Anti-Tumor And Immuno-modulating Effect of Honey in Normal And Tumor-bearing Mice

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الخلاصة

في دراستنا السابقة تم اثبات ان العسل لـ خصائص مضادة للتكاثر و حاثة للموت المبرمج في خطوط الخلا ا السرطانة لكل من Hep-2 و AMN-3 في الزجاج. و لقد صممت الدراسة الحالة للكشف عن تأثر اعطاء العسل داخل البر تون على الفئران المغروسة بسرطان الغدد اللبنة الفأري والتحري عن حجم و وزن الورم، معامل انقسام الخلا ا الجذع ة لنخاع العظم و كذلك مستوى كل من انترف رون- كاما interferon-ϒ و الانترلوكن-٢ في المصل.

اظ رت النتائج تثبطاً معنوياً في نمو الورم في الفئران المعاملة بالعسل حـ ث اختزل حجم الورم و وزن بحوالي ٧٨.٥% و ٤٣.٦% على التوالي. كذلك ازداد معامل الانقسام بشكل معنوي في المجامع المعاملة بالعسل للفئران الطبع ة (١٠.٣ ± ٠.٨٥) و الفئران الحاملة للورم (١٤.٤± ٠.٨) بالمقارنة مع سطراتـ ا (١٠.٨ ± ٠.٨ و ٧.٤ ± ٠.٥٣) على التوالي. في حـ ن سببت المعاملة بالعسل ارتفاعا معنوا في مستوى الانترفرون- كاما فقط و لـ س في مستوى الانترلوكن-٢ في مصول الفئران الحاملة للورم.[a] ذه النتائج تشـ ر بوضوح الى ان العسل متلك تأثرا مضادا للورم اما من خلال فعل المباشر على كتلة الورم او بشكل غـ ر مباشر من خلال تنشـ ط للجـ از المناعي.

ABSTRACT

In our previous study, it has been reported that honey have anti-proliferative and apoptotic effects against Hep-2 and AMN-3 tumor cell lines in vitro. The present study was designed to investigate that the effect of intraperitoneal administration of honey against tumor growth in murine mammary gland adenocarcinoma-implanted mice, the volume and weight of tumor, mitotic index of bone marrow stem cells as well as serum IL-2 and IFN-ϒ were assessed. The results showed significant tumor growth inhibition in honey-treated mice by reducing tumor volume and tumor weight about 78.5% and 43.6% respectively.Moreover, mitotic index was significantly increased ($p<0.01$) in honey-treated groups of both normal (10.3 ± 0.85) and tumor-bearing mice (14.4 ± 0.8) in comparison to their controls (10.8 ± 0.8 and 7.4 ± 0.53) respectively. However, the honey treatment caused significant elevation ($p<0.01$) in the level of IFN-ϒ only but not for IL-2 in the sera of tumor-bearing mice. These results indicated that honey has anti-tumor effect either through its direct activity against tumor mass or indirectly through enhancing the immune system.

INTRODUCTION

In recent years there is a growing interest in nutraceuticals which provide health benefits, diseases prevention and substitution of modern medicine (1). As many as 89% of patients with cancer or other chronic conditions use nutrceuticals, some of them show potential as adjuvants with conventional oncology treatment, others may be used to eliminate the side effects of conventional treatment (2). Although honey is an age-old remedy from the time of Egyptian civilization, very recently it has been found a place in a modern medicine (3). Honey is a supersaturated solution, mainly fructose (38%), glucose (31%), sucrose (1%), water (18%), other sugars (7%) plus amino acids, organic acids, enzymes, anti-oxidants, vitamins as well as minerals (4). Because of its high

viscosity, acidic PH, hydrogen peroxide, high osmolarity and rich nutritional properties honey can inhibit bacterial growth and enhance healing (5-7).

Very few studies had been carried out to investigate the anti-tumor properties of honey, Gribel and Pashiniskii demonstrated that honey revealed a moderate anti-tumor and pronounced anti-tumor activity of 5-flurouracil and cyclophosphamide against five strains of rat and murine tumors (8). It was found that honey is an effective agent for inhibiting the growth of T24, RT4, 253J and MBT-2 bladder cancer cell lines in vitro, and it is also effective when administered intralesionally or orally in the MBT-2 bladder cancer implantation models (9). Furthermore, the development of tumor implantation at the surgical wound following cancer surgery was markedly decreased by application of honey pre- and post- operatively (10, 11).

