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Original Article

Pharmaceutical Importance of Components of Tea in Preventing Cardiovascular Diseases (CVDs), Pathways and Genes Involved in CVDs and Strategy to Enhance the Absorption of Tea Components by Human Body

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Abstract

The beneficial effects of consumption of tea on prevention of Cardiovascular diseases (CVDs) is established by several *in vitro* and *in vivo* experimentations., Biochemical constituents of tea are reported to upregulate several key genes involve in prevention of CVDs. Few other contemporary research findings also reported that tea polyphenols down regulate several genes associated with occurrence of CVDs. Tea components are reported to modulate several pathways relevant to CVDs. Experimental *in vitro* studies, 2D and 3D cell culture studies, supported by relevant in-vivo studies, may generate valuable insights on role of tea polyphenols on regulation of different relevant genes and pathways. Tea components, including the most important epigallocatechin-3-gallate (EGCG) and theaflavin (TF), are poorly absorbed by human digestive tract. Recent reports on the possible roles of gut microbiota on absorption of tea components and better absorption of methylated form of EGCG (3" ECGF) pave the ways of microbial, as well as, biochemical interventions that may improve the absorption efficiency of tea polyphenols. This manuscript reviews the most recent findings on how polyphenols from tea regulate the expression of genes and pathways associated with occurrence of CVDs, mechanism involve and how absorption of polyphenols by human digestive tract can be enhanced by microbial and chemical modifications. .

Introduction

Cardiovascular Diseases (CVDs), the leading cause of premature death and is responsible for around 38% of the premature death worldwide [1]. CVDs are group of disorders and known as coronary heart disease, as cerebrovascular diseases, as peripheral artery diseases depending on the organs affected or it may be due to other damage to the heart valve and muscles caused by the rheumatic fever. Except the CVDs caused by the inborn heart defect (congenial heart diseases), many of the CVDs are caused by the improper life style and external stimuli and can be prevented by following healthy life style with good behavioral habits. Individuals diagnosed with dyslipidemia , overweight and obese are more susceptible to CVDs [2]. Several factors *viz*. smoking, excessive drinking, age, pollution, exposure to stress *etc*. stimulate production of excess of reactive oxygen species (ROS) over the endogenous defense system that results in oxidative stress [3] and the oxidative stress is a proven causal factor of CVDs. Several biomarkers *viz*. PT, BC, HDL-C, LDL-C, levels of glucose and insulin on fasting, C-reactive protein(CRP), Tumor Necrosis Factor (TNF-α) *etc*. are reported as significant factors associated with risk of CVDs [4-6] . Strategy and intervention that may result in reduction of these biomarkers may be considered as an adequate approach to be included in the dietary guidelines to address this global problem.

Tea, *(Camellia sinensis (L).O.Kuntze)*, a globally important cash crop, is used to process the most consumed non-alcoholic beverage and considering its significance, the United Nations (UN) designated 21st May as "International Tea Day" to celebrate the tea industries around the world. Due to the difference in the manufacturing process of different kinds of tea (Figure 1) , the concentration of catechins ranges from 150 mg/g to 200 mg/g in green tea,

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from 40 mg/g to 60 mg/g in black tea and oolong tea in between. In black tea, due to the oxidation/fermentation process, the catechin is converted to theaflavin (5-20 mg/g) and thearubidin (60-80mg/g) [7].

Polyphenols, the end products of the plant flavonoid biosynthetic pathway, have the potentiality to affect many biological processes related to metabolism and CVDs [8]. Cao, et al. recently reviewed the epidemiological studies reported from several countries from Asia, Europe and America and most of these studies, except few, established a promising role of tea and its bioactive compounds on reducing the risk of occurrence of CVDs and the mortality caused by CVDs [9]. The impact of tea drinking habit on prevention of CVDs, however, depends on several physical factors *viz*. age, sex, body-mass index, hypertension, hip ratio along with socio-economic factors like lifestyle pattern, food habit, smoking, alcohol addiction [10] and the gut microbiota [11]. Lau, et al. [5] critically reviewed the 7 most relevant literatures how green tea catechin (GTC) affects cardiovascular health in humans. GTC, when supplemented at a dose of 300 - 1500 mg/day may have effect on one or more of the CVDs risk factors such as body weight, blood plasma factors associated with CVDs and systolic and diastolic blood pressure. Polyphenols have been reported to scavenge ROS, RNS, peroxidation products of biomolecules [12] and can prevent formation of highly damaging OH by acting as metal ion chelators [13]. In animal husbandry also, polyphenols have been widely used as antioxidants for treating diseases [14]. But, considering the low bioavailability of plant polyphenol, the assumption that polyphenols have direct role in preventing CVDs through their antioxidant properties is very unlikely. Further investigations highlighted the other effects of polyphenols, for *eg*., as signaling molecules, and may play a pivotal role in preventing CVDs [13,15]. Endothelium is a site of synthesis of vasoactive substances which may lead to vasorelaxation (such as endothelial NO synthase and prostacyclin) or may lead to vasoconstriction (such as endothelin-1, ET-1) and thereby regulating blood pressure [16]. Plant polyphenols, including polyphenols from tea, could inhibit release of the vaso-constricting peptide ET-1 with a simultaneous increase in eNOS expression [10,17]. Flow-mediated vasodilation (FMD) value, an indicator of the future cardiovascular events, reported to be increased by 2.6% on consumption of 500 mL of tea per day which was equivalent to 2–3 cups of tea per day [18].

Key pathways associated with CVD

The complex signal transduction network that responds to inflammation and oxidative stress (ROS) promotes the expression of senescence properties, as well as, aging. CVD pathophysiology is strongly correlated with these signaling cascades. Signal transduction performs crucial physiological and pathological roles in the highly complex but well-organized cardiovascular system [19].

