

Full Length Research Paper

Modulation of programmed cell death by honey bee in human prostate adenocarcinoma

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The search for new anti-cancer drugs from natural products is one of the most prominent research areas of treatment of cancer. Honey finds a vital role in various applications using its anti-inflammatory, antioxidant, antibacterial and antitumor properties. We investigated the potential of honey to induce cytotoxic and antiproliferative effects in cultured human prostate cancer cell line (PC-3) in comparison to non-malignant (L929) cells. Both cells were cultured in RPMI medium and treated with the selected crude honey sample at various concentrations (5, 10 and 15%) for three consecutive days. The results revealed that the cell viability decreased in a concentration and time- dependent manner in the malignant cells treated with honey in comparison with non-malignant cells. Honey showed anti-proliferative activity with the IC₅₀ value 4, 10 and 14% after 24, 48 and 72 h, respectively in PC-3 cell line. Overall, it can be concluded that higher concentrations of honey is safe for L929, but it exerts anti-tumor cytotoxicity and anti-proliferative effects in a prostate cancer-derived cell line. Thus, it is considered as a potential chemotherapeutic agent against prostate cancer.

Key words: Anti-proliferative, programmed cell death, Honey bee, human prostate adenocarcinoma, MTT.

INTRODUCTION

Prostate cancer is the most common diagnosed malignancy and the second leading cause of male death in Western countries (Crawford, 2003) and produces killing about 200,000 men annually throughout the world. Mortality from prostate cancer results from metastases to the bones and lymph nodes. Clinically, prostate cancer is usually diagnosed in men over 50 years of age; with increasing life expectancy, the incidence of prostate cancer is likely to increase worldwide (Peter et al., 1995).

Cancer therapy is generally classified into three categories: surgery, radiation therapy and chemotherapy. Chemotherapy is the administration of drugs that can regulate the uncontrolled proliferation of abnormal cancer cells. Tumor cells very often develop resistance to

chemotherapy. On the other hands, approximately 75% of patients have surgically incurable disease at the time of diagnosis. There are few drugs which achieve a complete recovery in cancer patients and the failure of conventional chemotherapy to induce a major reduction in mortality indicates that the development of more effective chemotherapeutic drugs is essential for treatment of cancer worldwide. Thus, there is a promising opportunity for its intervention using cancer chemopreventive compounds that can prevent or slow the progression cancer disease (Tsao et al., 2004), (Jain et al., 1999).

Several epidemiological studies have shown a strong correlation between consumption of diets rich in natural products and vegetables and a lower risk of various cancers (Norman and Kerri, 2003). For a variety of reasons naturally occurring botanicals and natural dietary sub-stances are gaining increasing attention as cancer chemopreventive agents. Important for prostate cancer chemoprevention is the fact that in recent years, the

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use of natural dietary substances and botanical products is showing sustained increase by prostate cancer patients. In prostate cancer a fine balance between cell proliferation and apoptotic death is lost which contributes to increase cellular mass and tumor progression. In this regard, for prostate cancer chemoprevention at the present time there is considerable emphasis in identifying novel natural products that selectively induces apoptosis and growth arrest of prostate cancer cells without our less producing cytotoxic effects on normal cells.

Honey bee finds a role in both domestic and medicinal applications. It has been widely used as sweetener since ancient times. Composition of honey varies depending upon the geographical and the nectar sources of a region. The quality of the honey bee depends upon its physiochemical and sensory properties. Hence knowledge about its constituents is essential in judging its quality (Al-Khalifa and Al-Arif, 1999).

Recent studies revealed that phenolic compounds present in the honey can act as potent antioxidants in comparison with other constituents like Vitamins C and E (Nagendran et al., 2006; Elisabete et al., 1998). Consumption of certain dietary components has been related to several protective effects against cancer and also several other disorders such as diabetes and rheumatoid disease (Wollgast and Anklam, 2000). Honey is thought to exhibit a broad spectrum of therapeutic properties including antibacterial, antifungal, cytostatic and anti-inflammatory activity (Burdock, 1998; Ijaz et al., 2008). Honey has been used for the treatment of abdominal wound disruption, gastric ulcers, gastroenteritis and burns and for the storage of skin grafts (Lutfi et al., 2006).

