

Natural Honey and its Effect on the Activity of the Cells

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Abstract

Natural honey was assessed in cell culture system for its anticancer activity. Human leukemic cell line, HL 60 as treated with honey, cultured for 5 days and then cytotoxicity was calculated by MTT assay. Honey showed cytotoxicity with CC_{50} value of 174.20 $\mu\text{g/ml}$. Radical modulation activities was assessed by lipid peroxidation assay using egg lecithin. Honey showed antioxidant activity with EC_{50} value of 159.73 $\mu\text{g/ml}$. In addition, treatment with HL60 cells also resulted in nuclear DNA fragmentation, as seen in agarose gel electrophoresis. This is a hallmark of cells undergoing apoptosis. Confirmation of apoptosis was performed by staining the cells with Annexin V and FACS analysis. Apoptosis is an active, genetically regulated disassembly of the cell form within (inside the cell) Disassembly creates changes in the phospholipid content of the cytoplasmic membrane outer leaflet. Phosphatidylserine (PS) is translocated from the inner to the outer surface of the cell for phagocytic cell recognition. The human anticoagulant, annexin V, is a Ca^{2+} -dependent

phospholipid protein with a high affinity for PS. Annexin V labeled with fluorescein can identify apoptotic cells in the population It is a confirmatory test for apoptosis. Annexin V-positive cells were defined as apoptotic cells. Since honey shows both antioxidant activity and cytotoxicity at almost the same concentration, it can prevent the free radical induced cancer as prophylactic agent and kill the cancer cells by apoptotic process as a chemotherapeutic agent. Everyday intake of honey can prevent the cancer induction.

Keywords—Anticancer, cells, DNA, honey, Apoptosis.

I. INTRODUCTION

Honey is a natural product that has been widely used for its therapeutic effects. It has been reported to contain about 200 substances. Honey is composed primarily of fructose and glucose. It also contains fructo-oligosaccharides [1], many amino acids, vitamins, minerals and enzymes [2]. The composition of honey varies depending on the plants on which the bee feeds. However, almost all natural honey contains flavonoids (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin), phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids), ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), Millard

reaction products and peptides. Most of those compound work together to provide a synergistic antioxidant effect [3]-[7].

Human. use honey in the treatment of diseases dates back to ancient times. The Egyptians as well as old Greeks, Romans, Chinese and Indians used Honey. The material of Bee honey has a thick texture and aromatic sweet taste. Bees collect nectar then it transmutes it into honey with its own thick texture. In order for one bee to produce 1 kilogram of honey they move between flowers at a distance equivalent to 11 times the distance of the Earth's circumference around the equator. They produce Ddifferent types of honey in terms of Color, taste, smell and susceptibility to density and alkaline depending on the source of nectar. There are other factors that also affect the qualities of honey Such as soil type and weather factors for example two samples can vary greatly even if the source of the flower nectar is the same. [8],[9],[10]. honey color is also influenced by the temperature so we can notice that bee honey tends to be black in color if the temperature is high during the nectar season. The environment also changes some of the honey components so some types of honey such as the sidr and cumin honey proved to contain certain vitamins that are found in fruits and vegetables. There have been numerous scientific studies conducted on honey[11] during the past few years, there was a study in the British medical journal "BMJ". The study has proven that giving honey to children suffering from diarrhea would actually speed the healing process of those children. Another study in the British Journal of Surgery in 1988 was conducted about the use of honey for infected wounds and sores that did not respond to antibiotics and honey has also been proven to be effective.

In November 1992 a study by some Australian researchers in which honey was used in the treatment of 15 women after a caesarean section.

They say the researchers: "The honey put on wounds was effective and inexpensive and abolished the need for re-stitching the wounds and exposing the patient further surgery".

Also at another study as 20 people were suffering from a type of gangrene called "Fournier's Gangrene"; honey was used for the treatment of gangrene. Another group was treated using the conventional method in addition to those who were treated using honey. The researchers found that honey put on gangrene had given a clear advantage to conventional treatment, and patients were more responsive to honey which contained *Salmonella*, *Shigella*, *Escherichia coli* [3]-[15]. *Helicobacter pylori* [9], etc. In an inflammatory model of colitis, honey was as effective as prednisolone treatment [16]. Research has also indicated that honey may possess anti-inflammatory activity and stimulate immune responses within

a wound [Al-Waili and Boni (2003)] as it was noticed after ingestion of honey [19]. Honey, interestingly, has been shown to prevent reactive oxygen species (ROS)-induced low density lipoprotein (LDL) oxidation in some *in vitro* studies, thus exhibiting beneficial cardiovascular protection [20],[21]. Honey also had antineoplastic activity in an experimental bladder cancer [22] This article has reviewed important traditional and modern uses of natural honey in human diseases. Honey is natural food rich in essential nutrients.

