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Honey as a Source of Dietary Antioxidants: Structures, Bioavailability and Evidence of Protective Effects Against Human Chronic Diseases

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Abstract: In the long human tradition honey has been used not only as a nutrient but also as a medicine. Its composition is rather variable and depends on the floral source and on external factors, such as seasonal, environmental conditions and processing. In this review, specific attention is focused on absorption, metabolism, and beneficial biological activities of honey compounds in human. Honey is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%), containing also minerals, proteins, free amino acids, enzymes, vitamins and polyphenols. Among polyphenols, flavonoids are the most abundant and are closely related to its biological functions. Honey positively affects risk factors for cardiovascular diseases by inhibiting inflammation, improving endothelial function, as well as the plasma lipid profile, and increasing low-density lipoprotein resistance to oxidation. Honey also displays an important antitumoral capacity, where polyphenols again are considered responsible for its complementary and overlapping mechanisms of chemopreventive activity in multistage carcinogenesis, by inhibiting mutagenesis or inducing apoptosis. Moreover, honey positively modulates the glycemic response by reducing blood glucose, serum fructosamine or glycosylated hemoglobin concentrations and exerts antibacterial properties caused by its consistent amount of hydrogen peroxide and non-peroxide factors as flavonoids, methylglyoxal and defensin-1 peptide. In conclusion, the evidence of the biological actions of honey can be ascribed to its polyphenolic contents which, in turn, are usually associated to its antioxidant and anti-inflammatory actions, as well as to its cardiovascular, antiproliferative and antimicrobial benefits.

Keywords: Antioxidant capacity, antimicrobial action, bioavailability, cancer, cardiovascular disease, honey.

INTRODUCTION

It is known that oxidative stress, caused by an imbalance between the production of highly reactive molecules and antioxidant defences, causes structural and functional damage to lipids, proteins and nucleic acids leading to many biological complications including carcinogenesis, aging and atherosclerosis [1, 2]. Therefore, exogenous antioxidants from diet can counteract the deleterious effects of free radicals, reducing oxidative damage [3]. Many epidemiological studies show in fact that a diet rich in polyphenols is often associated with a lower incidence of several chronic pathologies, such as obesity, infections, cardiovascular and neurologic diseases, and cancer [4-6]. In the long human tradition, honey has been used not only as a sugar but also as a medicine: it has been employed in many cultures for its medicinal properties, including as a remedy for burns, cataracts, ulcers and wound healing [7, 8]. Only recently, scientific research focused its attention on the therapeutic effects of honey, in particular on its capacity to protect against cardiovascular diseases [9, 10], cancer [11-14] and microbial infections [15, 16]. These health-protective and therapeutic impacts of honey depend on the presence of various antioxidant components, especially phenolic compounds, such as flavonoids and phenolic acids, most of which express relevant antimicrobial, antioxidant, anti-inflammatory, antimutagenic activities capacities both *in vitro* and *in vivo* [17]. Besides phenolic compounds and sugars, honey is a source of proteins, free amino acids, minerals, enzymes, and vitamins, representing therefore a good healthy choice.

This review focuses on the nutrient and phytochemical contents of honey and on its antioxidant capacity. An overview on the bioavailability and metabolism of the most abundant honey phytochemicals after consumption is also presented, and the currently hypothesized health benefits related to honey consumption is reviewed, with particular attention given to recent evidence on its impact on cardiovascular health, cancer prevention, hyperglycemias regulation and antimicrobial activity.

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1. COMPOSITION

The composition of honey is rather variable, depending on the floral source and other external factors, such as seasonal and environmental conditions and processing. Honey contains a variety of approximately 180 compounds, such as sugars, proteins, free amino acids, essential minerals, vitamins and enzymes as well as a wide range of polyphenolic phytochemicals. This range of compounds will be discussed below, with specific focus on the most significant components with beneficial effect on human health, essentially flavonoids and phenolic acids.

1.1. Nutrients

Concerning its nutrient profile (Table **1**), honey represents an interesting source of natural macro and micronutrients. First of all, it is an important source of calories, since 100 g of honey provide approximately 300 kcal and a daily dose of 20 g covers about 3% of the recommended daily intake of energy (RDI)[7]. Carbohydrates represent 95% of its dry weight: approximately a total of 26 sugars (mono- and disaccharides) have been identified in honey (Table **2**) [18] with fructose (~ 40%) and glucose (~ 30%) as major sugars. It is important to note that most of these sugars do not occur in nectar, because they are the results of enzymes added by the honeybee during the ripening of honey or by chemical action in the concentrated form. To a lesser extent, honey contains proteins (roughly 0.5%), mainly enzymes and free amino acids. The amount of nitrogen in honey is low, approximately 0.04%, though it may reach 0.1% with 40 to 65% as protein form and the rest as free amino acids. The total of free amino acids in honey corresponds approximately to 1% (w/w) of total nitrogen and ranges between 10 and 200 mg/100 g, according to its origin (nectar, honeydew or floral origin), with proline as the major contributor, corresponding to approximately 50% of total free amino acids [19]. Since pollen is the main source of honey amino acids, their profile could be characteristic and indicative of their botanical origin [20]. In addition to classical amino acids also b-alanine (b-Ala), a-alanine (a-Ala), g-aminobutyric acid (Gaba) and ornithine (Orn) have been found and identified in honey [19-22].

Honey also presents a variable number of mineral elements, which varies according to geographic region, soil type and floral

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Table 1. Chemical Composition of Honey*.

Proximates and carbohydrates		Mineral content		Vitamins content	
Water (g)	17.1	Calcium (mg)	4.4-9.20	Ascorbic Acid (C) (mg)	2.2 -2.4
Energy (kcal)	304	Potassium (mg)	13.2-16.8	Thiamin (mg)	< 0.006
Carbohydrates (total) (g)	82.4	Copper (mg)	0.003-0.10	Riboflavin (mg)	< 0.06
....Fructose (g)	38.5	Iron (mg)	0.06-1.5	Niacin (mg)	< 0.36
....Glucose (g)	31.0	Magnesium (mg)	1.2-3.50	Pantothenic acid (mg)	< 0.11
....Maltose (g)	7.20	Manganese (mg)	0.02-0.4	Pyridoxine (B6) (mg)	< 0.32 mg
....Sucrose (g)	1.50	Phosphorus (mg)	1.9-6.30		
Proteins, amino acids, vitamins and minerals (g)	0.50	Sodium (mg)	0.0-7.60		
		Zinc (mg)	0.03-0.4		
		Se (µg)	1.0-2.91		

*Amount in 100 g of honey

Table 2. Carbohydrates Composition in Honey[18].

Trivial nomenclature	Systematic nomenclature
Disaccharide	
Cellobiose [2]	O-β-D-glucopyranosyl-(1 -> 4)-D-glucopyranose
Gentiobiose [2]	O-β-D-glucopyranosyl-(1 -> 6)-D-glucopyranose
Isomaltose [2]	O-α-D-glucopyranosyl-(1 -> 6)-D-glucopyranose
Isomaltulose [4]	O-α-D-glucopyranosyl-(1 -> 6)-D-frutofuranose
Kojibiose [1]	O-α-D-glucopyranosyl-(1 -> 2)-D-glucopyranose
Laminaribiose [3]	O-β-D-glucopyranosyl-(1 -> 3)-D-glucopyranose
Leucrose [4]	O-α-D-glucopyranosyl-(1 -> 5)-D-fructofuranose
Maltose [1]	O-α-D-glucopyranosyl-(1 -> 4)-D-glucopyranose
Maltulose [2]	O-α-D-glucopyranosyl-(1 -> 4)-D-fructose
Melibiose [4]	O-α-D-galactopyranosyl-(1 -> 6)-D-glucopyranose
Neo-trehalose [3]	O-α-D-glucopyranosyl-β-D-glucopyranoside
Nigerose [2]	O-α-D-glucopyranosyl-(1 -> 3)-D-glucopyranose
Palatinose [2]	O-α-D-glucopyranosyl-(1 -> 6)-D-fructose
Saccharose [1]	O-α-D-glucopyranosyl-β-D-frutocfuranoside
Turanose [1]	O-α-D-glucopyranosyl-(1 -> 3)-D-fructose
Trisaccharide	
kestose [4]	O-α-D-glucopyranosyl-(1 -> 4)-O-α-D-glucopyranosyl-(1 -> 2)-D-glucopyranose
1-kestose [4]	O-α-D-glucopyranosyl-(1 -> 2)-β-D-fructofuranosyl-(1 -> 2)-β-D-fructofuranoside
Erlose [1]	O-α-D-glucopyranosyl-(1 -> 4)-α-D-glucopyranosyl-β-D-fructofuranoside
Isomaltotriose [2]	O-α-D-glucopyranosyl-(1 -> 6)-O-α-D-glucopyranosyl-(1 -> 6)-D-glucopyranose
Isopanose [2]	O-α-D-glucopyranosyl-(1 -> 4)-O-α-D-glucopyranosyl-(1 -> 6)-D-glucopyranose
Laminaritriose [4]	O-β-D-glucopyranosyl-(1 -> 3)-O-β-D-glucopyranosyl-(1 -> 3)-D-glucopyranose
Maltotriose [2]	O-α-D-glucopyranosyl-(1 -> 4)-O-α-D-glucopyranosyl-(1 -> 4)-D-glucopyranose
Melezitose [2]	O-α-D-glucopyranosyl-(1 -> 3)-O-β-D-fructofuranosyl-(2 -> 1)-α-D-glucopyranoside
Panose [2]	O-α-D-glucopyranosyl-(1 -> 6)-O-α-D-glucopyranosyl-(1 -> 4)-D-glucopyranose
Rafinose [2]	O-α-D-galactopyranosyl-(1 -> 6)-O-α-D-glucopyranosyl-β-D-fructofuranoside
Teanderose [2]	O-α-D-glucopyranosyl-(1 -> 6)-α-D-glucopyranosyl-β-D-fructofuranoside

1- Majority 2- Minority 3- Traces 4- not confirmed

origin, representing approximately 0.2% of its dry weight. The amount of minerals and trace elements in honey is small and their contribution to the RDI is marginal (Table **1**). Minerals and trace elements play a key role in biomedical activities associated with food, since these elements have a multitude of known and unknown biological functions, such as the maintenance of intracellular oxidative balance [23, 24]. Recently, the presence of Se content in Portuguese unifloral honeys was reported [25]. Se is an essential trace element, and its role in the metabolism is largely related to its incorporation into selenoproteins [26]. As an essential component of

antioxidant enzymes GSH-Px and thioredoxin reductase, this element may promote endogenous enzymatic capacity to protect against excessive generation of free radicals [27]. Honey also contains choline (0.3-25 mg/kg) and acetylcholine (0.06-5 mg/kg). Choline is essential for cardiovascular and brain function as well as for cellular membrane composition and repair, while acetylcholine acts as a neurotransmitter [7].

Finally, the vitamin content in honey is low. Vitamins such as thiamin (B1), riboflavin (B2), pyridoxin (B6) and niacin have been reported in honey but in general their amount is small and the corresponding contribution of honey to the RDI is very limited [7, 8].

