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Evaluation of the antioxidant activity of honey in the peroxidation of rat liver microsomes

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ABSTRACT

Honey is a substance produced by bees and other social insects, from nectar or molasses that they gather on living plants and that transform or elaborate by evaporating water and action of enzymes, segregated by them, being stored in the alveoli or honeycomb cells. A prior investigation according to the literature indicated that the total antioxidant activity of honey is primarily provided by its phenolic composition. Polyphenols mainly exert their antioxidant activity by neutralizing free radicals. The objective of this study was to investigate the antioxidant effect of honey on the peroxidation of hepatic microsomal membranes. Rat liver microsomes were incubated with different concentrations of honey (25, 50, 100, and 200 mg/ml) in an *in vitro* non enzymatic ascorbic acid—Fe^{+2} system to determine the oxidative effect on membranes and to quantify the peroxidation level in standardized conditions. The microsomal peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in counts per minute (cpm), using microsomal membranes without honey as control. Analyzing the effect of honey was observed that the total cpm/mg protein originated from light emission, chemiluminescence, was statistically lower in samples obtained from honey group than in the control group; the antioxidant effect found was concentration dependent, and the results show the mean and its standard error of the averages of the cpm of the control sample 790,25 ± 223; of control + ascorbate 2232 ± 630 and of different concentrations used of honey: 25 mg/ml 1684 ± 475; 50 mg/ml 1383 ± 390; 100 mg/ml 895,5 ± 253 and 200 mg/ml 854,75± 241. These results indicated that honey may act as antioxidant, protecting rat liver microsomes from peroxidative damage.

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Introduction

Honey is a natural sweet substance produced by honeybees through foraging of different plants, transforming the collected material in their bodies into a liquid, and depositing the liquid in the cells of honeycombs. The properties of honey are shaped to a large extent by the plants foraged by the honeybees [1]. Honey is a high-quality natural food product with nutritional value that has been used in medicine traditionally from all over the world for its properties such as healing, antibacterial, and anti-inflammatory [2]. Honey contains 95% carbohydrates such as glucose and fructose, and it has 0.05% of proteins formed mainly by the amino acids: proline, lysine, glutamic acid, and aspartic acid [3]. Honey possesses enzymes such as alpha-glucosidases, amylases, and catalases. It has the presence of polyphenols, flavonoids, carotenoids, and phenolic acids, compounds to which the antioxidant power of honey is attributed [3]. The concentration of these compounds will depend on the floral species from which honey is made [3] Total phenolic content and total flavonoid content were analyzed to all honey types as reported; the total phenolic content values ranged from 8.01 to 20.31 mg GAE/100 g and decreased following the order: Biligirirangana Hills (multifloral type honey) > eucalyptus (unifloral type honey) > ginger

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(unifloral type honey) > neem (unifloral type honey) > Pan India honey (multifloral type honey) > lichi (unifloral type honey) > Kashmiri white (multifloral type honey); the phenolic content was in accordance with the previous studies on Indian honeys as well as with those of honeys from different geographical regions ; and the values for total flavonoid content ranged from 4.68 to 13.76 mg QE/100 g with the highest and lowest content in eucalyptus and lichi, respectively [4]. It was observed that the honey types had recorded variation in phenolic acids and flavonoid content: Biligirirangana Hills and lichi were found to be rich in phenolic acids and poor in flavonoids in contrast to eucalyptus, neem, ginger, and lemon [4]. The variation in phenolic and flavonoid content among the honey was likely due to variation in botanical sources, geographical location as well as season of collection [4]. In general, biologically active compounds in honey can be divided into two groups: antibacterial and antioxidant [5].

