Therapeutic Strategies of Plant-derived Compounds for Diabetes Via Regulation of Monocyte Chemoattractant Protein-1

Magdalena Czemplik¹,*, Anna Kulma², Yu Fu Wang³ and Jan Szopa²,4,5

¹Department of Physico-Chemistry of Microorganisms, Institute of Genetics and Microbiology, Faculty of Biological Sciences, University of Wrocław, Wrocław, Poland; ²Faculty of Biotechnology, University of Wrocław, Wrocław, Poland; ³Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha City, China; ⁴Department of Genetics, Plant Breeding and Seed Production, Wrocław University of Environmental and Life Sciences, Wrocław, Poland; ⁵Linum Foundation, Wrocław, Poland

Abstract: Background: Monocyte chemoattractant protein-1 (MCP-1) is a member of the CC chemokine family that plays a key role in the inflammatory process. It has been broadly studied in the aspect of its role in obesity and diabetes related diseases. MCP-1 causes the infiltration of macrophages into obese adipose tissue via binding to the CCR2 receptor and is involved in the development of insulin resistance.

Methods: We reviewed the available literature regarding the importance of plant metabolites that regulate MCP-1 activity and are used in the treatment of diabetic disorders. The characteristics of screened papers were described and the important findings were included in this review.

Results: This mini-review provides a summary of functions and therapeutic strategies of this chemokine, with a special focus on plant-derived compounds that possess a putative antidiabetic function via a mechanism of MCP-1 interaction. The highlights of this review include the roles of MCP-1 in development of diabetes, the evaluation of plant metabolites that specifically or non-specifically inhibit MCP-1 overproduction, and the molecular mechanisms of this activity. Among these metabolites, we particularly focused on phenolic acids and their derivatives, flavonoids, stilbenes, anthocyanins, capsaicin, alkaloids, plant sterols, terpenes, saponins, unsaturated fatty acids and plant-derived extracts.

Conclusion: Regarding the increasing number of diabetic patients yearly, the recent progress in the putative therapies needs to be summarized. This article underlines the significance and involvement of the chemokine MCP-1 in the development of obesity, type 2 diabetes, and diabetic complications, with an emphasis on the role of plant metabolites in the regulation of this chemokine and thus the role in the prevention or therapy of diabetes. We suggest that MCP-1 might be a molecular marker of type 2 diabetes.

Keywords: Diabetes, monocyte chemoattractant protein-1 (MCP-1), CCR2, plant extracts, plant metabolites.

1. MCP-1 CHEMICAL STRUCTURE

Monocyte chemoattractant protein-1 (MCP-1) is produced by different cell types crucial for the immune response, such as fibroblasts, epithelial, smooth muscle, astrocytic, monocyte, mesangial and microglial cells. Nevertheless, monocytes or macrophages are thought to be the major source of MCP-1 chemokine [1-3]. MCP-1 is the first discovered human chemokine, which has been intensively studied recently, as it was shown to be a crucial factor for the treatment of various diseases such as atherosclerosis [4], rheumatoid arthritis [5] and diabetes [6]. It was primarily purified from human cell lines on the basis of its monocyte chemoattractant properties. It is a member of the chemokine-
beta family of cytokines, which tend to attract and activate monocytes [7]. The human mcp-1 gene is located on chromosome 17 (chr. 17, q11.2), and the MCP-1 protein is composed of 76 amino acids and is 13 kDa in size [3, 8]. It contains structural domains typical for all chemokines: a flexible N-terminal domain, constrained by disulfide bonds; a long loop that leads into three antiparallel β-pleated sheets; and an α-helix that overlies the sheets [9]. Zhang et al. showed that the amino-terminal region is necessary for MCP-1 activity, and the carboxyl-terminal α-helix is not required for signaling per se but is required for maximal specific activity [10]. The presence of four cysteine residues that form intramolecular disulphide bridges enables the peptide folding to be stabilized. Two regions of the primary structure have been identified as crucial for the activity of MCP-1, namely the sequence Thr-Cys-Cys-Tyr (amino acids 10-13) and Ser-34 and Lys-35. Point mutations of Thr-10 to Arg and Tyr-13 lead to significantly lowered MCP-1 activity, and insertion of a Pro between these two residues, or their substitution by the sequence Gly-Pro-His, caused nearly complete loss of MCP-1 activity [11]. It was shown that MCP-1 forms dimers at physiological concentrations and wild-type homodimers interact with the MCP-1 receptor and generate a signal [12].

MCP-1 is glycosylated in human and murine cells, which affects the biological activity of this chemokine. The glycosylated form of MCP-1 shows lower chemoattractant activity for monocytes and lymphocytes, but exhibits enhanced functional stability compared with nonglycosylated MCP-1 [13, 14].