In the pathophysiology of cardiovascular disease, the MAPK signaling cascades are most likely to have a crucial impact. Even though considerable amounts of studies have been conducted on the MAPK pathway, it is unclear how individual signaling proteins involved in the pathway contribute to the pathophysiology of different CVDs [20]. Several investigations used potential pharmacological inhibitors to investigate the involvement of the Raf-MKK-ERK downstream pathway in the emergence of cardiomyocyte hypertrophy. Treatment of cardiomyocytes with the U0126 (PD184352), an inhibitor of MKK1/2 significantly inhibited phenylephrine- and endothelin-1-induced protein synthesis [21]. In several studies, MAPKs have also been implicated in pathological cardiac remodeling. This context has been extensively studied in relation to p38 MAPK in particular. [22]. According to a contemporary theory of cardiac remodeling due to myocardial infection, activation of p38 MAPK along with JNK1/2 downstream signaling encourages fibrosis in the infarct region, as well as, in intact myocardium. It also promotes apoptotic processes resulting in subsequent infarct enlargement, and dilatation of the left ventricular chamber [23]. Recent studies have explored the function of MAPK signaling in the development of foam cells *in vitro* [24]. As explained by one study, 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole, a specific inhibitor of p38α and p38β, functions by inhibiting macrophage scavenger receptor CD36 expression and the consequent uptake of lipids, leading to inhibition of foam cell formation. According to these findings, in presence of oxLDL, p38 MAPK control PPARγ specifically or indirectly leading to the expression of CD36 [25]. These findings link the p38 MAPK downstream pathway to cell migration and formation of foam cell, implying a crucial function for p38 MAPK suppression in the pathogenesis of atherosclerosis.

The pathological processes of the majority of CVDs rely significantly on NF-kB activation. NF-kB downstream activation is implicated in all phases of atherosclerosis progression. Ox-LDL, TNF-α, IL-1, ROS, and AGEs (advanced glycation end products) are a few examples of the pro-atherogenic chemicals that activate NF-kB [26-29]. NF-kB stimulated expression of cytokines as well as chemokines are a result of TLR activation by Ox-LDL in vascular endothelial cells, accelerates atherosclerotic progression of the blood vessel's inner coat [30-32]. Pre-clinical studies also provided preliminary evidence that NF-kB influences the onset and development of heart disorders. A mouse model that constitutively expresses the active form of IKK2 demonstrated inflammatory infiltration, fibrosis, and atrophy of myocytes in hearts. Inhibition of NF-kB increased expression of IkBα actually prevented the onset of the disease, signifying the pivotal influence of NF-kB in this phenomenon [33]. Numerous clinical studies showed a significant correlation between active NF-kB signaling and heart failure. Many clinical investigations also reported that failing human hearts have NF-kB in its active state [34, 35].

Several cardiovascular disorders are also linked to dysregulation of JAK-STAT signaling [36]. The JAK-STAT signaling can be set off by a variety of stimuli, like myocardial infarction (MI), mechanical stretch, pressure overload, as well as, those that promote hypertrophic expansion of cardiac myocytes or offer cardiovascular protection [37-39]. Interferons and cytokines are significantly involved in the JAK-STAT signaling cascades that are linked to atherosclerosis. Atherosclerosis is exacerbated by IFN-γ (interferon gamma), which sends signals via Jak1/2 [40-42]. IL-6, belonging to the family of IL-6-type cytokine, is known to have direct pro-, as well as, anti-atherogenic actions and potentially contribute to human atherosclerotic events by activating JAK family members and STAT family members [43]. Several cellular processes, such as the activation of JAK- and Ras-mediated signaling, are triggered when IL-6 binds to its receptor. Activation of STAT transcription factors, STAT3 and SHP2 can be activated by phosphorylation by JAKs. After phosphorylation, STAT3 forms dimer and enables its migration into the nucleus and prompts transcription of the downstream

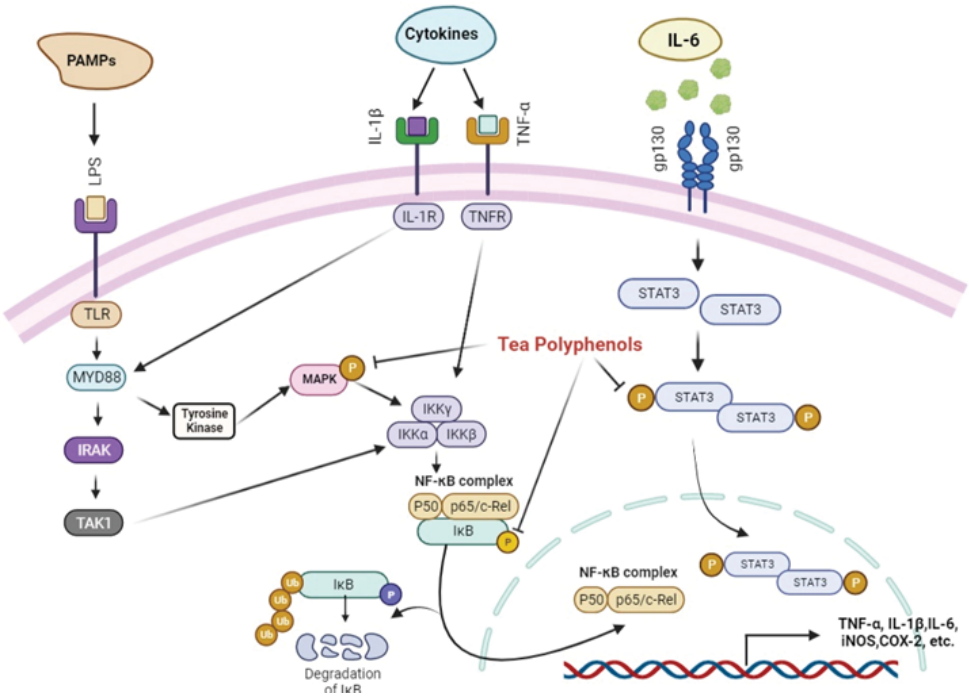

Figure 1

genes [44,45]. Cardiac myocytes, fibroblasts as well as, endothelial cells play roles in atherogenesis or myocardial diseases by regulating expression of the ;of the STAT family [46].