Recent studies by Gribel and Pashiniski indicated that honey possessed moderate antitumor and pronounced antimetastatic effects in five different strains of rat and mouse tumors (Gribel and Pashiniski, 1990). Furthermore, honey potentiated the antitumor activity of chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide (Wattenberg, 1986). Honey contained many biologically active compounds including caffeic acid (Mohamed and Mona, 2010), caffeic acid phenethyl ester (Mohamed and Mona, 2010) and flavonoid glycones (Yen et al., 2003). These compounds demonstrated to have an inhibitory effect on tumor cell proliferation and transformation by the down regulation of many cellular enzymatic pathways including protein tyrosine kinase, cyclooxygenase and ornithine decarboxylase pathways (Jim et al., 2003). Since honey is one of the common foods for humans, it prompted us to investigate it as a potential candidate for cancer treatment.

MATERIALS AND METHODS

Materials

3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl (MTT) was purchased from Bioseen Technology, Inc. RPMI 1640 was purchased from Igbo BRL (Grand Island, NY, USA). Fetal bovine serum was

purchased from PAA Laboratories GmbH, Austria.

Honey

Pure unfractionated honey bee was purchased from AliBaba (India) and diluted with RPMI-1640 medium to prepare concentrations of 0 to 15% (0, 5, 10, and 15%) to be used in the *in vitro* studies.

Cell lines

Two different cell lines were used in this study. The human prostate cancer cell lines designated as PC-3 and mouse fibroblast cell line designated as L929 were obtained from the Pasture Institute in Tehran, Iran.

Cell culture

Human prostate cancer cell line (PC-3) was maintained as monolayer cultures in RPMI-1640 supplemented with 10% fetal calf serum, 1% glutamine and 1% each penicillin and streptomycin. The cells were cultured in a 96-well tissue culture plates for 24 h. The culture medium was then aspirated and a 5, 10 and 15% dilution of honey in RPMI-1640 was added. The plates were maintained at 37°C in 5% CO₂ in humidified air for 72 h and then examined by phase contrast microscopy.

MTT assay

After 72 h incubation of PC-3 cells in various dilutions of honey (0 to 15%) in 96-well tissue culture plates, 10 mL of MTT reagent (1 mg/mL) was added to each well, and the plates were incubated for 3 to 4 h. The medium was then aspirated, and 100 µL DMSO (100%) was added. The highly colored DMSO-soluble Formazan product was assessed spectrophotometrically by employing a microplate reader.

Morphological studies

Morphological studies by using normal inverted microscope were carried out to observe the morphological changes of cell death in malignant (PC-3) and nonmalignant cell (L929) lines elicited by different concentration of honey. Concentration of (5, 10 and 15%) of honey was used for the morphological studies. The untreated cells served as the negative control. The morphological alterations of the cells were visualized under the normal inverted microscope after 24, 48 and 72 h post-treatment.

Determination of IC50

Plots of percent cytotoxicity index (%CI= [1- (OD570–630 treated/OD570–630 control)] ×100) versus concentrations were drawn from the experiments. IC₅₀, honey concentration resulting in 50% cytotoxicity was determined from the graph.

Statistical analysis

Significant level was ascertained by one way analysis of variance, followed by Tukey multiple comparison tests. Results were expressed as the mean ± SEM. A p value of <0.05 in Tukey test was considered significant. Statistical procedures were performed with SPSS software.