2. MONOCYTE RECRUITMENT MECHANISMS

The general mechanism of chemokines’ activity combines their secretion in response to signals, and the consequent selective recruitment of monocytes, neutrophils, and lymphocytes. Once induced, they direct migration of the cells that express the appropriate chemokine receptors, which occurs along a chemical ligand gradient Fig. (1). As a consequence, the cells move toward high local concentrations of chemokines [15]. MCP-1 chemokine activity is mediated by the CCR2 receptor, which is expressed in different leukocyte populations of cells. CCR2 consists of two isoforms, CCR2A and CCR2B, derived from a single gene with alternatively spliced carboxyl tails [16]. CCR2B is the dominant form in human monocytes, and CCR2A in mononuclear cells and vascular smooth muscle cells [17]. If the CCR2 expression is enhanced, the MCP-1–induced monocyte chemotaxis increases several folds, which accelerates monocyte accumulation. The CCR2 expression is highly regulated and depends on different factors. It was demonstrated that proinflammatory cytokines such as IFNγ, IL-1, or TNF decreased CCR2 gene expression, which retained monocytes at sites of inflammation and prevent their reverse transmigration [18]. Macrophage maturation exhibited similar effects on CCR2 expression, and differentiated macrophages could not migrate in response to MCP-1. In contrast, IL-2 increased the expression of CCR2 and augmented the chemotactic capacity of resting natural killer cells and T lymphocytes [19]. It is also suggested that HDL may inhibit monocyte CCR2 expression and reduce the chemotaxis mediated by MCP-1. Although the exact mechanisms by which lipoproteins affect CCR2 gene expression is still unclear, but intracellular cholesterol is thought to play a regulatory function. Incubation of monocytes with LDL or free cholesterol increased CCR2 expression, and lowering the level of cellular cholesterol reduced its expression. There is also a significant correlation between plasma LDL levels and monocyte CCR2 expression, but only in individuals with low HDL levels. High levels of HDL (50 mg/dl) significantly reduced the LDL-induced expression of CCR2 in hypercholesterolemic subjects [20]. In contrast, monocyte CCR2 biosynthesis was not affected by triglycerides.

It was found that CCR2 decreases after the monocytes have differentiated into macrophages [21]. MCP-1 recruits monocytes to the inflammation site [22]. The putative mechanism of this action assumes that MCP-1
dimerizes and associates with glycosaminoglycan in tissues, which establishes a gradient for redirection of monocytes towards sites of inflammation. When the amino acid substitutions in MCP-1 were performed to prevent dimerization or association with glycosaminoglycan, the recruitment processes were impaired [23]. MCP-1 plays a critical role in the recruitment of monocytes to sites of immune responses, which was confirmed by an MCP-1-deficient mouse study [24].

3. KEY ASPECTS OF TYPE 2 DIABETES ETIOLOGY

95% of diabetic cases are classified as type 2 diabetes, and its etiology is closely related to obesity and insulin resistance.

Insulin resistance involves the decreased ability of tissues to respond to insulin activity. Adipose tissue is an insulin-responsive tissue, but in the event of excessive accumulation of triacylglycerol in adipose tissue, insulin resistance occurs. Free fatty acids (FFAs) play an important role in induction of insulin resistance. FFAs concentration in blood is increased in obese people. Excessive accumulation of FFAs inhibits glucose uptake, as it impairs the functioning of signal pathways regulated by diacylglycerol. It was shown that chronic exposition of beta cells of the pancreatic islets on high FFAs concentration leads to insulin resistance and to the inhibition of insulin release [25]. Moreover, alteration in functioning of nuclei PARP receptors, as well as alteration in secretion of adipokines or proinflammatory cytokines, are suggested to contribute to insulin resistance [26, 27]. As a result of metabolic overload, the functioning of mitochondrial enzymes is impaired. The increased concentration of non-esterified fatty acids (NEFA) leads to the alterations in lipid metabolism and accumulation of acetyl-CoA in the cells, which influences key enzymes of Krebs cycle activity and inhibits glycolysis [28]. Insulin resistance may lead to severe effects, as insulin enables storage of triglycerides in adipose tissue by promoting the differentiation of preadipocytes to adipocytes, increasing the uptake of glucose and fatty acids derived from circulating lipoproteins and lipogenesis in mature adipocytes, and inhibiting lipolysis [29].

One of the crucial factors contributing to the development of type 2 diabetes is obesity-associated systemic inflammation. In humans, it is characterized by the infiltration of macrophages into adipose tissue and the liver in combination with an increase in body weight [30], which is positively correlated with insulin resistance. This state is characterized by increased circulating concentrations of pro-inflammatory cytokines and chemokines as well as the activation of several kinases that regulate inflammation such as including c-Jun NH2-terminal kinase (JNK), IκB-kinase β (IKKβ)/nuclear factor xB (NF-κB), and mammalian target of rapamycin (mTOR)/S6 kinase (SK6), which affect insulin metabolism in adipocytes and hepatocytes [26]. A crucial role in obesity-associated systemic inflammation is played by the adipose tissue macrophages, which are a prominent source of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, that can block insulin action in the liver, adipose tissue, and skeletal muscle via autocrine and/or paracrine signaling. Consequently, this causes systemic insulin resistance [31].

Reactive oxygen species (ROS) are known to play a crucial role during the pathogenesis of type 2 diabetes. They are produced under diabetic conditions in various pathways: during the non-enzymatic glycosylation reaction [32], the electron transport chain in mitochondria [33], and membrane-bound NADPH oxidase [34]. It is known that membrane-bound NADPH oxidase is one of the major sources of ROS in the vasculature and that NADPH oxidase-derived ROS play a critical role in the development of atherosclerosis. Under diabetic conditions, ROS are also involved in an increase of pancreatic β-cell dysfunction. These cells are vulnerable to ROS due to the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase. The production of ROS decreases insulin gene expression and secretion and finally brings about apoptosis of β-cells. Moreover, it has been reported that ROS are involved in the progression of insulin resistance [35]. ROS disrupted insulin-induced cellular redistribution of insulin receptor substrate-1 (IRS-1) and phosphatidylinositol-3-kinase (PI 3-K), and thus impaired insulin-induced GLUT4 translocation in 3T3-L1 adipocytes [36].

