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HARNESSING NATURE FOR CARDIOVASCULAR HEALTH: A REVIEW OF NATURAL PCSK9 INHIBITORS

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Abstract

Background *Cardiovascular diseases (CVD) constitute a major global health burden, with its progression closely related to dyslipidemia and atherosclerosis. The lack of specific therapeutic interventions to target the CVD at several phases leads to challenges for effective treatment. However, lipid-lowering medications contribute substantially to cardiovascular health but still, there is a debate about their precise effect on CVD outcomes. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a liver secretary enzyme that modulates the density of LDL receptors (LDLR) on hepatocyte surface and regulates plasma level of low-density lipoprotein cholesterol (LDL-C). Studies have confirmed that PCSK9 inhibitors with remarkable lipid-lowering effects and clinical efficacy substantiate the need for further research for their additional role in the managing cardiovascular diseases.*

Purpose *This review aims to explore the mechanism and relationship between lipid metabolism, cardiovascular diseases, and PCSK9. Moreover, it also assesses the effects of natural PCSK9 inhibitors in managing cardiovascular health conditions and offers updated therapeutic protocol guidance.*

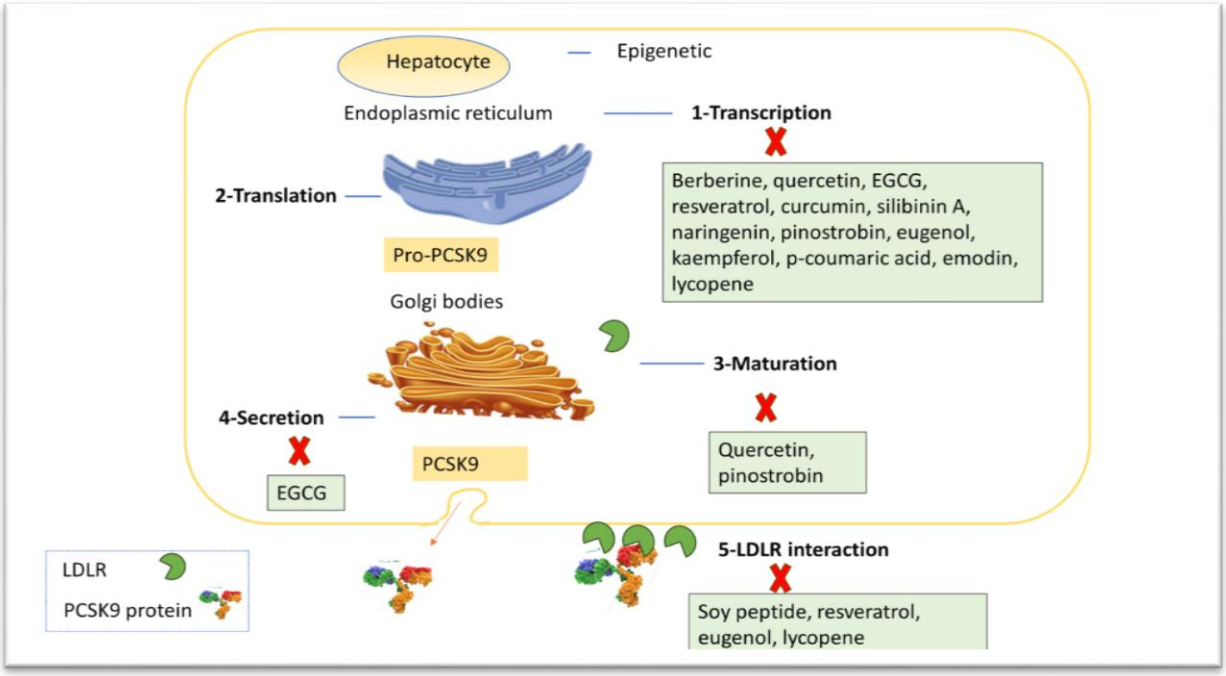
Method *Using multiple electronic databases such as PubMed, Scopus, Google Scholar, and Science Direct, covering studies from 2013 to 2023, a comprehensive literature search was conducted. Keywords such as PCSK9 inhibitors, dyslipidemia, cardiovascular diseases, medicinal plants, and natural PCSK9 inhibitors in vitro and in vivo were employed.*

Results *PCSK9 plays a substantial role in lipid regulation through both LDLR-dependent and independent pathways influencing cardiovascular health. Clinical studies revealed a positive relation between increased PCSK9 levels and CVD severity showing positive implications for lipid regulation. PCSK9 inhibitors are shown to be effective in decreasing LDL-C levels and improving lipid profiles, which may decrease the progression of atherosclerosis. In vivo studies also exhibited the potential of PCSK9 inhibitors to reduce cardiovascular diseases. Moreover, medicinal plants and compounds such as berberine, curcumin,*

resveratrol, quercetin, pinostrobin, eugenol, kaempferol, and other bioactive compounds are recognized as natural PCSK9 inhibitors and show potential cardiovascular benefits. These benefits may occur due to transcriptional inhibition, reduction in secretion, or other pathways.

Conclusion *PCSK9 plays a crucial part in pathophysiology of cardiovascular diseases and PCSK9 inhibitors have shown promising effects in the management of cardiovascular diseases in clinical and preclinical studies. Medicine plants and bioactive compounds can result in positive cardiovascular outcomes owing to their antioxidant, anti-inflammatory, and PCSK9 inhibition properties. PCSK9 inhibition can be employed as an auspicious therapeutic strategy for cardiovascular disease.*

Graphical Abstract



INTRODUCTION

Proprotein convertase-subtilisin/kexin type-9 (PCSK9) is an enzyme playing a critical role in regulation of low-density-lipoprotein receptors, and LDL-cholesterol levels[1]. PCSK9 belongs to subtilisin protease family and is its 9th number. It had been detected in the brain by the *Montreal Clinical Research Institute, Canada,* in 2003, and was first known as neural apoptosis-regulated convertase-1 (NARC-1) prior to being renamed PCSK9 through the conventional nomenclatural procedure [2]. PCSK9 protein is formed on chromosome 1P32 in humans, adjacent to the 3rd genomic site, the signal peptide, pro region, catalytic-domain, and C-terminal end which is primarily synthesized in the intestine, liver and kidney [3]. Like other proto-protein invertases, PCSK-9 is a secreted protein that is released entering the bloodstream forming a heterodimer following self-catalytic intramolecular degradation of a pre-protein in the hepatocytes. The precursor region of EGF of the LDL uptake receptor (LDL-R) is then bound to and internalized by the LDL-R [4], modulating its transit along hepatocytes surface to regulate the level of LDL lipids (LDL-C). Since the LDL uptake receptor-PCSK9 interaction cannot pass through normal blood vessels or reach lysosomes for destruction, their attraction for one another escalates

in acidic environments causing conformational modifications after binding[5]. PCSK9 mediates inflammatory pathway as well as SR-B receptors by binding with Toll-like receptors that activate platelets and stimulate thrombosis [6]. Very-low-density lipoprotein receptor (VLDL-R), low-density lipoprotein receptor-related protein 1 (LRP1), Apolipoprotein E receptor-2 (ApoER2), along with other receptors might encourage vascular endothelial proliferation and elevate lipoprotein concentrations. A significant amount of PCSK9 is produced by hepatocytes, where it is subjected to a self-catalytic degradation within the endoplasmic-reticulum (ER) that permits the transfer of activated PCSK9 transitioning from the ER towards the Golgi [7, 8]. The family of transcription factors including sterol-regulatory element (SRE) binding- protein (SREBP) controls 33 genes, including PCSK9 [9]. The PCSK9 promoter activity increases, triggering transcription when cell cholesterol levels drop or when intracellular production is inhibited [10]. HNF1, like other transcription factor, controls PCSK9 [11, 12]. After being secreted, PCSK9 interacts with the LDL receptor's EGFA-like repeat homology domain A by means of its catalytic domain. This process prevents LDLR from recycling on the cell surface and promotes its destruction in lysosomes. By decreasing the quantity of LDLR on hepatic cells this degrading action additionally hampers the liver's ability to absorb circulating LDL particles. Due to this association between mutation in PCSK9 gene resulting in increased function and hyperlipidemic situations, its pharmacologic suppression has been proposed as a novel mode of cardiac disease prevention [13-15].

Two strategic approaches comprising monoclonal antibodies as well as antisense oligonucleotides have been developed to lower PCSK9 serum levels or inhibit its interaction with LDLR [16]. Orally bioavailable small compounds with anti-PCSK9 suppression effect may be developed, however, as an ideal pharmacological method to inhibit PCSK9. The importance of finding naturally existing chemical substances offering therapeutic capabilities has been strongly supported by the history of pharmacology. For this reason, in the current study, we have compiled the most recent information on herbal substances or extracts that have conclusively inhibited PCSK9.

Methodology

In this review, we used a careful approach to gather information and evaluate studies. Searching for relevant literature and systemically reviewing the selected studies helped us ensure that methods were unbiased and accurate. This methodical approach enhances the reliability and credibility of the review's conclusions.

Definitions

PCSK9 inhibition was characterized as averting the PCSK9 peptide from interacting to the LDL uptake receptors, hence preventing LDLR disintegration, increasing the amount of LDLR that is restored to the liver cell surface for LDL-C absorption, along with decreasing the level of LDLc in the blood. Proteins or changes in gene expression associated with atherosclerosis are referred to be atherogenesis biomarkers. The term "natural products" refers to compounds or chemicals derived from plants. Biologically active substances present in plants are referred to as plant bioactive chemicals.

Search Criteria

Searches of the scientific literature in the Scopus, Science Direct databases, and PubMed were carried out between 2013-2023. The gene discovered as the third to be connected to autosomal dominant hypercholesterolemia, PCSK9, is the starting year. The finalized databases were searched starting on August 2 and going through August 30, 2023. "Proprotein Convertase Subtilisin-Kexin 9 Inhibitor" or "PCSK9 inhibitor", cardiovascular diseases, dyslipidemia "medicinal plants", and "natural pcsk9 inhibitors" were used to search for articles, and were considered. To restrict the years of publication search, a temporal filter was used from 2013 to 2023.

Inclusion and Exclusion Criteria

The original articles of biomarker levels, PCSK9 protein or gene expression, in-vitro, along with in-vivo investigations, as well as clinical trials, were all included in the research. The PCSK9 biomarkers were chosen and added explicitly. Studies using

atherogenesis biomarkers that lacked PCSK9 were disregarded. On the basis of their content and mode of publishing, the importance as well as relevance of the chosen publications were assessed. Studies were eliminated if (i) they were not written in English, (ii) studies included other type of PCSK for instance PCSK-1 and PCSK-8 (iii) they utilized PCSK9 to investigate the effects beyond lipid reduction in different ailments, (iv) they were not published, and (v) they comprise editorials, commentaries, or unpublished papers.