1.2. Enzyme and Organic Acids

One of the characteristics that distinguish honey from all other sweetening agents is the presence of enzymes. They may originate from the bee, pollen, nectar, even from yeasts or micro-organisms present in honey. Three main enzymes can be found: invertase, diastase and glucose oxidase. Invertase splits sucrose releasing its simple constituents; during this action, other groups of more complex sugars have been found in small amounts, explaining in part the complexity and variability of the minor sugars of honey. Invertase remains in honey and retains its activity for some time, completing its activity when honey is ripened. Despite this, the sucrose content of honey never becomes zero. Since invertase also synthesizes sucrose, the final low value for the sucrose content of honey probably represents an equilibrium between splitting and forming sucrose, an aspect which is often taken into account when measuring the maturity and quality of honey [28]. Diastase or amylase digests starch to simpler compounds. The origin of this enzyme in honey is controversial and it is not known for sure if it comes from nectar, pollen or bee, or what its functions are because starch is not present in honey. Alpha-amylase randomly breaks the down starch chains, producing dextrins and beta-amylase which divides the reducing sugar maltose from the terminal starch chains. Diastase activity is used as an important indicator of honey quality: the higher the content of this enzyme, the higher is the quality of honey [28]. Despite its discrete contribution to the human diet, the supplementation of diastase by honey can be interesting and helpful in increasing the metabolism of sugars, especially related with carbohydrate digestive disorders. Finally, glucose oxidase (GOx) is of interest because it is related to honey antibacterial properties. GOx converts dextrose to a related compound, a gluconolactone, which in turn forms gluconic acid, the principal acid in honey, and hydrogen peroxide the main agent responsible for antibacterial activity in most honeys. GOx amount varies in different honeys and since it was found in the pharyngeal gland of the honey bee this is probably the most likely source of this enzyme [28, 29]. Other enzymes reported in honey are catalase and acid phosphatase. It is important to note that honey enzymes can be destroyed or weakened by heat caused by careless handling during industrial processing or storage.

Finally, honey contains also a series of organic acids corresponding to 0.17-1.17% of the total acids, representing less than 0.5% of solids [28] which contribute to the flavor and in part are responsible for its excellent stability against microorganisms and are also associated with honey antibacterial activity [30]. Among these acids, gluconic acid has been identified as the most important one. Other organic acids in honey are formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, a-ketoglutaric, glycollic, citric, malic, 2- or 3-phosphoglyceric acid, α- or β-glycerophosphate, and glucose 6-phosphate.

1.3. Phenolic Phytochemicals in Honey

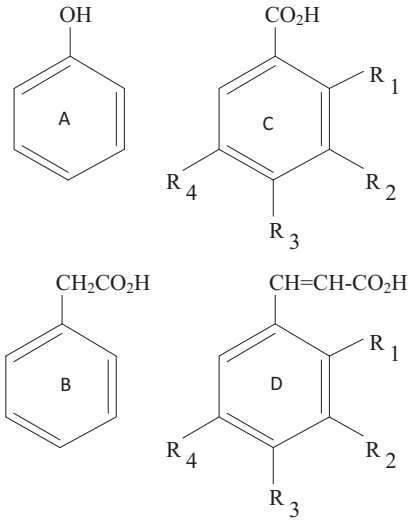
Honey phytochemicals are mainly represented by the extensive class of phenolic compounds depending on honey origin [31-36] and, therefore, expected to have different biological activities and huge biological potentialities in humans [17]. The major class of phenolic compounds in honey is represented by flavonoids (flavonols, flavanols and flavones) followed by phenolic acids (benzoic acids, phenylacetic and hydroxycinnamic acids). The most common phenolics acids and flavonoids identified in honey are shown in (Table **3**).

Table 3. Most Common Phenolic Acid and Flavonoids Identified in Honey.

Phenolic acid	Flavonoids
4- dimethylaminobenzoic acid	Apigenin
Caffeic acid	Genistein
p-coumaric acid	Pinocembrin
Gallic acid	Tricetin
Vallinic acid	Chrysin
Syringic acid	Luteolin
Chlorogenic acid	Quercetin
	Quercetin 3-methyl ether
	Quercetin-diglycoside
	Quercetin-3-O-rutinoside
	Quercetin-O-rhamnoside
	Kaempferol
	Kaempferol 8-OMe
	Kaempferol 3-OMe
	Kaempferol-7-O-rhamnoside
	Kaempferol-3-O-glycosyl
	Kaempferol-7-O-glycosyl
	Galangin
	Pinobanksin
	Myricetin
	Myricetin 3-OMe
	Myricetin 3,7,4',5'-OMe

Phenolic phytochemicals are the largest group of phytochemicals ubiquitous in plants and are incorporated into the honey via nectar / pollen from plants visited by the honeybee. Simple phenols are those with a C_6 carbon structure (such as phenol itself, cresol and thymol) (Fig. **1A**). Phenolic acids are derived from benzoic acid, phenylacetic and hydroxycinnamic acid (Fig. **1B**, **1C** and **1D**, respectively) where the hydroxyl (OH) groups can be substituted in the aromatic ring. Some of them have a C_6-C_1 structure (*e.g.* gallic, vanillic and syringic acids) and aldehydes (*e.g.* vanillin). Others have a C_6-C_2 structure, such as phenylacetic acids and acetophenones. Phenylpropanoid derivatives of a C_6-C_3 structure are mainly represented by hydroxycinnamic acids such as *p*-coumaric, ferulic and caffeic acids and their respective derivatives [37, 38]. Simple phenols, phenolic and phenylacetic acids can be found free and have been identified in several floral honeys,the most frequent ones being*p*-coumaric, ferulic and caffeic acids [31-36, 39]. In honey of *Leptospermum scoparium* and *Leptospermum polygalifolium* from New Zealand and *Eucalyptus ssp*. from Australia, gallic acid was identified as the most predominant phenolic acid [40, 41]. Other phenolic acids havealso been reported: chlorogenic, syringic, vanillic and *p*-hydroxybenzoic acids as the agents responsible for the antioxidant activity exhibited by the extracts of honey from different botanical origins (*Leptospermum polygalifolium, Epilobium angustifolium; Nyssa aquatica; Schinus terebinthifolius; Glycine max; Melilotus spp y Robinia pseudoacacia*) [39, 40].

Flavonoids are low molecular weight compounds that share a common skeleton of diphenyl propanes, formed by two benzene



in **C**:

i) R_1-H,R_2,R_3,R_4-OH→ gallic

ii) R_1,R_4-H,R_3-OH,R_2-OCH_3→ Vanillic

iii) R_1-H,R_3-OH,R_2,R_4-OCH_3 → Syringic

iv) R_1,R_2,R_4-H,R_3-OH→ *p*-hydroxybenzoic

in **D**:

i) R_2,R_3,R_4-H,R_1-OH→ *o*- coumaric

ii) R_1,R_3,R_4-H,R_2-OH→ *m*- coumaric

iii) R_1,R_2,R_4-H,R_3-OH → *p*- coumaric

iv) R_1,R_4-H, R_2,R_3-OH→ caffeic

v) R_1,R_4-H,R_2-OCH_3,R_3-OH→ ferulic

Fig. (1). Chemical structures of simple phenols with structure C_6 (**A**), phenylacetic acid, C_6-C_2 (**B**), benzoic acids, C_6-C_1 (**C**), and hydroxycinnamic acids, C_6-C_3 (**D**). The different structures of benzoic acids and hydroxycinnamic acids are shown in C and D, respectively.

rings joined by a linear three-carbon chain (C_6-C_3-C_6). Often, the carbons of the propane connecting the phenyl rings may form a closed pyran ring together with one of the benzene rings thus forming a structure of 15 carbon atoms arranged in three rings, labeled A, B and C (Fig. **2A**). These compounds generally have at least three phenolic OH groups and are generally combined with sugars to form glycosides, with glucose as the major sugar, but also galactose, rhamnose and xylose can be found, as free aglycon. At the same time, flavonoids are further divided and classified according to the degree of oxidation of the C ring into flavones, flavonols, flavanones, flavanonols, flavanols or catechins, isoflavones, anthocyanins and anthocyanidins. Within these groups, flavonols (Fig. **2B**) (*e.g.* quercetin, myricetin and kaempferol), flavones (Fig. **2C**) (apigenin, luteolin, diosmetin, chrysin) and flavanols or catechins (Fig. **2D**) (catechin, epicatechin, epigallocatechin, epigallocatechin gallate) are the most abundant in honey. Flavonols are characterized by an unsaturation between the C_2 and C_3 carbons of the C ring, a ketone group in C_4 and by the presence of a hydroxyl group in position 3 of the ring, while flavones do not exhibit this latter group. Flavanols present a hydroxyl group on carbon 3 [38, 42]. These flavonoids may appear in the *O*-glycosylated form, in which one or more hydroxyls are linked to a sugar, thus forming a *O*-C bond, with glucose as the most common glycosidic unit, even if other examples include glucorhamnose, galactose, arabinose, rutinoside and rhamnose. Glycosylation can also occur froma direct link between the sugar and the flavonoid nucleus, forming a strong bond *C*-C with the formation of *C*-glycosides. This type of glycosylation occurs only at positions C_6 and/or C_8. The objective of glycosylation seems to form a flavonoid less reactive and more soluble in water; the glycosylation can be seen as an essential form of protection of plants to avoid the cytoplasmic damage since flavonoids can accumulate in vacuoles [43]. Most flavonols are in the shape of *O*-glycosides and rarely of *C*-glycosides, while the flavones are often found in nature, both as *O*-glycosylated and as *C*- glycosylated [44].

2. BIOAVAILABILITY AND METABOLISM OF HONEY POLYPHENOLS

Because evidence of the potential health-promoting and disease-preventing effects of honey continues to accumulate, it is becoming more necessary to understand the nature of absorption and metabolism of polyphenolic compounds, as these plays an important role in healthy beneficial effects. Current knowledge on the absorption and metabolism of polyphenols has been elucidated through several *in vitro* methods, in situ animal experiments, and some *in vivo* studies [17, 45].

Currently there are very few studies on bioavailability of honey polyphenols in humans. The most significant study, by Schramm *et al.*, [46], reported that after consumption of 1.5 g of honey/kg body of two honey types in 40 subjects, the plasma total-phenolic content increased ($P < 0.05$) similarly to antioxidant and reducing capacities of plasma ($P < 0.05$). These data supported the concept that phenolic antioxidants from honey are bioavailable, and that they increase plasma antioxidant activity by improving the defenses against oxidative stress. However, although the honey used in this investigation provided mg quantities of 4-hydroxybenzoic and 4-hydroxycinnamic acids per kg of body weight, the plasma concentration of these acids could not be verified by HPLC analysis. According to the authors this could be due to (i) less than one-third of these compounds were absorbed, (ii) these compounds could have been distributed quickly into body compartments other than plasma, or (iii) the monophenols underwent first pass metabolism in the human body.

