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Green tea catechins: defensive role in cardiovascular disorders

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[ABSTRACT] Green tea, *Camellia sinensis* (Theaceae), a major source of flavonoids such as catechins, has recently shown multiple cardiovascular health benefits through various experimental and clinical studies. These studies suggest that green tea catechins prevent the incidence of detrimental cardiovascular events, and also lower the cardiovascular mortality rate. Catechins present in green tea have the ability to prevent atherosclerosis, hypertension, endothelial dysfunction, ischemic heart diseases, cardiomyopathy, cardiac hypertrophy and congestive heart failure by decreasing oxidative stress, preventing inflammatory events, reducing platelet aggregation and halting the proliferation of vascular smooth muscle cells. Catechins afford an anti-oxidant effect by inducing anti-oxidant enzymes, inhibiting pro-oxidant enzymes and scavenging free radicals. Catechins present anti-inflammatory activity through the inhibition of transcriptional factor NF-κB-mediated production of cytokines and adhesion molecules. Green tea catechins interfere with vascular growth factors and thus inhibit vascular smooth muscle cell proliferation, and also inhibit thrombogenesis by suppressing platelet adhesion. Additionally, catechins could protect vascular endothelial cells and enhance vascular integrity and regulate blood pressure. In this review various experimental and clinical studies suggesting the role of green tea catechins against the markers of cardiovascular disorders and the underlying mechanisms for these actions are discussed.

[KEY WORDS] Green tea catechins; Reactive oxygen species; Vascular cell adhesion molecule-1; Nitric oxide; Cardiovascular disorders

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1 Introduction

Tea is one of the most widely consumed beverages in the world. The increasing health benefits of tea has led to the inclusion of tea extracts in dietary supplements and functional foods. Green tea (non-oxidized), Oolong tea (partially oxidized), and Black tea (oxidized), three major categories of tea obtained from the plant *Camellia sinensis* (L.) Kuntze, belonging to the family Theaceae, differ in terms of their manufacturing and chemical composition [1-2]. Tea catechins exert a variety of physiological actions, which may be primarily responsible for the health benefits of green tea. There is surfeit of literature which correlates the potential of green tea catechins with their chemistry. Green tea is comprised of proteins, including enzymes, amino acids, carbohy-

drates, lipids, such as linoleic and α-linolenic acids, sterols, vitamins (B, C, E), pigments, such as chlorophyll and carotenoids, volatile compounds, such as aldehydes, and minerals and trace elements [3]. The major components of green tea are the polyphenols, which represent 36% dry weight of green tea. Polyphenols present in green tea are flavonoids. Non-fermented green tea contains more than 80% of flavonoids, while fermented black tea has only 20%−30% of this phyto-constituent [4]. The major flavonoids of green tea are catechins, which include (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-(EGC) and (−)-epigallo-catechin-3-gallate (EGCG), (+)-catechin (C), (+)-gallocatechin (GC), (+)-catechin gallate (CG), and (+)-gallocatechin gallate (GCG) [5-6]. EGCG is the most abundant green tea catechin (GTC), and is thought to be responsible for the majority of the biological activities of green tea [7]. Green tea contains 21 mg·L^{-1} of C, 98 mg·L^{-1} of EC, 90 mg·L^{-1} ECG, 411 mg·L^{-1} EGC, and 444 mg·L^{-1} EGCG [8]. Green tea consumption is associated with a variety of physiological functions, which may be primarily responsible for possible beneficial effects. The versatility of catechins and their active metabolites for potential therapeutic interventions is due to the diverse ac-

tions being performed at different sites. Many clinical and epidemiological studies have examined these actions of catechins in the form of anti-carcinogenic [9], anti-tumorigenic [10], anti-mutagenic [11], anti-diabetic [12] and anti-obesity effects [13].