Several other investigators studied the protective effect of honey against the most common complications that result from cancer therapy, for instances, febrile neutropenia which is a serious side effect of chemotherapy was decreased by using life-Mel honey (12), it was also used to prevent the mucositis, which is the most common acute complication of radiotherapy in the head and neck region (13, 14).

On the other hand, it had been reported that manuka honey increase IL-1β, IL-6 and TNF-α production from human monocytes (15, 16), and the active component was 5.8kDa, which increase production of these cytokines via TLR4 (17). In addition, it was reported that oral intake of honey augmented antibody productions in primary and secondary immune responses against thymus-dependent and thymus-independent antigen (18). Recently, it was found that Jungle honey (JH) possessed chemotactic activity for neutrophils that causes a decrease in the incidence and weight of tumor in JH-injected mice (19). In our previous studies we found that natural honey at a concentrations of 0.6% cause an elevation in the protein content of lymphocytes culture that lead to increase their proliferation rate with clear anti-inflammatory action in mice by using formalin-induced inflammation model (20), in addition to anti-proliferative, anti-adhesive and apoptotic effects against human and murine cancer cell lines HEP-2 & AMN-3 (21).

The purpose of this study was to investigate the effects of natural honey on immune function and anti-tumor activity in mice.

MATERIALS & METHODS

Preparation of Honey

Natural unprossessed and multifloral origin raw honey was collected from Khan Dhari, Baghdad, Iraq. The initial solution of honey in a ratio of 6% (wt/v) was prepared by dissolving 6 grams of honey in 100 ml phosphate buffer saline (PBS), then sterilized by filtration through 0.22μm filter.

Preparation of Tumor-bearing mice

Females (6-8 week-old, 20-30gm) mice were obtained from animal house of Iraqi Center for Cancer and Medical Genetics Researches. All animals were fed a standard diet and water ad libitum. Spontaneous mammary adenocarcinoma AM3 established by Al-Shammery, 2003 was transplanted in female mice. A tumor-bearing female mouse (Source) was anesthesized and needle gage-18 is inserted into tumor mass to aspirate 3-6 ml of tumor content that is transferred into a sterile beaker. Equal volume of a sterile PBS was added and the mixture was transferred into a sterile tube and centrifuged at 1000 rpm for 10 min in cooling centrifuge (18-20˚c). The supernatant discarded and the tumor cells resuspended in an adequate amount of PBS.

Mammary adenocarcinoma cells (1×10^6 cells/ml) were injected subcutaneously into 6-8 weeks aged female mice by inserting needle gage-18 starting from inguinal region

until reaching to cervical region. After appearance of tumor mass, the mice become ready to be used in the therapeutic experiment.

Therapeutic experiment

Female mice were divided into two major groups, normal and tumor-bearing mice, each of them was subdivided into two groups (n=5), honey-treated and control groups. Honey-treated groups were injected with honey intraperitoneally at a dose of 600 mg/kg body weight and continued for 28 alternating days, while control groups were injected with PBS in the same manner. The development of tumor was detected twice a week and the tumor volume was measured by using Vernier calipers and calculated from the following formula (23):

Tumor volume = $4/3\pi a^2.b/2$ (Whereas a is the minor diameter, b is the major diameter).

The relative tumor volume RTV was calculated from the following equation (24):

RTV = Tumor volume at day x / tumor volume at day Zero

After the last dose mice were left for 48h, then injected with 0.1 ml colchicin (1 mg/ml) intraperitoneally and left for 3-4 hr, then anesthesized to obtain the blood, Bone marrow and tumor mass specimens. Bone marrow mass obtained from femur and the mitotic index MI of bone marrow cells was determined according to Verma & Babu procedure (25). The tumor mass was removed and weighed, the percentage of tumor growth inhibition (TGI %) was calculated by the following equation (26)

TGI % = (1-B/A) X 100 (Whereas A is the average tumor weight of the control group, **B** is that of honey-treated group).

Blood has aspirated by heart puncture and the serum was obtained and stored at -20˚c to be used later in determination of IL-2 & IFN-ϒ. The levels of IL-2 and IFN-ϒ in the collected sera were determined by using Eliza kit for mouse IL-2, 3441-1A.6 and mouse IFN-ϒ, 3321-1A.6, purchased from MAB TECH, USA.

