Honey and Atherosclerosis

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Honey, a natural sweetener has been used universally as a complete food and in complementary medicine since early antiquity. Honey contains over 180 substances, including sugars mainly fructose and glucose, water and a plethora of minor constituents such as vitamins, minerals and phytochemicals. Atherosclerosis is a chronic disease occurring in the inner lining of arterial walls due to the progressive plaque formation. Multiple risk factors are implicated in the pathogenesis of atherosclerosis, including oxidative stress, inflammatory responses, hypercholesterolemia, hypertension, diabetes and cigarette smoking.

Honey composition antioxidants atherosclerosis inflammation oxidative stress cholesterol

1. Introduction

Atherosclerosis is a chronic disease occurring in the inner lining of arterial walls due to the progressive plaque formation [1]. Multiple risk factors are implicated in the pathogenesis of atherosclerosis, including oxidative stress, inflammatory responses, hypercholesterolemia, hypertension, diabetes and cigarette smoking [2][3] (**Figure 1**). The factors are interrelated and their interactions may intensify the chronic disease [4]. Different strategies developed to relieve the risk factors covering gene therapy, synthetic antioxidants, vitamins and drugs, but atherosclerosis is still a leading cause of death worldwide [1].

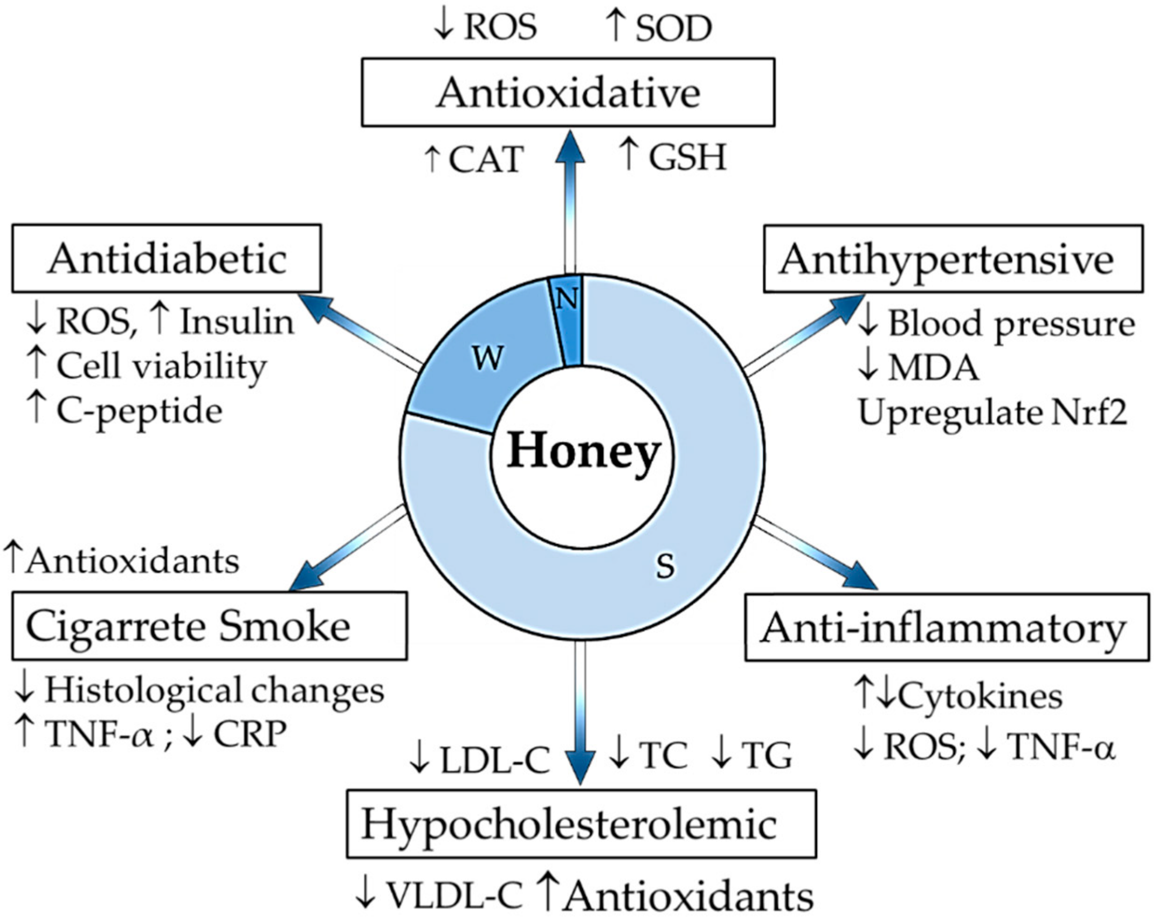

Figure 1. Summary of honey composition and its protective effects against risks in the pathogenesis of atherosclerosis. S: sugar components, W: moisture content, N: non-sugar components, ↓: decrease; ↑: increase; ROS: reactive oxygen species; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; MDA: malondialdehyde; Nrf2: nuclear factor erythroid 2-related factor 2; TNF-α: tumour necrosis factor alpha; LDL-C: low density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides, VLDL-C: very low density lipoprotein cholesterol; CRP: C-reactive protein.

2. Honey Composition and Antioxidant Activity

2.1. Honey Composition