However, the absorption of flavonoids seems much more complex, fundamentally due to its chemical characteristics. Fig. **(3)** illustrated the proposed mechanisms for the absorption and metabolism of polyphenolic compounds in the small intestine. The available literature suggests that not only the bacterial enzymes in the intestine [17, 47, 48], are responsible for beta-hydrolysis of sugar moieties in the *O*-glycosides flavonoids. Two β-endoglucosidases capable of flavonoid glycoside hydrolysis have also been characterized in human small intestine, namely lactase phlorizin hydrolase (LPH), acting in the brush border of the small intestine epithelial cells [48, 49] and a cytosolic β-glucosidase (CBG) as an alternative hydrolytic step within the epithelial cells [50, 51, 52]. LPH exhibits broad substrate specificity for flavonoid-*O*-β-D-glucosides, and the released aglycone may then enter the epithelial cells as a result of its increased lipophilicity and its proximity to the cellular membrane [53]. It is has also been proposed that for CBG-catalyzed hydrolysis to occur, the polar glycosides must be transported into the epithelial cells, possibly with the involvement of the active sodium-dependent glucose transporter 1 (SGLT1) [54]. Published studies on the bioavailability and pharmacokinetics have demonstrated that some flavonoids can inhibit the non-Na^+ -dependent facilitated diffusion of monosaccharides in intestinal epithelial cells [55]. Thereby, the parallel concentrative Na^+ -dependent transport

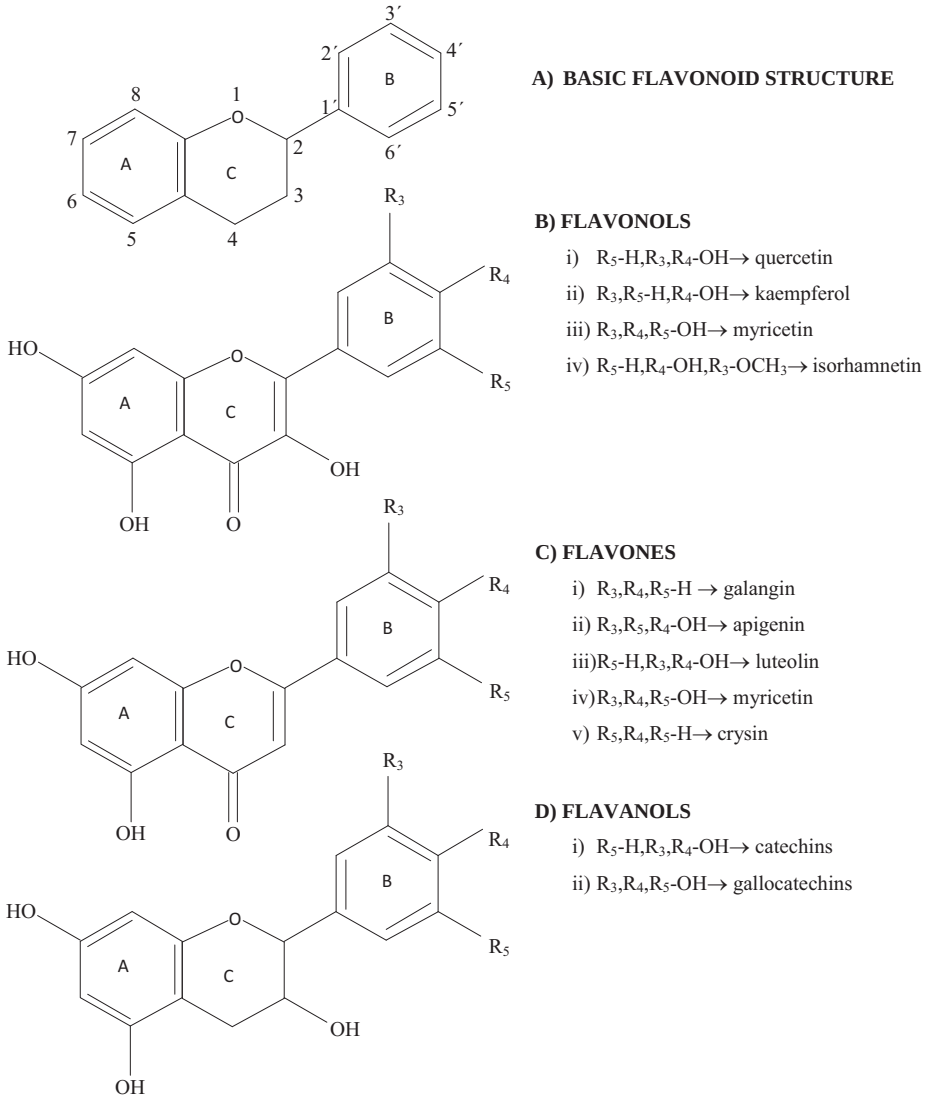


Fig. (2). Chemical structures of the more common flavonoids in honey.

ATPase for monosaccharides is benefited [56]. Therefore, the two possible routes by which the glycoside conjugates are hydrolyzed and the resultant aglycones cross into enterocytes are LPH/diffusion and transport/CBG [57]. To these, in the case of honey, the presence of the enzyme glycosidase in the bee salivary glands [58, 59] should also be added producing a hydrolysis of the glycosylated flavonoids and releasing the aglycon form. This explains, in part, the fact that unlike other phenolics present in foods or beverages, flavonoids in honey have been identified mostly as a form of aglycons and not in their glycosylated form. Phenolic aglycons are more readily absorbed through the gut barrier than their corresponding glycosides by passive diffusion [60] and, therefore, flavonoids present in honey may be more readily bioavailable. It has been also proposed that after release of the glycosides from the aglycone about 15% of the flavonoid aglycons are absorbed with bile micelles into the epithelial cells and passed on to the lymph [47, 48]. Despite this, the influence of the presence, location and structure of the sugar moiety in the bioavailability and metabolism of glycosylated flavonoids has also been highlighted [57].

Once absorbed by the intestinal epithelium and before crossing into the bloodstream, flavonoids undergo some degree of phase II metabolism with the generation of different conjugated products, predominantly sulphates, glucuronides and methylated derivatives through the action of sulfotransferases (SULTs), uridine-5`-diphosphate glucuronosyltransferases (UGT), and catechol-O'Methyltransferase (COMTs), respectively [57]. Besides the metabolic biotransformation of flavonoids, which occurs by the intestinal microflora and the gut-liver pathways, their bioavailability and cell/tissue accumulation have been closely associated with the multidrug-resistance-associated proteins like MRP-1 and MRP-2 (*i.e.* ATP-dependent efflux transporters), also named phase III metabolism [38] and with their tissue distribution and substrate affinity in the various organs. It has been proposed that MRP-2, localized on the apical membrane of cells of the small-bowel epithelium, transports the already intracellular flavonol back to the intestinal lumen, thus modulating the actual intestinal importation of these compounds. On the contrary, MRP-1, situated on the vascular pole of enterocytes, favors transport of the flavonoid from inside the cells into the blood [61, 62]. Moreover, it is has been proposed that MRP-3 and the glucose transporter GLUT2 are also implicated in the efflux of metabolites from the basolateral membrane of the enterocytes [63]. Once in the portal bloodstream, metabolites rapidly reach the liver: in hepatocytes, aglycones are transferred to the Golgi apparatus and possibly also to the peroxisomes, being oxidatively degraded and subjected to further phase II metabolism [57, 64]. These conjugate forms can retain their antioxidant properties, while others such as quercetin quickly enter the cell regaining their active, nonconjugated form [65, 66]. Finally, some flavonoid conjugates with sugar moieties resistant to the action of

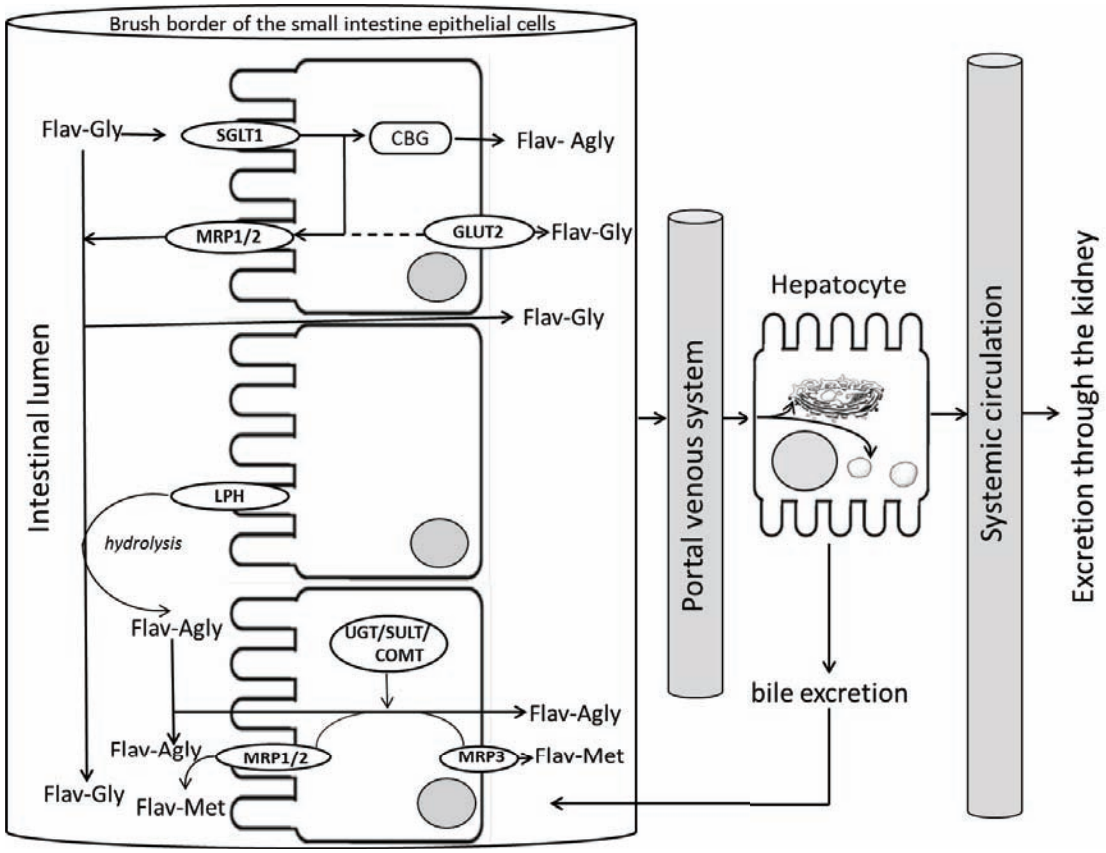


Fig. (3). Mechanisms for the absorption and metabolism of flavonoid compounds in the small intestine. CBG, cytosolic *β*-glucosidase; COMT, catechol-*O*-methyl transferase; GLUT2, glucose transporter; LPH, lactase phloridzin hydrolase; MRP1-2-3, multidrug-resistant proteins; Flav-Agly, Flavonoid aglycone; Flav-Gly, Flavonoid glycoside, Flav-Met, Flavonoid sulfate/glucuronide/methyl metabolites; SGLT1, sodium-dependent glucose transporter; SULT, sulfotransferase; UGT, uridine-5´-diphosphate glucuronosyltransferase.