Statistical Analysis

All values are expressed as mean ± S.E. The validity was tested by linear correlation between the treatment and parameter. Comparisons between control and honey –treated mice were made with the un-paired Student's t-test. Any p-values < 0.05 were considered statistically significant.

RESULTS

Both honey-treated and non treated tumor-bearing mice showed significant direct correlation between tumor volume and the time of follow up ($r = 0.46$, $p < 0.04$ and $r = 0.64$, $p < 0.03$ respectively) (figure 1). The relative tumor volume (RTV) at the end of fourth week as well as tumor weight were used to calculate the percentage of tumor growth inhibition (TGI %). Honey treatment demonstrated significant reduction in TGI % about 78.5 % in respect to tumor volume and 43.6 % in respect to tumor weight.

Figure 2 showed significant differences ($p < 0.01$) in mitotic index values between treated groups (14.4 ± 1.13 and 10.8 ± 0.8) and their corresponding controls (10.3 ± 0.85 and 7.4 ± 0.53) of both normal and tumor-bearing mice respectively.

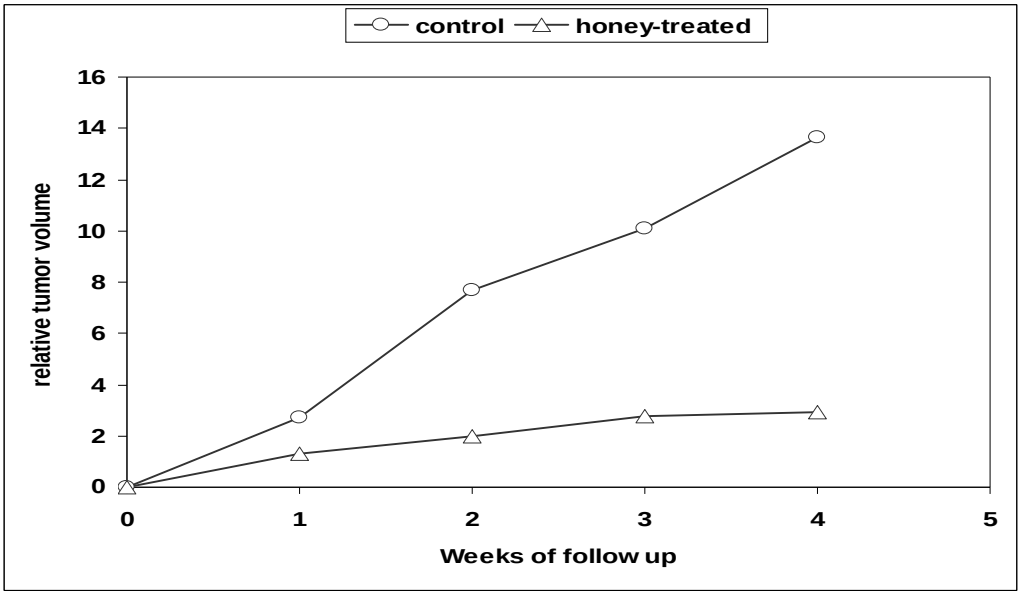

Figure 1: Follow up of the change in relative tumor volume in mice.