Honey consists of over 180 components, including sugars, water and non-sugar components (**Table 1**) [5]. The sugar components in honey are mainly monosaccharides, particularly fructose (to 40%) and glucose (35.0%) in some honey types from Asia, Europe and Turkey, followed by a small quantity of disaccharides and higher sugars (<10%) [6]. Fructose and glucose in honey are derived from the chemical conversion of disaccharides in floral nectar by bee-secreted enzymes, where fructose is the highest proportion of any sugars in almost every honey type [7]. Sugars determine the physicochemical properties of honey such as viscosity, crystallization, thermal and rheological behaviour [8]. Sugars in honey provide an energy value of 300 kcal/100 gram honey, which is equivalent to 15% of recommended daily intake of energy [5]. Significantly, fructose contributes the highest proportion in almost every honey types (up to 45.0%) and it is a sweetest sugar among the natural sugars [7]. However, fructose has a lower glycaemic index (GI), compared to sucrose and glucose (GI at 15, 65 and 100, respectively) [9][10][11]. Since carbohydrate-containing foods are rated according to their GI, where low GI foods are absorbed more slowly from the gastrointestinal tract, fructose-rich honey varieties may be considered as a beneficial alternative to high GI sweeteners in management of diabetes and cardiovascular diseases [5][12].

Table 1. Chemical composition of honey per 100 g [5].

Proximates (g)		Minerals (mg)		Vitamins (mg)	
Fructose	38.2	Calcium	3–31	Ascorbic acid	2.2–2.5
Glucose	31.3	Potassium	40.0–3500.0	Thiamin	0.0–0.01
Sucrose	0.7	Copper	0.02–0.60	Riboflavin	0.01–0.02
Other disaccharides	5.0	Iron	0.03–4.00	Niacin	0.1–0.2
Water	17.1	Magnesium	0.7–13.0	Pantothenic acid	0.02–0.11
Organic acids	0.5	Manganese	0.02–2.0	Pyridoxine (B6)	0.01–0.32
Proteins, amino acids	0.3	Phosphorus	2.0–15.0		
		Sodium	1.6–17.0		
		Zinc	0.05–2.00		
		Se	0.001–0.003		

The non-sugar components are at minor quantities, but they define a particular type of honey and bioactives, depending on the level of vitamins, minerals, antibiotic-rich inhibine, carotenoids, free amino acids, enzymes, proteins, Maillard reaction products and phenolic compounds present in honey composition [13][5]. Enzymes including invertase (saccharase), diastase (amylase), glucose oxidase and catalase play a critical role in honey formation. Particularly, invertase converts sucrose into monosaccharides, glucose oxidase catalyses hydrogen peroxide formation and catalase (CAT) supports the oxygen and water formation from hydrogen peroxide.