An interesting relationship of the effects of electromagnetic fields (EMFs) with diabetes has been reported. It was shown that EMFs frequently can stimulate glandular activity in the short term, but long-term exposure is often harmful for the glands. Glands are thought to be particularly sensitive to radiation as their secretions are normally produced in internal membrane systems, which can be damaged by electromagnetic fields. If these lesions are severe, the activity of the whole gland can be affected, which is serious for the glands of the endocrine system (those that coordinate our bodily functions), since it can affect many aspects of the metabolism and may be partially responsible for
the development of obesity. Thyroid gland exposed to power line frequencies underwent deterioration [37] and lost its capacity for producing thyroid hormones [38]. The expected consequences of this hypothyroidism are fatigue and obesity. Moreover, the prolonged exposure to EMFs limits the release of hormones related to appetite regulation such as ghrelin and peptide YY, which increases the risk of obesity. Although there is no sufficient literature data concerning how MCP-1 is associated with obesity-induced electromagnetic fields, but there are some reports on up-regulating expression of MCP-1 by EMFs [39]. Therefore, we suggest the interesting subject of further research might be the elaboration if EMFs could putatively contribute to the development of diabetes by up-regulating MCP-1. The application of a number of electrical devices in living and working environments caused that humans are exposed to different levels of EMFs and its putative hazardous effects on human health have been recently widely discussed.

Obesity can also be regarded as a result of microbiome alteration in humans. However, no evidence concerning participation of MCP-1 molecule in gut microbiome alteration has been reported so far, but it is worth mentioning that these alterations might play a role in 2 diabetes pathophysiology. Recent studies have shown that gut microbiota differ in composition between obese and lean subjects. Different ratios of Bacteroidetes and Firmicutes, the two dominant intestinal bacteria, were found in a leptin-deficient ob/ob mouse model [40]. Similar differences in this ratio were found in humans [41]. Products of intestinal bacteria play a role in type 2 diabetes pathophysiology. Butyrate, acetate and propionate are short-chain fatty acids (SCFAs), which are absorbed in the intestine and have both metabolic and epigenetic effects. It was suggested that propionate affects hepatic lipogenesis and gluconeogenesis, whereas peripherally acetate functions as a substrate for cholesterol synthesis [42]. SCFAs were also shown to reduce inflammation, and butyrate improved insulin sensitivity and increased energy expenditure by enhancing mitochondrial function in mice [43].

4. MCP-1 – A CANDIDATE MOLECULAR MARKER OF TYPE 2 DIABETES

MCP-1 is involved in the development of diabetes. It participates in the recruitment of monocytes, and thus it may contribute to the development of inflammatory state in the adipose tissue. Moreover, MCP-1 and other pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IL-6, influence glucose and lipid metabolism. MCP-1 exhibits a direct effect on angiogenesis of endothelial cells [44] and therefore it may be involved in the expansion and remodeling of the adipose tissue during development of obesity. It is suggested that MCP-1 may have a broader role in adipocyte physiology than only inflammatory cell recruitment, as it was shown to be also an insulin-responsive gene. Sartipy and Loskutoff showed that insulin induces expression and secretion of MCP-1 in vitro in insulin-resistant 3T3-L1 adipocytes and also in vivo in insulin-resistant obese mice [45]. It was also shown that MCP-1 may promote insulin resistance in differentiated adipocytes due to prolonged exposure to MCP-1. The differentiated 3T3-L1 adipocytes were treated with low concentrations of MCP-1. As a result, the insulin-stimulated glucose uptake was severely diminished by MCP-1 [45]. Similar mechanisms have been suggested to have relevance in humans, as circulating MCP-1 has been found significantly increased in patients with type 2 diabetes [46].

Many studies have shown a correlation between high glucose concentrations and MCP-1 level. Hyperglycemia initiates the increase of MCP-1 release in the endothelium of diabetic patients in vitro and elevated expression of vascular cell adhesion molecule-1, which might cause initiation of atherosclerosis [47]. The endothelial dysfunction is known to be related to the vascular inflammatory process and can lead to diabetic vascular disorders. Elevation of the intracellular glucose level causes the overproduction of adhesion molecules, which is suggested to be one of the earliest events of the vascular inflammation process. Intracellular adhesion molecule-1 and MCP-1 are up-regulated through the interaction between monocytes and endothelial cells by activation of nuclear factor-kappa B (NF-κB). Human endothelial ECV304 cells exposed to high glucose for 24 h caused an increase in MCP-1 and intercellular adhesion molecule-1, and promoted cell adhesion between monocyte and ECV304 cells [48]. Another study investigated the effect of high glucose level on chemokines and signaling mechanisms in human aortic smooth muscle cells. Exposure of these cells to a high glucose level resulted in an increase of fractalkine and MCP-1 expression and the activated mitogen-activated protein kinase signaling pathway [49]. Similarly, 7-day incubation of human umbilical vein endothelial cells with high glucose increased mRNA expression and the production rate of MCP-1 in a time- and concentration (10–35 mM)-dependent manner, through upregulation of reactive oxygen species (ROS) generation and subsequent activation of p38 MAPK (Takaishi et al.,
MAPK (Takaishi et al., 2003). These findings suggest that MCP-1 contributes to the initiation and progression of hyperglycemia and diabetes.

It is known that pro-inflammatory cytokines such as MCP-1, but also ICAM1 and TGF-β1, are elevated in diabetic kidneys, and thus renal inflammation can contribute to the development of diabetic nephropathy [50, 51]. Activated nuclear factor-κB (NF-κB) translocates from the cytoplasm to the nucleus and induces the expression of its target genes including MCP1, ICAM1, and TGF-β1, resulting in induction of inflammation, which leads to glomerulosclerosis and tubulointerstitial fibrosis [52, 53].

