Honey – Biological Activity - A Review

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Abstract

Honey, considered a functional food with a complex chemical composition, has been revered since ancient times for its nutritional and therapeutic properties. In addition to its high sugar content, honey contains a wide variety of bioactive components, especially phenolic acids and flavonoids, which gives it a high antioxidant and anti-inflammatory activity. In addition, results obtained by in vitro and in vivo testing of different types of honey have demonstrated the potential effects of this product in the prevention, progression and therapy of cancer. The antitumor effects of honey are generally attributed to different mechanisms, such as blocking the cell cycle, activating the mitochondrial pathway, permeabilizing the outer mitochondrial membrane, inducing apoptosis and, on the other hand, modulating oxidative stress, ameliorating inflammation and inhibiting angiogenesis. These aspects have demonstrated excellent preclinical potential in a variety of conditions and physiological systems, an aspect that can guide future research in thoroughly delving into the mechanisms of action of this proven "superfood" that can be optimized for the benefit of humanity.

Key words: honey, sanogenic properties, flavonoids, antioxidant activity, cancer

1. Introduction

Honey is produced by bees, by collecting the nectar of flowers and processing it through digestion and repeated regurgitation. The acidic pH of the bee stomach, together with invertase, diastase and enzymatic activities, give rise to a supersaturated aqueous product composed of 80% sugars, mainly fructose and glucose, with minor amounts of sucrose, maltose and other complex sugars. A multitude of substances such as water have been identified in the chemical composition of honey [1,2], proteins (enzymes), organic acids, vitamins, minerals, pigments, phenolic compounds and a variety of volatile compounds [3]. Nitrogen is present, for the most part, in the structure of amino acids and peptides, the most abundant amino acid in honey being represented by proline, followed by glutamic acid, alanine, phenylalanine, tyrosine, leucine, isoleucine and others. The honey produced by *Apis Mellifera* also contains small amounts of proteins, respectively 0.1-1.5%, the most abundant being defensin-1 and royal jelly protein, but also enzymes such as glucose-oxidase, diastase (amylase), α-glucooxidase, catalase and acid phosphatase. In addition, honey contains 0.57% organic acids, mainly gluconic acid, derived from glucooxidase activity, and citric acid, which gives it an average pH of 3.9, as well as small amounts of vitamins, especially the complex of B vitamins (especially vitamin B6,

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thiamine, niacin, riboflavin and panthenic acid) and ascorbic acid. At the same time, honey also contains a wide range of minerals (calcium, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), in percentages varying from 0.04% to 0.2%, reflecting the mineral content of the soils where the plants from which the nectar comes from grow. Of the total minerals, potassium represents about a third. The quality of honey depends on the type of flowers from which bees collect nectar [4-6], the climatic conditions in which plants grow and its processing and storage conditions [7]. Bee honey is a natural product, used not only as a nutritional product, but also as a medicine. The therapeutic potential of honey is due to its phytochemical, anti-inflammatory, antimicrobial, antioxidant, anti-allergic, hepatoprotective, antiproliferative, anticarcinogenic and antimetastatic properties. Responsible for the sanogenic properties of honey are compounds with an antioxidant role, such as phenolic acids, flavonoids, sugars, vitamins and enzymes, content in H_2O_2, minerals, especially copper and iron [8,9], but also a series of physical parameters such as pH, osmotic pressure, factors that can act singly or synergistically. The most important compounds of honey, with an antioxidant role, which confer its medicinal properties, are phenols and polyphenols. Despite their variability in the chemical composition of honey, the most abundant flavonoids are apigenin, quercetin, chrysin, luteolin, kaempferol, galangin, genistein, pinobanksin and pinocembrin, while the more abundant phenolic acids are represented by gallic acid, chlorogenic acid, syringic acid, p-coumaric acid, caffeic acid, vanillic acid and p-hydroxybenzoic acid [10]. Currently, these compounds are of particular interest for medical and food research, mainly due to their functional properties. Besides being considered to be strong agents of peroxide radicals, mainly due to the presence of high mobility of hydrogens in their molecular structures [11], polyphenols could be effective immune modulators, but also inhibitors of hormone action. Therefore, the quality of honey and its therapeutic properties depend on its chemical composition which, in turn, is dependent on the geographical area, the floristic composition, the season, the environmental factors but also the beekeepers' practices (processing, handling, storage).