Interestingly, during nectar and pollen forage, honey bees transform phytochemicals from floral nectars of host plants into honey. The diversity of secondary metabolites in plants attributes to the variance phytochemical profiles in honey composition [14]. Phytochemicals in honey are mainly phenolic acids, flavonoids and their derivatives. Phenolic acids (e.g. caffeic, chlorogenic, coumaric, ellagic, ferulic, gallic, homogentisic, phenyllactic, protocatechuic, syringic and vanillic acids) comprise hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids exert antioxidant capacity (AOC) based on the positions of OH groups in the aromatic ring, with gallic acid (3, 4, 5-trihydrozybenzoic acid) as the most effective antioxidant in this group [15]. Hydroxycinnamic acids present greater free radical scavenging ability because of the unsaturated chain bonded to the carboxyl group, imparting stability to the phenoxyl radical group. Hydroxycinnamic acids offer multiple hydroxyl groups to combat free radicals. In addition, the electron donor groups present in the benzene ring provide a greater number of resonant structures and increase the stability of the acrylic radicals in cinnamic acids [15][16].

Flavonoids (apigenin, chrysin, galangin, hesperetin, kaempferol, luteolin, myricetin and quercetin) consist of two aromatic rings A and B, joined by a 3-carbon link, usually in the form of a heterocyclic ring C [15]. Variations in the ring C result in different flavonoid classes, including flavonols, flavones, flavanones, flavanols, isoflavones, flavanonols and anthocyanidins. Substitutions in rings A and B generate diverse compounds in each flavonoid class [17]. Depending on the molecular structures, phenolic compounds exert antioxidant capacity (AOC) in different action modes such as metal chelators, free-radical scavengers or gene modulators of enzymatic and non-enzymatic systems regulating cellular redox balance [18]. The presence of a specific phytochemical or combination thereof in honey may potentially serve as a marker for geographical and botanical origin of honey [19][20]. For examples, methylglyoxal is in manuka honey, hesperetin in citrus honey, quercetin in sunflower honey and luteolin in lavender honey [21][20][22][23]. The structures of common phenolic compounds in honey are presented in **Figure 2**.