LPH/CBG are not absorbed in the small intestine and pass to the colon and are therefore excreted with the faeces, while enterohepatic recirculation may result in some recycling back to the small intestine through bile excretion [67]. Others, after metabolic transformation, are secreted by organic acid transporters into the blood and subsequently excreted through the kidneys [68-70].

More studies on the bioavailability and pharmacokinetics of polyphenols in humans are necessary. However, recently reports are encouraging, revealing that flavonoids can be incorporated in lipoprotein domains and plasma membranes, which generally serve as targets for lipid peroxidation, suggesting a protective interaction of flavonoids with lipid bilayers [50, 71] and they can also accumulate in the nucleus [72] and mitochondria [66] affecting diverse cell metabolic functions.

3. ANTIOXIDANT PROPERTIES

The antioxidant properties of honey have been associated to the ability and potential of reducing oxidative reactions within the food systems resulting as attractive/beneficial for human health. These oxidative reactions can cause deleterious reactions in food products and adverse health effects, includingchronic diseases and cancers [73, 74]. The antioxidant capacity (AOC) has been proposed as an indicator of the presence of beneficial bioactive compounds in honey when it was identified as a dietary source of natural antioxidants. The AOC varies greatly depending on the honey floral source, possibly due to the differences in the content of plant secondary metabolites as polyphenolics and enzyme activities [75-80]. It has been found that several constituents of honey play a significant role in AOC as glucose oxidase, catalase, ascorbic acid, organic acids, Maillard reaction products, amino acids, proteins, phenolic acids and flavonoids. Several research groups have studied the AOC of honey using different methods to determine alternatively (i) the capacity to scavenge active oxygen species (*e.g*. the superoxide anion, peroxyl and hydroxyl radicals) [81, 82] and (ii) enzymatic or non-enzymatic capacity of lipid peroxidation inhibition [78, 83]. Honey is a complex biological matrix that gives high variability in measurements and makes it very difficult to obtain standardized AOC indexes. Moreover, when reliable techniques are applied, it can be demonstrated that honey has an *in vitro* AOC similar to those of many fruits and vegetables on a fresh weight basis [73]. Honey has been shown to protect food against microbial growth [84] and deteriorative oxidation reactions, such as lipid oxidation in meat [85], enzymatic browning of fruits and vegetables [86], providing also an effective protection against chemically induced lipid peroxidation in rat liver, brain and kidney homogenates [78, 83]. This particular model of lipid peroxidation just cited is interesting because it has the advantage of including and mimicking several of the mechanisms responsible for the generation and/or modulation of lipid peroxidation occurring *in vivo:* therefore, it offers the possibility of identifying antioxidant compounds able to mitigate lipid oxidative damage. It has been reported that honey presents important radical scavenging capacities (Fig. **4**). Several studies demonstrated that honey is capable of scavenging hydroxyl and superoxide radicals [30, 78, 87-89]. The ability of honey to scavenge free radicals and to protect against lipid peroxidation may contribute to preventing/reducing some inflammatory diseases in which oxidative stress is involved, offering an interesting preventive and therapeutic option.

The AOC of honey, which depends on polyphenol contents, is also correlated with its color [26, 77, 80]. Frankel *et al.* [90] suggested that the color intensity in honey is related to pigments

(flavonoids, carotenoids, *etc*.). Actually, the dark honeys have shown the highest AOC, as well as, phenolic, flavonoid and carotenoid concentrations while the light-colored honeys are characterized to have the lowest values, with linear positive correlations between color *vs* phenolic and flavonoid content *vs* radicals scavenging activity and protection against lipid peroxidation ($P< 0.05$) [73, 75-78, 79, 80, 83, 88, 90].

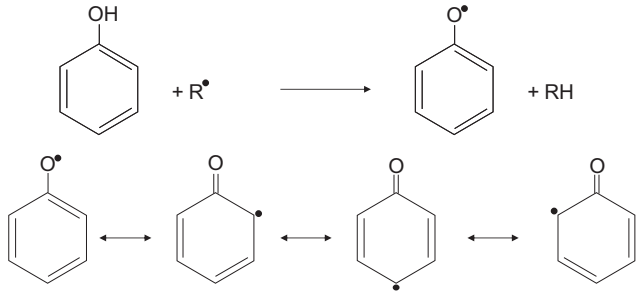


Fig. (4). Radical scavenging mechanism of phenolic compounds.

3.1 Polyphenols as the Principal Contributors of Honey AOC

Since polyphenols are considered as mostly responsible for AOC in honey, the mechanisms by which these compounds contribute to its antioxidant properties are considered as the most medically useful properties of honey. These positive characteristics seem to be ascribed to their efficacy as metal chelators and as excellent free-radical scavengers, as well as gene modulators able to influence enzymatic and non-enzymatic systems that regulate cellular redox balance [17, 38, 91].

Among the phenolic acids, benzoic acid is a weak antioxidant. This capacity is increased in the case of dihydric or trihydric derivatives, where the antioxidant effect depends on the relative positions of OH groups in the aromatic ring. Thus, gallic acid (3, 4, 5-trihydroxybenzoic acid) is the most potent antioxidant within all the hydroxybenzoic acids. Contrary to their homologs derived from benzoic or phenylacetic acid, hydroxycinnamic acids exhibits greater free radical scavenging ability. This property appears to be related to the inclusion of the unsaturated chain bonded to the carboxyl group as a distinctive structure which provides stability by resonance to phenoxyl radical, even offering additional sites for the attack of free radicals [92]. Furthermore, the existence of several electron donor groups in the benzene ring structure (as hydroxyl or methoxy groups in structures) also provides a greater number of resonant structures and increases the stability of the arylic radical in cinnamic acids, thereby favoring their antioxidant behavior.

It has been widely demonstrated that flavonoids are very effective as scavengers of reactive oxygen species (ROS) peroxyl, alkyl peroxide, hydroxyl and superoxide radicals, as well as against reactive nitrogen species (RNS) likenitric oxide and peroxynitrite, protecting against the oxidative damage induced by these molecules [49, 93-95]. This activity is attributed basically to three chemical features in flavonoid structure, namely an *ortho*-dihydroxy structure in the B-ring [96-99], and the presence, in the C-ring, of a 2, 3 double bond and/or of a 4-oxo function [91].

Hydroxyl groups on the B-ring donate a hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals,stabilizing them and giving rise to relatively stable flavonoid radicals. Oxidation of a flavonoid occurs on the B-ring when catechol is present [100], yielding a fairly stable *ortho*-semiquinone radical [101] through facilitating electron delocalization [50]. Other hydroxyl configurations are less clear, as the A-ring substitution, where the increasing total number of OH groups correlates little with AOC [91]. Moreover, the heterocyclic character of some flavonoids plays an important role in antioxidant activity by the presence of a free 3-OH and allowing conjugation between the aromatic rings, the closed C-ring itself may not be critical to the activity of flavonoids[102]. Flavonoids with a 3-OH and 3`, 4`- catechol are reported to be 10- fold more potent than ebselen, a known RNS scavenger against peroxynitrite radical [91, 98]. Examples are flavonols: the superiority of quercetin in inhibiting both metal and nonmetal-induced oxidative damage is partially ascribed to its free 3-OH substituent [50, 91], which is thought to increase the stability of the flavonoid radical, while the substitution of 3-OH by a methyl or glycosyl group decreases the AOC of this flavonol [97].

A distinguishing feature among the general flavonoid structural classes is the presence or absence of an unsaturated 2-3 bond in conjugation with a 4-oxo function. It has been demonstrated that flavonoids which do not exhibit one or both features exhibit a lesser AOC than those with both elements. The conjugation between the A - and B rings permits a resonance effect of the aromatic nucleus leading stability to the flavonoid radical [103]; this conjugation is critical in optimizing the phenoxyl radical-stabilizing effect of the 3`,4`-catechol [104]. The fact that flavonols have a higher free radical scavengers capacity than flavones [104, 105] may be associated to the greater number of hydroxyl groups and substituents 3-OH present in their structure.

4. HONEY AND HEALTH

The biological activities of honey have long been studied using several *in vitro* and animal models studies, as well as human epidemiologic and interventional studies (Table **4**).

4.1 Honey and CVD Risk

Studies *in vitro* and *in vivo* have shown that honey can positively affect risk factors for CVD by inhibiting inflammation, improving endothelial function [106], improving the plasma lipid profile and increasing low-density lipoprotein (LDL) resistance to oxidation [73].

The oxidative modifications of lipoproteins play an important role in the pathogenesis of atherosclerosis [107, 108]. In an *in vitro* model a significant correlation between AOC and inhibition of lipoprotein oxidisability from human serum by honey was reported [73, 109], providing initial useful evidence about the protective effect of honey against the oxidative damage of these molecules. Besides this, the improvement of endothelial function by honey has also been demonstrated. Beretta *et al.*[106] reported, using native honey, a significative quenching activity against lipophilic cumoxyl and cumoperoxyl radicals, with significant suppression/prevention of cell damage, complete inhibition of cell membrane oxidation, decrease of intracellular ROS production as well as intracellular GSH recovery in endothelial cell cultures (EA.hy926). Moreover, the phenolic fraction isolated from these honeys was used to pretreat the same endothelial cells, exposed later to peroxyl radicals from AAPH and to hydrogen peroxide, and indicated as the main cause of the same protective effect. In another *in vitro* study, reported by Ahmad *et al.* [110], the effect of honey on bovine thrombin-induced oxidative burst in human blood phagocytes was studied. The results reported that phagocytes activated by bovine thrombin and treatment with honey showed effective suppression of oxidative respiratory burst [110]. These results suggest that, through the synergistic action of its antioxidants, the antioxidant compounds present in honey could be beneficial in the interruption of the pathological progress of cardiovascular disease,could play a cardioprotective role and contribute to reducing the risks and effects of acute and chronic free radical induced pathologies *in vivo*.

Although blood is not strictly part of the cardiovascular system, it is in any case closely related to it functionally, and its alterations may be a predisposing factor for CVD. Recently, an interesting area of research which discusses the protective effect of food polyphenols in red blood cells (RBC) against oxidative damage has

Table 4. Effects of Honey Consumption on Health.

Diseases	Effect on health	References
Cardiovascular diseases (CVD)	Reduction of cardiovascular risk factors	9
	Inhibition of inflammation	106
	Improvement of endothelial function	106
	Improvement of plasma lipid profile	73
	Increase of low-density lipoprotein (LDL) resistance to oxidation	73,109
	Inhibition of Red Blood Cells (RBCs) hemolysis	118, 120
	Improvement of eritrocytes uptake capacity	119
	Protection of RBCs against intracellular depletion of GSH and SOD activity	118
	Decrease of the susceptibility of RBCs lipid membrane against oxidative damage	71, 111, 113,118,120
	Maintenance of the body weight in overweight or obese subjects (no increase)	9
Hypertension	Reduction of systolic blood pressure and MDA levels	128
	Ameliorament of susceptibility of kidneys to oxidative stress	129
Cancer	Antimutagenic capacity	145
	Induction of apoptosis	147, 149
	Antiproliferative effect	12, 149, 150, 152
	Citotoxic effect on several cancer cell lines	148, 152
	Antimetastatic effect	151
Diabetes	Reduction of glycaemia	169, 172, 173, 174, 177, 178, 179, 180, 181
	Reduction of serum fructosamine	170
	Reduction of glycosylated hemoglobin concentration	171
	Attenuation of post-prandial glycemic response	176
	Increase serum insulin concentration and reduce insulin resistance	170, 183, 186
Microbial infection	Inhibition of microorganisms of clinical relevance	80, 212, 218

reported interesting results [111-117], in which flavonoids from honey have also been studied [118-120].