5. THERAPEUTIC STRATEGIES OF PLANT-DERIVED COMPOUNDS FOR DIABETES

All the previously presented factors that contribute to the development of type 2 diabetes could be potential targets for prevention and therapy using plant metabolites or plant extracts via interaction with MCP-1. This is a result of some important properties and activities of plant metabolites. The most important plant metabolites are known antioxidants and are able to attenuate ROS activity. Their role in scavenging free radicals has already been broadly described [54, 55]. Apart from the ability to scavenge ROS, plant-derived phenolic compounds can regulate ROS-related enzymes. They can decrease the cellular level of free radicals either by inhibiting the activity or expression of free radical generating enzymes such as NADPH oxidase and xanthine oxidase (XO) or by enhancing the activity and expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) [56, 57]. Plant-derived phenolics are also immunomodulatory, which makes them attractive agents that modulate the complex immune system response in the prevention of infections, as most of the immunomodulators are cytotoxic drugs. Plant phenolics cause many different effects on the immune system: among others, they down-regulate T-helper cell cytokines and IL-4 production [58], reduce levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α [59, 60], inhibit T lymphocyte proliferation [61], downregulate TNF-α, IL-6, iNOS, COX-2 and upregulate IL-10 by inhibition of the expression of toll-like receptors and activation of NF-κB, and inhibit ICAM-1 and VCAM-1 activity [62, 63]. The effect of plant-derived phenolic compounds on MCP-1 was also described, and in the aspect of involvement in type 2 diabetes is described below. Furthermore, plant phenolics are involved in the development of the micro-biome. It was reported that polyphenols can stimulate the growth of commensal and beneficial microbiota, while pathogenic strains are inhibited. For instance, the growth of Clostridium perfringens, C. difficile and Bacteroides spp. was significantly inhibited by tea phenolics and their derivatives, while commensal anaerobes such as Clostridium spp. and Bifidobacterium spp. and probiotics such as Lactobacillus spp. were less severely affected [64]. Similarly, the consumption of red wine polyphenols significantly increased the numbers of Enterococcus, Prevotella, Bacteroides, Bifidobacterium, Bacteroides uniformis, Eggerthella lenta, and Blautia cocoides-E. rectal group members, while the quantity of Lactobacillus spp. was unaltered [65]. Plant phenolics can interact with certain proteins, thus influencing the development of type 2 diabetes. Phenolics possess anti-glycated properties, one mechanism of this activity being via binding to the protein molecule with a non-covalent bond, and in this way make glycation targets on the protein molecule (usually amino acids such as lysine) unavailable to take part in the glycation reaction [66]. Vlassopoulos et al. reported that pre-treatment of albumin with phenolic acids inhibits fructosamine production, especially in the presence of oxidative stress or oxidative damage [67]. Protein glycation is implicated in the development of several chronic diseases, particularly diabetic micro- and macro-vascular complications.

In light of the evidence, we suggest that the role of plant-derived phenolics might be crucial in the prevention of type 2 diabetes, as they may influence many metabolic processes involved in the development of this disease.

6. PLANT METABOLITES INTERACTING WITH MCP-1 ACTIVITY (TABLE 1)

According to the literature data, the number of publications indicating the pathological role of MCP-1 in obesity and diabetes has grown. Therefore this chemokine seems to be an accurate target for a type 2 diabetes prevention strategy. Some efforts have been made to inhibit MCP-1 over-production and ameliorate obesity-related syndromes, such as insulin resistance, diabetic nephropathy and type 2 diabetes, and an important portion of the studies was on plant metabolites, most of them phenolic in nature, and plant-based extracts. Here we present data confirming the activity of several selected components, from different classes of chemical compounds – phenolic acids, flavonoids, alkaloids – as well as some data on crude plant extract activity (Table 1).
### 6.1. Phenolic Acids and their Derivatives

Caffeic acid phenethyl ester (CAPE) is a known active component of propolis characterized by antibacterial, anticancer, and anti-inflammatory properties [68-70]. It was reported that CAPE can suppress the differentiation of 3T3-L1 into adipocytes and inhibit the production of adipocytokines during adipogenesis [71, 72]. Moreover, its interaction with MCP-1 was reported by Juman et al., who studied its suppressive effect on LPS-induced production of pro-inflammatory cytokines in a macrophage cell line, RAW264.7, and found that CAPE inhibits gene expression of key pro-inflammatory cytokines, MCP-1, IL-1-beta and TNF-α. Additionally, secretion of these pro-inflammatory cytokines from RAW264.7 macrophages also decreased with CAPE pre-treatment [73]. It is therefore suggested that CAPE is able to attenuate chronic and low-grade inflammation occurring via macrophage infiltration in obesity.

The cardiac protective effects of caffeic acid (CA) and ellagic acid (EA) in diabetic mice were studied. Dietary supplementation of these compounds in diabetic mice resulted in a significant decrease in glucose level and reduced the levels of pro-inflammatory cytokines IL-1beta, IL-6 and TNF-alpha as well as MCP-1 levels. Additionally, CA or EA treatment significantly up-regulated mRNA expression of catalase, SOD and GPX1, and down-regulated IL-1beta, IL-6, TNF-alpha and MCP-1 in cardiac tissue of diabetic mice [74]. Thus these plant metabolites could protect cardiac tissue against the progression of diabetic cardiomyopathy via these triglyceride-lowering, anti-oxidative, and anti-inflammatory effects.

