

# Honey bee is a potential antioxidant against cyclophosphamide-induced genotoxicity in albino male mice

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**Abstract:** The protective effects of honey bee (HB) and pollen grains against cyclophosphamide (CPM) -induced cytotoxic and genotoxic effects in mice were investigated. This was achieved through study the effects of CPM and HB on oxidative status, chromosomal aberrations and gene expression of the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL-6), interleukin-1 $\beta$  (IL1 $\beta$ ), interleukin 17A (IL-17A) and interferon-gamma (IFN- $\gamma$ ) in mice. In addition, the levels of reduced glutathione (GSH) and malondialdehyde were determined. The results of this study revealed that CPM decrease in GSH level and increase in malondialdehyde (MDA) level in the liver and kidney tissues. Moreover, CPM induced sperm abnormality, chromosomal aberrations and down regulated the expression of the studied cytokine genes. HB treatment in association with CPM ameliorates GSH, MDA, chromosomal aberrations and regulated the expression of IL-1  $\beta$ , IL-17A, IL-6, TNF- $\alpha$  and IFN- $\gamma$ . Thus, HB inhibits the cytotoxic and genotoxic risks associated with CPM treatment in mice.

**Keywords:** Cyclophosphamide, chromosomal aberrations, honeybee derivatives, cytokines, gene expression, antioxidant.

## INTRODUCTION

Chemotherapeutic agents not only kill cancer cells but also harm rapidly dividing cells (Oršolić *et al.*, 2008a, Simon *et al.*, 2009). Additionally, chemotherapeutic agents are gonadotoxic and can cause infertility (Brydøy *et al.*, 2008). Alkylating agents impair cell function by forming covalent bonds with amino, carboxyl, sulfhydryl and phosphate groups in biologically important molecules, including DNA (Takimoto *et al.*, 2008). Moreover, these drugs add methyl or other alkyl groups to nitrogenous base, which affect normal base pairing and leads to the miscoding of DNA. Cyclophosphamide (CPM) is an alkylating agent used in the management of many malignant diseases. CPM is bioactivated to phosphoramidate mustard by the hepatic cytochrome P450 enzyme (Selvakumar *et al.*, 2006). CPM is also cytotoxic to normal cells (Qiu *et al.*, 2008).

CPM alkylates DNA bases, preventing the replication of DNA and RNA transcription. Moreover, CPM induces mismatched base pairing of nucleotides, leading to DNA mutations. Numerous studies have shown that CPM exposure can disrupt the redox balance (Selvakumar *et al.*, 2006). Therefore, the intake of nutraceutical during chemotherapy may urgent necessity.

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Foods enriched with bioactive compounds reduce the risk of cancer and the side effects associated with the administration of chemotherapeutic agents (Drăgan *et al.*, 2007). Honey bees (HB) contain many biologically active compounds, including caffeic acid, caffeic acid phenethyl ester (CAPE) and flavonoid glycones (Oršolić and Bašić, 2007). These compounds have been demonstrated to display antitumor effects due to the down regulation of many cellular pathways, such as the tyrosine kinase, cyclooxygenase and ornithine decarboxylase pathways (Oršolić and Bašić, 2007). Additionally, HB flavonoids possess free radicals (FR) scavenging activity, thereby inhibiting FR-induced DNA damage (Chen *et al.*, 2004). Moreover, Michail *et al.* (2007) indicated that the abundance of hydroxymethylfurfural in HB might be associated with antitumor potential. Therefore, consumption of HB may decrease the side effects of chemotherapeutics (Oršolić and Bašić (2004a, b). Further studies are required about to confirm these data.

HB derivatives have been shown to exert an anti-proliferative effect in several types of cancer, including colon cancer (Jaganathan and Mandal, 2009 a, b), prostate cancer (Von Low *et al.*, 2007), bladder cancer (Swellaam *et al.*, 2003) and endometrial cancer (Burton and Wells, 2002). The estrogenic activity of certain types of HB may prevent cancer-related processes (Tsiapara *et al.*, 2009),

and HB from various floral sources mediate estrogen effects via modulation of estrogen receptor activity (Moutsatsou, 2007). It has been shown that the HB component CAPE can induce apoptosis (Lee *et al.*, 2003), while ellagic acid protects against CPM-induced genotoxicity (Muneeb *et al.*, 2012).