Erythrocytes are the most abundant cells in the human body and due to their structural and functional characteristics they are targets for continuous oxidative stress damage. Since oxidative damage to the erythrocyte membrane is generally associated with an increased lipid peroxidation process, causing malfunctioning of the membranes by altering its fluidity as well as the membrane-bound enzyme and receptor function, it has been proposed as a general mechanism involved in cell injury and death, leading to erythrocyte haemolysis [121-123]. In particular, *in vitro* tests have ascribed antioxidant and antihemolytic properties of dietary flavonoids to their localization in the membrane bilayer and to formation of selective bindings with RBC membrane lipids and proteins, which may exert a significant inhibition of lipid peroxidation, and enhance membrane integrity against several chemical and physical stress conditions [121, 122]. This mechanism appears to partially explain how polyphenol extracts from honey were able to inhibit RBCs oxidative hemolysis [118, 120], reduce the extracellular ferricyanide [119], protect against intracellular depletion of GSH and SOD activity and to decrease the susceptibility of RBC lipid membrane to peroxidation [118]. Another mechanism that may be involved in RBC protection by honey flavonoids seems to be erythrocyte uptake capacity of these molecules. Previous uptake studies in human RBC showed an excellent incorporation of honey phenolics in RBC [119]. Flavonoid uptake by RBCs and their interactions with membrane were confirmed using quercetin as a reference standard model because it has been widely identified in honey, is efficiently incorporated into erythrocytes, and finally, different studies have reported its involvement in protecting RBCs membranes against oxidative damage [71, 111, 113, 118, 120].

Epidemiological studies suggest that hypertension is a major public health concern because of its high prevalence, besides also being an important risk factor for the development of CVD, that end-lasts in renal disease, stroke, and death [124, 125]. Research has demonstrated a close relationship between oxidative stress and hypertension, causing a vast interest in therapeutic approaches or nutritional interventions to preventively decrease oxidative stress or to treat hypertension itself [126, 127]. As a model for humans, Erejuwa*et al.*[128] evaluated the effect of honey supplementation on elevated systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) as well as the capacity of honey to ameliorate oxidative stress in the kidney of SHR as a possible mechanism of its antihypertensive effect. Their findings demonstrated that honey supplementation significantly reduced SBP, and malondialdehyde (MDA) levels in SHR. Recent studies indicate also that honey may ameliorate susceptibility of kidney to oxidative stress in rats with chronic renal failure or hypertension via up-regulation of Nrf2 activity or expression [129]. In another study, the protection of honey on the cardiovascular system was evaluated by Rakha *et al.* [130], using a model of induction of hyperadrenergic activity in urethane-anesthetized rats by epinephrine. Acute administration of epinephrine for 2 hours induced several cardiac disorders and vasomotor dysfunction. The results showed that pretreatment with natural wild honey (5 g/kg) for 1 hour prior to the injection significantly reduced the incidence of epinephrine-induced cardiac disorders and vasomotor dysfunction in anesthetized normal rats. Moreover, post-treatment with natural wild honey, following the injection with epinephrine for 1 hour, showed several ameliorative outcomes to the electrocardiographic parameters and vasomotor dysfunction of anesthetized stressed rats. From the results of this study it has been hypothesized that honey may exert its cardioprotective effects against epinephrine-induced cardiac disorders and vasomotor dysfunction directly, via its AOC and its great wealth of both enzymatic and non-enzymatic antioxidants involved in cardiovascular defense mechanisms; also the contribution of substantial quantities of mineral elements should be taken into account such as magnesium, sodium, and chlorine, and/or indirectly, the enhancement of nitric oxide release, the endothelium-derived relaxing factor, through the influence of ascorbic acid (vitamin C) [130].

Despite the results obtained using *in vitro* and animal models, data from interventional studies in humans with CVD risk are few. In a study of 55 overweight or obesepatients, supplementation of 70 g of natural honey against fructose-supplemented control for 30 days caused a mild reduction in body weight (1.3%) and body fat (1.1%), a more consistent reduction of total cholesterol (3%), LDL-C (5.8%), triacylglycerides (11%), and C-reactive protein (3.2%), and an increase of HDL-C (3.3%) in subjects with normal values. Meanwhile, in patients with impaired values, honey caused reduc-

tion in total cholesterol (3.3%), LDL-C (4.3%), triacylglycerides (19%) and C-reactive protein (3.3%) [9]. Another study evaluated the effects of the ingestion of 75 g of natural honey compared to the same amount of artificial honey (fructose plus glucose) or glucose in normal subjects and in both hypercholesterolemic and hypertriglyceridemic patients. Results showed that honey consumed for 15 days decreased cholesterol (7%), LDL-C (1%), TG (2%), C-reactive protein (7%), homocysteine (6%), plasma glucose level (6%), and increased HDL-C (2%) in normal subjects. In patients with hypertriglyceridemia honey decreased TG while in subjects with hyperlipidemia it decreased cholesterol (8%), LDL-C (11%), and C-reactive protein (75%) after 15 days of consumption [131]. These results support the hypothesis that honey reduces cardiovascular risk factors, particularly in subjects with elevated risk factors, and it does not increase body weight in overweight or obese subjects [9].

The consumption of honey could affect pathways related to cardiovascular health by several mechanisms. The actions of polyphenols appear to be the main relevant mechanisms. The mains flavonoids, quercetin and kaempferol, are reported as promising pharmaceutical molecules in the treatment of cardiovascular diseases, and may in some way help to understand the mechanisms by which honey exerts its positive action against CVD.Quercetin is rapidly conjugated with glucuronic acid and/ or sulfate during a first metabolism step and a portion of the metabolites are also methylated, reaching the blood stream as methylated, glucuronidated and sulfated products [132]: for example, it has been recently reported that glucuronidated and sulfated metabolites of quercetin exhibited a protective effect on endothelial dysfunction [133]. In cultured human endothelial cells quercetin has been shown to both up-regulate eNOS gene expression [134] and stimulate the NO/cGMP pathway [135, 136]: these findings are of utmost interest since currently the endothelial NOS gene is a candidate in investigations on CVD genetics by the established role of NO in vascular homeostasis. Other studies *in vitro* and in animal models have also shown that quercetin can increase the activity of eNOS and stimulate arterial relaxation [137], possibly via activation of Ca^{2+}-activated K^+ channels [138]. Moreover, Shen *et al.*[139] reported that quercetin and its major *in vivo* metabolites can protect vessels against hypochlorous acid-induced endothelial dysfunction in isolated arteries, presumably mediated in part, by an adenosine monophosphate-activated protein kinase (AMPK) pathway. AMPK activation leads to subsequent eNOS activation and increased NO production, suggesting that beneficial effects of quercetin on endothelial cell functions are in part mediated via AMPK pathway.

In animal studies it was found that chronic treatment with dietary quercetin lowers blood pressure and restores endothelial dysfunction in hypertensive animal models. In a model using spontaneously hypertensive rats (SHR) (5 weeks old), animals were treated with quercetin (10 mg/kg) for 13 weeks. In these animals quercetin reduced blood pressure and heart rate, and enhanced the endothelium-dependent aortic vasodilation induced by acetylcholine. These findings suggest that enhanced eNOS activity, and decreased NADPH oxidase-mediated superoxide anion generation associated with reduced p47 expression appear to be the essential mechanisms for the improvement of endothelial function and the antihypertensive effects of chronic quercetin intake [139]. In a recent research Panchal *et al.* [140] reported that rats supplemented with quercetin presented a higher protein expression of nuclear factor (erythroid-derived 2)-related factor-2 (Nrf2), heme oxygenase-1, and carnitine palmitoyltransferase 1 and lower expression of NF-κB, compared with the control group. Moreover, the supplemented animals showed less abdominal fat and lower systolic blood pressure along with attenuation of changes in structure and function of the heart and liver compared with control rats. Thereby, quercetin treatment attenuated most of the symptoms of metabolic syndrome, including abdominal obesity, cardiovascular remodeling, with the most likely mechanisms being the decrease in oxidative stress and inflammation. These data are in accordance also with the results published by Jung *et al.* [141] where quercetin reduces fat accumulation in C57B1/6 the liver of mice that have undergone a high-fat diet, due to its capacity to regulate the lipogenesis at transcription level.

Another common flavonoid in honey with a putative important role in the treatment of cardiovascular diseases is kaempferol. Some researchers have elucidated a group of mechanisms by which kaempferol exhibits its beneficial properties to the cardiovascular system. The protective effect of kaempferol against endothelial damage seems to be correlated with its ability to improve NO production and to decrease asymmetric dimethylarginine (ADMA) levels. Experiments have been performed using aorta and plasma from C57BL/6J control and apolipoprotein E-deficient (ApoE−/−) mice treated or not with kaempferol (50 or 100 mg/kg, intragastrically) for 4 weeks, as well as human umbilical vein endothelial cells (HUVECs) pretreated or not with kaempferol (1, 3 or 10 μM) for 1 h and then exposed to lysophosphatidylcholine (LPC) (10 μg/mL) for 24 h [142]. Treatment with kaempferol improved endothelium-dependent vasorelaxation, increased the maximal relaxation value, and decreased its half-maximum effective concentration concomitantly with an increase in nitric oxide plasma concentration, and a decrease in ADMA and MDA plasma concentrations. Moreover, these compounds caused an increase in the expression of aortic endothelial NOS as well as of dimethylarginine dimethylaminohydrolase II (DDAH II) in ApoE−/− mice. Finally, it was found that kaempferol abolished the reduction of NO production, the increase in ADMA concentration and the decreased expression of eNOS and DDAH II in HUVECs caused by LPC [142]. In an *in vitro* study using isolated porcine coronary artery rings the vascular effects of kaempferol were studied by Xu *et al.*[143]. The results demonstrated that kaempferol enhanced the relaxation produced by bradykinin, isoproterenol and sodium nitroprusside in endothelium-intact porcine coronary arteries. It was concluded that kaempferol in a dose dependent mode, has the ability to enhance endothelium-dependent and endothelium-independent relaxations, which is not related with its antioxidant properties.