It is used as a medicine for the treatment of many diseases due to its medicinal value. It has been reported to contain over 400 substances. More than 95% of honey mainly consists of sugar and water in varying proportions .

It also contains proteins amino acids and enzymes. The antimicrobial action of honey was studied for the first time; Many researchers believe that the effect of honey could be due to various factors. The high acidity and osmolality are among the factors which contribute to its antimicrobial activity. Organic acids, volatiles and hydrogen peroxide are also important factors that can provide antimicrobial activity to honey. Mechanisms of antimicrobial activity of honey are different from antibiotics which destroy the bacteria's cell wall or inhibit intracellular metabolic pathways. The antibacterial activity is related to four properties of honey. First, honey draws moisture out of the environment and thus dehydrates bacteria. The sugar content of honey is also high enough to hinder the growth of microbes, but the sugar content alone is not the sole reason for honey's antibacterial properties[32]. Second, the pH of honey is between 3.2 and 4.5, and this acidity is low enough to inhibit the growth of most micro- organisms. Hydrogen peroxide produced by the glucose oxidase is the third and probably the most important antibacterial component, although some authors believe the non-peroxide activity to be more important. Lastly, several phytochemical factors for antibacterial activity have been identified in honey. [33],[34] .Hydrogen peroxide, glucose oxidase, catalase and phytochemical factors have been described as non-peroxide antibacterial factors (24). In addition

volatiles, organic acids, lysozyme, beeswax, nectar, pollen and propolis are important chemical factors that provide antibacterial properties to honey [22]-[35],[36]. Honey also contains oligosaccharides in small quantities. Shin & Ustunol (2005) relates the sugar composition of honey from different floral sources to the growth inhibition of various intestinal bacteria [37],[38]. Moreover, it is reported that a part of the antibacterial activity might be attributed to the components of plant origin [35]. All these physical and chemical factors give honey unique properties as a wound dressing. It has a rapid clearance of infections, rapid debridement of wounds, rapid suppression of inflammation, minimization of scarring and stimulation of angiogenesis as well as tissue granulation and epithelium growth [38], [39]

II. MATERIALS AND METHODS

A polysaccharide extracted from the leaf of Rhizosphere apiculate (RAP) assessed in cell culture systems, for its activity against human and simian immunodeficiency viruses. RAP inhibited HIV-1 or HIC-2 or SIV strains in various cell culture and assay systems. It blocked the expression of HIV-1 antigen in MT-4 cells and abolished the production of HIV-1 p24 antigen in peripheral blood mononuclear cells. The 50% effective concentration (EC50) of RAP in HIV-1 infected MT-4 cells and PBMC was 10.7 and 25.9 mg/ml, respectively. RAP (100mg/ml) completely blocked the binding of HIV-1 virions to MT-4 cells. RAP also reduced the production of viral mRNA when added before virus adsorption. RAP inhibited syncytium formation in co-culture of MOLT-4 cells and MOTL-4/HIV cells. RAP did not prolong activated partial thromboplastin time (APTT) up to 500 mg/ml. These properties may be advantageous should RAP be considered for further development.

III. OTHER EFFECTS OF HONEY

Positive effect of honey as an ant carcinogenic agent is reported in some studies [23],[24],[25] Honey has showed antineoplastic activity in the experimental bladder cancer [22]. Natural honey plays an important role in the treatment of chestpain, fatigue and vertigo. This is probably due to the highnutritional energy content of honey which provides immediateavailable calories after consumption [26]Benefits of honey also have been seen in tooth extraction pain and infection orcaries due to radiation-induced xerostomia [23], [24],[25]. It has been shown that honey is a very effective

agent for splitthickness skin graft fixations and can be easily used. [14] In asurvey that was performed in central Burkina Faso, it was found that local inhabitants use honey therapeutically for treatment of respiratory ailments, measles, period pains,postnatal disorders, male impotence and pharyngitis due to its antibacterial and anti-inflammatory effects [26]Honey has also been reported to exhibit anti-leishmanial effects *in vitro* [13]. In one study, daily consumption of honey showed a variety of beneficial effects on hematological indices, blood levels ofminerals and enzymes and endocrine system [27].In another study, oral honey stimulated antibody production duringprimary and secondary immune responses against thymus-dependent and thymus-independent antigens [13].

