

RESEARCH ARTICLE

# *In vivo* activity assessment of a “honey-bee pollen mix” formulation

Esra Küpeli Akkol<sup>1</sup>, Didem Deliorman Orhan<sup>1</sup>, İlhan Gürbüz<sup>1</sup>, and Erdem Yesilada<sup>2</sup>

<sup>1</sup>Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, Besevler, Ankara, Turkey, and <sup>2</sup>Yeditepe University, Faculty of Pharmacy, Kayisdagi, Istanbul, Turkey

## Abstract

Honey-bee pollen mix (HBM) formulation is claimed to be effective for the treatment of asthma, bronchitis, cancers, peptic ulcers, colitis, various types of infections including hepatitis B, and rheumatism by the herb dealers in northeast Turkey. In the present study, *in vivo* antinociceptive, anti-inflammatory, gastroprotective and antioxidant effects of pure honey and HBM formulation were evaluated comparatively. HBM did not show any significant gastroprotective activity in a single administration at 250 mg/kg dose, whereas a weak activity was observed after three days of successive administration at 500 mg/kg dose. On the other hand, HBM displayed significant antinociceptive ( $p < 0.01$ ) and anti-inflammatory ( $p < 0.01$ ) activities at 500 mg/kg dose orally without inducing any apparent acute toxicity or gastric damage. HBM was also shown to possess potent antilipidperoxidant activity ( $p < 0.01$ ) at 500 mg/kg dose against acetaminophen-induced liver necrosis model in mice. On the other hand, pure honey did not exert any remarkable antinociceptive, anti-inflammatory and gastroprotective activity, but a potent antilipidperoxidant activity ( $p < 0.01$ ) was determined. Results have clearly proved that mixing pure honey with bee pollen significantly increased the healing potential of honey and provided additional support for its traditional use. Total phenolic and flavonoid contents of HBM were found to be 145 and 59.3 mg/100 g of honey, which were estimated as gallic acid and quercetin equivalents, respectively.

**Keywords:** Honey-bee pollen mix; honey; antinociceptive; anti-inflammatory; gastroprotective; antioxidant activity; phenolics; flavonoids

## Introduction

As honey bees gather nectar to make into honey from flowers, pollens from the male organs of flowers stick to the bee's body and legs, particularly it is deposited in hairy cavities on the hind legs. In order to obtain this pollen mass, hive-keepers place a wire mesh in front of the hive which bees have to pass through to reach the hive; the pollen grains remain stuck to the mesh. This sticky pollen mass is then collected as “bee-pollen” and is the mixture of pollens gathered from different plant species in the environment by the honey bee; the composition may vary depending upon the origin (Alcamaraz-Abarcas et al., 2007).

Analysis of bee pollen pellets generally revealed that polysaccharides (50%), simple sugars (4–10%), proteins (6–28%), amino acids (6%) fats and lipids (1–20%) are the

main constituents of bee pollen, in addition to a variety of secondary metabolites such as flavonoids, carotenoids and terpenes, among others (Alcamaraz-Abarcas et al., 2007). Bees are vegetarians and use pollen as a source of protein and vitamin to feed larvae, while nectar gathered from flowers is used as a source of carbohydrates in the comb. It possesses a high nutrient value particularly rich in vitamins, amino acids, proteins and polysaccharides and marketed worldwide as a tonic food particularly for the elderly to ameliorate the asthenic effects of ageing (Ozcan et al., 2004). Bee pollen is also believed in folk medicines for centuries to be a remedy against various types of diseases.