Ferulic acid (FA) was studied regarding its protective effect in the development of diabetic nephropathy in type 2 diabetic rats. Diabetic nephropathy is a major complication associated with type 2 diabetes and is a leading cause of end-stage renal disease [75]. It was demonstrated that FA treatment in diabetic rats effectively improved blood glucose levels, increased insulin sensitivity and insulin secretion of beta cells. As MCP-1 plays a crucial role in mesangial cell proliferation, glomerulosclerosis and renal fibrogenesis [76], the treatment with FA significantly reduced MCP-1 level in FA-treated diabetic rats compared to the diabetic control group [77]. Moreover, FA treatment reduced ROS production and suppressed MCP-1 expression in cultured podocytes, which indicates strong antioxidant and anti-inflammatory effects of FA in diabetic nephropathy. Hence it is suggested that FA may be a potent

### Table 1. Chemical structures of chosen plant metabolites interacting with MCP-1 activity.

<table>
<thead>
<tr>
<th>Plant metabolite/s</th>
<th>Structure</th>
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<td>Phenolic acids</td>
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<tr>
<td>Flavonoids</td>
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<td>Stilbenes</td>
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<td>Anthocyanins</td>
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<td>Capsaicin</td>
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<tr>
<td>Alkaloids (Nicotine)</td>
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<td>Plant sterols</td>
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<tr>
<td>Saponins</td>
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<tr>
<td>Terpenes</td>
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<tr>
<td>Polyunsaturated fatty acids</td>
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(Examples: Linoleic acid (Linoleic acid), Limonene)
new therapeutic drug for diabetic nephropathy in type 2 diabetic patients

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, is thought to be the main active ingredient derived from the root of turmeric. Curcumin exists as a mixture of two tautomeric structures, the diketone and keto–enol form. The enol form is more energetically stable in the solid phase and in solution. Many therapeutic activities of curcumin are reported due to its structure, and thus there is a wide range of molecular targets. Curcumin can modulate several transcription factors, cytokines, growth factors, and kinases [78-80]. However, its wide biological activities are limited due to poor bioavailability. Curcumin is an unstable molecule that decomposes rapidly in neutral and basic conditions and has been reported to undergo extensive metabolism through oxidation, reduction, glucuronidation, and sulfation [81-83]. Curcumin is able to inhibit macrophage accumulation in adipose tissue [84] and can stimulate human adipocyte differentiation [85]. The putative mechanism is due to the suppression of NF-κB activation, which results in reducing TNF-α and nitric oxide (NO) and inhibits the release of MCP-1 from 3T3-L1 adipocytes [86]. Curcumin is suggested to possess anti-inflammatory properties. Curcumin supplementation decreased blood levels of MCP-1, IL-6, and TNF-α, hyperglycemia, and oxidative stress in U937 monocytes and in a diabetic rat model in response to high glucose (35 mM) treatment [87]. Moreover curcumin inhibits degradation of IκBα and NF-κB activity, which might reduce macrophage infiltration and prevent proinflammatory cytokines (TNF-α and IL-1β) from releasing and downregulating ICAM-1, MCP-1, and TGF-β1 protein synthesis in diabetic nephropathy.

6.2. Flavonoids

Luteolin, (3′,4′,5,7-tetrahydroxyflavone) is one of the most common flavonoids present in edible plants and in plants used in traditional medicine to treat a wide variety of pathologies [88]. Luteolin possesses anti-inflammatory properties in the obesity-associated inflammatory response due to its ability to regulate obesity-induced inflammation. Luteolin anti-inflammatory activity results from its structural features – a double bond at positions C2–C3 of the C ring and OH groups at positions 3′ and 4′ of the B ring [89, 90]. Luteolin exhibits anti-insulin-resistance properties in adipocytes as it significantly increased the response of glucose uptake to insulin stimulation in these cells [91]. The putative mechanism is an interaction of luteolin with glucose transporters on the cell membrane or by indirectly affecting insulin action and the levels of gene expression of glucose transporters. Luteolin was shown to significantly reduce gene expression of MCP-1 and other pro-inflammatory cytokines such as TNF-α, IL-6, cyclooxygenase-2 and resistin in a dose-dependent manner. Moreover, it enhanced the mRNA levels of gene expression for two key adipokines, leptin and adiponectin, in 3T3-L1 adipocytes and primary mouse adipose cells [91, 92]. Adiponectin levels are reported to be negatively correlated with obesity and insulin resistance, due to its anti-inflammatory function [93]. Addition of luteolin to a high-fat diet increases these levels. Therefore luteolin inhibits pro-inflammatory cytokines and adipokines implicated in the development of insulin resistance [94]. Additionally, it was suggested that luteolin increased the expression of IRS-1/2, a molecule participating in the insulin signaling cascade, in a dose-dependent manner [92].

Apigenin (4′,5,7-trihydroxyflavone) is a flavonoid abundantly present in common fruits and vegetables. It possesses health-promoting activities due to its low toxicity and high chemopreventive effects [95]. Apigenin inhibits the LPS-induced increase of pro-inflammatory cytokines, such as IL-1β, IL-6 and MCP-1, and the two adhesion molecules ICAM-1 and VCAM-1. Moreover, it prevents an LPS-induced decrease of the anti-inflammatory cytokine IL-10 [96]. The regulatory effects of apigenin and other flavonoids, chrysin and quercetin were evaluated on adhesion molecules and pro-inflammatory cytokine expression in endothelial cells. High glucose and TNF-α resulted in the augmented expression of ICAM-1, VCAM-1 and MCP1. The subsequent incubation with examined flavonoids resulted in strong inhibition of expression of the above-mentioned VCAM-1 and MCP1 genes. Apigenin and chrysin significantly inhibited the gene expression of VCAM-1 and MCP1 at 50 μM, quercetin inhibited the expression of ICAM1 and MCP1 at 50 μM (but enhanced that of VCAM-1 at 30 μM), and kaempferol inhibited the expression of MCP1 and VCAM-1. From the structure and inhibitory activity of these flavonoids, it was suggested that a double bond in the C-ring of flavonoids and the third hydroxyl group in the A-ring were required for the inhibition of gene expression [97].