According to a recent research, the PI3K/Akt is crucial to the onset and progression of atherosclerosis [47,48]. Activated receptors directly stimulate and activate PI3K, causing phosphatidylinositol-3,4,5-triphosphate (PIP3) to accumulate in the plasma membrane and attract proteins associated with PH domain such as phosphoinositide-dependent kinase-1 (PDK1)-a AGC protein kinase that activate PI3K signaling pathway and protein kinase B (PKB) that regulate cardiac metabolism and contractility. Among the several PI3K isoforms, PI3Kγ is abundantly expressed in hematopoietic cells and consequently plays a vital part in the inflammatory process in atherosclerosis [49]. PI3Kγ expression is shown to be elevated in both atherosclerosis patients and mice models [50]. $ApoE^{-/-}$ and $LDLR^{-/-}$ mice mutants exhibited dramatically reduced leukocyte recruitment to the atherosclerotic lesion and improved plaque stability in response to PI3Kγ inhibition [50,51]. Alterations in Akt signaling are critical for numerous pathological cardiovascular processes, including cardiac hypertrophy atherosclerosis, and vascular remodeling. Activation of PI3Kα and PI3Kγ by GPCR can result in phosphorylation of Akt and suppression of GSK3β signaling, which ultimately leads to cardiac hypertrophy [52]. Constitutively activated Akt can accelerate cardiac angiogenesis. This could cause adaptive cardiac hypertrophy at first, but leads to cardiac hypertrophy and cardiac failure in the long run [53].

Key genes associated with CVD

The availability of complete sequence of human genome and identification of genes associated with CVDs open up new possibilities for treating CVDs. A convincing examples is the understanding the role of LDL receptor on hypercholesterolemia, a Nobel-Prize–winning discovery that led to the LDL cholesterol lowering therapies to treat CVDs [54] . Biochemical constituents of green tea, as well as, black reported to upregulate several key genes (*viz*. glucose transporter, LDL receptor, interleukin-10, genes in lipid metabolism *etc*.) involve in prevention of occurrence of CVDs and downregulate several genes (*viz* genes coding tumor necrosis factor, liver X receptor, fatty acid synthase, sirtuin1, transcription factors-kappa B *etc.*) associated with occurrence of CVDs. During the last 10 years hundreds of loci associated with cardiovascular diseases are identified. Christopher et al [54] reviewed the scientific reports on Genome Wide Association Studies (GWAS) made over different ethnic groups to identify loci associated with common CVDs *viz.* myocardial infarction (> nine loci), coronary artery diseases (>20 loci), ischemic stroke (*NINJ2*), heart failure (*LRIG3*), Intracranial aneurysm (*9p21 and SOX17*), dilated cardiomyopathy (*HSPB7 and BAG3*), total and LDL cholesterol (*SORT1 and HMGCR*) *etc*. Other such genes related to common causes of CVDs are: for hypertension (*CACNB2 and SH2B3*), for blood pressure (*SLC4A7 and PLCE1*), for triglycerides (*ANGPTL3 and JMJD1C*), myocardial infarction (SNP for 8 loci, 9p21 and SORT1) and for sudden cardiac arrest (*GPC5 and BAZ2B*) [55-59] *etc*. Tamariz, et al. [60] reported many SNPs associated with sudden cardiac death with the strongest association seen in SNPs related to intracellular calcium

handling. Large Genome Wide Association Studies for CVDs risk factors carried out in 2010 and 2011 reported association 95 gene loci with lipid traits, more than 30 gene loci with each of type 2 diabetes mellitus, body-mass index, obesity, blood pressure and hypertension [54]. They also showed that in human genome, except chromosome pairs 16, 18, 20, 22 and sex chromosomes, all pairs has one or more gene/s associated either with myocardial infarction and/or heart failure.

In an integrated approach to identify the genes associated with CVDs, Miao, et al. [61] utilized two gene expression profiles of datasets of CADs to identify the Differentially Expressed Genes (DEG) and concluded two genes- interleukin 1 beta (IL1B) and C-C motif Chemokine ligand (CCL-2) are crucial in occurrence of CVDs in human . Sun, et al. [62] investigated the plasm concentration level of HDL-C and of CCL2 in patient with CVDs. The authors concluded that the levels of HDL-c were reduced whereas level of CCL-2 were elevated in patients with coronary artery disease (CAD) and CAD is negatively correlated with mature HDL-C levels CCL 2-CCR2 signalling axis was also found to be associated with several CVDs related disorders *viz*. atherosclerosis, acute liver failure, pulmonary hypertension and diabetics. The gathered information may lead to development of effective CVDs therapies based on chemokines and their receptor modulators [63]. In CC-Chemokine Receptor 2 (CCR2)-deficient mutant, the reduction in atherosclerotic lesion was quite evident which was a clear indication of pathogenic role of CCR2 in atherosclerosis [64]. The Changes in gene expression pattern in peripheral mononuclear cells (PBMCs) upon high-polyphenol cocoa intake was investigated [65] and a moderate differential expression pattern of genes regulating inflammation - converging three major regulatory networks, was observed. The effected regulatory networks were: (1) decreased ROS production exhibiting differential expression of Hemoglobin Subunit Alpha ½ genes, FPR1, IL8, Sestrin 3, *CD36*, (2) Ca2+ modulation exhibiting differential expression of *ADRB2*, *IL8*, *IL8RA*, *IL8RB*, *FPR* genes and (3) inflammatory response modulation exhibiting differential expression of *IL8, IL8RA* and *IL8RB*, *PTPRC, TPT1*,*TIGIT*, *FPR1* genes.