Honey also contains a number of components; these include flavonoids, phenolic acids, amino acids, proteins, enzymes (glucose oxidase, catalase, peroxidase), carotenoids, vitamins (C and E) (Baltrusaityte et al.,

Address for Correspondence: E. Yesilada, Yeditepe University, Faculty of Pharmacy, Kayisdagi 34755, Istanbul, Turkey. Tel: +90-216-5780068. Fax: +90 216 5780068; E-mail: yesilada@yeditepe.edu.tr

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2007). Due to the rich nutritious contents of honey and bee pollen, herb dealers in Turkey combine these hive products and marketing the mixture as “honey-bee pollen mix” (HBM) formulation, which has been widely proposed for the treatment of various symptoms or diseases. These include infectious diseases (bronchitis, tuberculosis, hepatitis B, etc.), inflammatory disorders (cylgia, rheumatism, asthma, and colitis), immune (eczema, cancers) and gastrointestinal ailments (peptic ulcer) as well as infertility problems (personal communication Herb dealer Mehmet Güneysu, Hemsin, Rize). Previous studies have demonstrated that honey serves as a source of natural antioxidants, effective in reducing the risk of heart diseases, cancers, immune deficiencies, cataracts and various inflammatory disorders (The National Honey Board, 2003). However, the potential antinociceptive, anti-inflammatory, gastroprotective and antioxidant effects attributed to honey-bee pollen mixture have not been evaluated so far (The National Honey Board, 2003).

In order to evaluate these health claims, the objective of the present study was to investigate the potential *in vivo* antinociceptive, anti-inflammatory, gastroprotective and antioxidant effects of HBM formulation in rats and mice. Moreover, total phenol and flavonoid contents of HBM formulation were determined in order to find a correlation between the biological activities, particularly for antioxidant activity by using Folin-Ciocalteu and aluminium chloride reagents.

## Material and methods

### Test material

Honey (multifloral, Binbirçiçek, Ankara, Turkey) was purchased from a local market (Ankara). “Honey-bee pollen mix” formulation was obtained from a local dealer, Deva. (Hemşin, Rize, Turkey).

### Determination of total phenolic and flavonoid contents

An aliquot of honey or HBM formulation (5 g) was diluted to 50 mL with distilled water and passed through Whatman No.1 filter paper. The solution (100  $\mu$ L) was treated with 0.2 mL Folin-Ciocalteu reagent, 2 mL of H<sub>2</sub>O, and 1 mL of 15% Na<sub>2</sub>CO<sub>3</sub>, and the absorbance of the solution was measured at 765 nm after 2 h at room temperature. The mean of three successive measurements was used for estimation and the total phenolic content was expressed in mg of gallic acid equivalents (GAE) per 100 g of honey or HBM formulation (Gao et al., 2000).

Flavone and flavonol content in HBM formulation and honey was expressed as quercetin equivalent. Quercetin was used to make the calibration curve [0.04,

0.02, 0.0025 and 0.00125 mg/mL in 80% ethanol (v/v)]. A sample solution (0.5 mL) was mixed with 1.5 mL 95% ethanol (v/v), 0.1 mL 10% aluminum chloride (w/v), 0.1 mL of 1 m/L sodium acetate and 2.8 mL water. The volume of 10% aluminum chloride was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The mean of three readings was used and the total flavonoid content was expressed in mg of quercetin equivalents (QE) per 100 g of honey or HBM formulation (Kosalec et al., 2004).

## Pharmacological procedures

### Animals

Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara). Sprague-Dawley rats of either sex (140-200 g) purchased from the Animal Breeding Laboratories of Gülhane Military Academy of Medicine (Ankara). The animals were allowed two days for acclimatization to animal room conditions were maintained on standard pellet diet and water *ad libitum*. The food was withdrawn the day before the experiment, but the animals were allowed free access to water. To prevent coprophagy in the ulcer experiments the rats were kept in wire-bottomed cages. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals (Gazi University, Commission of Animal Ethics).

### Preparation of test samples for bioassay

Honey or HBM formulation samples were administered in 250 and 500 mg/kg doses after suspending in 0.5% carboxymethyl cellulose (CMC)/distilled water. Test samples were administered in 5 mL/kg volume. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Misoprostol (0.4 mg/kg) for antiulcer, indomethacin (10 mg/kg) for anti-inflammatory or aspirin (100 and 200 mg/kg) for antinociceptive activity studies were used as reference drugs and all were suspended in 0.5% CMC.