Naringenin (49,5,7-trihydroxyflavanone), an aglycone of naringin, is produced in several plants, especially in grapefruit, and has been reported to possess various pharmacological effects, such as antioxidant,
that resveratrol inhibited TNF-α-induced MCP-1 secretion in part due to altering MCP-1 expression. It was shown that resveratrol inhibited TNF-α-induced adipocyte lipolysis [100]. Naringenin suppressed high-fat-diet-induced accumulation of macrophages during the 14-day administration period and also caused suppression of MCP-1 expression in adipose tissue [101]. Naringenin was also shown to regulate fat deposition in adipose tissues via MCP-1 action, as it inhibited the production of TNF-α, MCP-1, and nitric oxide in a dose-dependent manner in RAW264 macrophages and co-culture of 3T3-L1 adipocytes and RAW264 macrophages stimulated by LPS [102]. Therefore, naringenin may be beneficial for ameliorating the inflammatory changes in the obese adipose tissue.

Puerarin is a isoflavone glycoside, abundantly present in root of Pueraria lobata, a native plant in Southeast Asia. It is known to have antioxidant, anticancer and antihyperglycemic properties [103-105]. Puerarin could significantly increase glucose tolerance in C57BL/6J-ob/ob mice, an animal model of type 2 diabetes mellitus [106]. This metabolite could also inhibit the upregulation of MCP-1 gene expression, which was induced by high glucose treatment in vascular endothelial cells. Moreover, the epigenetic mechanism of this inhibitory effect was examined. It was found that a few key epigenetic chromatin markers such as H3K4me2/3, H3K4 HMTs and LSD1 of the MCP-1 gene promoter, which are involved in high-glucose-induced up-regulation of MCP-1 gene expression in endothelial cells, could be modulated by puerarin [107].

6.3. Stilbenes

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a stilbenoid, a naturally occurring polyphenol present in red wine and berries. The main bioactive effect of resveratrol is associated with its heart-protective effect, but other biological effects have also been well documented in the literature, such as anti-oxidative, anti-inflammatory, anti-aging, anti-cancer, and anti-viral activity [108-110]. It is also implicated in the health-promoting effects in diabetes and obesity. Oral administration of resveratrol was shown to improve insulin sensitivity in mice fed a high-fat diet. The favorable roles of resveratrol in improving insulin sensitivity and preventing atherosclerosis are suggested to be at least in part due to altering MCP-1 expression. It was shown that resveratrol inhibited TNF-α-induced MCP-1 secretion by altering the gene expression of this chemokine in 3T3-L1 adipocytes in a dose-dependent manner [111]. Moreover, the regulatory effect of resveratrol on MCP-1 transcription was examined by promoter luciferase activity analysis. The HEK293 cells were transfected with the MCP-1 promoter that was fused to the luciferase gene as a reporter. It was revealed that resveratrol inhibited TNF-α-induced luciferase expression in a dose-dependent manner ranging from 10 to 50 µM [111]. It was also assumed that these effects were mediated by directly repressing NF-κB binding activity, and also indirectly inhibiting NF-κB dependent transcription activity through activation of Sirt1 (NAD(+)-dependent protein deacetylase). These literature data indicate that resveratrol might be an important plant metabolite in preventing obesity-related pathologies.

6.4. Anthocyanins

Anthocyanins belong to the family of phenolic compounds and are the largest group of water-soluble pigments in plants. The basic structures of the anthocyanins are anthocyanidins, which consist of an aromatic ring bonded to a heterocyclic ring containing oxygen, which is then bonded by a carbon–carbon bond to a third aromatic ring [112]. Their glycoside forms are known as anthocyanins. They are known to possess strong antioxidative, anti-inflammatory and anti-hyperlipidemia effects [113].

A study of the effect of anthocyanins (cyanidin-3-O-β-glucoside chloride [C3G] and cyanidin chloride [Cy], an aglycon of C3G) on hyperglycemia-mediated cholesterol accumulation and inflammation, as well as on their molecular mechanism of action in HK-2 cells, was performed. It revealed that these anthocyanins are able to inhibit high-glucose-induced cholesterol accumulation and also reduced the inflammatory process by inhibiting the production of MCP1, ICAM1, and TGFβ1, both intracellularly and extracellularly. It was also suggested that the anthocyanin-mediated inhibition of high glucose-induced cytokine production was likely mediated by the NFκB pathway [114]. Similarly, the anti-diabetic properties of purple corn anthocyanins were reported by Kang et al. The anthocyanin-rich purple corn extract (PCA) could suppress monocyte activation and macrophage infiltration. In the diabetic kidney, high glucose promotes mesangial production of MCP-1, IL-6, and TNF-α, which, together with adhesion molecules, leads to the recruitment of leukocytes and their adhesion to endothelial cells. This interaction is crucial in activating monocytes to migrate from the
circulation to the kidney in the early stages of diabetic nephropathy [115]. PCA lowered the expression level of MCP-1 and chemokine macrophage inflammatory protein 2, both involved in monocyte chemotaxis and macrophage infiltration, in diabetic kidney, and thus antagonized the infiltration and accumulation of macrophages [116]. It is therefore considered that anthocyanins might contribute to the prevention of renal vascular diseases in type 2 diabetes.

6.5. Capsaicin

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a capsaicinoid and is the most abundant metabolite produced by pepper plants. It is an important ingredient in spicy foods consumed throughout the world. The favorable role of this metabolite has been widely described, especially its role in oxidative stress and cancer. It also plays a crucial role in inflammation. It was demonstrated that capsaicin significantly lowered the expressions of MCP-1 and IL-6 mRNAs and protein release in the mesenteric adipose tissues of obese mice. Moreover, it enhanced the expression of adiponectin mRNA and protein release. It exhibited an inhibitory effect of capsaicin on macrophage migration induced by obese mice mesenteric adipose tissue-conditioned medium or MCP-1 [117]. Additionally, the anti-diabetic effect was investigated in in vivo experiments on C57BL/6 obese mice fed a high-fat diet. It was revealed that dietary capsaicin protected against obesity-induced glucose intolerance, as it significantly increased the mRNA expression of insulin receptor substrate-1 and glucose transporter-4. Furthermore, it markedly decreased the levels of TNF-α, MCP-1 and interleukin IL-6 mRNAs and proteins in adipose tissue and liver. Additionally, capsaicin was reported to inhibit macrophage infiltration into obese adipose tissue by ~25% [118].