Study Identification and Selection

Following identification, the papers were added to the EndNote X20 program (*Thompson Reuters, Philadelphia, PA, USA*) after which duplicate manuscripts were eliminated. Titles and abstracts were utilized for the first screening of papers according to the qualifying criteria. Author(s), cell lines utilized, year of publication, studied plant biologically active compound (PBC) or natural product (NP), assessed biomarkers, and expression at the protein and gene levels were all extracted from the paper using a data extraction form. All of the identified studies were compiled into a summary.

Results

Online database searches for literature produced 900 research papers. 489 research papers were rejected as having no relation to the review after 105 titles and abstracts had been reviewed and the duplicated research articles had been eliminated. 207 possibly pertinent references with complete research papers were chosen for further investigation. After reading the full paper, 99 research articles were eliminated. The requirements for inclusion were satisfied by 207 publications.

PCSK9 Inhibitors and Current Limitations

To inhibit PCSK9, numerous strategies have been established comprising monoclonal antibodies (mAbs), gene-silencing or modification techniques including small interfering RNA (siRNA)s; antisense oligonucleotides, small-molecule inhibitors; along with CRISPR/Cas9 platform; engineered binding proteins comprising mimetic peptides; adnectins; as well as immunization [17] [18]. The mAbs tailored to bind plasma PCSK9 and prevent PCSK9/LDLR binding have received the greatest attention. Monoclonal antibodies that inhibit PCSK9 have been proven in studies to considerably lower LDL-C levels and CVD prevalence [19, 20]. So far, 2 mAbs known as (alirocumab as well as evolocumab) have been authorized by the *European Medicines Agency (EMA)* and the Food and *Drug Administration (FDA)* in 2015 in view of addressing individuals with elevated cholesterol levels and have been used effectively in clinics alone or in combination with statins [21, 22]. The debate over the expense of present PCSK9-blocking antibodies (priced at $14,500 and $14,100 annually for evolocumab and alirocumab, respectively) has gotten worse, given that these expensive antibodies only yielded a fifteen percent drop in relative risk [23]. For patients currently diagnosed with familial hypercholesterolemia, the cost-effectiveness ratio may be considered appropriate; however, it is not as advantageous for patients with common vascular disorders [24]. The most often used cholesterol-lowering drugs, statins have a variety of pleiotropic effects [25]. These medications work by raising the expression of PCSK9 and hepatic LDLR, which suggests their impact on PCSK-9 might reduce the medicinal benefit of statins[26]. Additionally, the EMA and FDA recently authorized Inclisiran, a new PCSK9 silencer based on siRNA, which has been shown to lower LDL-C in individuals predisposed to cardiac illnesses [27]. siRNAs work to silence intracellular PCSK9. However, these two drug classes can only be administered via the parenteral route, which is uncomfortable and restricts their application, particularly due to the chronic nature of LDL-C-associated ACVD [28]. Patients may find it challenging to adhere to treatment requirements because of the aforementioned characteristics. The treatment method of giving anti-PCSK9 antibodies has a number of potential downsides as well. The need for numerous injections at high dosages, neutralizing antibody generation, and brief duration of mAbs in body may be difficult for patients psychologically and financially [29]. They are typically proteins. As a result, complying with chronic usage, pathologic as well as physiological tolerance to them may develop due to enhanced reticuloendothelial system clearance or adaptive immune system-induced hypersensitivity, respectively [28]. Considering all of this, certain nutraceuticals would be well considered as supplementary lipid-lowering therapy. Traditional

Chinese Medicine has been shown in multiple earlier research to be a method of preventing and treating atherosclerosis [30].

Berberine

The Berberis genus (Family: Berberidaceae) has approximately 550 different species throughout the world. Studies have demonstrated that Berberis has been used traditionally to treat metabolic illnesses including diabetes and hyperlipidemia[31]. Alkaloids, anthocyanins, polyphenols, flavonoids, and other bioactive substances have all been discovered in Berberi's species. Berberine (BBR, C20H18NO4+), a type of quaternary amines obtained from alkaloids with isoquinoline framework, is frequently utilized in Ayurvedic as well as Chinese medicine. Berberine's chemical name is 5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo [5,6-] quinolizinium. The highly bioactive substance discovered in Berberis species, berberine, is thought as very beneficial in treating diabetes and other metabolic illnesses[32]. Berberine may also be found in the stem, roots, bark, and rhizomes of other flowering plant species including *Coptis rhizomes* and *Hydrastis Canadensis*. It relates to the group of protoberberines, which can be identified in a variety of plants, including *Coptis Chinensis*, *Berberis aristata*, and *Berberis vulgaris*, or synthesized entirely[33]. BBR seems to be among the most intriguing natural compound-based medicines for curing cardiac, metabolic and renal illnesses, according to the most recent developments in pharmacological research [34]. About seven hundred Chinese plants were examined having possible initiation effects on expression of LDLR, and the mechanism underlying lipid-lowering impact of berberine was discovered[35]. Berberine exhibited the most substantial effectiveness in raising LDLR expression among the substances examined, indicating a mechanism resembling that of statins, which are HMG-coenzyme A reductase suppressors. But berberine raises mRNA, protein, in addition to hepatic LDLR activity without affecting intracellular cholesterol levels. Therefore, the effect of berberine does not require the LDLR overexpression, which is accomplished through the activating the SREBPs [36]. Subsequent studies into the biological impacts of berberine revealed that this organic substance roughly triples the mRNA stability of LDLR.

Experimental investigations were conducted to determine if PCSK9 played a role in the mechanism that activates berberine after the impact of PCSK9 on LDLR was discovered. As previously mentioned, SREBP plays a major part in controlling the PCSK9 gene's transcription [37]. However, important SRE motifs are frequently located close to Sp1(specific proteins) or NF-Y (nuclear transcription factor-Y) binding sites, along with SREBPs interact employing these coactivators to promote complete transcriptional activation. The PCSK9 gene promoter holds a distinctive sequence in this context, possessing an HNF1 binding region just next to the SRE serving as a crucial regulating motif sequence. In fact, HNF1 is SREBP2's primary collaborative partner in the modulation of the PCSK9 gene[38].

Based on the significant structural variations between the PCSK9 and LDLR promoters, it has been demonstrated that berberine significantly lowers the levels of PCSK9 mRNA in a time- as well as dose-dependent manner [39]. Although associated with HNF1, this suppressing impact is not dependent on the SREBP pathway. What's more intriguing is that berberine blocks PCSK9 protein synthesis and negates the statins' activating effects. In fact, berberine considerably decreased the HNF1 expression (by about 60%) and barely reduced SREBP2 [38]. This impact is required to inhibit PCSK9 gene transcription with no interfering with LDL uptake receptor expression. Given that SREBP2 is essential for LDL-R transcription, the interaction among SREBP2 as well as HNF1 is advantageous for LDLR gene expression [40]. As berberine raises LDLR-protein levels in each of culture and animals, it is likely the effects are balanced favoring LDLR mRNA stabilization [41]. The pathway behind the restrictive impact of berberine on HNF1-driven PCSK9 gene transcription was examined in further detail.

The mechanism supporting berberine's inhibitory impact on HNF1-mediated PCSK9 transcription was examined in greater depth. Dong et al. showed that berberine accelerate the disintegration of HNF1 via proteasome pathway utilizing the proteasome antagonist bortezomib. As a result, berberine action is countered by inhibiting the proteasome, leading to a rise in PCSK9 levels along with a reduction in LDLR

expression [12]. Evidence from hamster studies implies that berberine's effect on LDLR as well as plasma cholesterol arises mostly through a systemic effect instead of a restriction of gut cholesterol uptake. These results are centered on the findings that oral berberine did not raise fecal lipids and that peritoneal berberine (20 mg/kg) had a higher lipid-reducing impact compared to oral berberine (100 mg/kg) [39]. These findings are especially significant in light of the fact that berberine's estimated oral bioavailability is 0.37% [42]. Following a single 400 mg oral dosage, the highest concentration (Cmax) of berberine in the human bloodstream was found to be 0.4 ng/mL [24]. It is believed that the main obstacle to berberine's oral bioavailability is intestinal first-pass elimination, and that berberine's high absorption along with distribution in the hepatocytes may be additional significant variables that contribute to its reduced plasma concentration in rats. Subsequent to intragastric dosage, berberine is extensively dispersed throughout the body, considering liver as the most prominent organ, where the average concentration of berberine measured almost seventy times higher compared to plasma [23]. It is also widely distributed throughout the heart, spleen, kidney, lungs, and even the brain. Unfavorable physicochemical qualities are one of the causes limiting its oral bioavailability. Another element influencing berberine's oral bioavailability is the existence of membrane transporters, such as P-glycoprotein (P-gp) as well as multidrug resistance protein 1 (MRP1)[43]. These carrier proteins prevent berberine from being absorbed by pushing it out of the intestine's lining cells. There are at least four metabolites of berberine, according to further research. Phase I metabolites amongst them include M1, produced by demethylation, and M2, produced by demethylenation. Jatrorrhizine, also known as M3, is another phase I metabolite. These phase I metabolites produce phase-II metabolites, which constitute mostly glucuronide conjugates of M1, M2, in addition to M3. These metabolites are distributed throughout the body in a variety of tissues, with unconjugated forms predominating, including the liver, heart, and kidney. It's interesting to note that after oral delivery, the phase I metabolites' glucuronides are the most prevalent forms found in the plasma [44].

In-vitro studies

Hepatoma cell lines HepG2 or Huh7 are typically used in in-vitro models to anticipate the hypocholesterolemic effects of nutraceuticals. The first study examining berberine's impact on PCSK9 found that at a dose of 15 g/mL (44 mM), berberine decreased PCSK9-mRNA levels by seventy seven percent. Chronological studies showed that berberine significantly decreased PCSK9-mRNA in eight hours of incubation, at 12 and 24 hours (65% and 61%, respectively). After being exposed to 15 g/mL of PCSK9, HepG2 cells produced 87% less of the protein in their medium[12]. In the same experiment setting, berberine enhanced the expression of LDLR-mRNA by 1.9- and 2.1-times, correspondingly, after 12 and 24 hours [12]. Li et al. reported very similar outcomes, with PCSK9 levels in berberine-treated cells significantly decreasing over the course of 48 hours from 31% at 12 hours (30%) to 23% at 20 uM (6.7 g/mL)[38]. The antagonistic action of berberine on statin-mediated concentration of PCSK9 mRNA was also supported by these findings. As the research was expanded to additional genes implicated in maintaining cholesterol levels, it was discovered that berberine lowered the concentration of HMG-CoA reductase-mRNA by 39% while having no appreciable impact on the mRNA levels of farnesyl-diphosphate synthase (FDPS) along with 7-dehydrocholesterol reductase (DHCR7), are enzymes responsible for cholesterol biosynthesis. The PPAR and SREBP2-mRNA levels of non-SRE-containing fat metabolism genes were elevated by berberine by 39% (p 0.05) as well as 74% (p 0.05), correspondingly [39].