Bincoletto *et al.* (2005) showed that prostaglandin inhibitors are crucial in the prevention of tumors. Al-Waili (2007) observed that prostaglandins, nitric oxide, FR and chronic inflammation play a major role in tumorigenesis. It is possible that the reduction of prostaglandins may be important in the stimulation of immune cells (Al-Waili 2007, Oršolić and Bašić, 2008 a). HB derivatives stimulate macrophage activity to produce factors capable of regulating the function of B-cells, T-cells and natural killer cells (Al-Waili, 2003 a, b). In addition, oral intake of HB derivatives augments antibody production in primary and secondary immune responses (Al-Waili and Haq, 2004). HB products possess anti-inflammatory, antioxidant, anti-tumor and immunomodulatory functions (Guo *et al.*, 2007).

CPM-treatment induced disruption of mucus layer, hemorrhage, edema, and infiltration of inflammatory cells and accumulation of mast cells (Rezvanfar *et al.*, 2009). Normal wound healing is a complex process in which damaged tissue is removed and gradually replaced by restorative tissue (Falanga, 2005) through a series of events including coagulation, inflammation, cell proliferation and tissue remodeling. HB facilitate increases in lymphocytes and phagocytes and aid in the release of cytokines, such as tumor necrotic factor- $\alpha$  (TNF- $\alpha$ ), and interleukins, including interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6), by monocytes, thus stimulating the healing process (Tonks *et al.*, 2003 and 2007). Yet, no enough data available about this issue.

Gonadal dysfunction is one of adverse effects of chemotherapy (Brydøy *et al.*, 2008). It has been reported that HB improve male reproductive status (Nakaya *et al.*, 2007). Furthermore, Abdelhafiz and Muhamad (2008) reported that administration of HB products can be used as a treatment for infertility. Further studies are required to address this issue. Therefore, the aim of this work is to evaluate the protective role of HB derivatives (honey bees, and pollen grains) as protective agent against cytotoxic and genotoxic effects of CPM in male mice. This was achieved through study the effects of HB (1 gram/kg) on chromosomal aberrations and gene expression of the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL-6), interleukin-1 $\beta$  (IL1 $\beta$ ), interleukin 17A (IL-17A) and interferon-gamma (IFN- $\gamma$ ) in mice treated with CPM (10 mg/kg). In addition, the levels of reduced glutathione (GSH) and malondialdehyde were determined in the liver and kidneys.

## MATERIAL AND METHODS

### Chemicals

Cyclophosphamide was purchased from Sigma. Honey bees from different times of the year were employed in this study. Pure pollen grains were used as the raw material for obtaining HB derivatives (HB). The remaining chemicals were of analytical grade.

### Animals

Male adult Swiss albino mice, aged 6-7 weeks and weighing 20-22 g, were acquired from the animal house at the College of Pharmacy at King Saud University, Riyadh, KSA. The mice were maintained at room temperature (22 $\pm$ 2°C) with a 12h light/dark cycle, and pathogen-free mice were housed in a specific pathogen-free environment and fed standard rodent chow and water *ad libitum*. The Institutional Animal Care and Use Committee approved all procedures.

### Experimental design

A total of 40 adult male Swiss albino mice were used in the present study. The animals were divided into 4 groups of 10 animals each, as follows:

- Group 1: Mice did not receive any treatment and served as a working control.
- Group 2: Mice were injected with a single dose of CPM (10 mg/kg); referred to as the CPM10 treatment group.
- Group 3: Mice received only HB derivatives (1 gm/kg) for 2 weeks as HB group.
- Group 4: Mice were injected with a single dose of 10 mg/kg CPM concurrently with HB derivatives (1 gm/kg) and then continued to receive HB for 2 additional weeks;

The daily food intake was recorded, and animals from each group were weighed. Dead animals from every group were recorded, and the total mortality at the end of experiment was tabulated.

**Table 2:** Mortality percent of experimental mice treated with CPM and/ or HB or their combination

| Trial  | No. of treated animal | No. of dead animal | % of dead animal |
|--------|-----------------------|--------------------|------------------|
| C      | 10                    | 0                  | 00               |
| CPM    | 10                    | 4                  | 40               |
| HB     | 10                    | 0                  | 00               |
| HB+CPM | 10                    | 0                  | 00               |

At the end of the experiment, the mice were euthanized via cervical dislocation, and mature sperm was harvested from the cauda epididymidis and vas deferens. Bone marrow was collected and incubated in physiological saline (0.9% NaCl), and the spleen was removed, weighed and homogenized in saline (9% NaCl).