The recent development in dissecting the mechanism of cardiovascular pathophysiology achieved through genetic discovery of key genes regulating the pathways associated with occurrence of CVDs provides new opportunities for prediction, prevention, as well as, treatment of CVDs by developing medicine and nutraceuticals on broad basis.

Regulation/alteration of different pathways associated with CVDs by tea components

Plant polyphenols, unlike drugs or micronutrients, have pleiotropic consequences and affect several biological processes correlated with metabolism well as CMDs. In a computational-based analysis of polyphenol versus protein interactome, Lacroix, et al. [9] showed that 5 pathways of the studied pathways, which constitute 55%, were highly enriched by the polyphenol.

Tea consumption appears to have a preventive impact against cardiovascular diseases. Consumption of black and/or green tea has been coupled with decreased risk of CVDs based on epidemiological evidence [57,58]. Though encouraging experimental and clinical evidence suggests the shielding effects of several tea polyphenols on cardiovascular health, not much is known about how these positive benefits of tea polyphenols are actually on a cellular level. Major flavonoids in tea includes epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) and their oxidized outcomes, theaflavins and thearubigins [60-62].

Epigallocatechin gallate (EGCG) protects the cardiovascular system owing to its anti-inflammatory, anti-oxidant, and anti-thrombogenic properties [63]. The phosphorylation activity of JNK 1/2, ERK 1/2, along with p38 in vascular smooth muscle cells (VSMC) caused by Ang II was dramatically diminished by EGCG. Additionally, EGCG also reduced the Ang II prompted expression of MAPK transcription factors like c-jun and c-fos [64]. Additionally, EGCG was shown to suppress the VMSCs proliferation elicited by homocysteine via the ERK1/2 and p38MAPK downstream signaling [66]. Furthermore, EGCG could impede the translocation of NF-kB into the nucleus by directly inhibiting the phosphorylation of IkB. IkBα degradation and NF-kB activity were both considerably reduced after receiving EGCG during reperfusion [67,68]. Interestingly, EGCG is also a strong antagonist of STAT-1 phosphorylation along with its activation. Suppression of STAT-1 phosphorylation by EGCG is reported to rescue cardiac myocytes from apoptosis induced by Ischemia/Reperfusion (IR) [69] . Fas receptor, a known pro-apoptotic target of STAT-1, was also suppressed by EGCG [70]. In addition, it was capable of preventing the activation of oxidized low-density lipoprotein-induced JAK2-STAT3 [71]. Another study reported that EGCG and zinc together prevented hypoxia/reoxygenation- stimulated cell death in rat H9c2 cardiac myoblast cells via activation of the PI3K/Akt signaling pathway [72]. A rat ischemia/reperfusion model also demonstrated that EGCG had cardioprotective effects, including prevention of ventricular arrhythmia and myocardial injury. It also inhibited mitochondrial DNA release and regulated the PI3K/Akt signaling pathway [73].

Theaflavins (TFs), on the other hand, are another significant group of polyphenols present in abundance in black and oolong teas. As reported by Chung, et al. TF3 was successful in reducing the phosphorylation of MAP kinase p38 [74]. Various studies on cancer cells reveal that the tea polyphenols regulates redox-sensitive NF-kB along with AP-1 [75]. However, there is a dearth of information on their effect on primary cells involved in the cardiovascular system. Although theaflavins' effect on NF-kB activity has not been extensively investigated in primary cells, evidence from macrophages showed that TF3 inhibited NF-kB [76]. However, it was established that EGCG and TF3 work synergistically to protect cardiomyocytes from oxidative stress independent of the activity of antioxidative enzymes [77].

Black tea also contains thearubigins, the most prevalent phenolic pigment class, which are synthesized during oxidative processing. [78]. Thearubigins and cardiovascular disease, however, have not yet been subjected to extensive research. An interesting observation suggested that thearubigins were effective at reducing inflammation and free radicals, making them a promising candidate for anti-inflammatory therapy [79]. According to another study, theaflavin-3,3-digallate was proven to be the most efficient inhibitor of IKK1 and IKK2, while EGCG and monogallated theaflavins indicated moderate IKK inhibitory action. However, the inhibition by thearubigins were relatively low [80]. Thearubigins was also shown to influence iNOS expression besides NF-kB suppression [81-83]. These findings suggest possible role of thearubigins in preventing development of cardiovascular disease that need further investigation.

Method

Whole tea powder of green tea, EGCG (the major component of green tea) and theaflavin (TF), the important component of black tea), play critical role in occurrence of CVDs by regulating expression of several associated genes. .

Whole tea powder

Consumption of Green tea polyphenol (GTP) affected the expression pattern of genes participating in lipid metabolism (notably peroxisome proliferator-activated receptor, PPary), in glucose uptake pathways, in proximal insulin signaling and anti-inflammatory modulator mRNA binding ZFP 36 protein (Tristetraproline, TTP) in cardiac tissue. GTP also reported to enhance the mRNA levels of *Ir* (insulin receptor), *Irs2*, regulatory subunit of phosphatidylinositol 3-kinase (PI3k)and High Fructose Diet (HFD) impaired expression of *Glut* (glucose transporter) *1* and *Glut4* significantly [15] . Chen, et al. [84] reported that in adipocytes of mouse adipocyte cell line 3T3-L1, green tea catechins, in the presence of norepinephrine, could enhance lipolysis via a PKA-dependent pathway and upregulated the expression of HSL and triglyceride lipase.