These findings showed that berberine had no consistent influence on the mRNA gene expression including or excluding an SRE. Therefore, the SREBP pathway is not involved in the lowering of PCSK9 mRNA level caused by berberine. Additionally, it was discovered that berberine reduced the promoter activity of PCSK9 while maintaining the mRNA stability of PCSK9 by employing actinomycin D [12]. The most potent berberine metabolite, berberrubine (M1) and its analogs can decrease PCSK9 in a manner dependent upon extracellular signal-regulated kinase (ERK)[45].

In-vivo studies

The 1st in-vivo study showing berberine's ability to decrease lipids was published in 2004, in which hamsters were given a high-fat along with cholesterol rich diet (10% egg yolk powder, ,10% lard, and 1% cholesterol) [46]. Meanwhile the dynamics of hepatocellular LDLR-driven LDL clearing have thoroughly been studied [47]. These hyperlipidemia hamsters were given berberine treatment, which resulted in a time- as well as concentration-dependent decrease in all-out and LDL cholesterol concentrations. The LDL particle kinetics indicated that an impact on LDL-cholesterol seemed to be noticed post seven days of therapy, and by day ten, berberine at concentration of 50 as well as 100 mg/kg/d lowered LDL-cholesterol by 26% and 42%, respectively. Raised LDLR-mRNA (3.5-fold) along with protein (2.6-fold) expression within the hepatocyte was linked to this impact [48]. However, the investigation carried out in hyperlipidemic C57BL/6 mice in reaction to inflammation triggered by LPS yielded the 1st in vivo investigation of berberine's action on PCSK9 [49]. The liver's PCSK9 mRNA levels, which were increased by LPS, were significantly and dose-dependently reduced when berberine was administered orally through gavage at doses of either 10 or 30 mg/kg per day. The LDLR mRNA significantly increased in response to this impact [49]. The findings supported the in-vitro research along with the idea that berberine decreases PCSK9 gene transcription, even if the animal model used unattainable to be considered ideal for evaluating the lipid-reducing effects of novel medicines.

The second research that used rats on a fat rich diet over six weeks and 47% calories sourced from fat, 20% calories derived from protein, and 33% calories taken from carbohydrates revealed different findings. Oral berberine 400 mg/kg/day effectively decreased LDL-cholesterol (45%) as well as elevated HDL-cholesterol (+45%), leaving all in total cholesterol (TC) levels constant. Unexpectedly, a considerable rise in plasma concentrations of PCSK9 was seen in reaction to a high-fat diet, and these values were further enhanced (nearly doubled) in response to berberine [50]. Simvastatin was used as the control treatment, and a similar pattern was seen.

A third investigation using a comparable hypercholesterolemic rat model was carried out in order to further examine the impact of berberine on PCSK9. Rats fed with fat-enriched diet containing 2% cholesterol, 20% lard, 0.3% bile salts, 5% egg yolk powder, as well as 0.2% Prothiucil over 4 weeks before being given berberine via gastric intubation once per day over eight weeks at a concentration of 156 mg/kg/day. By 68%, 83%, and 66%, respectively, berberine lowered total cholesterol (TC), and LDL cholesterol and triglycerides (TG). Unexpectedly, 8-hydroxydihydroberberine, a berberine derivative with a better bioavailability compared to berberine, had the similar lipid-reducing impact when given 25% of berberine's dosage [51]. In this research model, animals treated with berberine had much lower levels of PCSK9 in their livers than hypercholesterolemic control animals [51].

As a result, it is reasonable to draw the conclusion that the experimental animal models used revealed different results and may not be indicative of the human research, as there are noticeable variances in lipid metabolism.

Clinical studies

A 25% decrease in LDL cholesterol and a 35% reduction in TG were found in the first research study that examined the therapeutic effect of berberine within the Chinese population with raised cholesterol [46]. These effects seemed to be significantly greater in individuals not receiving treatment with additional lipid-reducing medications. The ability of berberine in lowering blood lipids was then assessed in at least three meta-analyses. These trials used a daily dosage of berberine ranging from 0.5 gram to 1.5 gram. The findings unmistakably showed that berberine lowers LDL-cholesterol in those with raised cholesterol and/or type-2 diabetes mellitus (T2DM) by about 25 mg/deciliter (dL). A considerable decrease in TG concentration and a little but remarkable rise in HDL-cholesterol levels were associated with this variant [31] [52].

The effects of a nutraceutical products tablet including policosanol (10 mg), berberine (500 mg), and red yeast rice (200 mg) were studied in a clinical trial by Pisciotta et al. in participants with primary hypercholesterolemia (HCH) who had previously experienced statin intolerance or had refused STs

treatment [53]. After a period of six months of monitoring, a nutritional supplement was shown to decrease LDL-C by 31.7%, that showed high efficacy and tolerated well compared to ezetimibe (EZE). In the same research, supplementing STs or STs + EZE with nutraceutical tablets led to a larger reduction in LDL-C (average decrease of 10.5%) in individuals with heterozygous hypercholesterolemia (HeFH). According to Pisciotta et al., the decrease (37.1%) surpassed the impact of doubling ST dosage and assumed to be related to non-direct, berberine-driven suppression of PCSK9[53].

A randomized, double-blind, placebo-controlled study found that a three month therapy with nutraceutical formulations including potential lipid-regulating ingredients (chitosan, , berberine 200 mg, red yeast rice) was efficient in decreasing plasma non-HDL-C (by 15%) as well as LDL-C (by 20%) in contrast to placebo. Notably, PCSK9 plasma levels in those with hypercholesterolemia were steady throughout the trial [54]. In addition, three negative effects—Epstein-Barr virus infection, duodenitis, and subclinical though significant elevation in creatine phosphokinase—were reported in the aforementioned experiment, which required hospitalization. The intervention, however, was highly accepted [54].

Pilot research by Formisano et al. found that a nutraceutical formulation including berberine (500 mg), monacolin-K (MonK)+KA (1:1), and silymarin was efficient as a lipid-reducing drug coupled with significant inter-individual fluctuation in reaction. The outcome was equivalent to what would have been obtained with 10 mg of atorvastatin [55]. In addition, a substantial +25.6% rise in blood PCSK9 was observed after eight weeks of nutraceutical therapy. When more readily accessible, they hypothesized that BBR activity might only partially inhibit the PCSK9 rise. As seen in human macrophages, NUT treatment ultimately prevented serum-mediated foam cell production (Formisano et al., 2020[24]). Ex vivo NUT therapy enhanced the biological properties of lipoproteins with potential antiatherogenic effects [55].

In spite of the fact that dosages up to 1 mg per day are generally well tolerated, berberine may cause stomach distension, constipation, a bitter taste as well as diarrhea. However, the majority of the trials that used the greatest dosages, these effects were seen [52]. It's also crucial to be aware that berberine has been reported to decrease CYP2D6, CYP3A4, and CYP2D9 activity when given to healthy persons over an extended time, effects that may be brought on by drug-drug interactions [56].

The bioavailability of various berberine formulations is another subject that requires more research. Although it appears that berberine supplements have beneficial impact on lipid processing, and is also known that enteric berberine uptake is frequently negligible and holds a high degree of interpersonal variability [44]. The effectiveness of the nutraceutical may vary greatly depending on this factor.

In this context, several initiatives have been made to increase the absorption of berberine, such as the development of synthetic derivatives and the use of drug-delivery nanotechnology [51, 57]. For instance, when just 25% of the original dose of berberine is taken, the synthetic derivative 8-hydroxy-dihydro-berberine could provide comparable lipid-regulating impact to berberine [58]. This suggests a superior metabolic profile. The development of a novel series of indole-containing tetrahydroprotoberberine established the foundation of a second strategy [57]. A novel drug with strong PCSK9 inhibitory action was discovered as a result of this investigation; it enhanced LDL-cholesterol absorption in HepG-2 cells in addition to having a 21.9% gut bioavailability. When given at a daily concentration of 30 mg/kg to rats administered a fat enriched diet (0.5% cholesterol), this chemical similarly demonstrated a strong in vivo hypolipidemic efficacy [57].

Ochin together with Garelnabi devised a novel preparation that encapsulates the drug inside PLGA-PEG nanoparticles that negatively impact PCSK9 in order to increase berberine bioavailability [28]. A clear contrast to berberine alongside in vivo proof of a greater gut bioavailability is currently lacking, despite the fact that this formulation was demonstrated to be effective in lowering PCSK9 expression in vitro. A rationally planned micelle (CTA-Mic) created for efficient berberine liver deposits has been found to have in vivo evidence of enhanced berberine action. However, the authors were unable to offer information on PCSK9 levels, this novel formulation exhibits good in vivo lipid-lowering efficacy [58].

Polyphenolic compounds

In addition to tea and red wine, phenolics are additional compounds formed by plants that can be present in fruits, herbs, vegetables, seeds, nuts, spices, stems, as well as flowers. Flavonoids, stilbenes, lignans, as well as condensed (flavan-3-ol polymers referred as proanthocyanidin) or hydrolysis sensitive (including tannic acid) flavonoid compounds are only a few examples of the wide variety of compounds that fall under this category [59]. Numerous epidemiological investigations and clinical trials have documented the numerous cardiovascular benefits attributed to polyphenols [60, 61]. These advantages are brought about by a variety of mechanisms of action, notably the plasma LDL-cholesterol-lowering effect. The majority of these compounds work mechanistically by activating the LDLR at the liver surface, as was the case with berberine. The investigation into the possible impact of polyphenols on PSCK9 was prompted by this evidence [62]. Although there is some information on the polyphenol's impact on PCSK9, it must be kept in mind that the main issue with studying polyphenolics in vitro particularly the proportions of the substances being studied are frequently higher compared to found in vivo, which limits the biophysical applicability of these findings. Additionally, it might be challenging to apply the results from in vitro studies to animal studies because of the extensive metabolism that gut microbes subject polyphenols through in vivo, which may produce bioactive chemicals. These elements were subjected to extensive debate after being thoroughly reviewed elsewhere [63, 64].

Epigallocatechin gallate

The most potent catechin present in green tea, epigallocatechin gallate (EGCG), has exhibited antihypercholesterolemic action through increasing LDLR mRNA levels as well as the expression of proteins in hepatocarcinoma cell lines in an ERK-signaling pathway. Furthermore, EGCG inhibited the synthesis of apo-B, the primary LDL protein moiety [65]. This impact was demonstrated to be unaffected by 67 kDa laminin receptors, the major EGCG receptors reported [66]. Li and colleagues gave more proof of EGCG's mechanism of action by demonstrating EGCG's ability to reduce endogenous cholesterol production through sirtuin-1/forkhead-box protein O1 (SIRT1/FOXO1) signal transduction pathway dependently suppressing SREBP2 [67].