6.6. Alkaloids

Berberine is an isoquinoline alkaloid (C_{20}H_{19}NO_5) and constitutes the main component of the ancient Chinese herb Coptis chinensis Franch, but also is present in Hydrastis canadensis (goldenseal), Cortex Phellodendri (huangbai), and Rhizoma Coptidis (huanglian). It has been used to treat diabetes for thousands of years, as it exhibits antihyperglycemic effects. Berberine significantly reduced levels of serum triglyceride and total cholesterol, as well as high- and low-density lipoprotein cholesterol (HDL-C and LDL-C) [119]. Such activity was similar to that reported elsewhere in vivo [120]. Thus it regulates glucose and lipid metabolism in diabetic patients. Several studies have demonstrated that the beneficial effects of berberine on metabolic disorders including cholesterol-lowering and hypoglycemic effects [120, 121] are associated with activation of AMP-activated protein kinase (AMPK). The adipogenic/lipogenic gene expression in the white adipose tissue of obese db/db mice fed with berberine was decreased. Moreover, the mRNA levels of pro-inflammatory genes, including MCP-1, TNF-α, IL-β, IL-6, iNOS, and cyclooxygenase-2 (COX-2), were decreased in the white adipose tissue upon berberine treatment [122]. It is also concluded that berberine suppresses pro-inflammatory responses in macrophages by inhibition of MAPK signaling and cellular ROS through AMPK activation. These reports indicate that berberine exhibits advantageous effects on metabolic disorders by, inter alia, attenuating the inflammatory state via MCP-1 regulation.

6.7. Plant Sterols

Plant sterols are plant metabolites that have a chemical structure similar to cholesterol, but with the presence of an extra methyl or ethyl group. The most abundant plant sterols in the human diet are β-sitosterol, campesterol and stigmasterol, while plant stanols are less abundant and consist mainly of sitostanol and campestanol [123]. Plant sterols are known to reduce circulating levels of cholesterol in humans and thus reduce the risk of coronary heart disease [124]. It is generally known that a daily intake of 2.5 g of plant sterols or stanols lowers serum cholesterol concentrations up to 10% [125]. It is suggested that they have a positive effect on obesity-associated metabolic disorders. Two phytosterols from Aloe vera, namely lophenol and cycloartanol, were suggested to diminished glucose levels in Zucker diabetic fatty rats. Treatment with those phytosterols was shown to reduce the serum MCP-1 level and increase the serum adiponectin level. Additionally, lophenol and cycloartanol decreased serum and hepatic lipid concentrations (triglyceride, nonesterified fatty acid, and total cholesterol) and lowered the expression levels of hepatic genes encoding gluconeogenic enzymes and lipogenic enzymes [126].

6.8. Saponins

Saponins are polycyclic aglycones that are attached to one or more sugar residues. The aglycone part is called sapogenin, and can be a steroid (C27) or a triterpene (C30). The aglycone may have steroid or triterpenoid structure according to which saponins are classified. Steroidal compounds are less common and usually
found among the monocotyledons, while triterpenoid saponins are more widely distributed and typical of the dicotyledons. Many studies have illustrated the beneficial effects of these metabolites on blood cholesterol levels, cancer, and stimulation of the immune system [29, 127, 128]. The soybean saponins possess anti-inflammatory properties as they are able to inhibit the release of MCP-1 and other pro-inflammatory mediators by LPS-stimulated peritoneal macrophages in a dose-dependent manner [129]. Saponins are the main active compounds of *Panax notoginseng*, which is used to promote the circulation of blood in Chinese medicine [130]. Saponins derived from *Panax notoginseng* were found to reduce the glucose and lipid levels in blood and also to attenuate the damage of diabetic kidney in a diabetic rat model. These effects were suggested to be a result of, among other things, regulation of inflammation via MCP-1. It was shown that saponins suppressed the expression of MCP-1 by up-regulating Sirt1 to deacetylate NF-κB and inhibit NF-κB signaling transduction in rat mesangial cells. Moreover, saponins reduced oxidative stress and suppressed renal fibrosis and inflammation in the kidney caused by high activity of MCP-1 [114].

### 6.9. Terpenes

Terpenes are biosynthesized from isoprene units (molecular formula C_{5}H_{8}) and are classified by the number of isoprene units in the molecule. They are volatile compounds produced by many plants, which often possess odors and flavors. Sesquiterpenes consist of three isoprene units and have the molecular formula C_{15}H_{24}. Sesquiterpene lactones were shown to inhibit inflammatory cytokines, such as MCP-1, IL-1β, IL-18 and MIP-1α, in human renal mesangial cells under hyperglycemic conditions. Another study revealed that synthesized sesquiterpene lactones could be a putative drug in treatment of diabetic nephropathy, as they could significantly decrease the high glucose-induced secretion of MCP-1, TGF-β1 and fibronectin at the concentration of 1 and 10 µM. In addition, the expression levels of MCP-1, TGF-β1 and FN were also diminished [131]. Other studies have demonstrated that parthenolide, a sesquiterpene which possesses anti-inflammatory activities *in vitro*, blocks MCP-1 mRNA and protein expression by inhibiting NF-κB activity in experimental glomerulonephritis [132]. Wang *et al.* reported that sesquiterpene lactones could inhibit MCP-1 expression induced by advanced oxidation protein products by increasing ROS-mediated activation of the NF-κB pathway in rat mesangial cells [133]. Similarly, sesquiterpene lactones were able to inhibit advanced oxidation protein product-induced MCP-1 expression in podocytes, and these effects were likely to be mediated by their anti-inflammatory properties through inhibition of the IKK/NF-κB pathway [134]. These findings suggest that sesquiterpene lactones can exert a beneficial role in the therapy of diabetic nephropathy.