### Chromosomal aberrations

The chromosomal aberrations in bone marrow cells were recorded and photographed. The mitotic index was calculated using the following equation: cells with visible chromosomes / the total number of cells visible (Yilmaz *et al.*, 2012)

### Diphenylamine assay of DNA fragmentation

The diphenylamine assay enables the quantization of degraded DNA as a percentage of fragmentation in bone marrow cells. DNA fragmentation was also confirmed via agarose gel electrophoresis with ethidium bromide staining (Dypbukt *et al.*, 1994, Burkitt *et al.*, 1996).

### RNA extraction, cDNA synthesis and RT-PCR

Total RNA was isolated from liver tissue homogenates using Trizol reagent (Invitrogen®) according to the manufacturer's instructions. The obtained RNA was quantified by measuring the absorbance at 260 nm, and RNA quality was determined by measuring the 260/280 nm ratio. cDNA synthesis was performed using the High-Capacity cDNA reverse transcription kit (Applied Biosystems®) according to the manufacturer's instructions. Quantitative analysis of the mRNA expression of target genes was conducted via real-time polymerase chain reaction (RT-PCR) amplification of cDNA in 96-well optical reaction plates in the ABI Prism 7500 real-time PCR system (Applied Biosystems) table 1.

### Sperm abnormalities

Mature sperm were harvested from the cauda epididymidis (by mincing the tissue) and vas deferentia (by squeezing the duct). Air-dried smears were fixed for 1 h in acetic alcohol (3 parts absolute ethylene alcohol, 1 part glacial acetic acid) and stained with 5% (w/v) aqueous eosin. Scoring and statistical analysis: 1,000 spread sperm per animal were examined to record the sperm abnormalities that occurred in these cells as a result of treatment. These aberrations were recorded and photographed.

### Reduced glutathione (GSH) and malondialdehyde (MDA) analysis

At the end of the treatment period, animals were sacrificed after an overnight fasting, by exsanguination

under ether anesthesia. The liver and kidney tissues of each animal were removed, cleaned, and processed for biochemical measurements.

GSH level was evaluated according to Nabavi (2012). The homogenate sample (720 µL) was diluted and then trichloroacetic acid (5%) was added to the reaction mixture for precipitation of protein content in tissue homogenates. Reaction was centrifuged at 10,000 g for 5 min and then the supernatant was taken. Ellman's reagent [5, 5'-dithiobis (2-nitrobenzoic acid) solution] was added to the sample. Finally, the absorbance of the sample was recorded at 417 nm.

Lipid peroxidation levels were measured as MDA which is one of the end products of thiobarbituric acid reactive substances (TBARS) according to Orun I (2008). Low-molecular-weight end products, probably malondialdehyde, that are formed during the decomposition of lipid peroxidation products. The analysis of lipid peroxidation was carried out as described with minor modifications (Beuge and Aust, 1978). The reaction mixture was prepared by adding 1 ml homogenate to 4 ml reaction solution (15% trichloroacetic acid: 0.375% thiobarbituric acid: 0.25 N NaOH, 1:1:1, w/v); these were heated to 100°C for 10 min. The mixture was cooled to room temperature, centrifuged (10,000g for 10min) and the absorbance of the supernatant was recorded at 532 nm.

### STATISTICAL ANALYSIS

The data are presented as the mean ± S.E.M for ten animals per group. The results were analysed using ANOVA followed by Tukey-Kramer analysis as a post-ANOVA test. P<0.05 was considered to represent a significant difference between each pair of groups.

### RESULTS

#### Percentage of dead animals (mortality %)

The results of the present study revealed that injection of CPM alone has lethal effects on adult male Swiss albino mice. The mortality rates associated with CPM (10 mg/kg) was significantly higher (40%) compared to the results obtained for control mice or mice treated with HB.