Hepatic cells subjected to 25 M EGCG showed much less PCSK9 production, with the greatest impact already being apparent after three hours of incubation. The same research found that EGCG might inhibit lovastatin's ability to induce PCSK9 secretion. Alteration in PCSK9 mRNA or level of precursor or mature protein inside the cell did not coincide with these effects [68]. Several researches discovered a strong correlation between consuming green tea along with decreased plasma levels of both total and LDL cholesterol. In one study, extracted EGCG has demonstrated antihypercholesterolemic abilities in healthy adults (LDL-C 9.29 %) [68]. Similarly, giving overweight and obese women green tea extract for 6 weeks reduced their LDL cholesterol by roughly 5% [69].

A study conducted by Chuan-Jue Cui revealed that by increasing nuclear FoxO3a and decreasing nuclear HNF1, EGCG inhibits PCSK9 synthesis, which increases LDL uptake receptors expression together with LDL absorption in hepatic cells. Consequently, liver and blood PCSK9 levels are inhibited, which leads to a reduction in LDL-C levels[70].

The EGCG bioavailability through oral ingestion in humans is low. The average Cmax was 275.4 g/mL after taking 300 milligram per day over 4 days, with an added 150 mg on the day 5 [71]. Individual variations were more than six times higher. The accelerated metabolic rate of ECGC, which mostly occurs within the hepatic and enterocyte and results in phenylvalerolactones and phenylvaleric acids that are subsequently glucuronidated, as well as methylated, sulfated, along with glucuronidated metabolites, is the cause of this variability[72]. The polymorphism of genes encoding the transporters facilitating the excretion as well as absorption of compounds, such as multiple drug resistance-associated protein 2 (MRP2) and organic anion-transporter peptide 1 B1 (OATP1B1), also affects the bioavailability of EGCG [73].

Resveratrol

Red wine, peanuts as well as grapes are some of the foods that contain resveratrol (3,5,4′-trihydroxy-trans-stilbene), a phenol that was initially discovered within

the roots of *Veratrum grandiflorum* (Maxim. ex Miq), a member of the Melanthiaceae family (Table 1). It has been proven in the past that polyphenols extracted from red wine increase LDL uptake receptor expression as well as activation while decreasing apolipoprotein B-100 production from HepG2 human cells. Following this revelation, scientists concentrated on the main bioactive polyphenol, resveratrol, and its mode of action. They discovered that resveratrol had a significant impact on the expression of the LDL uptake receptor gene in hepatocytes, particularly by the utilization of SREBP, although irrespective of the AMPK-dependent signal transduction pathway[74].

In steatotic hepatocytes, resveratrol also increased the levels of LDLR mRNA and protein through an action on the PCSK9 promoters that included SREBP1 c. 20 M resveratrol decreased PCSK9 expression and increased LDL absorption in the same cells, which has significant influence on the development of non-alcoholic steatosis (NAFLD), the main factor in hepatic dysfunction [75]. A naturally occurring precursor of resveratrol, polydatin (piceid), has been reported to have an upregulating effect on LDLR [76]. According to an in vitro screening study (Figure 1), polydatin demonstrated a possible impeding activity on the PCSK9 & LDLR binding [77]. This interaction is shown to be mediated by a number of hydrogen bonds via the immediate attraction of polydatin towards the activated binding site of PCSK9. The cited study's authors discovered that therapy with 20 M of polydatin reversed the impact of palmitic-acid's inducing action on PCSK9 protein concentration in insulin-resistant hepatocyte, raising the possibility that polydatin possesses beneficial effect on type 2 diabetes (T2DM)[76]. Employing glucosides rather than free-aglycone in these trials is a significant limitation.

Regarding humans, presently there is lack of information on resveratrol's impact on PCSK9, even the substance's ability to affect lipid profiles is still up for argument. The findings of a latest meta-analysis comprising 20 trials revealed no correlation between resveratrol supplementation and plasma concentration of LDL-cholesterol, indicating that the observed cardiac protective benefits of resveratrol might be caused by its impact over other parameters besides lipids [78]. Conversely, lengthier trials evaluating resveratrol effects (3 months) were shown to significantly lower plasma LDL-cholesterol, according to the findings of another meta-analysis [79]. Based on the information now available, larger, and longer research is still required to conclusively establish how resveratrol affects cholesterol levels.

The photosensitivity, low solubility, and quick metabolism of resveratrol have an adverse effect on its biological activities and bioavailability. Human plasma concentrations of resveratrol ranged from 1 to 5 nanograms (ng)/mL after consumption of a 25 mg dosage [80]. Resveratrol may accumulate in different organs together with tissues, including the gut, brain, and liver because of its lipophilic nature. Human plasma, tissues, and urine have been found to contain about 20 resveratrol-derived compounds. The most prevalent circulating metabolite from the liver amongst them is reportedly resveratrol-3-O-sulfate [81]. The gut bacteria may also transform resveratrol and its metabolites in the colon, producing dihydroresveratrol [82].

Quercetin

A flavonoid called quercetin, also referred as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one, is found in a wide variety of fruits and vegetables (Table 1). In hepatic cells, quercetin significantly increases the gene expression of the LDL receptors which increases LDL absorption. The stimulation of the transcription factor SREBP2 appears to be the pathway by which this impact is mediated [83]. According to in vitro research, a decrease of 20–30% in PCSK9 mRNA was observed when quercetin in its glycosylated state was given to HepG-2 cells at a dosage range of 1–10 uM. Additionally, researchers noted a 30–35% decrease in PCSK9 expression in the culture media and a 20–90% rise in cellular PCSK9 concentrations [84]. The latter resulted from Sortilin, which is a protein that stimulates the cellular release of PCSK9 via the trans-Golgi pathway towards the cell membrane, being negatively regulated [18]. It's important to note that quercetin 20 uM influences PCSK9 production in foam cell macrophage as well as in liver cells [85]. Since PCSK9 adversely regulates cholesterol absorption and inflammation in phagocytes, it may reveal a direct antiatherogenic as well as the impact of quercetin [86, 87]. In contrast to hepatic cells and macrophages, mouse pancreatic cells expressed more PCSK9 and LDLR when quercetin 3-

glucoside was present. This was viewed as having a positive effect, though. Indeed, cholesterol-dependent malfunction in these cells may be avoided due to the larger rise in PCSK9 compared to LDLR caused by quercetin [88].

The applicability of the aforementioned in vitro experiments is severely constrained by the elevated levels of quercetin and the utilization of the active component's glycosylated state than its free form. However, comparable action on PCSK9 observed in in vivo, where the microbial's enzyme-mediated cleavage generates an aglycon that may be absorbed. In fact, quercetin-3-glucoside administration (0.05 and 0.1% w/w) decreased PCSK9 circulating levels and boosted LDLR gene expression over liver cell surface in mice given a high-cholesterol diet. As seen in vitro, supplementation greatly raised pancreatic PCSK9 levels. In apo-E/ animals given a diet rich in fat, quercetin administration over 12 weeks led toa decrease in PCSK9 expression in the hepatocytes and aorta. These outcomes implies quercetin has various anti-atherosclerotic effects [89].

Specific clinical data is still lacking demonstrating quercetin's impact on PCSK9 levels in the blood. However, several human research certainly emphasized this flavonoid's ability to decrease cholesterol. An example is how a latest meta-analysis of studies with randomized control has demonstrated given that quercetin supplementation could decrease LDL-C concentration by about 12% [90]. In terms of pharmacokinetics, quercetin has poor oral absorption and low solubility in water, which results in physiological plasma values that are less than micromolar [91]. Further lowering its bioavailability is the fact that quercetin is identified as the substrate as well as regulator of the P-gp along with breast-carcinoma resistance-protein (BCRP) (Table 1) [92]. The primary type of quercetin present in nature, quercetin glycosides, are deglycosylated within the gut to produce the quercetin-free state, which is further a substrate for the hepatic enzymes that produce the metabolites quercetin-3-sulfate, quercetin-3-glucuronide in addition to quercetin-3′-sulfate [93]. Additionally, 3,4-dihydroxyphenylacetic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid, are produced by the gut bacteria during the metabolism of quercetin [93].

In order to increase polyphenols' solubility or stop them from degrading or being metabolized, several formulations have been developed [94]. A new quercetin formulation comprised of lecithin has been assessed on healthy individuals and demonstrated a notable increase in solubility and, thus, bioavailability [95].

Other polyphenolic compounds

Some additional polyphenolic compounds have been demonstrated to have an effect on PCSK9 based on preliminary animal or laboratory studies. The bioactivity of these chemicals with regard to PCSK9 needs to be further characterized, however, data are insufficient and more research is required. A drug-screening study, for instance, showed that a compound flavonolignan silibinin A is a modulator of PCSK9 promoter action (Figure 1 and Table 1) [96]. PCSK-9 mRNA concentration and protein gene expression decreased in HepG2 when silibinin A levels increased from 10 to 100 uM in a dosage-dependent way. The inhibition of the p-38 mitogen-stimulated protein-kinase (MAPK) cascade was necessary for this action. Significantly, silibinin-A showed to be capable of reducing the atorvastatin-triggered PCSK9, and a full neutralizing action was shown at a concentration of 50 uM, indicating that silibinin A is an interesting possibility to reverse the detrimental effects of statins on PCSK9 [96]. An ex vivo investigation has shown that silibinin may be processed through the gut microorganisms (producing de-methylated chemicals) as well as the hepatocytes, which synthesize sulfate along with glucuronide associates [97].

Pinostrobin

Pinus strobus L., *Cajanus cajan* (L.) Millsp. Belongs to Fabaceae family, *Boesenbergia pandurata* (Roxb.) Schltr., and *Boesenbergia rotunda* (L.) Mansf., and belonging to the Zingiberaceae family, are plants that contain the flavanone pinostrobin, which was investigated for its possible impact over PCSK9. Pinostrobin therapy of HepG2 with 20 and 40 uM decreased PCSK9-mRNA along with protein gene expression and its ability to catalyze in a dose-dependent manner, which boosted LDL uptake receptors expression and LDL absorption through the cells [98]. After intravenous and oral doses of

pinostrobin to rats, stereospecific variations in the drug's pharmacokinetic profile were found [99].

Naringin

The flavanone-7-O-glycoside naringin (also known as naringenin 7-O-neo-hesperidoside) that was derived out of grapefruit along with additional citrus varieties (Rutaceae) was given in dosage of 25, 50, or 100 mg/kg/day over eight weeks in obese mice. As a result, the LDL uptake receptor was stimulated. Naringenin has been reported to lower PCSK9 and LDL-cholesterol levels in the blood in a dose-dependent manner [100]. Naringin undergoes degradation to its counterpart aglycon naringenin employing hydrolase enzymes in addition to gut bacteria when administered orally. Partially absorbed, naringenin subsequently undergoes phase I as well as phase II metabolism. In the meantime, gut bacteria continue to break down, unabsorbed naringenin, as well as the metabolites are released through the enterohepatic dissemination to phenolic catabolites[101].