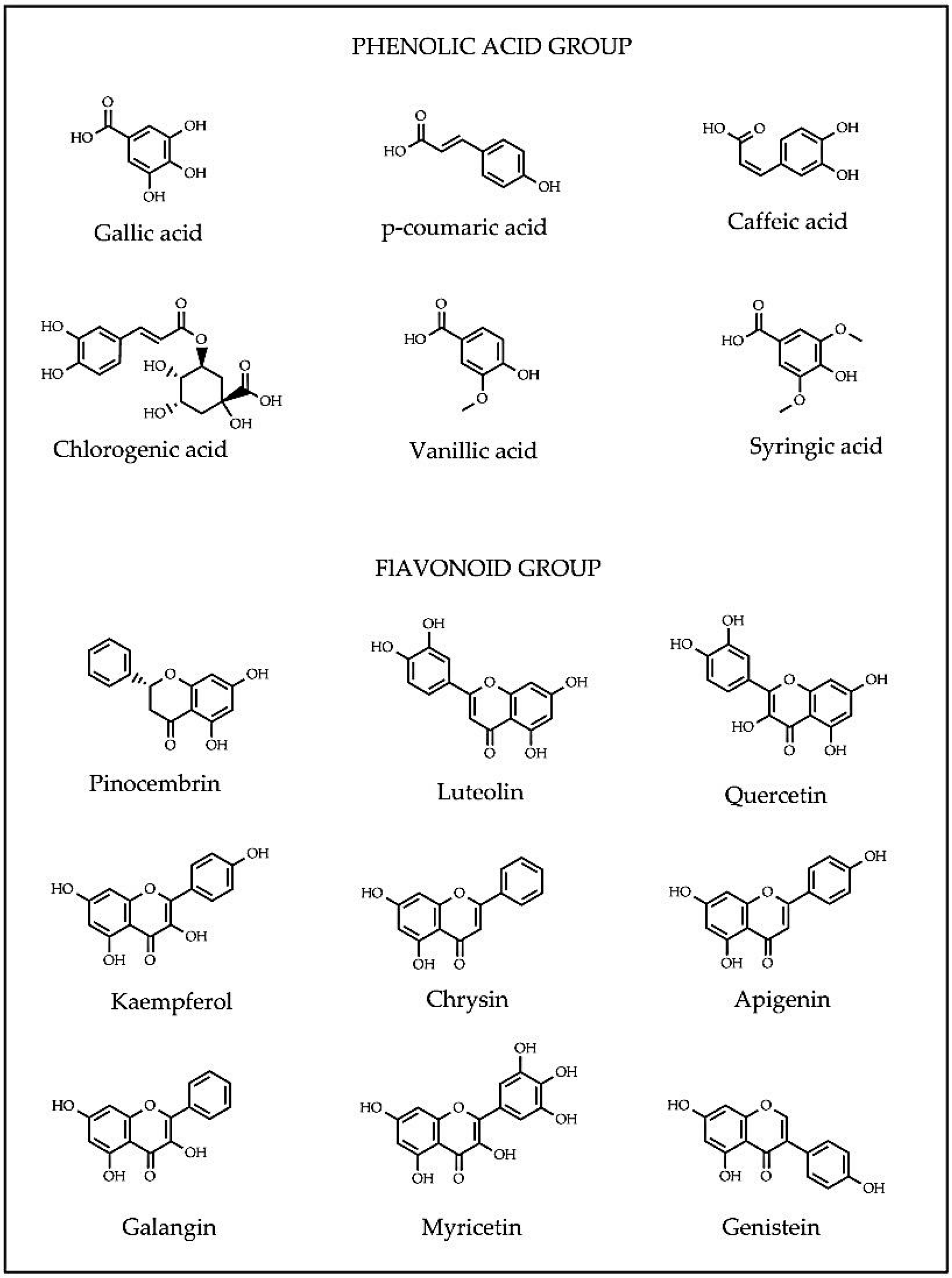

Figure 2. Common phenolic acid and flavonoid compounds identified in honey.

During pollen and nectar forage, bees are exposed to the vegetation, soil, climate and water conditions located approximately within seven km^2 in the vicinity of their hives [24]. The presence or deficiency of a particular element from the environment may be noticeable in the honey. Thus, the composition profile of honey not only reflects the quality and origin, it is also a bio-indicator of the environment [25].

To sum up, honey composition is complex and variable depending on its botanical and geographical origin. Each constituent has its nutritional, biological and technological functions. They synergistically contribute to the overall utility of honey, making honey unique and superior to other natural sweeteners in providing energy and health benefits.

2.2. Key Compositional Standards

The variations in honey's composition, bee species, seasonal and storage conditions highlight the need for the quality standardization of different honey types. Key compositional criteria have been specified as common quality norms for commercial honey in both European Directive and in the Codex Alimentarius standard [26][27] (**Table 2**).

Table 2. Key compositional standards of blossom honey [26].

Criteria	Values
Moisture content (%)	≤20.0
Fructose and glucose (Sum, g/100 g)	≥60
Sucrose (g/100 g)	≤5.0
Water-insoluble content (g/100 g)	<0.1
Electrical conductivity (mS/cm)	≤0.8
Free acid (meq/kg)	≤50.0
Diastase activity (Schade scale)	≥8.0
Hydroxymethylfurfural (HMF, mg/kg)	≤40.0

2.3. Antioxidant Capacity

The antioxidant capacity (AOC) of honey was reported to be the synergistic effect of mainly phenolic compounds along with other constituents in honey composition [28][29]. Considerable AOC values are well documented for a broad range of honey types from different botanical and geographical origins [30][31][23][32][33][34][35][36][37][38][39][40][41]. This notion was further supported by the fact that AOC value of honey is highly correlated to its phenolic content and colour intensity [42][43][29]. Interestingly, oxygen radical absorbance capacity (ORAC) value of honey was suggested to be equivalent to that of many fresh fruits and vegetables (3–17 µmol Trolox equivalent (TE)/g and 0.5–19 µmol TE/g fresh weight, respectively) [29].