**Table 1:** Primer sequences. IL, Interleukin; IL-1β, Interleukin-1 beta; IFN-γ, Interferon-gamma; TNF-α, Tumour necrosis factor-alpha; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase

| Gene   | Direction and Sequence          |                                   |
|--------|---------------------------------|-----------------------------------|
| IL-1β  | F: 5'-TCGTGCTGTCGGACCCATAT-3'   | R: 5'-GTCGTTGCTTGGTTCTCCTTGT-3'   |
| IL-6   | F: 5'-CCGGAGAGGAGACTTCACAG-3'   | R: 5'-GGAAATTGGGGTAGGAAGGA-3'     |
| IFN-γ  | F: 5'-TCTGGGCTTCTCCTCCTGCGG-3'  | R: 5'-GGCGCTGGACCTGTGGGTTG-3'     |
| TNF-α  | F: 5'-GCGGAGTCCGGGCAGGTCTA-3'   | R: 5'-GGGGGCTGGCTCTGTGAGGA-3'     |
| IL-17A | F: 5'-ATCCCTCAAAGCTCAGCGTGTC-3' | R: 5'-GGGTCTTCATTGCGGTGGAGAG-3'   |
| GAPDH  | F: 5'-CCCAGCAAGGACACTGAGCAAG-3' | R: 5'-GGTCTGGGATGGAAATTGTGAGGG-3' |

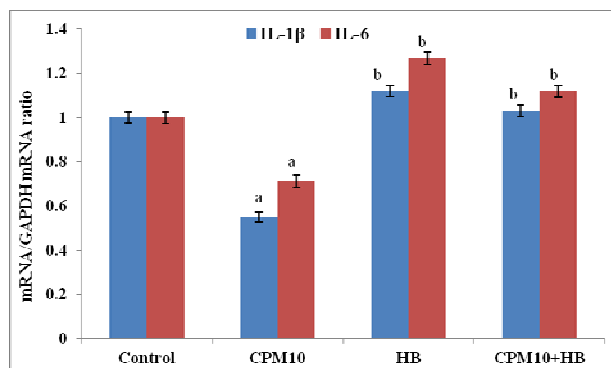
The lethal effect of the CPM drug was decreased in all mice treated with HB, table 2 displays these results.

### Chromosomal aberrations (CAs)

Table 3 provides data on the chromosomal aberrations as breaks, gaps, deletions, centromeric attenuation, endomitosis and other types of CAs observed in the different treatment groups. The results showed that CPM administered at a dose of 10 mg/kg significantly increased the total number of CAs compared with the control and HB with or without CPM treatment groups ( $p < 0.05$ ). Chromatid breaks were the most frequent form of damage induced by CPM in the present study. Treatment with HB derivatives alone did not significantly increase CAs compared with the control. An HB dose of 1 gm/kg was effective in reducing the percentage of total CAs induced by CPM treatment ( $p < 0.05$ ). Similar results were observed in terms of numerical aberrations in chromosomal endomitosis, polyploidy and aneuploidy.

### Mitotic index

The mitotic index was significantly decreased in the groups of mice that received CPM compared with control mice and mice receiving HB. The changes in mitotic index values that occurred due to CPM treatment were ameliorated by HB administration. Table 4 displays the results of the mitotic index analysis.



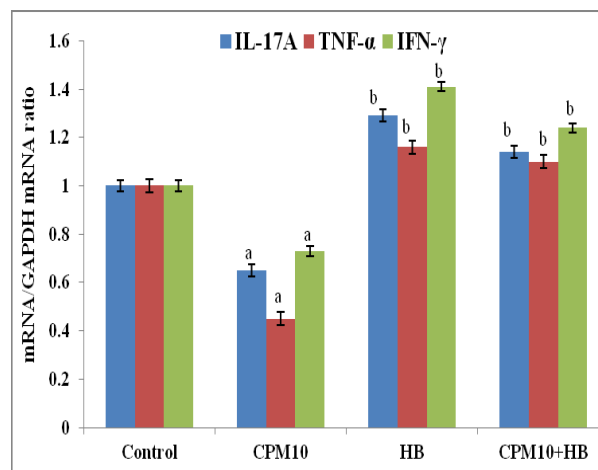
**Fig. 1:** Effect of CPM and HB on gene expressions of IL-1 $\beta$  and IL-6 expression in the spleen tissues. Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test.

### Sperm abnormalities

Treatment of male mice with CPM caused a significant decrease in the number and motility of sperm, and the number of dead and abnormal sperm was increased compared to the control group. Treatment with HB caused a significant increase in semen quality and minimized the toxic effects of CPM on sperm. The sperm aberrations observed in CPM treatment group included banana-like no-hock, no-head, small head and big head sperm. In addition, tail analysis showed a significant difference in CPM group compared with the control and HB with or without CPM groups. Table (5) depicted these results.