Besides its aglycon form, kaempferol has demonstrated protecting effects on CVD also in its glycosylated form. It was also demonstrated that kaempferol-3-*O*-sophoroside (KPOS) inhibited LPS-induced barrier disruption, expression of cell adhesion molecules, neutrophil adhesion and trans-endothelial migration of neutrophils to human umbilical vein endothelial cells (HUVECs). Further studies revealed that KPOS suppressed the production of TNF-α and the activation of NF-κB by lipopolysaccharides. These results suggest that KPOS possesses barrier integrity activity, inhibitory activity on cell adhesion and migration to endothelial cells by blocking the activation of NF-κB expression and production of TNF-α, thereby endorsing its usefulness as therapy for vascular inflammatory diseases [144]. Although these studies provide new insights on the potential functional benefits of honey antioxidant compounds, further investigations in animal and human models are needed to confirm the hypothesized vascular protective effects of honey.

4.2 Honey and Cancer

Currently, there are few studies reporting the effect of honey in cancer, although interest in this area is growing among researchers. A group of studies has been particularly focused on the efficacy of crude honey or its components in inhibiting mutagenesis or inducing apoptosis, and the transformation of different types cancer cell and proliferation *in vitro*; moreover, the mechanisms underlying the anti-tumorigenic effects of honey at the cellular and molecular levels are still limited. The major components of honey, *i.e.*, sugar, particularly glucose and fructose, have been reported to display both mutagenic and antimutagenic effects in different systems; antioxidants, in their turn, often show antimutagenic activity [145].

As a result, since honey is a rich source of dietary sugars, little is known about possible actual antimutagenic effects. In an *in vitro* model Wang *et al.*[145] studied the antimutagenic capacity of honeys from seven different floral origins against the encountered food mutagen Trp-p-1, comparing it to that of a sugar analogue and to individually tested simple sugars. The results showed that all honeys studied exhibited significant inhibition against Trp-p-1 mutagenicity. Interesting was the fact that sugars selected for analysis, either individually or in combination demonstrated a pattern of inhibition similar to that of honeys, where glucose and fructose were also similar to honeys and were more antimutagenic than maltose and sucrose. From these results it may be assumed that honey polyphenols are not solely responsibles for honey antimutagenic activity. It is known that sugars can display both mutagenic and antimutagenic effects in different systems [146], and since honey is a rich mixture of sugars, its use as a factor able to prevent mutagenesiscould result interesting.

The induction of apoptosis by honey is another capacity that has been recently highlighted. Jaganathan *et al.*, [147] tested the apoptotic effect of selected crude honey samples in colon cancer cell lines (HCT 15 and HT 29). Pretreatment of cells with honey produced a significant dose-dependent anti-proliferative effect, showing the increasing accumulation of hypodiploid nuclei in the sub-G1 phase of cell cycle that indicated apoptosis. In this cell model the same authors also reported that honey transduced the apoptotic signal via initial depletion of intracellular non protein thiols, consequently reducing the mitochondrial membrane potential and increasing reactive oxygen species generation. Honey induced apoptosis was accompanied by up-regulating p53 and modulating the expression of pro and anti-apoptotic proteins. Further, in HT 29 cells, honey elevated caspase-3 level and displayed typical ladder pattern, confirming apoptosis [147]. Another study tested the antiproliferative role of acacia honey and chrysin, as a major natural flavone found in this honey in human (A375) and murine (B16-F1) melanoma cell lines. The results showed that both compounds were able to induce an antiproliferative effect on melanoma cells in a dose- and time-dependent manner, mediating by G(0)/G(1) cell cycle arrest and induction of hyperploid progression [12]. Similarly, Tualang honey was investigated using human breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cell lines, as well as normal breast epithelial cell line (MCF-10A). After 72 h of incubation with increasing doses of honey (1-10%) an increase in lactate dehydrogenase (LDH) leakage from the cell membranes was evidenced, indicating a cytotoxic effect of honey to all the three cancer cell lines, while no cytotoxic effects was evidence in MCF-10A cell. Honey also reduced the mitochondrial membrane potential ($\Delta\psi(m)$) in cancer cell lines after 24h of treatment. Moreover, the activation of caspase-3, -7 and -9 was observed in all honey-treated cancer cells indicating the involvement of mitochondrial apoptotic pathway [148]. Tualang honey was also tested in oral squamous cell carcinomas (OSCC) and human osteosarcoma cells (HOS) [149]. In this study, the results in morphological appearance showed significant apoptotic cellular changes in both treated cell lines (rounded, reduction in cell number, blebbed membrane), as well as significative apoptotic nuclear changes (nuclear shrinkage, chromatin condensation and fragmented nucleus). Moreover, cell viability assay showed a time and dose-dependent inhibitory effect on both cell lines, where signals of early apoptosis were evident, with the percentage of early apoptotic cells which increased in a dose and time dependent manner [149].

Despite a consistentamount of results obtained using *in vitro* models, data from *in vivo* studies are still very limited. In bladder cancer cells, the antitumor effect of honey was examined both *in vitro* and *in vivo*. Three human bladder cancer cell (T24, 253J and RT4) and one murine bladder cancer cell lines (MBT-2) were used. The *in vitro* studies revealed significant inhibition of the proliferation of T24 and MBT-2 cell lines and of RT4 and 253J cell lines. A lower S-phase fractionwas also found, as well as absence of aneuploidy compared with control cells. Moreover, in the *in vivo* studies, researchers observed that intralesional injection of 6 and 12% honey and oral ingestion of honey significantly inhibited tumor growth [150]. Honey also exerted a pronounced antimetastatic effect in murine tumor models (mammary carcinoma (MCa) and a methylcholanthrene-induced fibrosarcoma (FS)) when administered before tumor cell inoculation (2 g/kg orally once a day for 10 consecutive days) [151]. The results presented by Attia *et al.* [152] have shown other possible routes by which honey can exert its antitumor capacity (EAT). In this study the antitumor effect of honey against EAC in mice and the possible mode of its antitumor action were investigated. Results evidenced that pre-oral administration of mice with different honey doses before intraperitoneal inoculation with EAT (1×10^6 cells) increased the number of bone marrow cells as well as peritoneal macrophages, but not peripheral blood leukocytes. An increase in phagocytic functions of macrophageswas also found as well as T- and B-cell functions. Moreover, *in vitro* studies on EAT cells demonstrated an inhibitory effect of honey on tumor cell proliferation, on the viability % of tumor cells as well as on the size of solid tumor. These results allow to hypothesize that honey antitumor activity may also occur *via* activation of macrophages, T- and B-cells [152].

Honey constituents, as polyphenols, have shown complementary and overlapping mechanisms of chemopreventive activity in multistage carcinogenesis [17]. Dietary flavonoids may be used as chemotherapeutics and preventatives against critical health conditions such as cancer. The large number of effects of flavonoids, like major polyphenolics in honey, on the metabolism of cancer cells is difficult to summarize in a few basic and specific mechanisms. Several biochemical structures and pathways related to carcinogenesis can be influenced by flavonoids like: (i) *cytoplasmic/nuclear* hormone receptors, as the most highly sensitive to flavonoids; (ii) *enzymatic*, by inhibition of several enzymes involved in the oncogenesis, while the reactions of some phosphatases and oxygenases are improved; (iii) *growth regulation*, by inhibition of pathways for the transmission of environmental signals to the genes as the steroid path via the cytoplasmic receptor and the protein kinase cascades; (iv) *energy metabolism*, by inhibition of glycolysis which leads to a depletion of ATP, especially in tumor cells with mitochondrial respiratory defects. These processes lead to a rapid dephosphorylation of the BAD molecules integrative of glycolysis-apoptosis in SER, a relocation of BAX to mitochondria and massive cell death, possibly due to removal of the inhibiting phosphate residue on the β-chain of the Na^+/K^+ pump [17].These effects were attributed to two fundamental properties of flavonoids: the electronic and the steric characteristics. The first one is due to the high mobility of the electrons in the benzenoid nucleus of flavonoids which accounts for both their antioxidant and free-radical scavenging properties; the second one is due to the structural similarity between the aglycone form of flavonoids and various compounds involved in normal cell biochemistry such as nucleic acid bases, coenzymes, steroid hormones, neurotransmitters, cytoplasmic/nuclear hormone receptors, as well as gene induction. Moreover, the high affinity of flavonoids for heavy metal ions allows to interfere with the action of enzymes and Zn^{2+} fingers in DNA-binding proteins [17].

Quercetin has been the subject of several studies that have shown a significant antiproliferative activity in different tumor lines, and since it is one of the mostfrequentlyflavonoids identified in honey, its presence could help, in part, to explain honey antiproliferative properties. Since it is rapidly absorbed and metabolized in circulating cells, its effects in several *in vitro* models of leukemia focused the attention of researchers. Kang *et al.* [153] investigated the role of quercetin in human promyelocytic leukemia cells (HL-60). Pretreatment of HL-60 cells with quercetin produced a dose-dependent inhibition on the activities of cytosolic protein kinase C

(PKC) and two pore K^+ (TPK). Cell cycle analysis indicated that quercetin suppressed in a dose-dependent mode the number of cells in the G2/M phase and decreased the population of G0/G1 cells. It was also found that quercetin repressed the complete activity of phosphoinositides like phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), and phosphatidylinositol 4, 5-bisphosphate (PIP2): it could be concluded that the inhibitory effect of quercetin on the growth of HL-60 cells may be related to its inhibitory effects on PKC and/or TPK *in vitro* and/or on the production of phosphoinositides. More recently, it was been reported that quercetin also induced fast ligand-related apoptosis in these cells by promotion of histone H3 acetylation [154]. In other cellular models, such as chronic myeloid leukemia (CML) cells, quercetin caused a depletion of the activity of telomerase [155], a decrease of the level of Notch1 protein [156] and inhibition of murine leukemia WEHI-3 cells when injected into BALB/c mice, as well as a promotion of macrophage phagocytosis and natural killer cell activity [157]. Studies conducted by Russo *et al.* [158] in B cells isolated from chronic lymphocytic patients (B-CLL) reported that quercetin enhanced sensitivity to anti-CD95 and rTRAIL treatment with an increase in cell death of about 1.5- and 1.6-fold, respectively, when compared with quercetin monotreatment, suggesting that quercetin supplementation may have the capacity to strengthen the efficacy of drugs widely used in the therapy of B-CLL, such as fludarabine [159].

The antitumor effects of quercetin are also reported in a large number of tumor cell models. Recent studies in breast cancer cell (MDA-MB-435, MCF-7) showed an inhibitory effect on cell growth in a time and dose dependent manner. The analysis of cell cycle in quercetin treated cells showed significant increase in the accumulation of cells at subG1 phase. Quercetin treatment also increased Bax expression but decreased the Bcl2 levels, while cleaved caspase-3 and PARP expression were increased [159, 160]. In human glioma cell cultures (U138MG) Braganhol *et al.* [161] reported that quercetin decreased cell proliferation and viability by necrotic and apoptotic cell death, arrest the G2 checkpoint of the cell cycle, and decreased the mitotic index. Furthermore, it was also found that quercetin was able to protect the hippocampal organotypic cultures from ischemic damage. Therefore, all these results allowed to hypothesize that the main routes by which quercetin is capable of regulating tumor cell growth are focused on its ability to induce apoptosis by decreasing the Bcl2 levels, increasing Bax expression, cleaved caspase-3 and PARP expression, stop cell cycle, and arrest the cell cycle in the G2/M phase, proving it to be an active compound with potential uses like chemotherapeutics and preventatives supplied by diet.