Since ancient times, honey has been not only considered as a food or sweetener but it was also used as a medicine for stimulating healing of wound and tissue regeneration and alleviating gastrointestinal disorders, gingivitis, and various other pathologies [6]. The therapeutic effect of honey results from the presence of various antioxidant molecules, including phenolic compounds, such as flavonoids and phenolic acids [6]. The activity of honey has been studied in various alterations; the research carried out highlights its ability to act as an antioxidant. It has been found that honey exhibits wide-spectrum therapeutic properties such as antimutagenic and speeds healing of wounds, antidiabetic, antiviral, antifungal, and antitumor [7]. Honey is highly and selectively cytotoxic against tumor or cancer cells while it is noncytotoxic to normal cells [8]. It can inhibit cancerogenesis by modulating or interfering with the molecular processes or events of initiation, promotion, and progression stages [8]. It could be purported as a natural cancer "vaccine" as it reduces chronic inflammation, improves healing of chronic ulcers and wounds, and improves immune status; its anticancer activity has been proved against various types of cancer: breast, colorectal, renal, prostate, endometrial, cervical, and oral [9]. Honey has the potential to reduce cardiovascular risk factors in normal healthy individuals. It causes to reduce systolic blood pressure and the level of triglycerides and very low-density lipoprotein in experimental animals [9]. An emerging and interesting aspect related to the use of honey is its employment as coadjuvant agent in several chronic pathological conditions [10]. Usually, honeys are classified as monofloral and polyfloral, and monofloral honey generally has better taste and higher economic value than polyfloral honey; therefore, the identification and nutritional evaluation of monofloral honey deserve a great attention [11]. The aim of this study was to evaluate the capacity of honey to protect liver microsomes against peroxidation.

Materials and Methods

Experimental

Female Wistar AH/HOK rats, 7 weeks old, weighing 120–137 g were used. All rats were fed commercial rat chow and water *ad libitum*. Female Wistar AH/HOK rats were obtained from the Laboratory Animal Facility, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Bovine Serum Albumin (BSA) (fraction V) was obtained from Wako Pure Chemical Industries Ltd., Japan. L (+) ascorbic acid, dimethyl sulfoxide, and methanol were purchased from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma-Aldrich.

Honey

Each honey sample (ten multifloral samples), collected from different regions of Buenos Aires, was analyzed and stored under dim light at 20°C. Due to the proximity of where the honey samples were taken, this work was done with a mixture of them.

Animals

The rats were euthanized by cervical dislocation, and the liver was rapidly removed, cut into small pieces, and extensively washed with 0.15 M NaCl. A 30 % (w/v) homogenate was prepared in a 0.25 M sucrose solution and 10 mM Tris-HCl pH 7.4 using a Potter-Elvehjem homogenizer. The homogenate was spun at 10,000 × g for 10 min in a centrifuge Kubota M6800 (Japan). The supernatant (30 ml) was applied to a Sepharose 4B column (1.6 × 12 cm) equilibrated and eluted with 10 mM Tris-HCl pH 7.4, 0.01% NaN3. The microsomal fraction appearing in the void volume (10–12 ml) was brought to 0.25 M saccharose with solid sucrose. All operations were performed at 4°C and under dim light. The quality of microsomal preparation is similar in composition with respect to concentrations and activities of certain microsomal enzymes to that obtained by ultracentrifugation [12].

Microsomal peroxidation

Rat liver microsomes were incubated with different concentrations of honey (25, 50, 100, and 200 mg/ml) in an *in vitro* nonenzymatic ascorbic acid – Fe+2 system to determine the oxidative effect on membranes and quantify the peroxidation level in standardized conditions. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR (Japan) by chemiluminescence in cpm. Microsomal membranes without honey were used as controls. Chemiluminescence and peroxidation were initiated by adding ascorbate to microsomes [12]. The microsomes (0.5 mg microsomal protein) with addition of honey (25, 50, 100, and 200 mg/ml) were incubated at 37°C with 0.01 M phosphate buffer pH 7.4, 0.4 mM ascorbate, obtaining a final volume of 1 ml. The phosphate buffer provides ferrous or ferric iron (the final concentration in the incubation mixture was 2.15 µM) for peroxidation [13]. Membrane preparations, without ascorbate, were carried out simultaneously. Membrane light emission was determined over a 180-minutes period, chemiluminescence was recorded as count per minute (cpm) every 10 minutes, and the sum of the total chemiluminescence was used to calculate cpm/mg protein.

Protein determination

Proteins were determined by the method of Lowry et al. [14] using BSA as a standard.