The AOC of a sample is the basis for the quality comparisons, controls and the treatment of associated diseases [44]. The AOC of honey has been extensively examined using a number of popular chemical assays such as total phenolic content, free radical scavenging using 2,2-Diphenyl-1-picrylhydrazyl, trolox equivalent antioxidant capacity and ORAC among the others [42][39][45]. Findings from the assays, however are indicative of limits in either elucidating the total AOC due to the complexity of chemical components and the unique action mode of antioxidants [29] or potential bioactivity under physiological conditions [46]. Therefore, *in vitro, in vivo* and clinical evidence are crucial for further understanding not only AOC but also other biological activities of honey in providing health benefits, particularly attenuating the pathogenesis of atherosclerosis.

3. Honey in Relieving Multiple Facets of Atherosclerosis

3.1. Oxidative Damage

Oxidative stress occurs as a pathological condition due to an excessive generation of radical species over antioxidant defence system [47]. The radical species are represented by superoxide anion radical, hydroxyl, alkoxyl and lipid peroxyl radicals, nitric oxide and peroxynitrite [48]. They attack the cells, oxidize and damage proteins, lipids and deoxyribonucleic acids (DNA) randomly under stress conditions and excessive levels. Organisms have developed self-defence mechanisms towards neutralizing free radicals including repairing, physical defence and antioxidant systems. Enzymatic antioxidants are represented by superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Non-enzymatic antioxidants include ascorbic acid, α-tocopherol, glutathione (GSH), carotenoids, flavonoids and other antioxidants. The balance between the defence systems and free radical species generation is critical for their vitality [4]. The honey's effects on oxidative stress have been the focus of several studies (**Table 3**). The mechanisms through which honey exerts the protection against oxidative damage resides in (i) antioxidant enzymes in its composition (such as catalase), (ii) phenolic compounds which chelate mental elements, trap or scavenge free radical species and induce cellular enzymatic and non-enzymatic antioxidant systems [43][21][18].

Table 3. Effects of honey on oxidative stress.

Honey Type	Research Model	Main Findings on Honey Effects	Reference(s)
Local honey	Rat kidney, brain, liver and lung homogenates	↓ Lipid hydroperoxides and malondialdehyde (MDA) value	[49]

Honey Type	Research Model	Main Findings on Honey Effects	Reference(s)
Christmas vine, Morning glory, black mangrove, linen vine singing bean honey	Rat liver homogenates	Highest radical scavenging capacity in linen vine honey ↓ Lipid peroxidation	[50]
Fireweed, tupelo, Hawaiian Christmas berry clover, acacia, buckwheat, soybean honey	Human blood serum	AOC is different among honeys, ↓ Lipoprotein oxidation (LPO) Correlation of ORAC value and LPO inhibition.	[29]
Acacia, coriander, sider and palm honey	Human LDL	High antioxidant activity in xanthine-xanthine oxidase system and LDL oxidation	[51]
Buckwheat honey	Human blood serum	↑ Serum antioxidant capacity	[52]
Multifloral honey	Human red blood cells (RBC)	↓ Lipid peroxidation	[53]
Multifloral honey	RBC	↓ Extracellular ferricyanide level	[54]
Christmas vine, linen vine honey	RBC	Protection of human erythrocyte membranes from oxidative damage ↑ Defence responses and ↑ cell functions	[50][55][56]
Native multifloral honey	Endothelial cell (EA.hy926)	Protection of EA.hy926 from hydrogen peroxide and peroxyl radical Synergistic effect of phenolic antioxidants in honey	[57]

Honey Type	Research Model	Main Findings on Honey Effects	Reference(s)
Gelam honey	Rat blood sample	↑ Antioxidant enzyme activities	[58]
Multifloral honey	Rat plasma and heart tissue	↓ Hypertriglyceridemia and pro-oxidative effects ↑ Plasma α-tocopherol and α-tocopherol/triglycerides, ↓ plasma NOx, ↓ peroxidation	[59]
Buckwheat honey	Human blood plasma	↑ Plasma antioxidant activity, ↑ defences against oxidative stress	[60]

AOE: antioxidant capacity, ORAC: oxygen radical absorbance capacity, LDO: lipoprotein oxidation, LDL: low density lipoprotein, RBC: Human red blood cells, TG: triglycerides, NOx: nitrogen oxides.