### Antinociceptive activity

*In vivo* *p*-benzoquinone-induced abdominal constriction test (Okun et al., 1963) was performed for determination of antinociceptive activity. According to the method, 60 min after the oral administration of test samples, the mice were intraperitoneally injected with 0.1 mL/10 g body weight of 2.5% (w/v)

*p*-benzoquinone (PBQ; Merck, Darmstadt, Germany) solution in distilled H<sub>2</sub>O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting 5 min after the *p*-benzoquinone injection. The data represent the average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Aspirin at 100 and 200 mg/kg doses was used as the reference drug in this test.

#### **Anti-inflammatory activity**

The carrageenan-induced hind paw edema model (Kasahara et al., 1985) was used with some modifications for determination of anti-inflammatory activity (Yesilada & Küpeli, 2002). Sixty minutes after the oral administration of test sample or dosing vehicle, each mouse was injected with freshly prepared (0.5 mg/25 µL) suspension of carrageenan (Sigma, St. Louis, MO) in physiological saline (154 mM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µL saline solution was injected into that of the left hind paw. Paw edema was measured every 90 min for 6 h after induction of inflammation. The difference in footpad thickness was measured by gauge calipers (Ozaki, Tokyo). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

#### **Anti-ulcerogenic activity**

##### **Ethanol-induced ulcerogenesis (Robert et al., 1979)**

A test sample (HBM formulation, 250 mg/kg or honey, 250 mg/kg) was administered orally with an intragastric gavage 15 min before the oral application of 96% EtOH (1 mL) to a group of six rats. The animals were sacrificed 60 min later with an overdose of ether. The stomachs were removed and inflated with 10 mL formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was then opened along the greater curvature, rinsed with tap water to remove gastric contents and blood clots and examined under dissecting microscope (20 × 6.3 ×) to assess the formation of ulcers. The sum of the length (mm) of all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition was calculated by the following formula:

$$[(\text{UI control} - \text{UI treated})/\text{UI control}] \times 100.$$

#### **Subacute administration of material on ethanol-induced ulcerogenesis**

HBM formulation (500 mg/kg) or honey (500 mg/kg) was administered orally to the fasted animals for three successive days in the mornings, who were then allowed free access to food. The last day, 60 min after the administration of last dose the same experimental procedures were followed as described above.

#### **Antioxidant activity**

##### **Acetaminophen-induced liver injury**

The animals were divided into groups (n = 6) and given the following treatments orally; Group 1: 0.5% CMC (vehicle), Group 2: 0.5% CMC and acetaminophen (600 mg/kg) (4-acetamidophenol, Sigma, St. Louis, MO), Group 3: HBM formulation (500 mg/kg) and acetaminophen, Group 4: HBM formulation (250 mg/kg) and acetaminophen, Group 5: Honey (500 mg/kg) and acetaminophen.

Treatments were followed daily for 5 consecutive days at doses of 250 and 500 mg/kg (HBM formulation groups), 500 mg/kg (honey group), and 0.2 mL/kg (vehicle control group). On the last day of treatment, and 30 min after receiving the last dose, the animals in groups 2, 3, 4, and 5 were given acetaminophen in 0.5% CMC at a dose of 0.6 g/kg. Group 1 was given the vehicle (Ali et al., 2001). Four hours after acetaminophen administration, the mice were anesthetized. Liver of each mouse was promptly removed and used to determine the tissue levels of thiobarbituric acid reactive substance (TBARS) and cellular glutathione (GSH).

##### **Determination of lipid peroxidation in liver tissue**

The modified method of Ohkawa et al. (1979) was used to determine lipid peroxidation in tissue samples (Jamall & Smith, 1985). Mice were sacrificed by an overdose of diethylether. The liver of each mouse was immediately excised and chilled in ice-cold 0.9% NaCl and then perfused via the portal vein with ice-cold 0.9% NaCl. After washing with 0.9% NaCl, 1 g of wet tissue was weighed exactly and homogenized in 9 mL of 0.25 M sucrose using a Teflon homogenizer to obtain a 10% suspension. The cytosolic fraction was obtained by a two-step centrifugation first at 1000 g for 10 min and then at 2000 g for 30 min at 4°C. A volume of the homogenate (0.2 mL) was transferred to a vial and was mixed with 0.2 mL of a 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 mL of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 mL of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4 mL with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water.