Statistical analysis

Results are expressed as means ± S.D. of five independent determinations. The data were statistically evaluated by one-way analysis of variance (ANOVA) and Tukey's test (SYSTAT Statistics). The statistical criterion for significance was selected at different p-values, which was indicated in each case.

Results

The incubation of rat liver microsomes in the presence of ascorbate – Fe+2 at 37°C for 180 minutes resulted in peroxidation of membranes as evidenced by light emission (chemiluminescence). When comparing the control group with the groups in the presence of honey, it was observed that, at higher concentrations of honey, there was a greater protection of the microsomal membranes (Fig. 1).

Table 1 shows the mean and its standard error of the averages of the cpm of the control sample—790,25 ± 223, control + ascorbate – 2232 ± 630, and different concentrations used of honey.

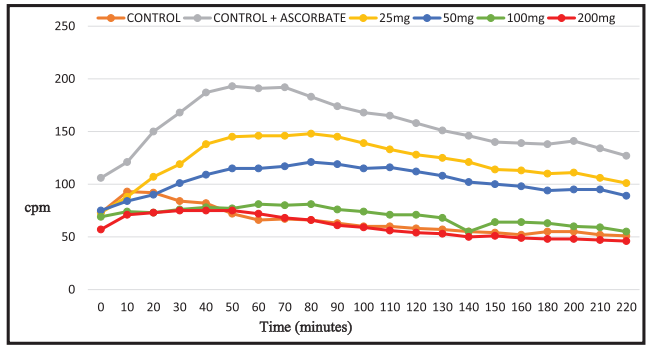

Figure 1. Antioxidant activity of honey in the peroxidation of rat liver microsomes.

Table 1. Light emission of rat liver microsomes during peroxidation (cpm) with different concentrations of honey (25, 50, 100, and 200 mg).

Microsomes	Average ± ES
Control	790,25 ± 223 [1,2]
Control + Ascorbate	2232 ± 630 [1, a, b]
25 mg	1684 ± 475 [2, c]
50 mg	1383,25 ± 390
100 mg	895,5 ± 253 [a]
200 mg	854,75 ± 241 [b, c]

Data are given as the mean ±SD of experiments. Statistically significant differences in honey concentrations and microsomal liver rat control and peroxidized are indicated by 1, a $p < 0.01$ and 2,b,c $p < 0.05$.

Discussion

Rat liver microsomes incubated with honey were protected against lipid peroxidation when compared to similar membranes from control group, as demonstrated by the results from chemiluminescence. The *in vitro* lipid peroxidation studies are useful for the elucidation of possible mechanisms of peroxide formation *in vivo* [15,16] since the composition of membranes causes susceptibility to peroxidative degradation [17]. Although considerable research has already been performed to characterize the changes in structure, composition, and physical properties of membranes subjected to oxidation [18–21], it is important to know how biological compounds with antioxidant properties contribute to the protection of specialized membranes against deleterious effects produced by reactive oxygen species and other free radicals. The antioxidant capacity of honey is given primarily by phenolic compounds, but enzymes, amino acids, and carotenoids also contribute to this ability [6]. Radical scavenging and protection against the lipid peroxidation of honey can reduce and prevent diseases and physiological situations

where oxidative stress plays an important role [6]. Other authors indicate that the chemical composition (content of individual sugars, ashes, nitrogen, and metal content) of honey can vary according to the floral species that is used as a source of the nectar [3]. As honey samples are different and may not contain all of the polyphenols described and because not all polyphenols exert the same protective effects, consumption of a wide variety of honey samples at any one time is recommended [22]. It is generally accepted that phenolics are important contributors to the antioxidant capacity of honey. Given that phenolic composition is greatly variable with respect to floral origin, honey is expected to show a wide range of antioxidant power [23]. Although considerable research has been done to determine the effects of honey on biological membranes, further studies are needed to determine if any differences exist among honey types.

Conclusions

In conclusion, our results are consistent with the hypothesis that honey may act as an antioxidant, protecting biological membranes from the deleterious effect of free radicals. This study is very important to know the properties of honey and to implement it to take care of health. However, further studies are needed to corroborate these observations.

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