### Gene expression

The expression of the IL-1 $\beta$ , IL-17A, IL-6, TNF- $\alpha$  and IFN- $\gamma$  genes was down regulated in the groups subjected to CPM compared to the control and other treated groups with HB and CPM with HB (figs. 1 and 2).



**Fig. 2:** Effect of CPM and HB on gene expressions of IL-17A, IL-6, TNF- $\alpha$  and IFN- $\gamma$  in the spleen tissues. Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test.

### Lipid peroxidation and GSH level

The changes in GSH and MDA levels in the liver and kidney tissues of all groups after treatment with CPM, HB and CPM + HB were evaluated. Treatment with CPM alone altered the levels of GSH and MDA ( $P < .05$ ). GSH levels significantly decreased, whereas MDA levels in the liver and kidney tissues of the CPM treated group significantly increased compared with the control group ( $P < .05$ ). However, there was a significant ( $P < .05$ ) alteration in GSH and MDA levels after HB treatment relative to the control group. GSH levels significantly increased, whereas MDA levels in HB treated group significantly decreased, compared with the control group ( $P < .05$ ). However, oral administration of HB with CPM reversed the GSH and MDA levels back to the control levels in the tissues (table 6 depict these results).

### DISCUSSION

The use of phytochemicals to protective against diseases and to mitigate the toxic effects of drugs was documented (Wollgast *et al.*, 2000). HB contains polyphenols, caffeic acid (CA), CAPE, sugars, vitamins, minerals and enzymes (Kelloff *et al.*, 2004). Therefore, honey exhibits antioxidant, anti-infective, anti-mutagenic and immunomodulation effects (Orsolic *et al.*, 2004, Al-Waili, 2003 a, b). As honey is a common food for humans, we investigated it as a potential candidate for chemo preventive treatment of CPM-induced cytotoxicity and genotoxicity in male mice.

**Table 3:** Frequencies of chromosome aberrations in bone marrow cell of control and mice treated with CPM, HB and CPM plus HB.

| Trial  | No. of metaphase | Structural aberrations | Polyploidy | Aneuploidy | Total aberrations        | %                 |
|--------|------------------|------------------------|------------|------------|--------------------------|-------------------|
| C      | 50               | 2.10±.02               | 1.05±0.05  | 1.80± 0.01 | 4.95 <sup>a</sup> ±0.13  | 9.9 <sup>a</sup>  |
| CPM    | 50               | 10.7±0.03              | 4.50±0.01  | 5.00±0.05  | 20.20 <sup>b</sup> ±0.39 | 40.4 <sup>b</sup> |
| HB     | 50               | 1.40±0.02              | 0.25±0.01  | 0.5±0.02   | 2.15 <sup>a</sup> ±0.06  | 4.3 <sup>a</sup>  |
| CPM+HB | 50               | 2.50±0.03              | 0.50±0.01  | 0.50±0.05  | 3.50 <sup>a</sup> ±0.19  | 7.0 <sup>a</sup>  |

Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test. Levels of significance was accepted at  $P<0.05$ . **a**) as compared to control group; **b**) as compared to cyclophosphamide (CPM). Each value indicates the mean  $\pm$  SE of ten animals/group.

**Table 4:** The mitotic index in bone marrow cells from each treated male mice after different types of treatments

| Trial  | No. of cells (Mean) | Mitotic Index $\pm$ SE   |
|--------|---------------------|--------------------------|
| C      | 1000                | 48.38 <sup>b</sup> ±2.15 |
| CPM    | 1000                | 29.25 <sup>a</sup> ±1.15 |
| HB     | 1000                | 60.37 <sup>c</sup> ±3.15 |
| HB+CPM | 1000                | 44.25 <sup>b</sup> ±2.10 |

Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test. Levels of significance was accepted at ( $P<0.05$ ). Each value indicates the mean  $\pm$  SE of ten animals/group.

**Table 5:** Percentage of sperm abnormalities in mice after different types of treatments

| Trial  | Head Abnormalities | Tail Abnormalities | Other ab. | Total ab. (%)                       |
|--------|--------------------|--------------------|-----------|-------------------------------------|
| C      | 1.7±0.03           | 0.8±0.04           | 0.3±0.07  | 2.8±1.09 <sup>a</sup>               |
| CPM    | 29.0±0.4           | 11±0.2             | 4.0±0.3   | 44 <sup>b</sup> ±1.6 <sup>a</sup>   |
| HB     | 0.3±0.02           | 0.2±0.0            | 0.5±0.05  | 1.0 <sup>a</sup> ±0.07 <sup>a</sup> |
| CPM+HB | 3.6±0.06           | 0.1±0.02           | 0.2±0.01  | 2.9 <sup>a</sup> ±0.57 <sup>a</sup> |

Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test. Levels of significance was accepted at ( $P<0.05$ ).