Ikarisoside

The dried aerial portions of *Epimedium koreanum* Nakai (Berberidaceae), also known as *Herba Epimedii*, were traditionally utilized as a tonic or as a remedy for rheumatic, hypertensive, and paralytic disorders as well as dementia [102]. This plant possesses lignans, prenylated flavonoids, and phenol glycosides [103]. Individual components, such as icariin and *E. koreanum* extracts, showed an array of biological actions, comprising anti-hepatotoxic, anti-tumor, anti-inflammatory, immunoadjuvant, and antiosteoporosis properties as well as improving sexual function[104]. *E. koreanum* aerial portions were subjected to phytochemical analysis utilizing the PCSK9 mRNA monitoring test. To clarify the structures of novel substances, MS, NMR, and other techniques were used in the study. In HepG2 cells, the inhibitory effect of each isolated drug was evaluated against PCSK9 mRNA expression. Among the 22 compounds isolated from the plant, it was investigated that compounds icariside I, icariin, ikarisoside A, korepimedoside A, kor, epimeoside C, caohuoside B, epimedokoreanoside I, epimedin L, and epimedoicarisoside A reduce PCSK9 expression of mRNA and protein. It was demonstrated that compound icariside I in particular increased LDLR mRNA expression[105]. Ikarisoside A may have a positive effect on cholesterol levels. Therefore, ikarisoside A may be worthy of further investigation both in vitro and in vivo.

Eugenol

Clove oil [*Syzygium aromaticum* (L.)] contains eugenol (4-allyl-2-methoxyphenol), a phenolic compound alongside documented hypo-cholesterolemia properties. It was deemed a risk-free dietary element, having a daily consumption limit for humans of about 2.5 mg/kg body weight. Eugenol appears to hold a preventive action against atherosclerosis and fatty liver disorders in animal experiments because it decreases blood cholesterol in addition to preventing lipogenesis within the liver [106]. A molecular docking investigation conducted more recently (Figure 1) found hydrophobic bonds between PCSK9 and its ligand, eugenol. Additionally, it was believed that eugenol decreased PCSK-9 expression in Jurkat cell lines [107]. The two chemicals may physically interact to provide this effect, or eugenol may indirectly block the SREBP pathway (Figure 1)[106]. Experimental models are the only ones that have looked at the pharmacokinetic characteristics of eugenol[108].

Phytosterols and plant derived proteins

Plant sterols/stanols are one of the dietary supplements/substituents for cholesterol-lowering that have the broadest range of applications [109]. However, there are no definitive data on these chemicals, and the data that do exist practically reveal no effect on PCSK9 levels. The significance of PCSK9 in the LDL-C-reducing action of plant stanol consumption has been examined by two groups [110]. In a placebo-controlled experiment in healthy as well as individuals with raised cholesterol, Simonen et al. evaluated the implication of consuming oil-based spread (20 gram/day) for 6 months, either with (plant-stanol group) or without it (control-group), with plant stanols (3 g/day) as an ester. Phytosterol derivatives are capable of decreasing LDL-C by preventing cholesterol uptake by interrupting PCSK9 metabolic pathway. According to research that found prolonged consumption of phytosterol esters decreased LDL-C by 7–10% free from changing PCSK9 levels in blood or hepatocyte LDL-R

expression [111]. De Smet et al. demonstrated that in mice, short-term consumption of phytosterol esters (0.25 mg cholesterol plus 50 mg of plant sterols esters dispersed in olive oil) increased the mRNA gene expression of the enteric genes PCSK9 along with LDLR as well as the primary transcriptional factor comprising SREBP-2, while hepatocyte activation of these genes decreased following 15 minutes. Reduced plasma LDL-C levels in addition to decreased enteric absorption of cholesterol happened simultaneously [112].

A number of dietary peptides from plant sources decrease cholesterol through interactions with bile acid micelles that formed [113]. However, they once more exhibit no PCSK9 activity. Instead, the instance of dietary peptides decreasing LDL-cholesterol through statin-like processes is more interesting. Both lupin as well as soy peptide combinations were successful in inhibiting HMG-CoA reductase activities by more than 50% at concentrations of 0.5 mg/mL [114]. For soy -beta-conglycinin, a similar process has been described [115]. Additionally, it appears that hempseed polypeptides (derived from *Cannabis sativa* L. Cannabaceae) exhibit cholesterol lowering effect through a statin-like [116]. On the basis of molecular docking studies and enzyme tests, the process of HMG-CoA reductase suppression was proposed for some peptides (TPMASD, PMAS and HFKW), which is consistent with their 3-dimensional similarities with statins [117]. The impeding effect was, although, much reduced compared to the nano-molar IC50 concentrations of recognized statins.

Soy protein

The majority of dietary proteins have been studied for their ability to regulate metabolism [118]. In a paper evaluation the impact of herbs and animal-based protein foods on lowering lipids, it was stated that proteins derived from Glycine constitute the prototype of plant-based proteins and have gained popularity [119]. LDL cholesterol is reduced between 3% and 10% when daily soy dosages are consumed; this impact is independent of fluctuations in PCSK9 circulatory levels [120].

Lupin

The four familial lupin species—*Lupinus albus* (white lupin),), *L. angustifolius* (sweet leaf lupin; Fabaceae), *L. mutabilis* (pearl lupin), and *L. luteus* (yellow lupin)—represent the lupin, a grain legume high in protein. For many years, studies have been conducted on lupin proteins primarily owing to their ability to lower plasma cholesterol, which is mostly attributed to an LDLR-activating pathway [121]. Lupin proteins have demonstrated hypolipidemia and a notable antiatherotic activity in animal models [122]. A beneficial impact on LDL-cholesterol as well as the LDL: HDL cholesterol proportion was revealed in two investigations, one constituting supplementation and the further with food enrichment, which was conducted clinically on hypercholesterolemic patients primarily [123].

Contrarily, cellulose and lupin protein mixtures had a notable hypocholesterolemic impact, which was accompanied by a drop in PCSK9 plasma levels (8.5% vs. control) [124]. The nutritional supplementation through lupin proteins resulted in an 8% decrease in LDL-cholesterol and a decrease of 12.7% (vs. baseline) in PCSK9 levels in individuals with metabolic syndrome [125]. A mechanism has just recently been proposed, according to which lupin proteins reduce the amounts of the proteins PCSK9 and HNF1 in HepG2 cells [126]. Presently, there is increasing attention in the newly discovered suppressing route of functional foods, which is connected to equally LDLR overexpression and potential PCSK9 antagonism, and which may result in novel strategies of cardiovascular protection.

Nutraceutical/Dietary nutrients

Lycopene

As a member of the carotenoids, belonging to family of antioxidants with lipid-soluble compounds, lycopene is mostly found in tomatoes as well as tomato-covering products, accounting for nearly 80% of all lycopene consumption. Carotenoids are also prevalent in other fruits and vegetables [127]. Lycopene appears to have a number of positive impacts on maintaining cardiovascular health and function, according to growing data. Lycopene is the carotenoid with the most antioxidant activity, but it also appears to have other cardioprotective qualities, including anti-inflammatory effects, the ability to prevent platelet aggregation and endothelium protection[128]. It was recently demonstrated in an in vivo investigation that lycopene treatment in hyper

triglyceridemic rats (with a dose of 5mg, 10mg, as well as 50 mg/kg body weight/day) reduced the expression of PCSK9-mRNA in the liver by twice and thrice via the ubiquitin-induced proteasomal breakdown of HNF1 [129]. The fact that lycopene treatment significantly lowered plasma levels of LDL-cholesterol, TG, and VLDL cholesterol, in addition to greatest effect occurring at the maximum dose (85.3%, 55.5%, along with 55.5%, correspondingly), partially accounts for the decreased plasma concentration of atherosclerotic lipoproteins in rats cured by lycopene. The authors proposed that the lycopene-mediated reduction in PCSK9 genetic expression in rats with raised cholesterol may be relevant to the blocking of lycopene-mediated inflammatory mediators as a result of the reported reciprocating regulation among PCSK9 and inflammatory cytokines [129] [130]. Treatment led to a significant reduction in the levels of permeating interleukin (IL)-1, IL-6, as well as (TNF), which was 45, 39.3%, Finally, the scientists showed through in-silico molecular modeling experiments that lycopene decreases PCSK9's affinity for the multifaceted EGFA (epidermal growth factor-A) of LDL-R.

Different research also showed that lycopene inhibits LPS-stimulated hepatocellular activation of PCSK9 within the rats through suppression of HNF1 expression and perhaps by overexpression of farnesoid-X-receptor (FXR) in addition to/or PPAR [131]. Again, the demonstrated decrease of PCSK9 expression in LPS-treated animals is probably responsible for the reported restoration of the inflammatory cascades (64.1%, 20.9%,25.7%, and 27.4% on permeating IL-6, TNF-1, CRP, in addition to IL-1, correspondingly, relative to the LPS- control rats).

The major issue stems from lycopene's limited bioavailability; solely 10–30% of lycopene in the human body absorbs in trans-isomeric form from dietary sources [132]. Its bioavailability is influenced by a number of variables, including the many biochemical variants of lycopene, lycopene sources, dosages, dietary co-ingestion, and hereditary factors. In fact, there are a minimum of 28 single-nucleotide variants in sixteen genes, including those encoding the membrane cholesterol carrier scavenging receptor belonging to type B, the molecular signal slit homologous sequence 3 (SLIT3), member-1 (SCARB1), in addition to steroid-degradation enzyme dehydrogenase/reductase (SDR family) member of type 2 (DHRS2). Recent research on novel approaches to solving bioavailability issues is used in animal rheumatoid arthritis (RA) models to evaluate nano drugs in a nano sized emulsion containing lycopene like an anti-inflammatory compound [133]. It's been observed that b,b-carotene 9′,10′-dioxygenase (BCO2, an enzyme, may accelerate a peculiar breakdown of each provitamins as well as non-provitamin A (carotenoids that make apo-10′-carotenoids, including apo-10′-lycopenoids derived lycopene, that has turned out to regulate a portion of its physiological functions of lycopene [134] [135].

Welsh Onion

The perennial Welsh-onion (scientifically known as *Allium fistulosum* L., from Amaryllidaceae) is cultivated extensively all over the world, although it is most popular in Asia. The ethanolic extract of Welsh onion believed to regulate the activation of many genes related to lipid as well as cholesterol metabolic processes in reaction to plasma depletion of lipids in HepG2 cells [136]. The extract substantially maintained the expression of the LDLR protein at concentrations ranging from 50 gram per milliliter to 200 grams per milliliter. A substantial decrease in PCSK9 mRNA levels was also seen in the same quantities, which is noteworthy since it suggests a deleterious effect on gene transcription. The authors found that both SREBP2 and HNF1 were significantly reduced, which is consistent with their theory [136]. Ethanolic extract of Welsh onion additionally decreased PCSK-9 protein gene expression as shown through western-blot examination of all-out protein extracts, with no appreciable alteration in LDL uptake receptor concentrations. These findings imply that Welsh onion is not involved in raising LDL-R genetic expression while having a considerable inhibitory impact on PCSK9. A detrimental impact on the SREBP or HNF1-mediated expression of PCSK-9 replication was further demonstrated by the ethanol extract's ability to inhibit the expression of PCSK9 through statins [136].