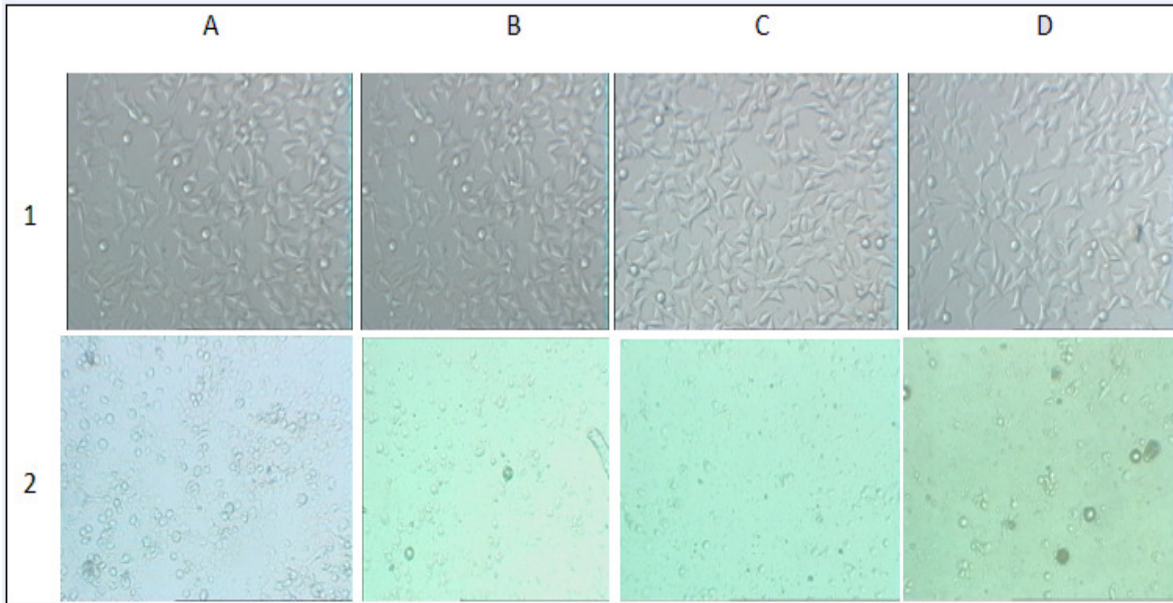


Figure 2. Comparison cytotoxicity effect of honey on cell viability of prostate cancer cell (PC-3) and non-malignant cell (L929) line after 24 h. Morphological changes of cells after treatment with different concentration of honey. 1: L929 cell line; 2: PC-3 cell line; A = 0%; B = 5%; C = 10% and D = 15% honey.

RESULTS

To discriminate between early and late effects of honey action, malignant (PC-3) and non-malignant control (L929) cells were exposed to increasing concentrations of honey for 24, 48 and 72 h.

Morphological evaluation of the effect of honey on cell viability

After 24 h co-culture of the cells with different concentration of honey (5, 10 and 15%), cell population was decreased as compared to control and also morphologic changes were observed in the prostate cancer cells versus L929 cells which is consisting of reduction in number of living cells, volume and rounding until the nucleus constituted the majority of the cellular volume. The reduction of malignant cells (PC-3) compared to L929 was statistically highly significant. This cytotoxicity was increased at higher concentrations (Figure 2). After 48 h of incubation with honey moderate cytoplasmic granulations were apparent and a large number of cells became rounded at each dose. On day 3, nearly 90% of surface in the control flasks were confluent, while in all honey concentrations about 20- 10 % of surface covered by PC-3 cells. So that, honey treated prostate cancer cells (5, 10 and 15 %) were damaged but there were no morphological changes in honey treated L929 cells at the same concentrations (Figure 3). After 24, 48 and 72 h, there was no clear morphological changes were detected in L929 cells at any dose of honey (Figures 2 and 3).

Effect of honey on cell viability

In order to evaluate the effect of honey on growth of prostate cancer cells and L929, the cells were incubated with different concentrations of honey (5, 10 and 15%) for 24, 48 and 72 h and their growth inhibitory effects were compared. MTT assay, a simple and reliable technique, which measures cell viability, can be used for screening of anti-proliferative agents. The assay was repeated five times; there was a concentration-dependant antitumor effect in prostate cancer cell line. Honey showed significantly high growth inhibitory effects on prostate cancer cell line in a concentration and time- dependent manner compared to the L929. As shown in Figure 1 honey (5, 10 and 15 %) decreased cell viability in malignant cells (PC-3) but not in non-malignant cells (L929) after 24 h. This toxicity was consistent with morphologic changes. However, honey at different concentrations could not significantly decrease cell viability in L929 cells. Table 1 presented the dose inducing 50% cell growth inhibition (IC_{50}). The IC_{50} values determined from different honey concentration on PC-3 cell lines. Honey (72 h) showed potent cytotoxic effects with the IC_{50} values of 4% in PC-3 line or 2.5 times more potent than 48 h exposure (10%) whereas honey (24 h) gave the IC_{50} values of 14 % in PC-3 cell line, which was about 3.5 times less potent than 24 h exposure.