3.2. Inflammatory Responses

Inflammation reflects a pathophysiological response of tissues characterized by signs of pain, heat, redness and swelling [61], however, prolonged inflammation is the cause of several chronic diseases such as diabetes, dyslipidaemia, hypertension, cardiovascular, obesity and pulmonary conditions. Under inflammatory conditions, mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) pathways are activated, triggering several important proinflammatory markers including cyclooxygenase-2 (COX-2), lipoxygenase 2 (LOX-2), C-reactive protein (CRP), interleukins (IL-1, IL-6 and IL-10) and tumour necrosis factor alpha cytokine (TNF-α) [62]. Honey was found to modulate the inflammatory response in the pathogenesis of atherosclerosis through distinct inhibitory paths of (i) proinflammatory markers such as cytokines, COX-2, CRP and TNF-α [63][64][65][66] and (ii) ROS generation [67].

It was reported that the anti-inflammatory activity of honey is contributed by phenolic compounds and other minor constituents in its composition [67][68][69][70]. Kassim et al. detected a range of phenolic compounds, including chrysin, quercetin, ferulic acid, ellagic acid, hesperetin in Gelam honey. This honey reduced cytokine (TNF-α, IL 1β and IL 10) and NO levels but increased heme oxygenase-1 levels. Thus, the honey was recommended to be further investigated for treatment of different inflammatory diseases [66]. Some phenolic compounds have been individually examined for their anti-inflammatory activity. Chrysin was reported to suppress lipopolysaccharide-induced COX-2 in Raw 264.7 cells [63]. Luteolin was found to reduce intercellular adhesion molecule-1 and TNF-α

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and endothelial leukocyte infiltration in tissues [65]. Quercetin was demonstrated to reduce human CRP expression and also serum amyloid A and fibrinogen which are cardiovascular risk factors in mice [64].

The findings are supportive to a study on the anti-inflammatory effect of a natural honey type on bovine thrombin-induced oxidative burst in human neutrophils and rodent macrophages. It has been known that the accumulation of phagocytes, ROS production and thrombin activation occur at the sites of endothelial damage [67]. It was demonstrated that bovine thrombin activated phagocytes to produce ROS which might amplify the inflammatory responses at the site of atheromatous plaques. However, honey treatment suppressed the thrombin-induced ROS generation by the phagocytes. The findings suggested a beneficial role of honey in the pathology of atherosclerosis, particularly in ROS-induced LDL oxidation and cell signalling [67].

3.3. Hypercholesterolemia

Cholesterol is an indispensable molecule in growth and development of animal and human cells. It fulfils vital functions such as a cell membrane component, a precursor for steroid hormones and bile acids and an activator in cell signalling pathways [71]. Cholesterol is combined with lipoproteins so that they are transported from one tissue to the others throughout the body. Lipoproteins are divided into high density lipoprotein (HDL), low density lipoprotein (LDL) and very low-density lipoprotein (VLDL), thus cholesterol (C) is classified accordingly into HDL-C (good cholesterol), LDL-C and VLDL (bad cholesterols) [72].

A high level of LDL-C is the main cause of plaque formation in blood vessels, which when occurred in coronary arteries, it results in blockages and heart attacks [73]. In addition, a marked elevation of lipid oxidation products and/or a reduction in plasma antioxidants promotes hypercholesterolemia [74]. Use of dietary antioxidants combined with physical exercises has been recommended as a premised lifestyle approach to control cardiovascular risks in general and cholesterol levels in particular [75].