White Tea is a non-fermented tea and White Tea Extract (WTE) was found to play a significant role in lipid transport and synthesis by upregulating genes in lipid transport and metabolism such as *FABP1* and *LIPIN1.WTE also upregulated* genes inhibiting cholesterol synthesis (*viz. INSIG1*), transport- (*viz. NPC1L1* and *ABCA3*) and genes for uptake of LDL-c (viz. *LDLR*) whereas downregulated genes for synthesis of triglyceride (*viz*. *FADS2*) cholesterol (*viz*. *CEL*), cholesterol transport (*viz*. *ABCG1*) and VLDL production (*viz. APOB* and *MTTP*) [85].

Cardiomyocyte (CM) Glycogen Synthase Kinase-3 beta (*GSK-3β*) is an important gene involve in cardiac dysfunction and obese knockout mutant of this gene exhibited a globally improved glucose tolerance and maintained normal cardiac function. Inactivation of *GSK-3β* by LiCl leads to accumulation of β-catenin, a key factor strongly associated with several cardiac pathologies [86]. EGCG induced β-catenin degradation by phosphorylation of the N-terminal end and pharmacological inhibition or depletion of *GSK-3β* did not evade the EGCG-mediated β-catenin degradation, indicating a mechanism independent of GSK 3 *β [87]*.

Unnatural activation of Toll-like receptor 4 (*TLR4)* is associated with several inflammatory diseases including CVDs. EGCG3″Me, a methylated derivative reported in selective germplasm in in tea, significantly attenuated *TLR4* expression. In adipose tissue, this methylated derivative of EGCG could inhibit the upregulation of TNF α induced by diet rich in fat and sucrose (HF/HS diet) and prevented hypertriglyceridemia and hyperinsulinemia triggered by HF/HS diet [88].

HF/HS diet increased the expression of Sterol Regulatory Element-Binding Protein *(SREBP-1), a transcription factor involved in CVD,* by 3.3 folds but, no significant increase in expression of *SREBP* was found when the HF/HS diet was supplemented with EGCG3.

Pu-erh tea is a highly fermented tea and Pu-erh tea extract when administered @0.5 g, 2 g or 4 g tea to SD male rats fed with HFD, in all the three cases, an improved mRNA levels of Hormone Sensitive Lipase (HSL) and significantly lowered levels of plasma TC, TG, LDL-C levels were detected, although, no significant effect on affect HDL-C level was observed [89]. Fujian Black Tea Extract (FTE) downregulated the expression of gene encoding Fatty Acid Synthetase, its transcription regulator *SREBP-1c*, *C/EBP-α* that convert pre-adipocyte to mature adipocyte , establishing the anti-obesity effects of FTE [90].

Polyphenol, when supplemented at safe optimum doses, could modulate the expression of miRNA *in vivo* and in apoE -/- mice, the expression was opposite to their expression in wild type ones. 5 miRNAs were differentially expressed by all nine polyphenols used in this investigation: the expression was upregulated in two, whereas, in three miRNA, the expression was downregulated Milenkovic et al [91].

Epigallocatechin gallate (EGCG)

EGCG when administered to obese mice fed with HFD, could reduce diet digestibility and promote fat oxidation via over expression of *UCP2* gene in liver. A marked reduction in the expression of two rate limiting genes in fatty acid biosynthesis pathways viz. *Fatty acid synthase* and α-*Acetyl-CoA carboxylase-1 (ACC 1) was also observed*. Further, EGCG, could participate in DNA methylation and thus, as epigenetic modulator, could participate in gene expression and miRNA activities[92] .

Expression of Heme oxygenase-1 (*HO-1*), an NF-E2-regulated gene, plays an important role in CVDs as expression of this gene found to prevent vascular inflammation, proliferation of vascular smooth muscle cell and promote vascular relaxation and prevention of expression of this gene resulted in vascular injury [88] The adhesion and transmigration of leukocytes to the interstitium is required during inflammation and vascular cellular adhesion molecule-1 (VCAM-1) participates in the this process. Human aortic endothelial cells (HAEC) line when treated with EGCG @ 2.5μM for 8 hrs could increase the expression of *HO-1* by 3 folds and decrease *VCAM-1* expression, indicating possible beneficial role of EGCG in prevention of CVDs. However this EGCG stimulated action was not found when p38MAPK or Nrf2 was inhibited or in P38MAPK knockout mutant HAEC line indicating the role of p38MAPK in EGCG mediated stimulation of *HO1 [93]*.

Intraperitoneal injection of EGCG to male Wistar rats fed with HFD brought all the biomarkers of CVDs to near normal level in comparison to the hyper-cholesterolemic values for the control rats [94]. In addition, the activities of anti-oxidant enzyme (CAT, SOD and GPx), activities of enzyme involve in lipid metabolism viz. *sirtuin 1* , activities of *eNOS* and AMP-activated protein kinase α(*p-AMPK α*) and morphology of myocardial tissues were improved in rats treated with EGCG indicating preventive role of EGCG on prevention of CVDs [95].

LDL receptor, when expressed in the cell surface of hematocyte, taken up the LDL into the cell and thus important in lowering the LDL level in blood plasma. . Patients with mutated LDLR gene are more prone to atherosclerosis due to elevated level of LDL cholesterol in blood plasma. In HepG2 cells, EGCG could suppress the expression of PCSK9 and up-regulated LDLR with an increase in mRNA level of LDLR [96].