Equal volumes of tissue blank or test sample and 10% TCA were transferred into a centrifuge tube and centrifuged at 1000 g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 UV-Vis system spectrometer, 4315395, CA, USA). Malondialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form a pink chromogen TBARS. The control experiment was processed using the same experimental procedure except that the TBA solution was replaced with distilled water due to the peroxidative effect of acetaminophen on tissue; livers of acetaminophen-treated mice were used as positive control. 1,1,3,3-Tetraethoxypropane (Sigma, Steinheim, Germany) was used as a standard for calibration of the curve.

#### Nonprotein sulfhydryl groups (Cellular GSH) in liver tissue (Sedlak & Lindsay, 1968)

The liver (200 mg) was homogenized in 8 mL 0.02 M ethylenediaminetetraacetic acid (EDTA) in an ice bath. The homogenates were kept in the ice bath until used. Aliquots of 5 mL of the homogenates were mixed in 15 mL test tubes with 4 mL distilled water and 1 mL 50% trichloroacetic acid (TCA). The tubes were centrifuged for 15 min at approximately 3000 g, 2 mL supernatant was mixed with 4 mL 0.4 M Tris buffer, pH 8.9, 0.1 mL Ellman's reagent, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), was added, and then the sample was vortexed. The absorbance was read within 5 min of the addition of DTNB at 412 nm against a reagent blank without tissue homogenate. Results were expressed as mol GSH/g tissue.

#### Toxicity assessment

##### Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were further observed during 48 h and morbidity or mortality was recorded, if it happened, for each group at the end of observation period.

##### Gastric-ulcerogenic effect

After the antinociceptive activity experiment, the mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleeding.

#### Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error ( $\pm$  SEM). Statistical differences between the treatments and the controls were evaluated by ANOVA and Students-Newman-Keuls post hoc tests.  $p < 0.05$  was considered to be significant (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

## Results and discussion

The antinociceptive activity of honey and the HBM formulation was studied by using the *p*-benzoquinone-induced writhing model in mice. A dose-dependent activity relationship was observed and HBM formulation significantly inhibited the chemically induced writhes at the 500 mg/kg dose without inducing any apparent acute toxicity or gastric damage. The activity of HBM formulation was twice as much of that of honey (Table 1).

The materials were investigated for their anti-inflammatory effect using the carrageenan-induced hind paw edema model in mice. As shown in Table 2, a dose-dependent anti-inflammatory activity was found for HBM formulation, which provided a significant inhibitory activity between 18.2% and 31.9% at the 500 mg/kg dose, while honey was found inactive against this model of inflammation.

Gastroprotective effect of the HBM formulation and honey was tested against EtOH-induced gastric lesions in rats. As shown in Table 3, HBM formulation was found completely inactive at 250 mg/kg dose on single dose administration, while honey exerted 24.3% (not significant) protection against EtOH-induced lesions. Then

**Table 1.** Effect of honey-bee pollen mix and honey against *p*-benzoquinone-induced writhings in mice.

| Test samples         | Dose per os (mg/kg) | Number of writhings $\pm$ SEM | Inhibitory ratio (%) | Ratio of ulceration |
|----------------------|---------------------|-------------------------------|----------------------|---------------------|
| Control              |                     | 45.7 $\pm$ 3.9                |                      | 0/6                 |
| Honey                | 250                 | 43.3 $\pm$ 2.7                | 5.3                  | 0/6                 |
|                      | 500                 | 36.3 $\pm$ 2.7                | 20.6                 | 0/6                 |
| Honey-bee pollen mix | 250                 | 39.8 $\pm$ 2                  | 12.9                 | 0/6                 |
|                      | 500                 | 27.5 $\pm$ 1.8*               | 39.8                 | 0/6                 |
| ASA                  | 100                 | 22.8 $\pm$ 2.1**              | 50.1                 | 4/6                 |
|                      | 200                 | 20.4 $\pm$ 1.9**              | 55.4                 | 5/6                 |

\* $p < 0.01$ ; \*\* $p < 0.001$  significant from the control value.