2. Antioxidant and anti-inflammatory properties of honey

Oxidative stress is defined as an imbalance between the protective activity of antioxidants and the activity of free radicals [12]. In cellular systems, free radicals and reactive oxygen species (ROS) are physiologically produced in various cellular biochemical reactions, such as aerobic energy production in mitochondria [13], fatty acid metabolism [14], drug metabolism [15] and during immune system activity [16], but also under the action of some exogenous factors such as pollution, UV rays, ionizing radiation, unhealthy lifestyle habits, being major oxidizing agents, involved in aging and the occurrence of many types of diseases [17]. Antioxidants are molecules capable of donating an electron to free radicals, with the effect of neutralizing, reducing, or eliminating their ability to damage cells and major biomolecules, such as nucleic acids, proteins, and lipids [18]. To reduce these negative effects of free radicals, cellular systems have defense mechanisms that consist in the synthesis of endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPX), alpha lipoic acid (ALA), coenzyme Q10, catalase, vitamins and others. In addition, for greater effectiveness, they also resort to a series of exogenous antioxidants (e.g. vitamin C and E, selenium, zinc, copper, manganese, β-carotene, phenolic compounds, etc.), taken from the fruit-rich diet, vegetables, but also other sources from the plant and animal kingdom [19]. Due to the different enzymatic and non-enzymatic antioxidant compounds in its composition, honey is considered a nutritional supplement, which can be used against oxidative damage. Responsible for the antioxidant capacity of honey are mainly phenolic compounds [20,21], there is a direct relationship between the concentration of total phenols in honey and its antioxidant properties. Numerous researches have demonstrated the existence of a correlation between the darker shade of honey color and a higher content in total phenols and, implicitly, a higher antioxidant capacity [22]. By scavenging free radicals and protecting against lipid peroxidation, honey can reduce and prevent various clinical conditions, such as chronic diseases, inflammatory disorders, and cancer [23,24]. In target tissues, the production of ROS is responsible for the induction

of inflammation and the release of pro-inflammatory cytokines. Honey modulates oxidative stress, inflammation and apoptosis by down-regulating of the expression of NF-kB, NRLP-3, p65, p38, ERK and JNKand also up-regulating . of Nrf2 and AMPK expression. The antioxidant capacity of polyphenols is determined by: the redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen saturation [25]; the possibility to form chelated compounds with metals [26], to react with free radicals and genotoxic or carcinogenic substances and is considered to be much more greater than essential vitamins, contributing significantly to the sanogenic effects manifested by the natural products in which they exist. Although the antioxidant capacity of honey (AOC) is mainly given by phenolic compounds, other products such as enzymes, amino acids and carotenoids also play an important role, the amount of these substances in honey being dependent on the geographical area and the climatic condition. For example, the flavonoid kaempferol can be found in rosemary honey and quercetin in sunflower honey. The total concentration of phenols in bee products depends a lot on the floral source, the place where nectar and pollen are collected. It has been established that the consumption of natural products, with a high content of antioxidants of phenolic origin, is associated with a reduced risk of cardiovascular diseases and some chronic diseases [27]. Alvarez-Suarez et al. [2012] determined the role of monofloral honey phenolics on human red blood cell membranes against oxidative damage. The results show that honey suppresses the oxidative damage of erythrocytes the most, probably due to its assimilation into the cell membrane and the ability to enter and reach the cytosol. Honey contains adequate antioxidants that are responsible for the biological activity, defense and increased functions of red blood cells [28]. A number of other compounds have been found in varying concentrations in various types of honey. Thus, gallic acid, an anti-inflammatory compound, and its derivatives, are present in a variety of products with diverse biological and pharmacological activities, including scavenging radicals, interfering with cell signaling pathways, and cancer cell apoptosis [29]. Protocatechuic acid is considered to be an active component of some traditional Chinese herbal medicines. It has been found to possess various pharmacological effects that may be closely related to its antioxidant activities. Similarly, 2,4-dihydroxybenzoic acid is an antioxidant compound [30]. Catechin, a bio-flavonoid, is known as a free radical scavenger, reported as a component of green tea, as an antitumor agent and as an insect repellent. The antioxidant activity of phenolic compounds can represent an alternative for maintaining and restoring the balance of the intestinal microbiota, since these compounds can stimulate the secretion of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR) that block ROS or stimulates the endogenous defense system [31,32]. Numerous studies [33,34] showed a high correlation between the total amount of polyphenols (TPC), respectively the total amount of flavonoids (TFC) and the antioxidant capacity of honey [35] The antioxidant capacity of honey is also influenced by the type of treatments applied for preservation. Akhmazillah, F.N., et al., [2014] reported that high pressure processing of Manuka honey results in a significant increase in antioxidant capacity compared to unprocessed honey. Also, thermal treatment, by heating to 45°C and 55°C, causes a linear increase in antioxidant capacity, but also a high correlation between TPC and antioxidant activity [36].