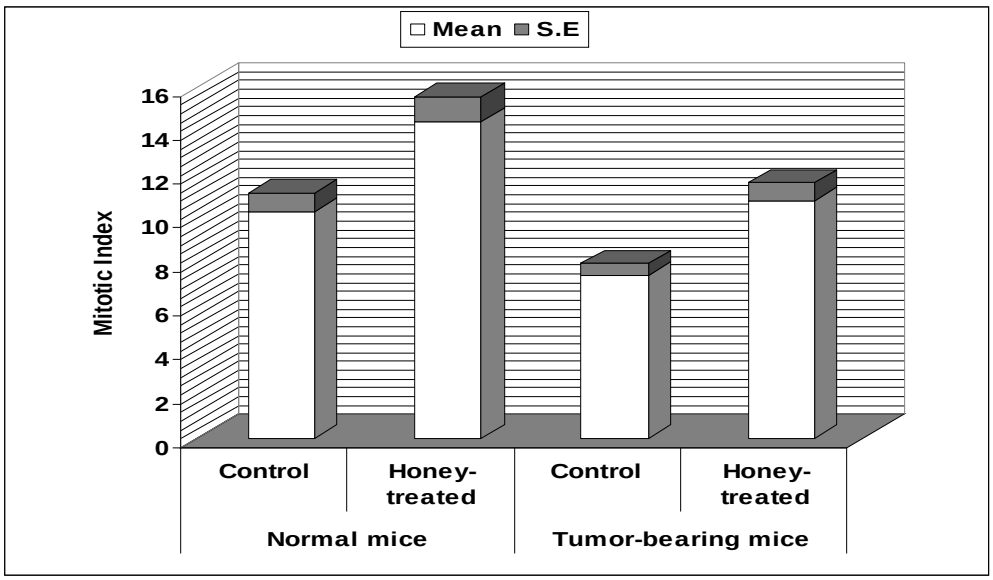

Figure 2: Effect of honey administration on the mitotic index of bone marrow cells in normal and tumor-bearing mice.

Honey-treatment didn't cause any significant differences in serum IL-2 level neither in normal mice (52.6 ± 1.9 pg/ml) nor in tumor-bearing mice (53.1 ± 2.9 pg/ml) in comparison to their corresponding controls (50.2 ± 1.4, 52.4 ± 3.8 pg/ml) respectively (figure 3). However the level of IFN-γ was significantly ($p < 0.01$) elevated from 495 ± 30 pg/ml (in control) up to 599 ± 40 pg/ml in honey-treated group of tumor-bearing mice but without significant differences between honey-treated group (647 ± 50 pg/ml) and its control (704 ± 77 pg/ml) in normal mice (figure 4).

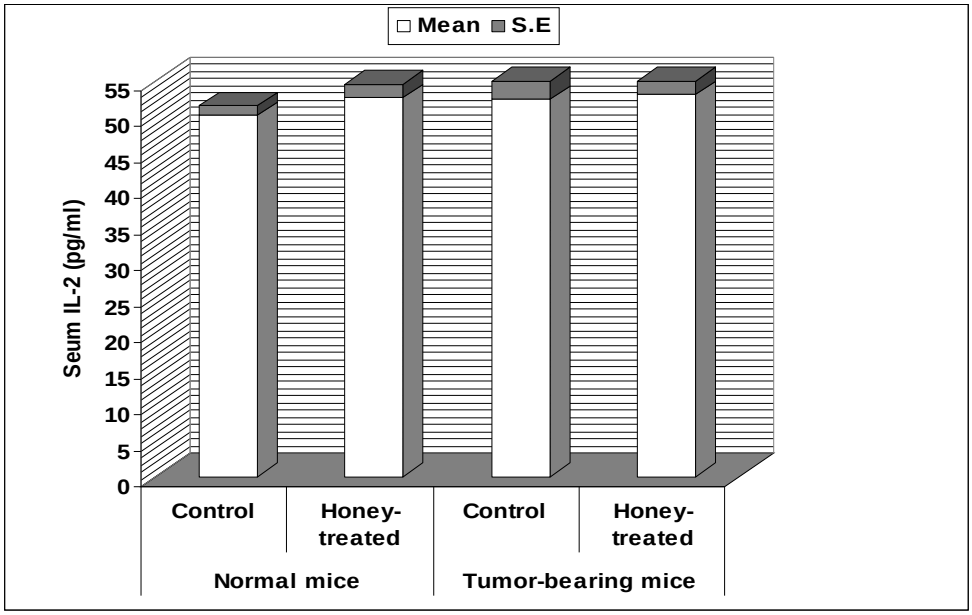

Figure 3: Effect of honey administration on serum IL-2 level in normal and tumor-bearing mice.