**Table 2.** Effects of honey-bee pollen mix and honey against carrageenan-induced paw edema in mice.

| Test samples         | Dose per os (mg/kg) | Swelling thickness ( $\times 10^{-2}$ mm) $\pm$ SEM (inhibition %) |                         |                         |                          |
|----------------------|---------------------|--------------------------------------------------------------------|-------------------------|-------------------------|--------------------------|
|                      |                     | 90 min                                                             | 180 min                 | 270 min                 | 360 min                  |
| Control              |                     | 46.2 $\pm$ 5.5                                                     | 54.2 $\pm$ 5.1          | 58.5 $\pm$ 4.7          | 63.2 $\pm$ 4.5           |
| Honey                | 250                 | 47.4 $\pm$ 3.2                                                     | 58.6 $\pm$ 3.2          | 61.8 $\pm$ 4            | 67.9 $\pm$ 3.9           |
|                      | 500                 | 41.5 $\pm$ 4 (10.7)                                                | 44.5 $\pm$ 3.5 (17.9)   | 46.5 $\pm$ 4.4 (20.5)   | 49.3 $\pm$ 5.5 (21.9)    |
| Honey-bee pollen mix | 250                 | 40.2 $\pm$ 3.1 (12.9)                                              | 45.4 $\pm$ 3.7 (16.2)   | 49.9 $\pm$ 4.2 (14.7)   | 48.2 $\pm$ 4.6 (23.7)    |
|                      | 500                 | 37.8 $\pm$ 4.8 (18.2)                                              | 39 $\pm$ 3.9 (28)*      | 40.3 $\pm$ 3.6 (31.1)** | 43 $\pm$ 3.3 (31.9)**    |
| Indomethacin 10      |                     | 34.5 $\pm$ 2.2 (25.3)                                              | 33.8 $\pm$ 2.8 (37.6)** | 34.5 $\pm$ 2.9 (41)***  | 37.8 $\pm$ 2.7 (40.2)*** |

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  significant from the control value.

twice the higher dose (500 mg/kg) of the test materials was administered for three consecutive days before the induction of ulcers. The level of gastroprotection provided by honey was 40.5% compared to 67.6% provided by HBM formulation at this dose, but the values were statistically not significant.

In order to evaluate the possible antihepatotoxic and antioxidant effects of HBM formulation and honey, acetaminophen-induced liver injury model in mice was used. As shown in Table 4, tissue lipid peroxidation (35.4%) level in the livers of acetaminophen group animals, as evidenced by TBARS determination, increased significantly when compared to the control group, while the content of GSH in liver decreased (15.5%). HBM formulation administered in 500 mg/kg dose reduced the acetaminophen-induced increase in liver TBARS level about 34.5%. On the other hand, in both test samples, HBM formulation and honey were found to be completely ineffective in normalizing the acetaminophen-induced reduction of hepatic GSH levels, and in fact worsened the situation.

**Table 3.** Effects of acute and subacute administration of the honey-bee pollen mix and honey against gastric lesions induced by EtOH in rats.

| Test samples            | Dose per os (mg/kg) | Ulcer index (mean $\pm$ SEM) | Prevention from ulcer <sup>a</sup> | Inhibition (%) |
|-------------------------|---------------------|------------------------------|------------------------------------|----------------|
| Acute administration    |                     |                              |                                    |                |
| Control                 |                     | 91.5 $\pm$ 13.8              |                                    |                |
| Honey                   | 250                 | 69.3 $\pm$ 17.7              | 0/6                                | 24.3           |
| Honey-bee pollen mix    | 250                 | 113.9 $\pm$ 37.6             | 0/6                                |                |
| Misoprostol             | 0.4                 | 0 $\pm$ 0*                   | 6/6                                | 100.0          |
| Subacute administration |                     |                              |                                    |                |
| Control                 |                     | 101.1 $\pm$ 31.8             |                                    |                |
| Honey                   | 500                 | 60.2 $\pm$ 19.3              | 0/6                                | 40.5           |
| Honey-bee pollen mix    | 500                 | 32.8 $\pm$ 12.7              | 0/6                                | 67.6           |
| Misoprostol             | 0.4                 | 0 $\pm$ 0**                  | 6/6                                | 100            |

\*p < 0.05; \*\*p < 0.01 significant from control; SEM, mean standard of error.