3. Anticancer activity of honey

Numerous studies have highlighted the effectiveness of honey and associated bioactive constituents as antitumor agents against some types of cancer. Currently, cancer is the second leading cause of death worldwide, causing approximately 10 million deaths annually. According to the International Agency for Research on Cancer, 19.3 million new cases were estimated in 2020 [37]. Cancer is a process that starts from a single transformed cell and then proceeds in several stages. The pathogenesis of cancer is characterized by a change in the capacities of cell proliferation, invasion and metastasis. This process is controlled by various transcription factors, protein kinases, cell cycle proteins, pro-apoptotic and anti-apoptotic proteins and other target molecules [38]. Currently, a series of effective drugs are used in the treatment of cancer, but they induce toxicity [39], an aspect that has led, in recent years, to the need to find

alternative sources, with an emphasis on the use of natural products. Studies carried out on tumor cell lines have demonstrated the role of honey both in reducing the tumorigenicity of some types of cancer, such as colorectal cancer [40,41], pulmonary [40,41], breast [42,43], cervix [44], renal [45], skin [46], prostate [47], as well as in improving the effect chemotherapeutic drugs, such as 5-fluorouracil and paclitaxel [40,46]. The antitumor effects of honey have been attributed to its role in inducing apoptosis [43,46,48], modulation of oxidative stress [40,46,49] and its anti-proliferative properties [40,43,46] and anti-metastatic [40, 43]. In addition, several studies have demonstrated that honey has the ability to modulate the immune system by inducing an anti-inflammatory effect against cancer [40, 45]. Polyphenols identified in the composition of honey, such as chrysin, galanginin, caffeic acid, caffeic acid phenyl ester, quercetin, acacetin, kaempferol, pinocembrin, pinobanksin and apigenin, may be promising pharmacological agents in the treatment of cancer, through their anti-proliferative and molecular mechanisms [10]. Research by Galijatovic et al., [2001] showed the effect of chrysin in preventing cancer, in a similar manner to anastrozole, a drug used to treat breast cancer. The mechanisms by which honey can exert its antiproliferative, antimetastatic and anticancer effects include, on the one hand, cell cycle arrest, activation of the mitochondrial pathway, permeabilization of the outer mitochondrial membrane, induction of apoptosis and, on the other hand, modulation of oxidative stress, amelioration of inflammation and inhibition angiogenesis [50]. Pichichero et al., [2010] demonstrated that both honey and its constituent, chrysin, have antiproliferative effects on human and murine melanoma cells by arresting the cell cycle in the G0/G1 phase [51]. Also, quercetin and kaempferol can stop the cell cycle in different phases such as G0/G1, G1 but also G2/M in human melanoma, renal, cervical and esophageal adenocarcinoma [52] [Figure 1].

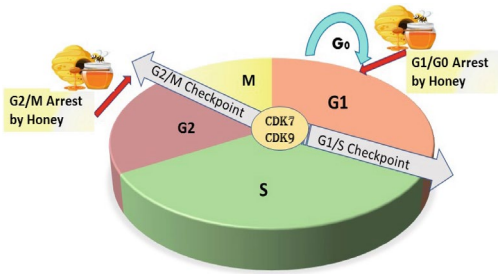

Figure 1 Schematic representation of cell cycle arrest in G0/G1 and G2/M, under the action of honey [https://link.springer.com/chapter/10.1007%2F978-981-15-7305-7_16]

The mitochondrial pathway involves a series of interactions between multiple stimuli, including nutrients, physical stress, oxidative stress, and injury, during which proteins such as cytochrome c can be released into the mitochondrial intermembrane space (IMS), leading to cell death [53]. Therefore, compounds such as flavonoids in honey are able to activate the mitochondrial pathway and thus can be considered as potential agents with a cytotoxic effect [54, 55]. Various apoptotic stimuli can induce a decrease in the mitochondrial membrane potential (MMP) which leads to the release from the external mitochondrial compartment of some pro-apoptotic or apoptogenic proteins (e.g. Cytochrome c – Cyto-C) to activate the mitochondria-dependent apoptotic pathway (intrinsic pathway) [56]. The integrity of the mitochondrial membrane potential (MMP) is regulated by the bcl-2 family of proteins, composed of pro-apoptotic (e.g. bax, bad, bak, bim and bid) and anti-apoptotic (e.g. bcl-2 and bcl-xL) members [57]. Activation of such apoptotic signaling pathways as p53-PUMA and death receptor pathways can disrupt the balance in the bcl-2 protein family in favor of pro-apoptotic proteins (eg bak, bax) leading to increased mitochondrial membrane permeability. As a result, cytochrome c, located in the mitochondria, will be released into the cytosol, where it will subsequently bind to the apoptotic protease activating factor 1 (Apaf-1) (Apoptotic peptidase activating factor - 1), resulting in a conformational change leading to oligomerization of Apaf-1, in the presence of cytosolic dATP (deoxyadenosine triphosphate) or ATP. Together with cytosolic procaspase-9, dATP and cytochrome c, oligomerized Apaf-1 can form a massive complex called apoptosome, with a role in the activation of