Besides quercetin, other flavonoids identified in honey such as kaempferol, are responsible for significant antiproliferative effects. Using the HL-60 cells, it was found that kaempferol caused cell cycle alterations, with a significant increase of cells in S-phase and a progressive accumulation in G2/M, while cells with apoptotic indices confirmed a heightened caspase-3 activity and a decreasing of anti-apoptotic Bcl-2 expression [162]. Kaempferol also showed the capacity of reducing the risk of ovarian cancer. Recently, Luo *et al.*[163] demonstrated that kaempferol in a time-dependently way inhibited vascular endothelial growth factor (VEGF) secretion, and suppressed *in vitro* angiogenesis in ovarian cancer cells. Kaempferol also down-regulated extracellular signal-regulated kinases (ERK) phosphorylation as well as NFκB and cMyc expression, but promoted p21 expression, suggesting a novel ERK-NFκB-cMyc-p21-VEGF pathway, which accounts for kaempferol angioprevention effects in ovarian cancer cells.

Among honey phytochemicals, phenolic acids like caffeic acid and its esters have also been associated to the chemopreventive effects of honey, appearing to function as an anticarcinogen at the initiation and post-initiation stages of tumor development in *in vitro* and *in vivo* experiments [164, 165]. The studies using HCT 15 and HT 29 colon cancer cells demonstrated that caffeic acid significantly inhibited the cell proliferation. The cell-cycle analysis in caffeic acid-treated cells indicated increasing accumulation of cells at sub-G1 phase, accompanied by an increasing ROS generation and reduction in the mitochondrial membrane potential, confirming a dose- and time-dependent apoptotic effect of caffeic acid [164-166]. Moreover, when caffeic acid phenethyl ester (CAPE) was explored on growth, cell cycle, apoptosis and beta-catenin/T-cell factor signaling in human colon cancer cell (HCT116 and SW480) it completely inhibited growth, and induced G1 phase arrest and apoptosis in a dose-dependent manner. In treated cells a dose-dependent and time-dependent loss of total beta-Catenin proteinwas also found, associated with a decrease of nuclear beta-catenin, as well as a reduction of the expression of cyclin D1 and c-myc [167]. The studies in other tumor cell lines also confirmed the antiproliferative effect of caffeic acid. A research in human cervical cancer cells (HeLa cells) conducted by Chang *et al.* [168] showed that caffeic acid significantly induces apoptosis in HeLa cells in a concentration-dependent manner by inhibiting Bcl-2 activity, leading to release cytochrome c and subsequent activation of caspase-3 and p53. These results indicate that caffeic acid and its esters are excellent inducers of apoptosis in tumor cell lines, allowing to hypothesize about the other possible mechanisms by which honey exerts its antitumor effects.

Results *in vitro* and *in vivo* are encouraging and demonstrate the potential uses of honey as a possible preventive agent against the development of degenerative diseases. Certainly, other potential mechanisms of honey anticancer activities, already investigated with others polyphenolic rich-food, remain to be evaluated, such as the ability to interact/interfering with an environmental carcinogenic uptake or activation, or the capacity of inhibiting matrix metalloproteinases and other enzyme families implicated in cancer metastasis, just to mention some of them.

4.3 Honey and Diabetes

Honey can positively affect the glycemic response by reducing blood glucose [169], serum fructosamine [170] or glycosylated hemoglobin concentration [171]. Animal studies demonstrated that honey supplementation significantly decreases glycemic values in both diabetic and non-diabetic rabbits [172], and it reduces blood glucose concentrations in alloxan-induced [169] and in streptozotocin-induced (STZ-induced) diabetic rats in a dose-dependent mode [173, 174]. The data referring to the effects of honey on fructosamine or glycosylated hemoglobin levels are still limited; however, it has been reported that chronic honey supplementation reduces glycosylated hemoglobin in non-diabetic rats [171], while in STZ-induced diabetic rats it decreases significantly serum concentrations of fructosamine [170]. Moreover, the combination of antidiabetic drugs, such as glibenclamide or metformin, with honey results in further reductions in serum concentrations of both glucose and fructosamine in STZ-induced diabetic rats [170].

Evidence from clinical studies showed that honey, compared with dextrose, sucrose or other sweeteners, attenuated post-prandial glycemic response in non-diabetic volunteers [175]. In healthy human subjects, it was found that natural honey consumption (1g/kg body weight) decreases glycemic post-prandial response compared to artificial honey and D-glucose which elevated blood glucose levels by 47% and 52%, respectively after 60 minutes [176]. In patients with diabetes mellitus, honey supplementation significantly reduced postprandial glycemic response causing a lower rise in plasma glucose compared with other sugars or sweeteners [176]. Similarly, honey decreased the concentrations of blood glucose in patients with type 2 diabetes mellitus [177,178]. Compared to sucrose, honey lowered glycemic and peak incremental indices in type 1 diabetic patients [179], while in children with type 1 diabetes mellitus honey reduced hyperglycemia [180]. A glucose-lowering effect of honey was also reported in subjects with impaired glucose

tolerance [181]. In this study subjects presented significantly lower plasma glucose concentrations after consumption of honey at all-time points of the honey tolerance test in comparison to the oral glucose tolerance test. Plasma glucose levels in response to honey peaked at 30-60 minutes and showed a rapid decline, when compared to glucose. Significantly, the high degree of tolerance to honey was recorded in subjects with diabetes, suggesting a lower glycemic index for honey. However, despite the evidence of honey hypoglycemic effect, some studies found no beneficial effect of honey on hyperglycemia in type 2 diabetic patients and non-diabetic rats [182, 183], possibly caused by the short duration of honey supplementation or feeding [184].

From the above mentioned articles, it can be evinced that honey consumption or its addition to dietary carbohydrates could be beneficial in individuals with diabetes. However, few studies have studied fructosamine or glycosylated hemoglobin in diabetic patients after honey supplementation, making it difficult to ascertain the actual effect of honey on these parameters in diabetic patients. The antidiabetic mechanisms by which honey exerts its glycemic control have also been associated with its capacity to modulate key glucose-regulating hormones, especially insulin [185]. Studies in healthy subjects demonstrated that honey supplementation, compared with glucose or the combination of glucose-fructose solution, produced significantly lower serum insulin and C-peptide concentrations [186]. In diabetic patients, honey supplementation increased insulin concentrations more than sucrose [186], while in type 2 diabetics reduced insulin resistance [183]. The effect of honey on glucose-regulating hormones and pancreas was also reported in animal studies using STZ-induced diabetic rats, where honey supplementation was associated with a considerable improvement in pancreatic islets as well as increased serum insulin levels [170].

Fructose and glucose, the main sugars present in honey, are involved in some mechanisms related to the glycemic control. Studies either in diabetic rodent models or healthy and diabetic subjects have shown that fructose reduces hyperglycemia [187-190]. Evidence suggests that fructose contributes in regulating blood sugar levels by slowing digestion [191], prolonging gastric emptying and slowing down the rate of intestinal absorption [192]. Besides fructose, glucose is the second major sugar constituent in honey [7]. A significant synergy has been reported between these molecules which actively influence their absorption, indicating that intestinal absorption of fructose is improved in the presence of glucose [193]. Fructose and glucose have different transporters, GLUT5 (and/or GLUT2) and SGLT1, respectively [194]; despite this, actually the mechanism by which glucose enhances fructose absorption remains unclear. The recruitment of GLUT2 carrier to the brush border membrane caused by increased intestinal fructose may contribute to the synergistic effect of glucose on the absorption of fructose [193]. The general schema of the absorption of glucose and fructose by enterocytes in the small intestine is shown in Fig. **(5)**. SGLT1 is expressed in the brush border membrane of the enterocyte, where it couples the transport of two sodium ions and one glucose molecule across the brush border membrane. The energy produced by the sodium electrochemical potential gradient across the brush border membrane is used to facilitate the glucose accumulation inside of enterocyte against its concentration gradient. The sodium ion that enters the cell along with glucose is then transported out into blood through Na/K-pump in the basolateral membrane, allowing to maintain the driving force for glucose transport. The accumulation of sugar into enterocytes generates a driving force to transport glucose from cells into the blood via GLUT2, expressed in the basolateral membranes of enterocyte. A fraction of the intracellular glucose seems to be taken up into endosomes, as glucose-6-phosphate, and then released into the blood by exocytosis through the basolateral membrane [195]. Otherwise, after ingestion of fructose, unlike glucose, an increase in the expression levels of GLUT5 mRNA was found [196]. It was also suggested that there may be a disaccharidase-related transport system which considers both fructose and glucose production from the enzymatic hydrolysis of sucrose [197]. Other evidence suggests that fructose is absorbed via a saturable carrier in the absence of glucose, while in the presence of glucose; fructose is absorbed via a disaccharidase-related transport system [197]. Besides this, passive diffusion across the intestinal epithelium has also been proposed as a possible mechanism [197]. Studies

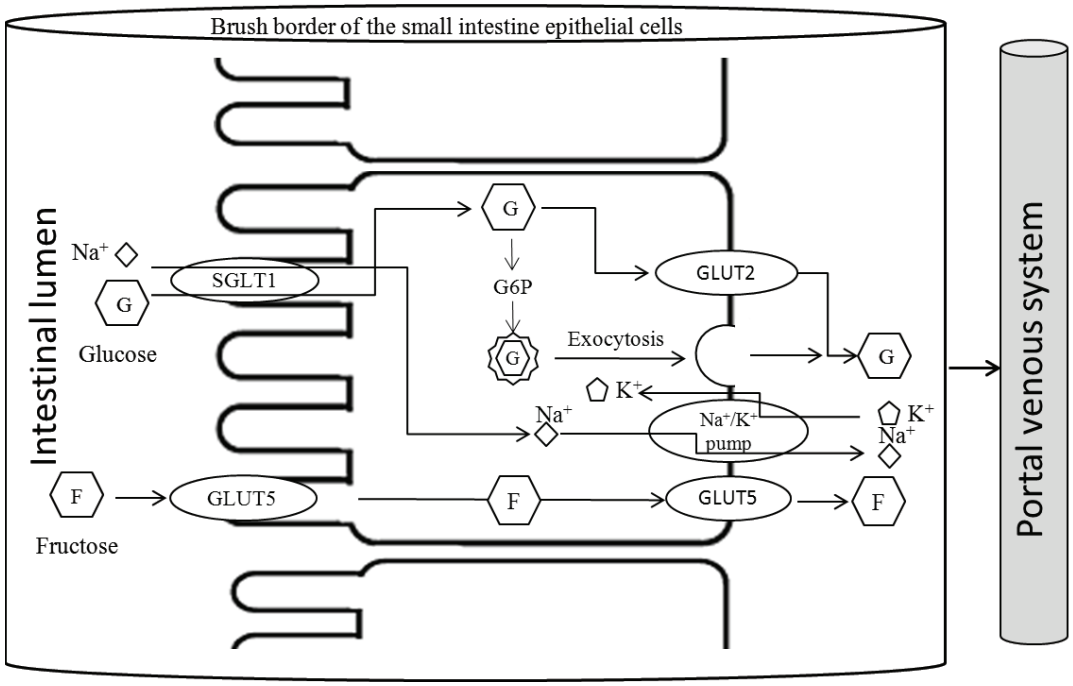


Fig. (5). Mechanisms for the absorption of glucose and fructose in the small intestine. F, fructose; G, glucose; GLUT2, glucose transporter; GLUT5, fructose transporter; G6P, glucose-6-phosphate; SGLT1, sodium-dependent glucose transporter.

have shown that glucose improves the transportation and absorption of fructose but not vice versa, increasing the amounts of fructose that reach the liver. According to the results of the investigations exposed above, the potential role of honey against diabetes mellitus is at an early stage, where more specific researches are needed to understand the mechanisms by which it can exert its hypoglycemic action. Even if these studies are still scarce, they have shown that honey is preferable to the most common sugars or sweeteners, because it is more tolerable both in healthy subjects and in patients with diabetes mellitus. Moreover, its consumption or its addition to other carbohydrates can be recommended in diabetic patients, because of its minimal incremental effect on blood glucose compared to other sweeteners or common sugars.