### 6.10. Polyunsaturated Fatty Acids (PUFA)

The beneficial effects of PUFA have been broadly investigated, and the results suggest that diets rich in PUFA are beneficial to health, providing a protective role against a range of diseases [135], but also play an important role in metabolic syndrome, which is an indicator of risk of type 2 diabetes and obesity [136]. Plant oils such as soybean oil, peanut oil and corn oil are a rich source of PUFA. Corn oil has one of the highest PUFA levels, and is composed of 59% polyunsaturated, 24% monounsaturated and 13% saturated fatty acid. A high intake of corn oil, with 58.6% fat-derived calories, improved health and longevity of aging mice. It also significantly reduced pro-inflammatory cytokines including MCP-1, IL-1β and IL-6 in the blood. Moreover, it lowered serum triglyceride, total cholesterol and LDL-C [137]. The effect of fish oil-derived n-3 PUFA on adipocytes in obese mice was investigated. It was observed that fish oil derived n-3 PUFA reduced secreted protein concentrations of MCP-1 (223%), IL-6 (242.6%), TNF-α (267%), macrophage inflammatory protein (MIP) (252%), MIP-1b (262%), and MCP-3 (219%). They also reduced activation of the inflammatory transcription factor NF-κB. Thus, a beneficial effect of n-3 PUFA on the obese inflammatory phenotype was reported [138].

### 6.11. Plant-derived Extracts

Procyanidins belong to flavonoids, and are oligomeric forms of catechins. They are abundant in red wine, grapes, cocoa, tea and apples. They are strong antioxidants and exhibit anti-inflammatory actions [139, 140]. It was demonstrated that grapeseed procyanidin extract has a protective role in obesity and insulin resistance and has a regulatory role in lipid synthesis, lipid degradation, glucose uptake, and adipocyte differentiation [141]. The exposure of adipocytes and macrophage-like cells to increasing doses of grapeseed procyanidin extract followed by an inflammatory stimulus was found to modulate IL-6 and MCP-1 expression levels. Grapeseed procyanidin extract at 100 mg/L concentration and higher caused a linear reduction of MCP-1 mRNA. Lower concentration was found not significant for this activity [142]. Grapeseed pro-
cyanidin extract could also modulate adiponectin gene expression *in vitro* at the concentration of 100 mg/L, but leptin mRNA induction was not detected [142].

An ethanol/water extract from fresh leaves and small branches of the bamboo *Phyllostachys edulis* was shown to lower MCP-1 concentration in mice fed a high-fat diet. Mice were fed a standard (10% energy from fat) or high-fat (60% energy from fat) diet with or without bamboo extract supplement (11 g dry mass per 17,000 kJ) for 6 months of treatment. The study showed that bamboo extract supplementation decreased the weight of mesenteric fat by 0.52 g, and could significantly attenuate MCP-1 secretion from this tissue and subsequently contribute to the decrease of MCP-1 in the circulation by 49% [143].

Unripe kiwi fruit methanol extract was applied to 3T3-L1 pre-adipocyte cells. It was found that kiwi extract could regulate gene and protein expression of MCP-1 and IL-6 by treatment with 30 and 100 µg/ml [144]. Moreover, the kiwi extract promoted differentiation of 3T3-L1 adipocytes, which contributes to lowering of the glucose level in the hyperglycemic/diabetic state. This was proved by the intracellular triglyceride content and activity of glycerol-3-phosphate dehydrogenase, an enzyme of adipogenesis.

Mulberry (*Morus alba* L.) leaf extract, which contains flavonoids including rutin, quercetin, isoquercitrin and quercitin 3-(6-malonylglucoside), exhibits a hypoglycemic effect [145]. It was demonstrated that mulberry leaf extract could reduce atherosclerotic lesions in apolipoprotein E-deficient mice by inhibiting lipoprotein oxidation [146] and inhibit TNF-α-induced nuclear factor κB activation and lectin-like oxidized low-density lipoprotein receptor-1 expression in vascular endothelial cells [147]. Mulberry leaf extract could modulate adipocytokine dysregulation and suppress macrophage infiltration, which are involved in the development of obesity. The expression of inflammatory adipocytokines, such as TNF-α and MCP-1, in white adipose tissue was markedly increased in db/db mice, while mulberry leaf extract decreased the expression of TNF-α and MCP-1 by 25 and 20% in db/db mice, respectively [148].

Flax fiber hydrophobic extract containing cannabidiol (CBD), phytosterols, and unsaturated fatty acids was shown to influence the expression of inflammation-related genes in skin cells. CBD is a well-known anti-inflammatory agent proposed to be responsible for most of the extract-induced expression changes. Real-time PCR showed inhibition of pro-inflammatory gene expression in skin fibroblasts, including interleukins 1β and 6, cyclooxygenase 2, and activation of the expression of suppressor of cytokine and chemokine signaling 1, which is a known anti-inflammatory gene. The altered expression of other genes showed a similar direction of extract activity, lowering migration, and adhesion of leukocytes (MCP1 and ICAM1), and these changes were correlated with the cannabidiol concentration in the extract [149].

**CONCLUSION**

In conclusion, this article reviews the significance and involvement of the chemokine MCP-1 in the development of obesity, type 2 diabetes, and diabetic complications, with an emphasis on the role of plant metabolites in the regulation of this chemokine and thus the role in the prevention or therapy of diabetes. In these studies, the downregulation of MCP-1 production was found to result in improvement of the symptoms of diabetes-related pathologies.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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