Kaempferol, p-coumaric acid, and quercetin were among the active ingredients found in the extract that greatly decreased the PCSK9 level in HepG2 cells

when lipid deprivation was present, although this impact was only seen at maximum laboratory concentrations (40 M). Ferulic acid, comparatively, had no discernible impact. Welsh onion ethanolic extract's hypolipidemic impact was studied in C57BL6/J mice given a diet high in fat that contained 60% fat, 20% carbs, and 20% protein [137]. The mice were given 400 mg/kg/day of Welsh onion extract, diluted in ordinary saline, orally for 6.5 weeks. With a considerable decrease in TG (46%), LDL-cholesterol (24%), and TC (11%), this supplementation significantly reduced body weight and food consumption. It's interesting to note that the authors also noticed a decrease in SREBP1c expression in the hepatocytes, corresponding to the in vitro evidence and indicating a potential impact on PCSK9, though this analysis has not been done [137] [136].

Pigeon pea

The pigeon pea, or *Cajanus cajan* (L.) Millsp., is an annual bean crop used in tropical as well as semi-arid tropical areas. *Cajanus cajan* L. turned out to be a traditional medicine in addition to being a dietary supplement [138]. Different portions belonging to Cajanus cajan L. have been discovered to exhibit ethnopharmacological effectiveness in addition to biological or pharmacologic properties, such as inflammation reducing, antioxidant, anti-atherogenic, anti-cancer, in addition to lipid lowering effects [139, 140]. pigeon pea leaves are abundant in flavones and stilbenes, according to chemical tests [141, 142]. Among these, cajaninstilbene acid (CSA), a kind related to stilbene, is mostly found within its leaves [143]. CSA is 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid. In an invitro analysis, cell subjected to MECC (0.05 along with 0.1mg/mL for 24 hours) remarkably ($p<0.05$) improved LDLR mRNA expression and LDLR protein on RT-qPCR as well as western-blot-analysis [144]. In rats with diet-induced hypercholesterolemia, the stilbene-enriched extract of pigeon pea decreased plasma lipid levels [145]. Heng et al observed that In HepG2 cells, MECC boosted LDLR expression, LDLR levels on the hepatic cell surface, and LDL absorption activity. They observed that cajaninstilbene acid, a stilbene present in pigeon pea is credited for the cholesterol reducing effect of Pigeon pea leaves. Moreover, MECC downregulated the PCSK-9 mRNA as well as protein levels but did not affect the expression of other lipids or lipid metabolic processes-related genes comprising HMG-CoA reductase (HMGCR), LDLR degradation inducer (IDOL), acetyl-CoA carboxylase (ACC1), fatty acid synthase (FASN), as well as liver-X receptor-α (LXR-α) within the HepG2 cell line. Additionally, MECC decreased hepatocyte nuclear factor-1 (HNF-1), a key transcriptional modulator as to activating the PCSK9 promoter, however, there is no nuclear sterol-responsive element binding protein-2 (SREBP-2) expression , in human hepatoma cells, thereby reducing the expression of the PCSK9 gene [144]. Apart from cajaninstilbene acid, it is necessary to investigate if additional stilbenes or flavanones contained within MECC possessing comparable effects or the combined impact of following substances might play a role in altering LDLR as well as PCSK9 gene expression to regulate cholesterol levels.

Allicin and Capsaicin

Garlic (known as *Allium sativum* L.) is extensively utilized in cuisine, spices, in addition to traditional remedies worldwide [146]. Garlic has previously been effective in treating infections, cardiac illnesses, rheumatism, raised blood pressure, diabetes, elevated cholesterol, as well as preventing arterial plaques and carcinomas [146]. Garlic is hypotensive, antimicrobial, hypolipidemic, along with antihyperglycemic [147, 148]. S-allyl-cysteine sulfoxide (alliin), diallyl sulfide (DAS), E/Z-ajoene, diallyl trisulfide (DATS), diallyl thiosulfonate (allicin), diallyl disulfide (DADS), as well as S-allyl-cysteine (SAC) are all organosulfur components present in garlic [149]. Allicin, the main active ingredient of garlic, is synthesized through alliinase from alliin, that gets released and stimulated by uncooked garlic [149].

Capsicum annuum (Solanaceae), a spicy condiment sometimes recognized as chili-pepper or red-pepper. The capsaicinoid chemicals found in *Capsicum annuum* include capsaicin, homocapsaicin, dihydrocapsaicin, and homodihydrocapsaicin [150]. Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) being the primary constituent of capsaicinoid, and it possesses

antioxidant, weight-loss, anti-inflammatory, as well as lipid regulating properties [151].

Nantiya et al. studied the LDLR expression in order to look at how allicin together with capsaicin affected the amount LDL receptor on cell-surface. Their findings demonstrated that capsaicin possessing no notable influence on the expression levels of LDL-R mRNA, but allicin (200 uM) considerably enhanced those levels. But in contrast to vehicle-treated cultures or the control group, capsaicin (200 uM) along with allicin (200 uM) considerably enhanced the LDLR protein by around 1.33 ($p < 0.05$)-fold as well as 1.86 ($p < 0.05$)-fold, accordingly [146]. In comparison to the control group, allicin (200 uM) and capsaicin (200 uM) markedly enhanced the levels of LDLR on HepG2 cell surface by around 182.33% ($p < 0.001$) as well as 147.22% ($p < 0.001$), correspondingly by flowcytometry analysis. Capsaicin (200 uM) and Allicin (200 uM) did not substantially alter the levels of LDLR protein compared to an atorvastatin (10 uM) positive control. Similarly, Capsaicin (200uM) and Allicin (200 uM) significantly improved LDL absorption into human hepatocarcinoma cells by about 4.82 and 4.64 folds respectively when investigated by Confocal laser scanning microscopy (CLSM) [146]. Additionally, in HepG2 cells, allicin (200 uM) remarkably reduced the PCSK9 mRNA expression, although capsaicin had no noticeable effect. However, PCSK9 protein expression and concentrations in the culture medium were markedly reduced by allicin and capsaicin. The positive control drug, atorvastatin (10 uM), on the other hand, markedly raised PCSK9 mRNA and protein expression levels[146].

In the same research, action of Allicin and capsaicin was observed on mRNA and protein expression of transcriptional factors HNF1α and SREBP that contribute in regulation of PCSK9 genes [50]. Allicin-200 uM together with capsaicin-200 uM considerably raised the SREBP2 protein expression compared to the control group by about 1.19-folds ($p < 0.01$) and 1.28-folds ($p < 0.05$), in specified order, despite not significantly altering the SREBP2 mRNA. Furthermore, comparative to vehicle-treated cells, capsaicin yet not allicin, remarkably lowered the mRNA expression of HNF1. Additionally, in contrast to cells that had been treated with a vehicle or the control group, allicin-200 uM along with capsaicin-200 uM dramatically reduced the concentrations of HNF1 protein expression. The mRNA and protein of SREBP2 and HNF1 expression were dramatically raised by the positive control atorvastatin (10 uM) [146].

Kenaf

Kenaf (known as *Hibiscus cannabinus* L., from Malvaceae) and refined kenaf seed-meal (DKSM) are economical agricultural wastes that have the potential to be used as hypocholesterolemic dietary ingredients with added value [152]. In contrast to typical eatable flours, such as wheat, sweet potato flour, rice, phenolic compounds, in addition to saponins are significant biologically active groups in DKSM offering greater protection from oxidative compounds. Recently, rats fed an atherogenic diet rich in fat and cholesterol that included either 15% or 30% DKSM had their hypocholesterolemic effect examined. Rats were either given 2.3 percent or 4.6 percent of the polyphenolic-saponin abundant extract (PSRE) from DKSM in addition to the same diet. P-coumaric acid, catechin, caffeic acid, as well as gallic acid were the most prominently active substances found in DKSM or PSRE [152].

In hypercholesteremic rats supplemented with DKSM and PSRE at equal dosages for 10 weeks, there was a significant reduction of atherogenic risk with decreased concentration of total as well as LDL cholesterol and raised levels of HDL cholesterol [152]. HMG-CoA reductase within the hepatocytes and, particularly, serum PCSK-9 expression was decreased by DKSM and PSRE. These outcomes are most likely because of the presence of saponin components along with polyphenolics. According to many animal models, p-coumaric acid, catechin, caffeic acid, as well as gallic acid all show anti-hypercholesterolemic effects [153]. Saponins are the substances that are most likely to have had an impact on PCSK9 expression since they appear to specifically interact with the SREBP transcriptional factor [154].

Curcumin

One out of the primary biologically active polyphenolic compounds found in the turmeric, curcumin is extracted out of the root of *Curcuma longa* L. (family: Zingiberaceae), a member of the ginger family. In a dose- as well as time-dependent manner,

curcumin enhanced the concentration of the LDLR as well as LDL absorption in human hepatoma cells [155]. The SREBP pathway was activated to cause this action, while additional research did not support this conclusion [156]. More recently, it was shown that curcumin had a stimulating impact on LDLR expression and activities. However, this rise wasn't followed by modifications to LDLR transcription or mRNA stability, indicating that the regulation was occurring at the transcriptional stage [157]. Curcumin-10 as well as 20 uM over twenty-four hours significantly lower PCSK-9 mRNA in addition to protein expression within liver cells. The action of curcumin on PCSK9 in this study was mediated via the transcription factor HNF1, not SREBP. Curcumin, however, nearly entirely reversed the PCSK9-stimulating effects of lovastatin, indicating that it may be able to block the impact of statins on circulating PCSK9 [158]. This finding also offers new opportunities for innovative naturopathic cholesterol-lowering combination strategies. The significance of these findings is diminished by the greater curcumin concentrations, which, despite being extensively utilized within the cultured cell systems, are greater than those attained in animal studies.

In 2017, curcumin was reported to inhibit PCSK9 in vivo for the first time. The authors propose that curcumin has an anti-endotoxemic effect that might enhance LPS clearance through the LDL uptake receptors. The writers found that, despite no change in mRNA, giving cirrhotic rats 200 mg/kg/day of curcumin for 12 weeks caused a rise in LDLR protein expression within their livers. It was happened as a result of curcumin's suppression of PCSK9 protein and mRNA levels[159].