caspase 9 [58,59]. The initiator caspase 9 activates, in turn, through the proteolytic activation of pro-caspases 3 and 7, the effector caspases 3 and 7, whose substrates are represented by poly (ADP-ribose) polymerase (PARP), lamin and fodrin, resulting in DNA fragmentation and the formation of apoptotic bodies [60] [Figure 2].

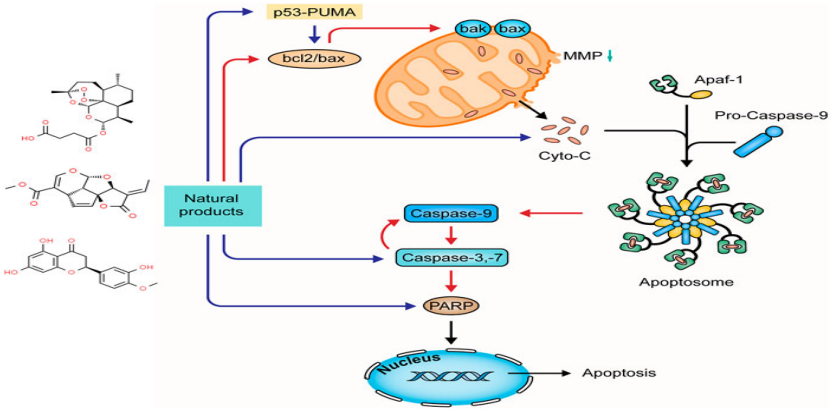

Figure 2. How natural products modulate apoptosis in intestinal epithelial cells, via the mitochondrial pathway [61]

Fauzi, A.N., et al., [2011] showed that honey treatment causes apoptosis of breast cancer cells by inducing the activation of caspases-3/7 and -9 [62] Also, recent studies have reported the adjuvant effect of honey in tamoxifen-induced apoptosis, by activating caspases -3/7, -8 and -9 [63]. In addition, the role of honey in inhibiting the activity of PARP, the enzyme involved in apoptosis and DNA repair, has been demonstrated [64]. Thus, inhibiting PARP activity [Figure 3] prevents DNA repair and therefore increases the cytotoxicity of honey in cancer cells. Through this study, it was demonstrated that the use of honey in cancer therapies induces the process of apoptosis by activating the caspase-3 pathway and by expressing the p53 and Bax proteins. Similar effects, inducing apoptosis, were obtained by using honey in breast cancer, murine melanoma but also on colorectal carcinoma cells [46], the apoptotic effect being mediated by the activation of caspase-9, - 3 and decreasing the expression of the antiapoptotic protein Bcl-2 [46]. Duo, J. et al., [2012] also report the inhibitory effect of quercetin, another honey compound, on pancreatic and breast cancer cells by upregulating the expression of the proapoptotic protein Bax and downregulating the expression of the antiapoptotic protein Bcl-2 [65].

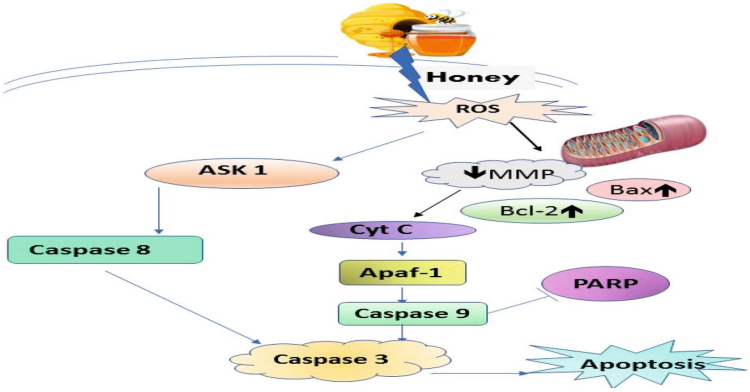

Figure 3. Schematic representation of the mechanism of apoptosis induction by the use of honey [https://www.hindawi.com/journals/ecam/2013/829070/fig1/]

In addition, recent research [66] reveals that chrysin, a major component of honey, exerts an antimetastatic effect in human breast cancer cells and induces apoptosis by activating caspase-3 and of Bax protein in melanoma cells [67]. All these data indicate the role of polyphenols in the composition of honey in the death by apoptosis of cancer cells.