The vascular function of EGCG in endothelial cell, in isolated vessels and in skeletal muscles was critically reviewed by Keske, *et al*. [97] . NO and ET1 are the two agents with opposing vasoactive actions (vasodilator vs. vasoconstrictor) and are in balance under normal healthy conditions whereas disruption

of the balance between NO and ET-1 production is believed to contribute to the development of risk factors of CVDs such as hypertension, type 2 diabetes and atherosclerosis [97]. EGCG promoted NO and simultaneously inhibited the production of ET-1 and may have the potential characteristics to consider as therapeutic agents to treat CVDs [98]. Hydrogen peroxide concentrations produced during auto-oxidation of EGCG, even at a very low level of 500 nM, could induce vasodilation in explanted aortic rings significantly [99].

Activation of NF-κB family genes, along with its downstream genes, initiates different pathological events associated with CVDs [100]. EGCG, although did not alter intra-nuclear phosphorylation levels of NF-κB-p65 or its expression , but regulated its suppression epigenetically by strongly repressing the DNA-binding ability of NF-κB-p65 [101]. Obesity contributes directly to incidence of CVDs and is an important cardiovascular risk factors [102]. Obesity is mainly characterized by deregulation of lipid metabolism, decrease glucose uptake and mitochondrial dysfunction [103]. EGCG, like insulin, increased the muscle lipid oxidation, stimulated glucose uptake by muscle in isolated myocytes, adipocytes and in culture myotube, augmented insulin-stimulated glucose metabolism in adipose cells, suppressed gluconeogenesis in cultured hepatocytes and thus played a potential role in lowering the blood glucose level [104].

In blood serum, an elevated level of Monocyte Chemoattractant Protein 1 (MCP 1) is considered as a reliable marker of inflammatory condition and TNF-a induces MCP-1. EGCG found to suppress the MCP-1 secretion induced by TNF-a, predominantly by inhibition NF-κB pathway and thus EGCG may be a potential candidate to treat atherosclerosis [105].

EGCG blocked phosphorylation of Platelet-derived growth factor-bb (PDGF-bb), , PDGFR-β and could significantly inhibit the switching of Vascular Smooth Muscular Cell (VSMC) to phenotype with high rate of proliferation [106].

Theaflavin/thearubigins

Naveeda, et al. [107] reviewed the vascular function enhancing effect of black tea *viz*. inhibition of clot formation, improve vasodilation, improve Flow Mediated Dilation (FMD) of brachial artery, lowering blood pressure, improve oxidation of LDL *etc* and epidemiological studies reported 10-20% reduction in occurrence of CVDs in groups consuming 3 cups of black tea (237 ml)/day regularly.

Luo, et al. [108] exhibited that that theaflavin, on intraperitoneal administration even for a short period, could significantly reduce the hepatocyte steatosis and, diminished the fat content in steatotic livers. The increase in the expression of the inflammatory cytokines viz. cytokines IL-6,TNF-α, , iNOS, OPN and interferon gamma (IFN-γ)in steatotic livers I was suppressed by pre-treatment with theaflavin. Theaflavin could inhibit the expression of Lipopolysaccharide (LPS)-induced pro-inflamatory mediators interleukin-6 *(IL-6*), *MCP-1* and *ICAM-1* in macrophages isolated from ICR mice through blockade of NF-κB and all MAPK signalling pathways [109]. In steatotic hepatocytes, theaflavin decreased the level of ROS and also the TNF-α level in LPS-stimulated macrophage cell line RAW264.7 [108] .

Theaflavin-3,3'-digallate (TF3) when added at the concentration range of 1–10 μM to Pathological Cardiac Hypertrophy (PCH) H9c2 cell induced by angiotensis-II, suppressed the expression of fatal Natriuretic peptide A (*ANP*) and Natriuretic peptide B (*BNP*) genes. Subsequently, a decrease in production of antioxidant enzymes SOD and CAT was also observed which might be due to the inhibitory effect on the expression of the regulatory genes [110].

Zhang, et al. [111] from their investigation established that theaflavin (TF)-1 has antiplatelet, as well as, antithrombotic activities and TF-1 acquired these characters by attenuating binding and spreading of fibrinogen and by attenuating formation of Thromboxane A2.

Mechanism of action

EGCG, has a double edged nature of activity and behaves as pro, as well as, anti-oxidant. At lower biological concentrations (1 μM up to 10 μM) EGCG produces smaller amount of intracellular ROS and stimulated multiple pathways associated with cellular protective mechanisms. However, at higher concentrations (>50 μM) it disrupt the radox balance mechanism and the pro-oxidant nature of EGCG predominates resulting in cytotoxicity [112]. The mechanism how tea polyphenol works on prevention of CVDs may be explained from their effect on several genes, enzymes and pathways associated with CVDs ,

1. Ability to phosphorylate nitric oxide synthase (eNOS) through modulating the kinase molecular signaling such as PI3-kinase/Akt pathway, as well as, through modulating intracellular Ca2+ [18]. Leung, et al. [113] exhibited that black tea, like estrogen treatment, has equivalent potentiality in restoring NO availability, basically by inducing the phosphorylation of eNOS. Saito, et al. [114] exhibited that a single oral dose of TF, promoted eNOS phosphorylation in the aorta, however, on pretreatment with 'Carvedilol-an adrenaline blocker, these changes were not evident.