<sup>a</sup>Number of stomachs completely prevented from any bleeding or lesion.

It was previously reported that the chemical composition and antioxidant capacity of honey products were largely dependent on the plant source, geographical, seasonal and environmental factors as well as the processing techniques employed in the preparation. Among the components of honey products, the total phenolic acid (caffeic acid, ferulic acid) and flavonoid contents are determined as the indication of several biological activities which are attributed to these products (i.e. antimicrobial, antioxidant activity) (Wahdan, 1998; Al-Mamary et al., 2002; Aljadi & Kamaruddin, 2004; Meda et al., 2005). In the present study, the total phenolic acid and flavonoid contents of HBM formulation and pure honey samples were measured and compared by using Folin-Ciocalteu and aluminium chloride reagents. The total phenolic contents of HBM formulation and honey were found to be 145  $\pm$  2.3 and 50.3  $\pm$  0.9 mg GAE/100 g of honey, respectively. On the other hand, the total flavonoid contents of these samples were found to be 59.3  $\pm$  2.7 and 17.7  $\pm$  0.3 mg QE/100 g of honey, respectively. These results revealed that HBM formulation possesses higher total phenolic and flavonoid contents as compared to the pure honey sample (Table 5). Studies have shown that composition of phenolics varies depending upon the origin of bee pollen. Campos and colleagues (Campos et al., 1997) conducted a comparative study on the flavonoid/phenolic profiles of bee pollen from Portugal and New Zealand by high performance liquid chromatography. Main flavonoids were found to be quercetin, kaempferol and myricetin glycosides, while the main phenolic acids were hydroxycinnamic acid derivatives. Consequently, they suggested that comparison of the phenolic profiles would be a useful parameter for the quality assessment of bee pollen. In terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers, phenolic compounds possess potent antioxidant capacity and accordingly a part of the therapeutic properties of honeybee products may be attributed to their antioxidant capacity (Buratti et al., 2007).

Results have clearly demonstrated that combination of honey with bee pollen (4 to 1 ratio, respectively) has

**Table 4.** Effect of honey-bee pollen mix and honey on TBARS and GSH levels against acetaminophen-induced hepatotoxicity.

| Test samples                      | Dose per os (mg/kg) | Tissue TBARS (nmol/g wet wt) | % change <sup>c</sup> | Tissue GSH (mol/g) | % change <sup>c</sup> |
|-----------------------------------|---------------------|------------------------------|-----------------------|--------------------|-----------------------|
| Control (vehicle)                 |                     | 312 $\pm$ 28.2               |                       | 64.9 $\pm$ 4.9     |                       |
| Acetaminophen <sup>a</sup>        | 600                 | 422.5 $\pm$ 37.6*            | +35.4                 | 54.9 $\pm$ 3.5*    | -15.5                 |
| Honey <sup>b</sup>                | 500                 | 319.8 $\pm$ 25.3*            | -24.3                 | 33.2 $\pm$ 2*      | -39.5                 |
| Honey-bee pollen mix <sup>b</sup> | 250                 | 354.8 $\pm$ 32.2*            | -16                   | 43.3 $\pm$ 3.7*    | -21.1                 |
|                                   | 500                 | 276.7 $\pm$ 16.6*            | -34.5                 | 34.7 $\pm$ 1.9*    | -36.8                 |

Results were expressed as mean  $\pm$  SEM.

<sup>a</sup>Compared to vehicle control (0.5% CMC).

<sup>b</sup>Compared to acetaminophen.

<sup>c</sup>(+) represents percentage of increase and (-) represents decrease in each value when compared to either vehicle (for acetaminophen) or acetaminophen (for test samples).

\*p < 0.001 significant from control (for acetaminophen) or acetaminophen (for test samples).