4.4 Antimicrobial Action of Honey

The antimicrobial activity of honeycan be divided into two fundamental mechanisms: (i) a non-peroxide antibacterial activity, mainly due to its high osmolarity and acidity as well as to methylglyoxal, bee defensing-1and flavonoid contents; (ii) a peroxide-associated antibacterial activity due to the specific hydrogen peroxide content [30, 198, 199].

The inhibition of microorganisms of clinical significance carried out by honey has been widely reported in many studies. Cooper *et al.* (2002) well established the antimicrobial activity of manuka and pasture honeys and made a comparison with an artificial honey solution using eighteen strains of methicillin-resistant *Staphylococcus aureus*, seven strains of vancomycin-sensitive enterococci, isolated from infected wounds, and 20 strains of vancomycin-resistant enterococci, isolated from hospital environmental surfaces. The authors reported that for all of the strains tested, the minimum inhibitory concentration of both honeys was below 10%, while the concentrations of artificial honey necessary to achieve equivalent inhibition *in vitro* were at least three times higher, thus confirming that the inhibition of bacteria by honey is not exclusively due to its osmolarity. In another study, the antimicrobial activity of five native monofloral Cuban honeys against four bacterial strains, two gram-positive (*Bacillus subtilis*, ATCC 6633 and *Staphylococcus aureus*, ATCC 25923) and two gram negative species (*Pseudomonas aeruginosa*, ATCC 27853 and *Escherichia coli*, ATCC 25922), was reported [80]. The results indicated that *S. aureus* was the most sensitive microorganism, while *P. aeruginosa* was the most resistant. Moreover, *B subtilis* and *E. coli* were moderately sensitive to the antimicrobial activity of honey. In general, the results of this study showed that Gram-positive bacteria were more sensitive to the honey antimicrobial action than Gram-negative bacteria. Other authors have also reported *S. aureus* as the most sensitive microorganism to honey antimicrobial action:since *S. aureus* has been reported as the causal agent of a range of illnesses from skin infections to life threatening diseases, such as pneumonia and meningitis, this is an important achievement that suggest to consider honey as a possible treatment against this agent [200-209]. Honey also presents inhibitory activity against *Pseudomoma aeruginosa*, *Bacillus anthracis* (anthrax), *Corynebacterium diphtherine* (diphtheria), *Klebsiella pneumoniae* (pneumonia), *Mycobacterim tuberculosis* (tuberculosis), *Salmonella typhi* (typhoid fever), *Vibrio cholerae* (cholera) [210].

As indicated above, the antibacterial activity of honey is due to the involvement of multiple compounds and to the contribution of individual components to its total antibacterial activity. Recently researchers have focused their attention on the presence of methylglyoxal, a component that contributes to honey non-peroxide antibacterial activity: methylglyoxal, (CH_3-CO-CH=O) is the aldehyde form of pyruvic acid formed by two carbonyl groups (Fig. **6**). This compound is formed from sugars during heat treatment or prolonged storage of carbohydrate-containing foods and beverages [211]. High levels of methylglyoxal have been found in manuka honey [212, 213], one of the components mainly responsible for its non-peroxide antibacterial activity [213]. Its high concentration in manuka honey is due to the conversion of dihydroxyacetone, which is present in great amounts in the nectar of*L. scoparium* flowers [214], and it occurs non-enzymatically at a slow rate during storage of honey. The study demonstrated a strong correlation between methylglyoxal levels and the potential of honey to inhibit the growth of *S. aureus*. In a different research, it was suggested that methylglyoxal may be fully responsible also for the non-peroxide antibacterial activity of manuka honey [212].

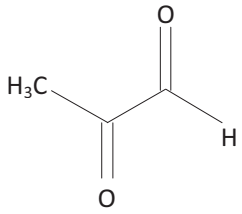


Fig. (6). Chemical structures of the methylglyoxal.

The amino- and carboxy-termini are labelled N and C, respectively

Another antibacterial component in honey is bee defensin-1 peptide (Fig. **7**). This peptide has been previously identified in honeybee hemolymph [215], honeybee head and thoracic glands [216] and in royal jelly [217], and a potent activity was reported against Gram-positive bacteria including *B. subtilis*, *S. aureus*, and *Paenibacillus larvae* [218]. Kwakman *et al.* [218] are the authors of the first report of this peptide in honey and they also investigated its antibacterial capacity in their recent analysis of unprocessed Revamil honey. Despite this interesting finding, the presence of bee defensin-1 in honeys has not been investigated systematically, and quantitative data on the concentration of this peptide in honey have not yet been established.

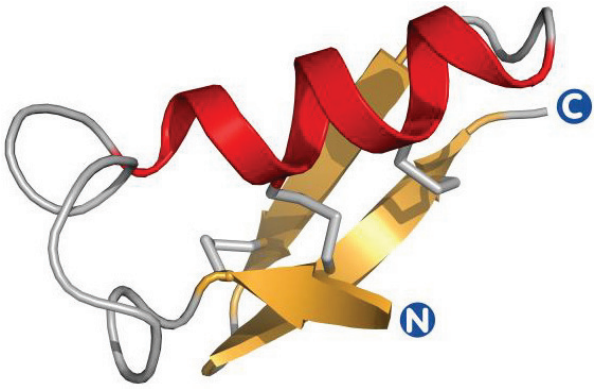


Fig. (7). Homology model of defensin-1 from *Apis mellifera*. The model of the mature protein (residues 44-94) was obtained using the experimentally resolved structure of lucifensin from *Lucilia sericata* (PDB ID: 2LLD) as a template. Alignment and modelling was performed using the SwissModel server (http://swissmodel.expasy.org). Disulfide bridges are shown as sticks.

Finally, the non-peroxide antibacterial activity of honey and its relationship with its own flavonoid composition have also been partially ascertained. Several antibacterial phenolic compounds have been identified in honeys [219-221], but their contribution to the antimicrobial activity of honey remains unclear. The use of flavonoids against bacterial infections has two purposes: (i) to kill the bacterial cells and (ii) to counteract the spread and the effects of the bacterial toxins [17]. The bactericidal effect of flavonoids appears to be the result of a metabolic perturbation related with ion channels, which are especially sensitive points of inhibition and likely targets of flavonoids [17]. Besides the active role that the flavonoids play in the destruction of infectants, they fortify loose connective tissues by inhibiting some of the enzymes that can hy-

drolyze their proteoglycan and protein meshwork, making the diffusion of infections through the tissue sterically difficult [17].

The incomplete knowledge of antibacterial compounds involved in the antibacterial activity of honey is an obstacle for wide clinical use of honey. In recent years, the knowledge on the antibacterial compounds in honey markedly increased. The results on honey antimicrobial properties are encouraging and demonstrate the potential uses of honey as an antibacterial agent, where its powerful activity against antibiotic-resistant bacteria could be an effective mode to counteract these agents.

CONCLUSIONS

Honeys are a natural source of phytochemical compounds mostly represented by polyphenols. The evidence of the biological action correlated to their polyphenolic content has been demonstrated. Honey compounds have been associated to antioxidant and anti-inflammatory actions, reporting cardiovascular, antiproliferative, and antimicrobial benefits. Although most health-promoting effects were initially observed with *in vitro* studies, there are increasing animal and clinical researches focused on translating the *in vitro* evidence into *in vivo* outcomes. A greater understanding of the mechanisms and factors governing the bioavailability of honey phytochemicals will be crucial to understanding the mechanisms by which honey exerts its beneficial effects on human health.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

AAPH	=	2,2´-azobis(2-methylpropionamidine dihydrochloride
ADMA	=	Dimethylarginine
AMPK	=	Adenosine monophosphate-activated protein kinase
AOC	=	Antioxidant capacity
ApoE−/−	=	Apolipoprotein E-deficient mice
Bcl2	=	B-cell lymphoma 2
CBG	=	Cytosolic β-glucosidase
COMTs	=	Catechol-O'Methyltransferase
CVD	=	Cardiovascular diseases
DDAH II	=	Dimethylarginine dimethylaminohydrolase II
EAT	=	Ehrlich ascites tumor
eNOS	=	Endothelial nitric oxide synthase
ERK	=	Extracellular signal-regulated kinases
GLUT2	=	Glucose transporter
GLUT5	=	Glucose transporter
GOx	=	Glucose oxidase
HDL-C	=	High-density lipoprotein
HeLa cells	=	Human cervical cancer cells
HL-60	=	Human promyelocytic leukemia cells
HUVECs	=	Human umbilical vein endothelial cells
KPOS	=	Kaempferol-3-*O*-sophoroside
LDL	=	Low-density lipoprotein
LDL-C	=	Low-density lipoprotein
LPC	=	Lysophosphatidylcholine
LPH	=	Lactase phlorizin hydrolase
MDA	=	Malondialdehyde
MRP-1	=	Multidrug-resistance-associated proteins
MRP-2	=	Multidrug-resistance-associated proteins
MRP-3	=	Multidrug-resistance-associated proteins
NF-κB	=	Nuclear factor kappa-light-chain-enhancer of activated B cell
NO	=	Nitric oxide
Nrf2	=	Nuclear factor (erythroid-derived 2)-related factor-2
OH	=	Hydroxyl group
PARP	=	Poly (ADP-ribose) polymerase
PKC	=	Cytosolic protein kinase C
RBC	=	Red blood cells
RDI	=	Recommended daily intake of energy
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
SBP	=	Systolic blood pressure
SGLT1	=	Sodium-dependent glucose transporter 1
SHR	=	Spontaneously hypertensive rats
SHR	=	Spontaneously hypertensive rats
SOD	=	Superoxide dismutase
STZ-induced	=	Streptozotocin-induced
SULTs	=	Sulfotransferases
TG	=	Triacylglycerides
TNF-α	=	Tumor necrosis factor-alpha
TPK	=	Two pore K^+
Trp-P-1	=	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole
UGT	=	Uridine-5`-diphosphate glucuronosyltransferases

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