2. Role of polyphenols as anti-inflammatory compound: Atherosclerosis plays a significant role in the development and progression of CVDs. Polyphenols, by its ability to decrease the production of pro-inflammatory molecules such as TNF-α, IL-6 and CRP- through downregulation of the NF-κB pathway , as well as, its ability to inhibit production of VCAM1 and ICAM 1, the adhesion molecule participating in the process of inflammation, could prevent inflammation[110]. Another investigation by Goszcz, et al. [115] showed that EGCG @ 1–100 μg could suppress production of the pro inflammatory cytokines such as IL-6 and IL-8, and, at the same time, could increase the production of the anti-inflammatory cytokine such as IL-10 in whole blood cultures.

3. Scavenging of ROS/RNS: Overproduction of ROS/RNS over a long period may cause substantial damage to the cellular structure and functions through inducing somatic mutations, pre-neoplastic and neoplastic transformations. Phenolic compounds and flavonoids have the ability to scavenge the ROS/RNS and thus terminated the chain reaction leading to impaired cell activity [18].

4. Induce production of enzymes associated with lipoprotein metabolism: Lipoprotein lipase (LP), Hormone Sensitive Lipase (HSL), hepatic lipase (HL), triglyceride lipase, carnitine palmitoyl transferase1 are the key enzymes responsible for lipoprotein metabolism and turnover of fatty acids in adipose tissue for utilization by different tissues [116] .Tea polyphenol found to have the potentiality

to upregulate the expression of the genes responsible for lipid metabolism [117]. Malondialdehyde (MDA), the end product of lipid peroxidation, is an indicator of onset of AS and plaque formation. Theaflavin treatment not only significantly reduced the levels of Serum lipid and MDA but also enhanced the activities of antioxidant enzymes such as SOD, CAT, and glutathione peroxidase [117, 118] .

5. By improvement in microcirculatory dilation: Fuchs, et al. [119] reported that tea components viz. EGCG and theaflavin exhibited similar sized effects in modest improvement in microcirculatory dilation as measured by Reactive Hyperemia Index (RHI) and plays a critical role in cardiovascular and metabolic diseases by regulating the release of an unusually diverse family of vasoactive compounds.
6. By decreasing the ER and oxidative stress: Angiotensin-II induced hypertensive male Sprague Dawley rats when orally administrated with black tea extract @ 15 mg/kg/day orally for 2 weeks, both ER stress and oxidative stress were reduced significantly[120]. Black tea (70% theaflavins) treatment (@15 mg kg^{-1} day^{-1}) inhibited the NADP (H) oxidase that produces large quantity of superoxide anion that scavenge NO and could reduce the oxidative stress in aortas of ovariectomized (OVX) rats[113] .
7. By stimulating the production of microRNA: In vascular endothelial cells of ApoE−/− mice fed with HFD and TF, TF found to upregulate Nuclear factor erythroid 2-related factor (Nrf2) /HO-1 (a downstream protein of Nrf2) signaling pathway via stimulating the production microRNA-24 (miR-24) and attenuates the progression of AS induced by HFD [118] .
8. By improving mitochondrial function: Theaflavin reported to have the potentiality to improve the mitochondrial function by attenuating mitochondrial damage caused by ROS and also, by promoting membrane potential of mitochondria, a crucial factor for the storage of energy during synthesis of ATP [121].

Means to improve absorption efficiency of tea components

Tea polyphenols, including EGCG, when ingested orally, the enzymatic transformation is started with the saliva in the gastrointestinal tract and major metabolic changes take place in small intestine and liver. Free catechins are absorbed in the small intestine whereas the conjugated catechins excreted by the bile is reabsorbed when degraded by the colonic bacteria [122,123]. The bioavailability of EGCG after ingestion is the crucial factor to realize the pharmaceutical importance of EGCG and several factors influence the bioavailability of EGCG[124].

A detailed understanding of inter- and intra-individual differences regarding absorption rate, distribution after absorption , metabolism and finally excretion (ADME) of (e)-epicatechin, and potential involvement of colonic microbiota in the formation of bioactive molecules from epicatechin is required to unravelling the role of this bioactive compound in human health [2].

The identification of the vehicle that will help the phenolic compounds to retain the important biochemical characteristics in the lumen of the gut is an important factor to ensure that the phenolic derivatives eventually reach the bloodstream. Addition of Human serum Albumin (HSA) was reported to have remarkable effect on preservation of catechins in aqueous solution and the recovery, even after 48 hrs of incubation, ranged from 29% (in case of EGCG) to as high as 85% (in case of EC) whereas the corresponding values in PBS (without HSA) were 0% and 5% respectively [125].

Naumovski, et al. [124] concluded that EGCG, when taken in encapsulated form in empty stomach after a fasting period of 8 hrs, the plasma concentration of EGCG was the maximum, suggesting water as a vehicle for administration and an empty stomach was found as the most efficient strategy for the oral delivery of EGCG [126] .

The hydroxyl groups in EGCG makes it highly reactive and thus has low bioavailability , however, methylated group in EGCG3″Me increase the transportation through biological membrane and increase the oral bioavailability. In comparison to EGCG, EGCG3″Me has higher intestinal absorption with low rate of disappearance in blood serum that improves its bio efficiency. Further, phosholipids (PL), when form complex with EGCG3''Me, the EGCG3''Me-PL complex have better stability in mimic gastrointestinal digestive conditions [127]. Recently, methylated form of (+)-catechin and (−)-epicatechin were synthesized chemically and thus open up the possibility of improving the absorption of EGCG through chemical modification [128]. Acetylation of EGCG also reduced the hydrophilic characters by acetylation of the OH groups, solubility of EGCG in fat and increased the antioxidant properties [129] .