According to Guerrini *et al* (2009) stingless bee honey acts as a protective agent against DNA damage and could represent interesting evidence in relation to the determined antioxidant capacity [24]. Kilicoglu *et al*(2008) examined the effects of honey on oxidative stress and apoptosis in experimental obstructive jaundice and found that honey diminished the negative effects of bile duct ligation on the hepatic ultrastructure. This effect might be due to its antioxidant and anti-inflammatory activities [28].In another study, administration of honey investigated on *N*-ethylmaleimide (NEM-induced) liver injury in rats. NEM is a sulfhydryl blocker which impairs the sulfhydryl dependent antioxidant system (mainly glutathione) in the body. The findings imply that depletion of glutathione concentration plays a causal role in NEM-induced liver injury, and that the hepatoprotective effect of honey may be mediated through sulfhydryl-sensitive processes [22] In hepatocellular carcinoma honey can be considered as promising with inhibition of the proliferation, protease activity and gelatinase activity of HepG2 cells in an independent manner [30].

Zaid *et al* (2010) showed that honey had beneficial effects on menopausal rates by preventing uterine atrophy, increased bone density and suppression of increased body weight. They concluded that honey could be an alternative to hormone replacement therapy [31].

Honey is relatively free of adverse effects. Topical application of honey may lead to transient stinging sensation. Otherwise it is described in different forms as soothing, pain reliever and it has non-irritating effect and a painless dressing change. Allergy to honey is rare, but there could be an allergic reaction to either pollen or bee proteins in honey. Excessive application of honey may lead to dehydration of tissues which can however be restored by saline packs. Theoretical risk of rise in blood glucose levels

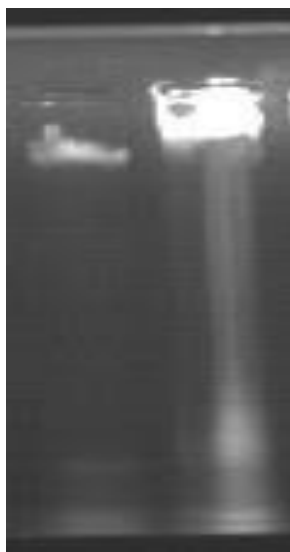
may always be there when applied to large open wound in diabetics. Risk of wound botulism, due to presence of spores of Clostridia can be minimized by gamma radiation which will kill the spores of clostridia without any loss of antibacterial activity [22]-[40].

IV. RESULTS AND DISCUSSION

Until Now, researchers pay more attention to medicines with natural origin and believe that natural products may be efficient therapeutics in comparison with the synthetic drugs. One of the most important natural products is honey, which has been used for different medicinal purposes since ancient times. In addition to the important role of honey in the traditional medicine, scientists also accept honey as a new effective medicine for many kinds of diseases. The most well-known effect of honey is antibacterial activity. Honey has also been reported to exhibit an inhibitory effect on yeast, fungi, leishmania and some viruses. Topical application of honey has been effectively used on mucocutaneous injuries such as genital lesions, superficial skin burns and post operation wounds. In addition, honey has been used in some gastrointestinal, cardiovascular, inflammatory and neoplastic states. The antioxidant capacity of honey which plays an important role in its useful effects, related to a wide range of compounds including phenolic, peptides, organic acids, enzymes, and Millard reaction products.

DNA fragmentation analysis

1 2

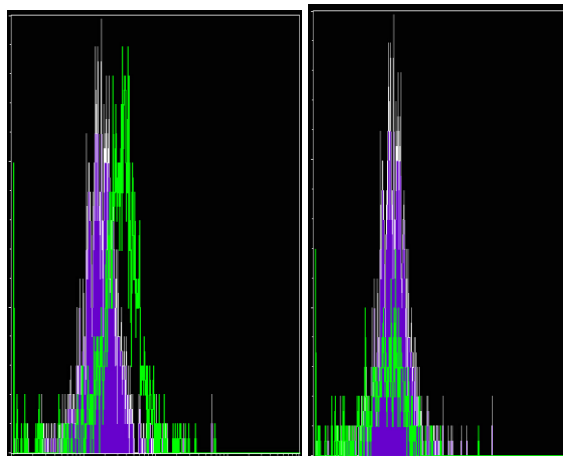


HL60 cells were treated with honey, and then the cellular DNA was extracted. Lane 1, cell control; 2, honey treated

FACS Analysis for Apoptosis Induction

A

B



HL60 cells treated with natural honey and then stained with FITC-Annexin V. Shaded histograms represent cellular fluorescence of the control cells and open histograms represent the cellular fluorescence resulting from the specific binding of FITC-Annexin V to apoptotic cells. A) cell control B) honey treated

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