**Table 5.** Total phenolic and flavonoid contents of honey-bee pollen mix and honey.

| Test samples         | Total phenolic content (mg GAE/100 g honey $\pm$ SEM) | Total flavonoid content (mg QE/100 g honey $\pm$ SEM) |
|----------------------|-------------------------------------------------------|-------------------------------------------------------|
| Honey                | 50.3 $\pm$ 0.9                                        | 17.7 $\pm$ 0.3                                        |
| Honey-bee pollen mix | 145 $\pm$ 2.3                                         | 59.3 $\pm$ 2.7                                        |

GAE, gallic acid equivalent; QE, quercetin equivalent; SEM, mean standard error; n = 3.

increased the total phenol and flavonoid content to almost three times higher concentrations. Consequently, higher antinociceptive, anti-inflammatory and antioxidant activities were observed for HBM formulation compared to those of pure honey. The effect of HBM was dose-dependent and the formulation exerts a significant effect at higher doses. Experimental results confirm that HBM formulation may be beneficial against inflammatory disorders, in particular in rheumatic and sciatic pain as well as in pathologies where antioxidant or antilipidperoxidative treatments may be helpful. However, honey and HBM formulation showed a weak effect in peptic ulcers, therefore they might only be advised as a complementary therapy in drug treatment.

Oryan and Zaker (1998) also observed that honey upon external application on wounds decreased the inflammatory response, i.e., less edema, less granular and mononuclear infiltration, less necrosis, and provided better wound contraction and lower glycosaminoglycan and proteoglycan concentrations. Al-Waili and Boni (2003) reported that natural honey inhibits prostaglandin concentrations in various biological fluids, such as plasma and urine. Since prostaglandins are well-known mediators of pain and inflammation, antinociceptive and anti-inflammatory activities of honey and HBM formulation might be due to this pathway. On the other hand, the prostaglandin inhibitory effect of honey may explain the weak effect of honey and HBM formulation on the peptic ulcer model, since prostaglandins comprise the main constituent of the inner lining of gastric mucosa. However, in previous studies by Ali (1991, 1995) it has been reported that natural honey provided a significant protection against acute and chronic gastric lesions induced by ethanol in rats, possibly by preventing depletion of glandular non-protein sulfhydryls.

Although the effect of honey on infectious diseases was not investigated in the present study, previous studies have shown that honey exerts a wide range of antimicrobial activities, against various Gram-negative and Gram-positive human pathogenic bacteria and *Candida albicans* (Al-Waili & Saloom, 1999) as well as anti-leishmanial (Zeina et al., 1997) and antiviral activities (*Herpes simplex* (Al-Waili, 2004) and rubella virus (Zeina et al., 1996)). On the other hand, the effect of HBM on immune disorders may be supported by the study of Al-Waili (2001) which reported immunostimulatory

effect of natural honey, by enhancing the antibody production against both thymus-dependent and thymus-independent antigens during primary and secondary immune responses.

In conclusion, experimental results have shown that combination of honey with bee pollen has significantly increased the healing potential of honey and inferentially the "honey-bee pollen mix" formulation may have beneficial effects against inflammatory, immune and infectious diseases as suggested by the herb dealers. On the other hand, we did not find any scientific evidence to sufficiently support the claimed healing effect of HBP mix on peptic ulcer.

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This paper is dedicated to the memory of Mehmet Güneysu (informant, Hemsin).

## Declaration of interest

The authors report no conflict of interests.