**Table 6:** Effect of CPM and HB n MDA and GSH in liver and kidney of mice after different types of treatments

| Tissue Parameter             | C                        | CPM                                   | HB                        | CPM+HB                    |
|------------------------------|--------------------------|---------------------------------------|---------------------------|---------------------------|
| TBARS (nmol MDA/g of tissue) |                          |                                       |                           |                           |
| Liver                        | 0.178±0.01 <sup>a</sup>  | 0.313 <sup>b</sup> ±0.01 <sup>b</sup> | 0.160± 0.008 <sup>a</sup> | 0.173±0.013 <sup>a</sup>  |
| Kidney                       | 0.255±0.01 <sup>a</sup>  | 0.484 <sup>b</sup> ±0.01 <sup>b</sup> | 0.232± 0.01 <sup>a</sup>  | 0.249±0.012 <sup>a</sup>  |
| GSH (mg/g of tissue)         |                          |                                       |                           |                           |
| Liver                        | 0.271±0.014 <sup>b</sup> | 0.162±0.01 <sup>a</sup>               | 0.301± 0.016 <sup>b</sup> | 0.265± 0.010 <sup>b</sup> |
| Kidney                       | 0.344±0.012 <sup>b</sup> | 0.198±0.011 <sup>a</sup>              | 0.403±0.014 <sup>b</sup>  | 0.384±0.013 <sup>b</sup>  |

Statistical analysis was done using one way ANOVA followed by Tukey-Kramer as post-ANOVA test. Levels of significance was accepted at ( $P<0.05$ ).

Despite is chemotherapeutic agent CPM, it the risk of mutagenicity in animals and humans (McCarroll *et al.*, 2008, Qiu *et al.*, (2008). CPM as alkylating agents work by three different mechanisms: 1) Attachment of alkyl groups to DNA bases. 2) DNA damage via the formation of cross- links which prevents DNA from being separated for transcription and 3). The induction of mismatching of the nucleotides leading to mutations (Lona *et al.*, 2002). All these unfavorable effects lead to DNA fragmentation, CAs and cytotoxicity.

Cellular thiol levels are important in determining the sensitivity of cellular damage produced by CPM-induced oxidative damage (Selvakumar *et al.*, 2006). In the present study, CPM treatment induced a significant decrease in GSH levels, while MDA level was increased in the liver and kidney tissues. Similarly, Ahmed 2005 and Sener *et al.*, 2007 reported significant decrease in GSH levels and increase in MDA levels of the liver and kidney tissues after CPM treatment. However, oral administration of HB at dose of 1 gm/kg of body weight for 2 weeks caused a significant increase in GSH levels

and decrease MDA production. This implying a reduction of CPM-induced oxidative damage. Similarly, Oršolić and Bašić, (2007) reported that antioxidant action of HB attributed to high level flavonoids. Moreover, HB inhibits ROS-generating enzymes (Chow, 2002; Ezz El-Arab *et al.*, 2006). Likewise, Giray *et al.* (2001) reported antioxidant provided significant protection against the elevation of MDA induced by CPM. HB flavonoids have the ability to correct a deficient thiol status in cells by increasing the GSH level and capturing FRs (Chen *et al.*, 2004). The increase in MDA levels in the liver and kidneys suggests enhanced lipid peroxidation leading to cytotoxicity, DNA damage and genotoxicity. Enhanced FRs formation by CPM responsible for the cytotoxic and genotoxic effects of this drug was concluded by Selvakumar *et al.* (2006).