Although there is currently little information on how curcumin affects PCSK9 in humans, various research has looked at the impact on LDL cholesterol levels. These research findings are debatable [160], revealing a modest impact or no change, which is what a systematic review of randomized-controlled trials' findings demonstrated [161]. The demographic investigated, the length of therapy, and the formulation type, which might affect bioavailability, could be the causes of this disparity.

Curcumin's therapeutic utility is indeed limited by its poor water solubility, limited bioavailability, and unfavorable pharmacokinetic characteristics. With a t1/2 of less than 10 minutes, curcumin in particular exhibits poor stability under physiological circumstances [162]. The gut microbiota further transforms curcumin and its hepatic-derived metabolites, which are mostly coupled with glucuronide, glutathione, and sulfate to produce more than 10 distinct compounds, including tetrahydrocurcumin, bisdemethylcurcumin, and demethylcurcumin etc. [163] [164]. Several formulation strategies that will need to be put to the test in the proper pharmacological trials have been developed to address the pharmacokinetic problems with curcumin [165].

Omega 3 fatty acids

The last double-bond in the hydrocarbons (acyl) chain, which is between carbons 3 and 4, is what distinguishes omega3 (n-3) unsaturated fats (PUFA), considering the final methyl carbon as number one. Eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and docosapentaenoic acid (DPA; 22:5n-3), which are present in substantial levels in fish oil, fatty fish, as well as different seafood, are examples of longer chain n-3 fats. By positively modifying a number of CVD risk variables, including blood lipids, heart rate, heart rate variability, blood pressure, endothelial integrity, inflammation, and platelet aggregation, these have a number of cardioprotective benefits [166]. Regarding cholesterol metabolism, DHA and EPA have been demonstrated to decrease the synthesis of VLDL particles and may accelerate the clearance of triglycerides-rich lipoproteins (TGRL), while also accelerating the clearance of LDL particles [167]. The suppression of the SREBP1-stimulated pathways, which include the stimulation of HNF4, liver X receptor (LXR), FXR, as well as PPARs, appears to be the mechanism behind these effects [168, 169]. Studies in experimental animal demonstrated that prolonged consumption of fish oil (10% in diet) enriched in n-3 PUFAs decreases PCSK9 expression in hepatocyte, leading to a considerable 84% decrease in LDL-cholesterol and that an omega-3 enriched feed decreases PCSK9 plasma concentration in conjunction with a 40% decrease in plasma VLDL- and HDL-cholesterol [170, 171].

Canola oil rich food enhanced by DHA (6%) decreased plasma PCSK9 as well as TG levels when

contrast to canola along with canola oleic feed in patients with one of the risk factors associated with metabolic syndromes. Plasma PCSK9 concentrations were discovered to be strongly as well as favorably correlated with LDL-cholesterol, apoB , and TG levels in the same research [172]. Additionally, postmenopausal, and premenopausal women's plasma PCSK9 levels were lowered about 9.8% and 11.4%, respectively, by daily ingestion of marine n-3 PUFAs (including 38.5% EPA, 6.0% DPA and 25.9% DHA) without influencing plasma LDL-cholesterol levels [173].

Long-chain PUFA consumption has a number of positive benefits, however, EPA as well as DHA were found to raise LDL-C levels, as DHA to be powerful compared to EPA [174]. Steadily, it was found that supplementing with DHA, as opposed to EPA, raised LDL-C (+3.3%; $p = 0.038$) in addition to the average LDL particle size, and decreased the amount of small LDL (23.2%; $p = 0.01$) in men as well as women at increased risk of heart ailments [175]. Regardless of the rise in LDL cholesterol, each of EPA and DHA lowered PCSK9 concentrations in a comparable way when compared to the control (EPA, -218.2 ng/mL; DHA, -225.0 ng/mL). Additionally, after DHA but not following EPA, mutation in PCSK9 were certainly associated with alteration in LDL apoB-100 concentrations and negatively correlated with modifications in LDL apoB-100 fractional degradation rate, indicating that PCSK9 may have played a role in the different activities of DHA along with EPA dietary consumption on LDL metabolic processes. Additionally, Allaire et al. noted that the serum PCSK9 content was higher in subjects sensitive to DHA and EPA than in non-responders at baseline, indicating that this protein may modulate the effects of n-3 PUFAs [176]. It has been proposed that a modification of SREBP2-mediated pathways triggers the pathway involving the association in omega-3 along with PCSK9 [177]. Recent research also found a significant relationship between PUFA consumption in Costa Rican Hispanics and the common PCSK9 type rs11206510 situated in the active part of PCSK9 gene, which was linked to early onset coronary artery disease (MI) by a genome-wide analysis (GWAS) [178]. This relationship is related to the reciprocal regulation relationship among long-chain n-3 PUFAs as well as PCSK9. When compared to non-carriers, those who have this mutation reported a reduced risk of non-fatal MI [179].

The US Food and Drug Administration (FDA) permitted the utilization of many omega-3 preparations that are organically enriched or isolated from fish oil in the management of chronically raised TG levels. A proportion of these preparations offer EPA along with DHA in the form of ethyl esters (EE), which must be broken down by the enzyme carboxylic ester- lipase (a bile salt-mediated lipase). Because a fat-rich diet triggers the production of bile salts, the absorption of EPA as well as DHA sourced from n-3 EE foods is therefore strongly reliant on this. Technologies have been created in this area to promote bioavailability and improve both DHA and EPA absorption [180]. Recent research in humans demonstrated that partially digested omega-3-sn-1(3)-monoacylglycerol fatty acids structure (OM3-MAG) possess a remarkably higher uptake at elevated medicinal dosages (2.9 gram/day) compared to popular omega-3-EE (3.1 gram/day) state utilized for TG elevation, indicating the administration of the prohydrolyzed OM3-MAG to be a highly effective treatment in chronic CV illnesses in which raised quantities of omega-3 are needed along with a low-fat diet [181]. Some of natural pCSK9 inhibitors with their metabolism and effect on PCSK9 expression are given ion table 1.

Table-1: Pharmacodynamic and pharmacokinetic properties of natural compounds with PCSK9 inhibitory activities

Natural comp.	Half-life time (h)	Bioavail %	Metabolism	Mechanism of action	Effect on PCSK9 and LDLR	Level of evidence

Berberine	28.6	0.37	Demethylating and glucuronidation	Suppress SREBP and HNF1α	Dec. PCSK9 mRNA and protein Inc. LDLR mRNA and protein	In-vtro, in-vivo and clinical
Polyphenolic Compounds						
EGCG	3.4	0.1	Liver: methyl, sulfate, along with glucuronide, Gut: Phenylvalerolactones & phenylvaleric acid	Inhibit SREBP	Suppress PCSK9 mRNA as well as protein. Inc. LDLR mRNA along with protein	In-vitro, In-vivo and clinical trials
Resveratrol	9.2	<1	Liver: Glucuronide and Sulfate; Gut microorganisms: dihydroresveratrol	Impede SREBP1c as well as interaction PCSK9-LDLR	reduced PCSK9 while maintain LDL receptor (LDLR) expression	In-vitro and In-vivo
Quercetin	2.1	<0.31	Liver: methyl glucuronide along with Sulfate; Gut microbiota: 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid, free aglycone; 3-(3-hydroxyphenyl) propionic acid, along with 4-hydroxybenzoic acid	Inhibits secretion (Sortilin) and SREBP,	Decrease PCSK9 expression	In-vitro and In-vivo
Pinostrobin	38.1	1.8	Glucuronide	Block transcription along with catalytic activity	Decrease PCSK9 expression and reduce LDL-C	In-vitro
Naringin	9.5	<1	Liver: Hydrolysis together with glucuronide, methylation; sulfate, Gut microbiota: Phenol derivatives	Inhibits SREBP	reduce PCSK9 expression	In-vivo
Ikarisoside	Not Known	0.17	Not known	Not known	Reduce PCSK9 and Increase	In-vitro

					LDLR expression	
Eugenol	14	<1	Phenol, glucuronide, and sulphate	Direct binding with PCSK9 as well as inhibits SREBP	reduce PCSK9 expression	In-vitro
Phytosterols and plant derived proteins						
Soy protein	Not known	Not known	liver: Hydrolysis, Glucuronide, Sulphate, Methylation, gut: Gut microbiota	Modulate HNF1α	Reduce PCSK9 protein expression	In vitro, also in-vivo
Lupin	Not known	Poor predicted	Proteases	Blocks interaction PCSK9-LDLR; Suppress HNF1α	reduce PCSK9 expression	In-vitro, In-vivo Clinical(negative)
Dietary nutrients						
Lycopene	235	34	Phase I, oxidation	Inhibits transcription and interaction PCSK9-LDLR		In-vitro and in Kenaf-vivo
Welsh onion	Not known	<1	liver: Hydrolysis, Sulphate, Methylation, gut: Gut microbiota	Inhibits SREBP2 and HNF1	Reduce PCSK9 and increased LDLR expression	In vitro and in vivo
Curcumin	7	<1	Liver: glucuronide, and reduction, sulfate, in addition to glutathione; Gut microbiota: demethylcurcumin, tetrahydrocurcumin, bisdemethylcurcumin etc.	Impedes HNF1α	Reduce PCSK9 and Increase LDLR	In-vitro and in vivo
Allicin and Capsaicin	Not known	0.55	liver: Hydrolysis, Sulphate,	Inhibits; HNF1α	Allicin: increased LDLR expression and	In-vitro

			Methylation, gut: Gut microbiota		Capsaicin has no effect.	
Kenaf	Not known	0.55	liver: Hydrolysis, Sulphate, Methylation gut: Gut microbiota	Unknown	Decreased PCSK9 expression	In-vivo
Vitamin K 7	60	2	Not known	Unknown	Not known	In vitro and in-vivo
p-Coumaric acid	0.25	24	Glucuronide and sulphate	Inhibit transcription	Not known	In-vitro
Kaempferol	Unknown	2.5	Glucuronide in addition to sulphate	Inhibits transcription	Not known	In-vitro
Other Inhibitors						
Protodioscin	20	0.2	Oxidation, deglycosylation and glucuronid	Inhibits transcription	Unknown	In vitro and in vivo
Emodin	8.6	low	sulphate along with glucuronide	Suppress SREBP; HNF1α	Unknown	In-vitro

Other Nutrients

Cashew nuts

A higher intake of fruits, legumes, low-carb vegetables, nuts, fish, vegetable oils, whole grains, as well as yogurt combined with reduced consumption of processed and red meats, salt, and foods containing processed carbohydrates, have been linked with a decreased likelihood of coronary artery diseases, according to the latest guidelines provided by the *European Atherosclerosis Society (EAS) and European Society of Cardiology (ESC)* [182]. These findings suggest that substituting polyunsaturated fatty acids (PUFAs) and vegetable sources of fat for animal fats may reduce the risk of cardiovascular disease (CVD). Four contradictory studies are the only clinical studies linking cashew nuts with elevated cardiac risk variables, such as LDL-C [183, 184]. In a randomized crossing experiment including a total of 42 people, the consumption of 42 grams of cashews daily was linked to a substantial decline in PCSK9 plasma concentration (270.8 ng/mL compared to 252.6 ng/mL) [185]. The chemical ingredient that inhibits PCSK9 is currently unknown, and this impact was not linked to any appreciable changes in LDL cholesterol.