Containing an abundant source of phenolic compounds [13][7][76], honey has been shown to improve lipid profile, particularly cholesterol levels (**Table 4**). The exact mechanism of honey in the improvement of this risk factor has not been clearly determined. However, phenolic compounds present in honey are reportedly associated with improvement of coronary vasodilation, prevention of blood clots and protection of LDL-cholesterol from oxidation [77]. Several natural phenolics have been reported to reduce cholesterol, including quercetin-3-β-D-glycoside, vanillin rich fraction and luteolin among the others. The phenolic compounds have been known to (i) decrease cholesterol level through the inhibition of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase which is a crucial rate limiting enzyme in cholesterol biosynthesis, and/or (ii) modulate plasma LDL-C via the upregulation of LDL-receptor (LDLR) expression, of which LDLR is a cell surface glycoprotein important to the hepatic uptake and removal of plasma cholesterol [78][79][80][81]. It has been demonstrated that honey is a potential alternative for sucrose intake in individuals with poor glycaemic control and/or coronary heart disease. In a study, the long-term 52 week consumption of honey did not result in any differences in LDL-C, triglyceride (TG) or total cholesterol (TC) levels among the rat groups. However, honey diet revealed a significant increase in HDL-C levels (16% to 21%) in honey diet rats, compared to sucrose ($p = 0.044$) or sugar-free diet group ($p = 0.006$) [82].

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Table 4. Effects of honey on lipid profile.

Honey Type	Research Model	Main Findings of Honey Effect	Reference(s)
Honeydew honey	Rat blood serum	Similar weight gain and body fat in honey and control group; ↓ HbA1c, ↑ HDL-C	[82]
Clover honey	Rat blood serum	↓ Weight gain and adiposity, ↓ TGs but ↑ non-HDL-C levels	[83]
Native honey	Rat blood samples	↓ glucose and lipids no deteriorated effects on hyperglycaemia and dyslipidaemia	[84]
Local honey	Rat blood serum	↑ Plasma TG, HDL-C and VLDL-C but ↓ plasma LDL-C and TC	[85]
Tualang honey	Rat heart tissue	↑ Antioxidant enzyme levels in heart tissue and ↓ lipoprotein oxidation (LPO)	[86]
Tualang honey	Rat blood serum, kidneys	↓ TC and TG compared to the control at 7 days; ↓ Serum creatinine level than no honey group after 48 h; No structural effect histologically in the HCD-fed rats	[87]
Gelam, Acacia honey	Rat blood serum, internal organs	↓ Excess weight gain and adiposity index; ↓ plasma glucose, TGs, TG and	[88]

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Honey Type	Research Model	Main Findings of Honey Effect	Reference(s)
		obesity at similar levels to orlistat drug group	
Malícia honey	Rat blood serum, liver	↓ Food consumption, ↑ glucose tolerance and SOD activity; ↓ TC, LDL and AST levels; ↑ beneficial bacteria and organic acids; Colon and liver was protected	[89]
Natural local honey	Healthy, diabetic and hyperlipidaemic human subjects, blood samples	↓ Blood lipids, homocysteine and C-reactive protein (CRP) in normal and hyperlipidaemic subjects; ↓ plasma glucose elevation in diabetics	[76]
Natural honey	Human plasma	↓ TC (3.3%), LDL-C (4.3%), TGs (19%) and CRP (3.3%) in elevated variable subjects; No increased body weight in overweight or obese participants	[90]
Natural unprocessed honey	Type 2 diabetes human subjects, weight and blood samples	↓ Body weight, TC, LDL-C, TGs ↑ HDL-C and HbA1C levels	[10]
Kanuka honey, formulated with cinnamon, chromium and magnesium	Type 2 diabetes human subject, weight and blood samples	↓ Weight Improve blood lipid profile	[91]

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HbA1c: Haemoglobin A1c, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, VLDL-C: very low density lipoprotein cholesterol, TC: total cholesterol, TGs: triglycerides, LPO: lipoprotein oxidation, HCD: high cholesterol diet, AST: aspartate aminotransferase, CRP: C-reactive protein.