The antitumor effect of honey, mediated by the increase in oxidative stress at the cellular level, associated with the increase in the generation of ROS. In vitro studies on HCT-15 and HT-29 colon carcinoma cells [68], showed that the anticancer effect of honey is mediated by increasing oxidative stress at the cellular level, associated with increased ROS generation. Honey-induced cell death was accompanied by DNA fragmentation, which was subsequently strongly inhibited by treatment with the antioxidant, N-acetyl-L-cysteine (NAC). The obtained results indicate the role of honey in inhibiting the development of cancer by modulating oxidative stress, respectively by the antioxidant or prooxidant mechanism, depending on the state of oxidative stress in cancer cells. In both cases, the pro-oxidant and antioxidant effects of honey lead to the death of cancer cells. The dual effect of honey on cancer cells is due to its rich phenolic content [69]. Thus, phenolic compounds can interact with transition metals, such as copper, leading to the release of ROS and, implicitly, to DNA damage and cell death. Some studies [70] showed that treating HIT-T15 cells with honey had the effect of reducing the expression of MAPK and NF-kB, and chrysin, a compound also found in honey, induced apoptosis in cells of melanoma B16-F1 and A375 [67] by modulating MAPK kinase. In addition, chrysin induced TNF (TRAIL)-mediated apoptosis in cancer cell lines [71]. The potential ***anti-angiogenic effect*** of honey in cancer cells was studied in vitro by Abdel Aziz et al., [2009] on HePG2 hepatocellular carcinoma cells [72]. Caffeic acid phenyl ester (CAPE), chrysin and other cytotoxic constituents in the composition of honey have been reported to have anti-angiogenic and anti-cancer effects [73]. Therefore, these data indicate that honey may exert anticancer effect by inhibiting angiogenesis.

The antitumor effect of honey, due to its immunomodulatory property, by inducing the release of proinflammatory cytokines by myeloid cells

Numerous studies have demonstrated the ability of honey to significantly reduce the release of reactive oxygen species (ROS) in tumor cells, a process accompanied by the improvement of the production of pro-inflammatory cytokines (eg TNF-α, IL-1β, IL-6) by macrophages. One possible mechanism of this immunomodulatory effect would be due to the increased levels of hydrogen peroxide in honey, which leads to a negative feedback on the release of ROS by tumor cells [74, 75]. The results of other studies have shown that manuka honey has an anti-inflammatory effect by inhibiting the effect of lipopolysaccharide (LPS) on macrophages [76], inhibiting the production of TNF-α as well as reducing the release of superoxide by neutrophils [77-79]. Ahmed S., et al., 2017, show that manuka honey determined the reduction of breast tumors, a process accompanied by an increase in the serum level of the cytokine IFN-γ, thus demonstrating its antitumor immunomodulatory effec[79].

Similarly, thyme honey has been shown to stimulate the activation of nuclear factor kappa B (NFκB) and AP-1 transcription factors in murine RAW 264.7 macrophages, leading to the production of PGE2, TNF-α and IL-6 [80].

4. Conclusions

In this review we presented recent data on the antioxidant, anti-inflammatory and anticancer activity, as well as the molecular mechanisms of these therapeutic properties of honey. The sanogenic properties of honey are conferred both by its chemical composition, especially phenols and polyphenols, but also by a series of physical parameters such as pH and osmotic pressure.

The antioxidant activity of phenolic compounds modulates oxidative stress by eliminating free radicals and protecting against lipid peroxidation, by stimulating the secretion of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), glutathione reductase (GR) and the endogenous defense system . The antitumor activity of honey is achieved either by stopping the cell cycle in its various phases, such as G0/G1, G1 but also G2/M, by activating the mitochondrial pathway of apoptosis, which involves the permeabilization of the outer mitochondrial membrane, stimulating the activation of cytochrome c, caspases 3/7 and -9, as well as

inhibition of PARP activity. The antitumor effect of honey is mediated by reducing the expression of MAPK and NF-kB at the cellular level. At the tumor level, the immunomodulatory properties of honey are manifested by reducing the production of ROS, inducing the release of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 by myeloid cells, but also by inhibiting the effect of lipopolysaccharide (LPS) on macrophages. In addition, it has been demonstrated that a series of bioactive components of honey intervene in tumor reduction by inhibiting the angiogenesis process.

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