In the present study, the increase of CAs and DNA fragmentation were consistent with previously results reported by (McCarroll *et al.*, 2008, Qiu *et al.*, 2008). Similarly, Qiu *et al.* (2008) showed that CPM causes DNA damage. In contrast, HB derivatives treatment did not increase DNA damage. These results were in agreement with the findings of Saravana and Mahitosh (2009), who demonstrated that HB products display chemo preventive effects. Similarly, Khalil (2006) reported that HB derivatives are protective against chemotherapy induced cytotoxicity. Tarek *et al.* (2003) described the anti-carcinogenic effect of HB derivatives. One potential mechanism of HB protection against CAs and DNA damage is the scavenging of FRs by polyphenols before FRs can interact with DNA. Our results confirm the findings of earlier studies in which honey was reported to scavenge FRs (Oršolić and Bašić, (2007). Moreover, HB inhibits ROS-generating enzymes (Chow, 2002; Ezz El-Arab *et al.*, 2006). Thus, HB derivatives can preserve thiol contents. It has been demonstrated that, flavonoids reduce the genomic damage induced by chemicals (Yilmaz *et al.*, 2012).

In the present study, abnormalities in sperm morphology were observed in CPM-treated mice, confirming previous findings showing that CPM induces alterations in sperm characteristics (Higuchi *et al.*, 2001, Selvakumar *et al.*, 2006). It has also been reported that CPM causes an increase in apoptosis at specific stages of the germinal cycle (Selvakumar *et al.*, 2006). Direct toxicity of CPM to the spermatogenic compartment may be responsible for the production of abnormal sperm (Selvakumar *et al.*, 2006). Moreover, oxidative stress induces sperm abnormalities (Agarwal and Saleh, 2002). CPM- impaired energy metabolism through a decrease in the activity of testicular citric acid cycle (Selvakumar *et al.*, 2005b).

HB derivatives pretreatment substantially attenuated the abnormalities in sperm morphology induced by CPM treatment. Similarly, Abdelhafiz and Muhamad (2008)

reported that HB derivatives decrease the risk of infertility. Furthermore, HB products inhibit negative effects on male reproductive status (Nakaya *et al.* 2007). Likewise, Hidaka *et al.* (2006) demonstrated that HB can alleviate the negative effects of chemotherapeutic agents on genital system. This may attributed to presence of CAPE as protective agent against chemotherapy induced oxidative stress (Armagan *et al.*, 2008).

Tumor progression is characterized by inflammatory reactions in the tumor microenvironment that initiated by various inflammatory mediator (Umansky and Sevko, 2012). These mediators are cytokines, chemokines, growth factors, ROS and prostaglandins (Umansky and Sevko, 2012). All those are responsible for the recruitment of immune cells at sites of malignancy and mediate pro- and anti-angiogenic effects (Rainczuk *et al.*, 2012). TNF- $\alpha$  and interleukin 6 (IL-6) are pro-tumorigenic cytokines, interleukin-1 $\alpha$  (IL1 $\alpha$ ), interleukin 17A (IL-17A) and interferon-gamma (IFN- $\gamma$ ), can influence all stages of tumor development (Grivennikov and Karin, 2011, Rainczuk *et al.*, 2012). CPM is commonly used as an immunosuppressive drug, and it has been employed to investigate the immune-restorative action of propolis (Sforcin and Bankova, 2011).

In the present study, the expression of the IL-1  $\beta$ , IL-17A, IL-6, TNF- $\alpha$  and IFN- $\gamma$  genes was down regulated by the administration of CPM in comparison to the control group. In contrast, the expression of these cytokine genes was normally regulated following HB derivative treatment alone or together with CPM. Administration of HB derivatives has been shown to upregulate IL-1 and IL-6 secretion by macrophages and spleen cells. The anti-inflammatory action of honey bee derivatives has been described by several researchers (Hu *et al.*, 2005; Paulino *et al.*, 2006). Honey bee derivatives administration in mice inhibits IFN- $\gamma$  production (Orsatti *et al.*, 2010b). Moreover, in mice treated with honey bee derivatives , inhibition of IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-2 and IL-10 production has been observed, suggesting the anti-inflammatory activity of this compound (Missima *et al.*, 2010). Honey has been found to lower prostaglandin levels and elevate nitric oxide end products. These properties might help to explain some of the therapeutic properties of honey (Al-Waili *et al.*, 2011). Fukuda *et al.* (2009) demonstrated that honey enhances immune functions and antitumor activity in mice.

## CONCLUSIONS

This study confirmed that CPM increase chromosomal aberrations, cytotoxicity and genotoxicity. These deleterious effects of CMP attributed to depletion of GSH as major cellular antioxidant agent and increase MDA as reactive aldehyde. Supplementation HB derivatives attenuate the cytotoxicity and genotoxicity induced-by

CMP. Thus, administration of HB derivatives may reduce the risk of CPM-induced cytotoxicity and genotoxicity.

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