3.4. Hypertension

Hypertension is closely implicated in the pathogenesis of atherosclerosis. Recent studies which reported honey reduced systolic blood pressure and MDA levels in hypertensive rats [92] and alleviated the susceptibility of rat kidneys to oxidative damage through upregulating the expression of erythroid 2-related factor 2 (Nrf2), an important transcription factor regulating antioxidant defences in chronic renal failure of hypertensive rats [93]. The results have indicated that the protective effect of honey on hypertensive rats is mainly contributed by its antioxidant and anti-inflammatory activity.

3.5. Diabetes

Diabetes is implicated in inflammation, oxidation and glycation. Therefore, strong antioxidant agents potentially limit the pathogenesis of diabetes and the associated complications [94]. Gelam honey extract has been found to protect pancreatic hamster cells from hyperglycaemic conditions. Significantly, this honey decreased ROS production, glucose-induced lipid peroxidation, increased insulin content and the cell viability under hyperglycaemic conditions [95]. The findings were supported by an investigation of Jujube honey for its role in modulation of the main enzymes participating in glucose metabolism namely glucokinase and glucose 6-phosphatase in rats. Jujube honey was found to reduce MDA levels while improving the total AOC in diabetic rats ($p < 0.05$). It also decreased heat shock protein (HSP70) and glucose 6-phosphatase expressions while increasing the glucokinase expression [96].

Moreover, a pilot study with 20 patients with type 1 diabetes and 10 healthy controls showed honey treatment reduced glycaemic index and the peak increment index in both patients ($p < 0.001$) and control ($p < 0.05$) groups compared to sucrose. In this study, honey significantly increased C-peptide level, compared to either glucose or sucrose in the control group. The results suggested honey may be used as a sugar substitute for patients with type 1 diabetes [97]. Collectively, the findings suggested potential effect of honey on diabetes management in animal models should be translated into larger clinical trials for type 1 diabetic patients.

3.6. Cigarette Smoking

The active or passive exposure to cigarette smoking is implicated in all stages of atherosclerosis and complicates cardiovascular events [98]. Tualang honey was examined for its protective effect on rats exposed to cigarette smoke. It was found that honey protected rat testis from oxidative stress caused by tobacco smoking. The honey decreased the histological changes and lipid peroxidation, but it increased the total antioxidant levels and recovered the activity of antioxidant enzymes, particularly glutathione peroxidase, SOD and catalase in the cigarette smoke-exposed rats [99].

The findings were further supported by a recent study which examined the effect of a 12-week honey administration on plasma inflammatory markers such as highly sensitive CRP, IL-6 and TNF-α among 32 non-smokers and 64 chronic smokers [100]. The study reported that TNF-α was significantly increased, but CRP

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expression was significantly reduced at post-intervention among smokers with honey group. These indicated that effects of honey on TNF-α and CRP are opposite, thus it raises the needs for further investigations on the inclusive effect of honey on inflammation among chronic smokers.

4. Adverse Effects of Honey

Despite the nutritional and medicinal values, honey is prone to microbial and non-microbial contaminations. Several microorganisms including bacteria, moulds, yeast from pollen, bee intestine, human, equipment, containers and dust may infect honey. However, honey has antimicrobial properties due to the synergistic contributions of saturated sugars (~80%), acidic pH, bee defensin-1, inhibines (hydrogen peroxide, flavonoids and the phenolic acids) and low water activity [101][102]. However, spore-forming bacteria can resist for over a year in honey at low temperature [103][104], particularly the *Clostridum botulinum* causing botulism poisoning was detected in many countries [103][105][106][107]. Thus, raw honey that was not sterilized or qualified should not be used for infants. It was also recommended that *Clostridia* spores need to be eliminated from honey using gamma irradiation, a sterilization process which is not interfered with antibacterial activity of honey [108].

In addition, honey may contaminate with traces of pesticides, herbicides, antibiotics or heavy metals due to the bee disease control and the exposure of honey bees to environment [104]. Honey also may contain poisonous compounds, particularly grayanotoxins found in mad honey which originates from *Andromeda* flowers [109]. Thus, honey needs to be subjected to quality analysis and labelling regulations. Moreover, honey production and processing have to comply with standard protocols and legislation to assure its safety.

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