The population of gut microbiota in the gut played a significant role in degradation of unabsorbed tea polyphenol that reached the large intestine [130] and in maintaining the host's health. Extract of black tea and green tea had a differential effect on the gut microbiota and black tea extract was found to have better influence in stimulating the gut microbiota in utilization of starch [131] . The tea components from green tea (EGCG) and black tea (Theaflavin digallate) stimulated the growth of health promoting bacteria such as *Bifidobacterium spp*., *Lactobacillus*, and *Enterococcus* and, at the same time, the growth of harmful bacteria such as *Prevotella* and *Fusobacterium* was slowed down [132,133]. The genus *Bacteroide*s is found abundantly in human gut microbiota and provide nutrition by metabolizing the complex saccharides. Long-term treatment with green tea phenolics was reported to enrich the *Bacteroides* in colon of rats and thus may be useful as food supplement [134]. Gut microbiota, in return, may metabolize tea components to produce smaller bioactive molecules involve in preventing inflammation, correct imbalance of gut microbial community, or control the production of harmful metabolites [132]. A multi-pronged approach involving metagenomics, metabolomics, proteomic and transcriptome is necessary to identify the key bacterial strain, key metabolites, target genes/protein and interplay of TP with gut microbiota to get a clear picture how tea polyphenol alleviate metabolic syndrome (MS) and recommending TP as functional ingredients in medical supplements.

Discussion

A successful strategy to control and prevent CVDs in China, the country that is the home of about 18.5 % of global population, may have high relevance in developing policy document to address this global health issue. Wu, et al., [135] highlighted the importance of research to develop a low-cost, simple, sustainable, and scalable interventions and, in

that context, developing a habit of drinking tea may meet the aforesaid criteria.

Among the passionate tea connoisseurs, always there is a question which tea is more health promoting: green tea, black tea or oolong tea? The effect of black tea component (TF3) @ 10 µM was comparable to major green tea component (EGCG) @ 50 µM in their efficacy to prevent cardiac problem, indicating a stronger preventive effect of TF3 than EGCG in preventing CVDs. Besides, considering the effect on cell viability, TF3 is considered as a safer and potential drug than EGCG (138). Non-fermented tea, like green tea, contains, along with EGCG, alpha Ɣ-amino butyric acid (GABA) and theanine found to reduce oxidative stress, antioxidant characteristic and improvement of post-stroke depression in mice [136]. White tea, another non-fermented tea, also a major source of theanine and GABA, found to improve the contractile function of heart by modulating cardiac glycolytic in prediabetic rats [137]. Theanine also , elevated glutathione level and activities of glutathione peroxidase that catalyzed reduction of hydrogen peroxide that improve antioxidant defense and reduced cell apoptosis caused by H_2O_2 [138].

EGCG attenuate lovastatin-induced proprotein convertase subtilisin kexin 9 (PCSK9) protein expression and upregulation LDLR expression in HepG2 cells by preventing LDLR degradation and the mode of action was distinct from the route reported in statins., Thus EGCG has high potentiality to be used as a supplement to statin therapy in treating atherosclerosis, particularly, in statin intolerant patients [96].

Considerable body of knowledge is generated through both *in vitro* and *in-vivo* studies support the preventive role of tea polyphenol on occurrences of CVDs, however, the exact mechanism why the tea polyphenol could not show constant and repetitive results over various trials is remain elusive [123]. A review of literature carried out by Murray et al [132] for the period 2005 to 2015 could not establish a statistically significant association between consumption of green tea and reduction of risk factors of CVDs, however, substantial evidences were found on improvement of LDL-C level on consumption of green tea. Because of the inherent limitations of classica; models such as standard 2D cell culture, minipigs and goats, three-dimensional (3D) culture systems, that mimic the *in-vivo* condition to a greater extent, gradually replacing the conventional systems and outcomes from such investigations seem to represent the response of cell in an intact tissue. The introduction of induced pluripotent stem cells and recent development in 3D cell culture model system to study the CVDs such as basic form "multicellular aggregate" to advanced "engineered heart tissue (EHT) may enable researchers to gather scientific information on the role of tea components on prevention of CVDs [133].

Polyphenols from tea is reported to have the capacity to reverse gene expression [91], LDL-c clearance [85], to induce Heme oxygenase-1 even at low dose [93] and thus may be an important ingredient in designing drugs.

Conclusion

The medicinal importance of tea was also discussed in depth with several references in a very old book on tea, written in 1831, and maintained as special collection in the museum at National Agricultural library, USDA, Maryland, USA [139]. The above recent references and continuously emerging new recent findings from clinical trials clearly demonstrates the role of components of tea in preventing CVDs that outweigh the potential harm from intake of tea polyphenol. However, the outcomes of the trials on human subjects are less satisfactory which may be due to improper research strategy and analysis of accumulated data [137]. Hartley, et al. [134] opined that there was a shortage of long term randomized controlled trials (RCTs) which was essential to minimize the error effect and to examine the exact effects of intervention of tea components on prevention of CVDs. It is very interesting to observe that, recently, Academy of Nutrition and Dietetics, USA, based on the moderate evidence of cadio-protective effect of flavan-3-ol, a compound abundant in tea, included it in the food based guideline [140]. A multi-pronged approach involving all the relevant disciplines and all the stakeholders of pharmaceutical industries may yield an effective tea based drugs to treat the CVDs in near future.

Author Contribution

First author (DB) deigned the review paper and wrote the abstract of the paper, sections on tea components regulate/alter expression of key genes associated with CVDs, Mechanism of action and designed the flow diagram showing the differences in manufacturing processes of popular teas. Second author (ZF) wrote the Key genes associated with CVD and means to improve absorption efficiency of tea components. AD wrote the sections on Regulation/alteration of different pathways associated with CVDs by tea components, discussion and the flow diagram of the pathways associated with CVD. YR prepared the introduction. RM reviewed the first draft and prepared the key pathways associated with CVD. Corresponding author wrote the conclusion, reviewed the completed MS and approved the submitted version.

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