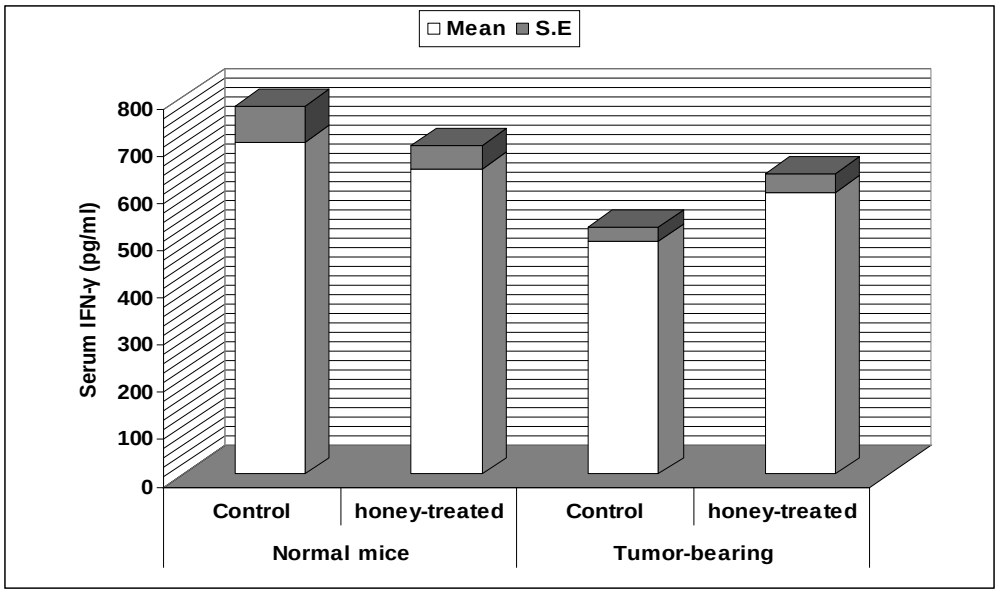

Figure 4: Effect of honey administration on serum IFN-γ level in normal and tumor-bearing mice.

DISCUSSION

This study demonstrated a clear therapeutic activity of intraperitoneal administration of honey in tumor-bearing mice. The therapeutic index of honey was based on its ability to reduce the weight and volume of tumor (figure-1), enhancing the mitotic index of bone marrow cells (figure -2) and elevation in IFN-Ϋ level (figure – 4). These results were confirmed our previous results, that reported an anti-proliferative activity of honey against Hep-2 and AMN-3 cell lines *in vitro* (21) and promote lymphocyte to secrete protein- materials in their culture (20). Some investigators demonstrated that the number of peritoneal cells and migration of neutrophils were increased by jungle honey which also has a proliferative effect on tumor growth (19). According to the results obtained from this preliminary study, it can be suggested that several factors influence the effectiveness of honey. Some of these factors enable honey to act as anti-tumor directly such as its osmotic properties, acidic PH, enzymes , minerals and vitamins that help repair tissue directly (3). However, earlier publications suggested that the phenolic acids and flavonoids found in the honey were the main causes of its protective effect against some pathological conditions and may act as an anti-oxidant (27) or apoptotic agents (21). Also sulphorafane component found in the honey, which is enzymatically released from the glucosinolate, play an important role in the anti-tumorigenic activity (28).

On the other hand, honey may act as anti-tumor indirectly through enhancing immune response by different mechanism. The first mechanism supposed that honey might partly inhibit either the synthesis or the release of certain protein molecules, which may be growth factors or cytokines, for instances, some epidermoid carcinomas secrete complex molecules “haptoglobin” to suppress the activity of natural killer cells (29), while other tumor cells secrete different cytokines such as IL-10 to suppress immune response against them (30, 31). The second mechanism focused up on the stimulation of lymphocyte to release certain cytokine that is associated in their proliferation, because many investigators indicated that honey can stimulate cytokine production from human lymphocytes such as TNF-1, IL-1 and IL-6 and the proliferation of peripheral blood B and T lymphocytes (15, 16, 17) Since infiltration of many neutrophils was observed at necrotic areas in honey-injected tumor tissue (19), the 3rd mechanism of anti-tumor activity of honey may be due to production of reactive oxygen species ROS by neutrophils into tumor tissues because it was reported that ROS produced by activated neutrophils has tumor cytotoxic properties (32).

It can be concluded from this study that honey has the ability to reduce tumor growth in tumor-implanted mice and enhance their immune response through increase the level of IFN-Ϋ and the mitotic index of bone marrow stem cells. However, it is necessary to investigate the type of immune cells that infiltrated into the tumor tissues and the apoptosis events that may occur in tumor cell to confirm the direct and indirect anti-tumor activity of honey.

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