DISCUSSION

The therapeutic activity of most anticancer drugs in

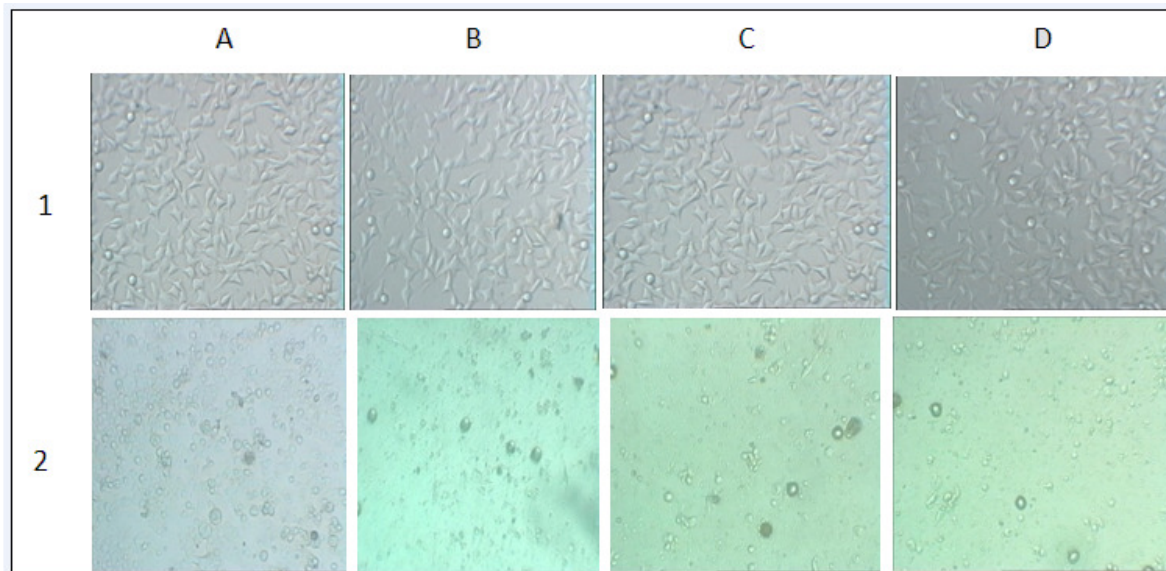


Figure 3. Comparison cytotoxicity effect of honey on cell viability of prostate cancer cell (PC-3) and non-malignant cell (L929) line after 48 h. Morphological changes of cells after treatment with different concentration of honey. 1: L929 cell line; 2: PC-3 cell line; A = 0%; B = 5%; C = 10% and D =15% honey.

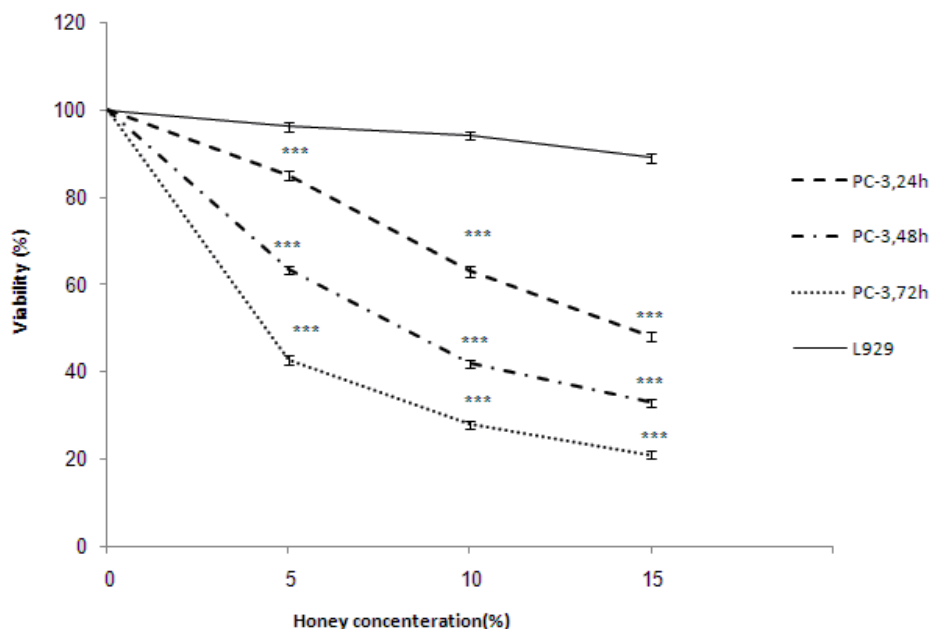


Figure 1. Effect of honey on cell viability of PC-3 cells. Cells were treated with different concentration of honey for 24, 48 and 72 h. Viability was quantitated by MTT assay. Results are mean \pm SEM. The asterisks are indicator of statistically difference obtained separately at different time points compared to their controls shown in figure as *** $p < 0.001$.

clinical use is limited by their general toxicity to proliferating cells, including some normal cells. Although, chemists continue to develop novel cytotoxic agents with unique mechanisms of action, many of these compounds still lack tumor selectivity and have not been therapeutically useful (Rao et al., 2005; Chari, 2008). New targets for cancer therapy focus on interfering with

specific targeted molecules needed for carcinogenesis and tumor growth in order to overcome the problems of traditional therapies (Goldman, 2003). Natural products are perceived as pure, and without side effects medication products (Montbriand, 2004). Many patients with cancer or other chronic conditions use alternative therapies, often herbal or natural products (Montbriand

Table 1. Doses inducing 50% cell growth inhibition (IC₅₀) of Honey extract against prostate cancer cell line (PC-3).