Vitamin K

Menaquinones, or vitamin K2, phylloquinone, or vitamin K1, are the two dietary forms of vitamin K. The major sources of vitamin K2 are fermented foods like cheese as well as "natto," a soybean product from Japan [186]. From MK-4 to MK-15, there are more than 12 distinct forms of MK-n, however "n" denotes the quantity of polyunsaturated isoprenoid moieties joined with the menaquinone. MK-7 has a better pharmacokinetic profile than MK-4, comprising an extended half-life as well as increased bio accessibility. It is mostly manufactured via submerged fermentation utilizing *Bacillus subtilis* [187]. After being absorbed through the intestinal wall, vitamin K is packed into chylomicrons by being dissolved in bile salts as well as pancreatic juice [188]. The Required Daily allowance (RDI) of vitamin K, ideally as a vitamin K2 (200 g/day for MK7), was proposed by European experts as the amount necessary for the optimum carboxylation of extrahepatic -GLA-proteins. An ancient investigation on patients with chronic kidney disease receiving peritoneal dialysis on a continuous basis gave rise to the hypothesis that vitamin K has a

hypocholesterolemic effect. The biochemical research revealed that the TC level at 12 weeks were much greater compared to those at 7 months or beyond after several months of receiving vitamin K2 at a relatively high dose (45 mg daily). On LDL cholesterol levels, similar effects were shown [189].

Recently, with the administration of the nutritional blend RenaTris®, which contains MK-7, Sucrosomial® Iron, and magnesium carbonate, we observed a decrease in TC levels in uremic rats [190]. It was discovered through in vitro tests using hepatoma cells that MK7 alone lowers cholesterol production, perhaps by interfering with an enzyme in the mevalonate cascade, which is prior to squalene synthase. Similar to statins, MK7 increases LDLR in response to the reduction of cholesterol production; this impact was neutralized via incubation accompanied by squalene [190]. The production and secretion of PCSK9 by hepatoma cells was found to be suppressed by MK7, in contrast to statin, which increases PCSK9 expression [190]. Thus, this is very comparable to what has been seen with berberine, even if the exact mechanism by which MK7 works is still a mystery.

Chitosan oligosaccharides

Chitosan-derived oligosaccharides, composed of straight chain polymers that consist of deacetylated and N-Acetyl-D-glucosamine, offer a variety of positive benefits, including antioxidant, anti-tumor, antibacterial, as well as anti-inflammatory properties [191]. Additionally, their lipid regulating impact were also examined. An investigation revealed that chitosan derived oligosaccharides may dramatically reduce PCSK9 mRNA as well as protein in hepatocyte. The LDLR receptor's protein levels increased following the chitosan treatment, while its mRNA levels remained the same. Following treatment with chitosan oligosaccharides, the expression of SREBP-2 and HNF-1 dropped whereas FOXO3 increased. Chitosan oligosaccharides increase FOXO3a levels, which lowers HNF-1's ability to bind to the PCSK9 promoter along with repressing PCSK9 expression while raising LDLR levels as well as lowering blood LDL-C. PCSK9 expression is repressed as a result of decreased SREBP-2 levels brought on by chitosan oligosaccharides (Yang et al. Citation2018). The Small interfering RNA (siRNA), in particular, serves as a potential therapeutic option for a number of illnesses, including cancer [192]. SiRNA may specifically inhibit the translation of a protein by causing either mRNA degradation or RNA interference [193].

Probiotics

The gut bacteria greatly influence the pathophysiology of cholesterol metabolism and atherosclerosis [194]. As a result, a novel treatment strategy for managing hypercholesterolemia has been proposed, involving the utilization of certain probiotics possessing unique biophysiological characteristics. Only one research provided information on PCSK9 levels in this context [195]. This clinical study assessed the effectiveness as well as safety consideration of a nutraceutical formulation having *Bifidobacterium longum* BB-536, niacin, fermented red rice extract, as well as coenzyme-Q10 in reducing LDL cholesterol alongside the effectiveness and safety profile of a group of clinical and research-based cardiovascular disease indicators. This randomized, double-blinded placebo-controlled trial's findings showed that a 3-months treatment regimen remarkably decreased TC (16.7%), apo B (17%), and LDL-cholesterol (25.7%) without affecting PCSK9 plasma levels. It was determined from the evaluation of the concentrations of lathosterol, a marker of cholesterol biosynthesis, as well as campesterol, a marker of enteric cholesterol uptake, how *Bifidobacterium longum* BB536 may inhibit the potential increase in cholesterol absorption caused by monacolin K, a substance found in red yeast rice. The rise of PCSK9 plasma levels seen in individuals taking statins may be suppressed by *Bifidobacterium longum* BB536, in a similar vein [195]. It is unknown how *B. longum* BB536 could control PCSK9 expression, though.

Emodin

Among bioactive anthraquinone compounds found in *Rheum palmatum* L. belonging to Polygonaceae along with few additional Chinese botanicals is emodin, also known as 6-methyl-1,3,8-trihydroxy-anthraquinone [196]. Emodin administration at doses of 40miligrams as well as 80 milligram/kg/day demonstrated an amelioration in lipid concentrations related to a decrease in SREBP transcription in C57BL6/J mice given a fa enriched diet over 3

months [197]. Emodin has also been demonstrated to prevent hypercholesterolemia in rats on high-fat diets by inhibiting the biliary acids-CYP7A1 cascade. Emodin lowers lipid levels by binding to bile acids and reducing their absorption, which causes cholesterol to be directed towards the synthesis of bile acids [198]. Recently, it was shown that aloe, which also possesses emodin, can lower TC as well as LDL levels in rats with diet-driven hypercholesterolemia when given 100 mg/kg per day. It's important to note that aloe reduces the amount of fat in the liver, whereas HepG2 cell in vitro experiments reveal a detrimental impact on SREBP and HNF1. As anticipated, PCSK9 was downregulated in response to the suppression of each of transcriptional factors, which was paired with a raise in the expression of the LDLR and LDL uptake [199]. It has been demonstrated that the majority of the particular drugs block the PCSK9 transcription factors SREBP and HNF1. Epigallocatechin gallate, also known as (EGCG), that influences PCSK9 release; resveratrol; soy peptides; eugenol; in addition to lycopene, which prevent PCSK9 from interacting along with the LDL receptor; and subsequently, quercetin along with pinostrobin, which prevent PCSK9 from undergoing autocatalytic processing and development in the endoplasmic network. Currently no proof exist that organic substances can influence PCSK9 in a translational or epigenetic manner.

Dioscorea

For over 30 years, China has employed hydrous extracts of the roots of *Dioscorea zingiberensis*, Wright along with *Dioscorea nipponica* Makino from Dioscoreaceae to prevent and cure atherosclerotic cardiovascular disease (CVD). These goods received Dutch approval in 2012 as well. *Dioscorea nipponica* can lower TC, TG, and LDLC concentrations, based on several clinical studies [200]. Dioscorea has shown substantial lipid-lowering and anti-atherosclerotic benefits in apoE/ mice given a diet rich in fats over eighteen weeks in a traditional mouse model of atherosclerosis [200]. More significantly, Dioscorea decreased circulating PCSK9 as well as downregulated PCSK9 mRNA in hepatocytes. Protodioscin, dioscin, and pseudoprodioscin were found in the *Dioscorea nipponica* rhizome extract after its chemical composition was examined. The primary active ingredients are thought to be these steroidal saponins. While their respective aglycones may be accessible, some dioscin terpenoids are bonded with polysaccharides and are not taken in at the enteric level. It's interesting to note that PCSK9 expression has been demonstrated to be suppressed by protodioscin, methylprotodioscin, and pseudoprotodioscin (Figure 1 and Table 1) [201]. The LDLR protein was induced in HepG2 cells as a result of this action, which was linked to the suppression of SREBP transcription factors [201].

The active part of PCSK9 is uncertain since it remains unknown that if in vitro condition facilitates the release of aglycone from protodioscin as well as pseudoprotodioscin. Indeed, it is well known that HepG2 cells exhibit incredibly low levels of a variety of xenobiotic metabolizing enzymes, which might lead to false findings in pharmacological testing with substances that call for biotransformation [202-204]. It's especially true for organic substances that must be stimulated by gut microbiota catalysts that are absent from cultivated cells.

Conclusion

PCSK9 is a fundamental lipid metabolism regulator along with an effective targeted compound for lowering plasma LDL-C levels. The clinical effectiveness of two monoclonal antibodies that have received FDA/EMA approval, evolocumab, and alirocumab, demonstrates the significance of PCSK9 being a novel molecular targeted approach for curing hypercholesterolemia as well as and related cardiac disorders [205]. The only anti-PCSK9 treatments now available, the monoclonal antibodies feature a number of drawbacks: Subcutaneous administration (poor compliance and accessibility); (i) extremely expensive (ii) possible autoimmunity with long-term therapy. Inclisiran, a gene silencing RNA (siRNA) created to regulate PCSK9 mRNA in hepatocyte, serves as a more contemporary substitute for anti-PCSK9 antibodies. This strategy still has several shortcomings, though, including an extensive pharmacokinetic parameter, intravenous delivery, and a not yet known safety record [206]. Thus, there is a tremendous demand for less expensive, orally ingestible small-molecule medications. The discovery of organic substances possessing lipid-regulating effect coupled with anti-PCSK9 suppressing effect may provide a solution to this problem. We uncovered several substances with potent anti-PCSK9 inhibitory

activity in this review study, mostly through regulating transcriptional pathway, and some instances involving the self-degradation secretion stage or PCSK9 contact accompanied by the LDL uptake receptor. The fact that many of these potentially effective PCSK9 inhibitors have limited oral bioavailability and that there has been little proof of their effectiveness in in-vivo studies is a crucial factor. To boost oral bioavailability, however, a range of drug delivery strategies including chemically altered natural molecules are being investigated. Additionally, it must be acknowledged that, as previously described [207], the following crucial factors are important to consider when evaluating a nutraceutical's effectiveness: (i) to uncover the proof of clinical effectiveness assessed by placebo, double-blinded trials [207]; (ii) to discover evidence of origin and purity variations between goods on the market; and (iii) to assess the impact of mixing active substances. In order to investigate safety and efficacy in humans, pre-clinical research on experimental animals must first be conducted, then data based on the mode of action of active components under laboratory conditions must follow [207]. For the natural substances discussed in this review, not all these details have always been given. Consequently, the chosen molecules may only be viewed as launching pads intended to the future advancement in orally available PCSK9 modulators.

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