## References

- Alcamaraz-Abarcas N, Campos MG, Avila-Reyes JA, Naranjo-Jimenez N, Corral JH, Gonzales-Valdez LS (2007): Antioxidant activity of polyphenolic extract of monofloral honeybee-collected pollen from mesquite (*Prosopis juliflora*, Leguminosae). *J Food Compos Anal* 20: 119–124.
- Ali AT (1991): Prevention of ethanol-induced gastric lesions in rats by honey and its possible mechanism of action. *Scand J Gastroenterol* 26: 281–88.
- Ali AT (1995): Natural honey exerts its protective effect against ethanol-induced gastric lesions in rats by preventing depletion of glandular nonprotein sulfhydryl. *Trop Gastroenterol* 16: 18–26.
- Ali BH, Bashir AK, Rasheed RA (2001): Effect of the traditional medicinal plant *Rhazya stricta*, *Balanitis aegyptiaca* and *Haplophyllum tuberculatum* on paracetamol-induced hepatotoxicity in mice. *Phytother Res* 15: 598–603.
- Aljadi AM, Kamaruddin MY (2004): Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chem* 85: 513–518.
- Al-Mamary M, Al-Meeri A, Al-Habori M (2002): Antioxidant activities and total phenolics of different types of honey. *Nutrition Res* 22: 1041–1047.
- Al-Waili NS (2001): Effects of honey on the primary and secondary immune responses due to thymus-dependent and thymus-independent antigens. *Hematol J* 1: 161S–165S.
- Al-Waili NS, Saloom KY (1999): Effects of topical honey on post-operative wound infections due to Gram-positive and Gram-negative bacteria following caesarean sections and hysterectomies. *Eur J Medical Res* 26: 126–130.
- Al-Waili NS, Boni N (2003): Natural honey lowers plasma prostaglandins concentration in normal individuals. *J Medicinal Food* 6: 129–135.
- Al-Waili NS (2004): Topical honey application versus acyclovir for the treatment of recurrent *Herpes simplex* lesions. *Medical Science Monitor* 10: MT 94–98.
- Baltrusaityte V, Venkutonis PR, Cekstertyte V (2007): Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chem* 101: 502–514.

- Buratti S, Benedetti S, Cosio MS (2007): Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis. *Talanta* 71: 1387-1392.
- Campos M, Markham KR, Mitchell KA, Cunha AP (1997): An approach to the characterization of bee pollens via their flavonoid/phenolic profiles. *Phytochem Anal* 8: 181-185.
- Gao X, Ohlander M, Jeppsson N, Björk L, Trajkovski V (2000): Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J Agric Food Chem* 48: 1485-1490.
- Jamall IS, Smith JC (1985): Effects of cadmium on glutathione peroxidase, superoxide dismutase, and lipid peroxidation in the rat heart: A possible mechanism of cadmium cardiotoxicity. *Toxicol Appl Pharmacol* 80: 33-42.
- Kasahara Y, Hikino H, Tsurufuji S, Watanabe M, Ohuchi K (1985): Anti-inflammatory actions of ephedrine in acute inflammations. *Planta Med* 51: 325-331.
- Kosalec I, Bakmaz M, Pepeliniak S, Vladimir-Knezevic S (2004): Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharmaceutica* 54: 65-72.
- Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG (2005): Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chem* 91: 571-577.
- National Honey Board (2003): Honey-Health And Therapeutic Qualities, The National. Honey Board, 390 Lashley Street, Longmont, CO 80501-6045, www.nhb.org.
- Ohkawa H, Ohishi N, Yagi, K (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytic Biochem* 95: 351-358.
- Okun R, Liddon SC, Lasagnal L (1963): The effect of aggregation, electric shock and adrenergic blocking drugs on inhibition of the "writhing syndrome". *J Pharmacol Exper Ther* 139: 107-109.
- Oryan A, Zaker R (1998): Effects of topical application of honey on cutaneous wound healing in rabbits. *Zentralblatt für Veterinarmedizin A* 45: 181-188.
- Ozcan M, Unver A, Ceylan DA, Yetisir R (2004): Inhibitory effect of pollen and propolis extracts. *Nahrung/Food* 48: 188-194.
- Robert A, Nezamis JE, Lancaster C, Hancher AJ (1979): Cytoprotection by prostaglandins in rats. *Gastroenterology* 77: 433-443.
- Sedlak J, Lindsay RH (1968): Estimation of total protein-band and nonprotein and nonprotein sulfhydryl group in tissue with Ellman's reagent. *Anal Biochem* 25: 192-205.
- Wahdan A (1998): Causes of antimicrobial activity of honey. *Infection* 26: 26-31.
- Yesilada E, Küpeli E (2002): *Berberis crataegina* DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J Ethnopharmacol* 79: 237-248.
- Zeina B, Othman O, Al-Assad S (1996): Effect of honey versus thyme on *Rubella* virus survival in vitro. *J Altern Complement Med* 2: 245-248.
- Zeina B, Zohra I, Al-Assad S (1997): The effects of honey on leishmania parasites: An *in vitro* study. *Tropical Doctor* 27: 36-38.

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