IC ₅₀	24 h	48 h	72 h
PC-3	14±1.0 (%)	10±0.9 (%)	4±0.05 (%)

Cells were treated with different concentration of Honey for 24 and 48 h. Viability was quantitated by MTT assay.

1995, 2000, 1994). Honey, which is one of the most complex mixtures of carbohydrates produced in nature (Roderick and Lise, 1999), has a long history as a medicinal substance. It is a known natural product with several biological activities. Some bioactive compounds have been found in honey such as chrysin which have been used to prevent cancer, in a similar fashion as anastrozole (a breast cancer drug), and treat conditions such as anxiety and inflammation (Galijatovic et al., 2000, 2001).

The objective of our *in vitro* study was to investigate the cytotoxicity and anti-proliferative activity of a range concentration of honey against human prostate cancer cell lines. Honey is also known as a dietary source for flavonoids (Marie et al., 1996) which have been demonstrated to have anti-carcinogenic and anti-inflammatory activities (Abdel et al., 2009). Although crude honey was reported by some authors as a proliferative agent that enhances the proliferation of both normal and malignant cells (Abuharfeil et al., 1999; Tonks et al., 2001), it was also reported as a promising antitumor agent with pronounced antimetastatic effect (Orsolčić et al., 2005). The proliferative effect of honey on tumor cells was suggested to be a nutritional effect rather than a carcinogenic effect, and the antitumor effect was reported to result from many activities such as the inhibition of DNA synthesis with no signs of cytotoxicity (Saravana and Mahitosh, 2009).

In vitro study showed that diluted honey affected the growth patterns of prostate cancer cells. The effect was observed to be dependent on the duration and concentration of honey used. According to the results of MTT cytotoxicity test and morphological evaluation, all concentrations of honey exhibited antiproliferative effect on PC-3 cells with no significant differences on L929 cells. The cytotoxic effect of honey can be explained by the fact that honey is a dietary source of phenolic compounds that are referred to as flavonoids. These compounds have been isolated from several types of honey and they have been reported as potent anticancer agents that can be extracted from their sources mainly by ethyl acetate (Yang et al., 2008). Caffeic acid (3,4-dihydroxycinnamic acid) ester derivatives, which are present in honey at levels of 10 to 20% (total phenolic compounds), are thought to exhibit a broad spectrum of activities that possibly include tumor inhibition (Wei et al., 2007). Several cellular components that have been associated with cell proliferation, such as polyamines and polyamine synthetic enzyme activities

including ornithine decarboxylase, are presents at high levels in proliferating normal and neoplastic cells (Karima et al., 1999; Susan, 2007). In addition many kinases, such as tyrosine protein kinase (TPK), mediate proliferative as well as metabolic signals in the cells (Lise and Richard, 1994). Eicosanoids, the metabolites of arachidonic acid through the lipoxygenase and cyclooxygenase pathways, exerts a variety of biological activities (Marta et al., 2005). The cytotoxic effect of chloroform extract of H1 may be attributed to the fact that honey may contain pyrrolizidine alkaloids which considered as toxic compounds that were extracted by chloroform (Deinzer et al., 1977).

Chloroform/methanol solvent was reported to extract lipids, including unsaturated fatty acids. It was reported that unsaturated fatty acids possess a broad spectrum of biological properties in both animals and plants and some of these compounds show biological activity, e.g., 9,10-dihydroxy-8-oxo-12Zoctadecaenoic acid exhibits cytotoxicity against HeLa cells and an inhibitory effect on tea pollen growth; it was also reported that the lipid fraction extracted from *Agaricus blazei* by chloroform/methanol retarded tumor growth (Takaku et al., 2001). Thus the cytotoxic effect of the chloroform/methanol extract of all tested honeys may be attributed to the presence of cytotoxic lipid contents of honeys and, probably, to other cytotoxic component(s). In our studies, treatment of cancer cell with honey resulted in a significant lowering of the proliferation compared to control cells, supporting our hypothesis about the anti proliferative and cytotoxic effect of honey in prostate cancer.

In conclusion, honey can be considered as promising antitumor agents where they inhibited the proliferation. Further investigations are needed to study in details the composition and mechanism of action of honey on PC-3 and other cell lines.

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