *Exp Toxicol Pathol* 2009; **61**(2): 123-32.

[44] Karadeniz A, Simsek N, Karakus E, Yildirim S, Kara A, Can I, et al. Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin. *Oxid Med Cell Longev* 2011; **2011**: 981793.

[45] Yildiz O, Can Z, Saral O, Yuluğ E, Oztürk F, Aliyazıcıoğlu R, et al. Hepatoprotective potential of chestnut bee pollen on carbon tetrachloride-induced hepatic damages in rats. *Evid Based Complement Alternat Med* 2013; doi: 10.1155/2013/461478.

[46] Bastos DHM, Santos MCM, Mendonça S, Torres EAFS. Antioxidant capacity and phenolic content of stingless bee honey from Amazon in comparison to *Apis* honey. *Acta Horticulturae* 2009; **841**: 483-5.

[47] da Silva IA, da Silva TM, Camara CA, Queiroz N, Magnani M, de Novais JS, et al. Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. *Food Chem* 2013; **141**(4): 3552-8.

[48] Alvarez-Suarez JM, Giampieri F, Battino M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. *Curr Med Chem* 2013; **20**(5): 621-38.

[49] Sendra JM, Sentandreu E, Navarro JL. Kinetic model for the antiradical activity of the isolated p-catechol group in flavanone type structures using the free stable radical 2, 2-diphenyl-1-picrylhydrazyl as the antiradical probe. *J Agric Food Chem* 2007; **55**(14): 5512-22.

[50] Küçük M, Karaoğlu Ş, Ulusoy E, Baltaci C, Candan F. Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chem* 2007; **100**: 526-34.

[51] Tamura S, KonoT, Harada C, Yamaguchi K, Moriyama T. Estimation and characterisation of major royal jelly proteins obtained from the honeybee *Apis mellifera*. *Food Chem* 2009; **114**(4): 1491-7.

[52] Ohta S, Fujimaki T, Uy MM, Yanai M, Yukiyoshi A, Hirata T. Antioxidant hydroxycinnamic acid derivatives isolated from Brazilian bee pollen. *Nat Prod Res* 2007; **21**(8): 726-32.

[53] Tsiapara AV, Jaakkola M, Chinou I, Graikou K, Tolonen T, Virtanen V, et al. Bioactivity of Greek honey extracts on breast cancer (MCF-7), prostate cancer (PC-3) and endometrial cancer (Ishikawa) cells: profile analysis of extracts. *Food Chem* 2009; **116**: 702-8.

[54] Fauzi AN, Norazmi MN, Yaacob NS. Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. *Food Chem Toxicol* 2011; **49**(4): 871-8.

[55] Jaganathan SK, Mandal M. Antiproliferative effects of honey and of its polyphenols: a review. *J Biomed Biotechnol* 2009; doi: 10.1155/2009/830616.

[56] Han B, Li C, Zhang L, Fang Y, Feng M, Li J. Novel royal jelly proteins identified by gel-based and gel-free proteomics. *J Agric Food Chem* 2011; **59**(18): 10346-55.

[57] Majtán J, Kovácová E, Biliková K, Šimúth J. The immunostimulatory effect of the recombinant apalbumin 1-major honey bee royal jelly protein-on TNF alpha release. *Int Immunopharmacol* 2006; **6**(2): 269-78.

[58] Suhail N, Bilal N, Khan HY, Hasan S, Sharma S, Khan F, et al. Effect of vitamins C and E on antioxidant status of breast-cancer patients undergoing chemotherapy. *J Clin Pharm Ther* 2012; **37**(1): 22-6.

[59] Petrick JL, Li N, McClain KM, Steck SE, Gammon MD. Dietary risk reduction factors for the Barrett's esophagus-esophageal denocarcinoma continuum: a review of the recent literature. *Curr Nutr Rep* 2015; **4**(1): 47-65.