Cardiovascular disease is the single largest contributor to global mortality today, and will continue to dominate mortality trends in the future [14]. Quite a significant amount of research has already been carried out in exploring various synthetic and plant-based interventions which can prevent this disorder. In this direction, some epidemiological and review studies have reported the positive relationship between GTC intake and reduced cardiovascular disorders [15-18]. The multiple mechanisms undergone by GTC for the prevention of coronary heart disease involve its anti-oxidative, anti-inflammatory, anti-proliferative, anti-platelet, and anti-thrombogenic effects [18-19]. In this review, the available data on the effect of green tea on cardiovascular performance and risk are presented. Oxidative stress, inflammation, vascular endothelial function, proliferation and platelet aggregation, and their management by green tea will be discussed.

2 Effect of GTC on Cardiovascular Risk Factors

2.1 *Anti-oxidant activity*

Oxidative stress plays a crucial role in the progression of various cardiovascular diseases, including atherosclerosis, hypertension, endothelial dysfunction, ischemic heart diseases, cardiomyopathy, cardiac hypertrophy and congestive heart failure. Increased formation of reactive oxygen species (ROS) and/or decreased antioxidant enzymes indicate oxidative stress in cardiac and vascular myocytes [20]. Oxidative stress-mediated ROS causes rapid depolarization of mitochondrial inner membrane potential and subsequent impairment of oxidative phosphorylation. Damaged mitochondria produce ROS, especially in the form of the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which further propagate ROS generation. Moreover the increased generation of ROS is due to its increased secretion by white blood cells, endothelial dysfunction, and auto-oxidation of catecholamines, as well as exposure to radiation or air pollutants [20-21]. Nox1, Nox2, and Nox 4 family members of NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoxygenases, and uncoupled nitric oxide synthase form and propagate ROS generation [22].

Mammalian cells are equipped with a variety of antioxidant enzymes to control ROS production and its further propagation. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GSR) are the major enzymes that represent a coordinated operating network of defenses against oxidative stress-induced tissue damage [23]. Depletion of these antioxidant reserves, either due to ROS or due to the exhaustion and/or changes in gene expression, plays a crucial role in vascular abnormalities [20].

GTC are important antioxidants, which can reduce oxidative stress mediated lipid peroxidation, endothelial dysfunction and improves antioxidant defense [3]. Heim *et al.* demonstrated that catechins uninterruptedly produced their antioxidant effects by scavenging ROS, chelating redox-active transition metal ions, and inhibiting lipid peroxidation [24]. EGCG, at different doses, reduced lipid peroxidation [25]. However, catechins also function circuitously by inhibiting the redox-sensitive transcription factors, nuclear factor-kappa B (NF-κB) and activator protein-1 responsible for oxidative stress. Further, GTC manage the generation of ROS by inhibiting "pro-oxidant" enzymes, such as inducible nitric oxide synthase (iNOS), lipoxygenase, cyclooxygenase and xanthine oxidase, and by inducing antioxidant enzymes, such as SOD, CAT and GPX [26-27]. EGCG protects heart against doxorubicin-induced myocyte injury by improving Ca^{2+} handling through scavenging reactive oxygen species [28]. Green tea extract prevented the development of atherosclerosis in apolipoprotein E-deficient mice through its potent anti-oxidative activity [29]. (Fig.1) The above evidence suggests that the antioxidant properties of the catechins contributes to the cardioprotective activity of green tea.

2.2 *Anti-inflammatory activity*

Atherosclerosis is normally considered a bland lipid storage disease, but actually involves an on-going inflammatory response [30]. Inflammation-induced monocyte adhesion to endothelial cells (ECs), followed by transmigration into the sub-endothelial intima, is one of the key events in the development of atherosclerosis [31-32].

In normal conditions, ECs, which form the innermost surface of the artery wall, resist adhesion by leukocytes. However, triggers of atherosclerosis, such as a high-saturated-fat diet, smoking, hypertension, hyperglycemia, obesity, and insulin resistance, can initiate the expression of adhesion molecules by ECs, thus allowing the attachment of leukocytes to the arterial wall [30]. NF-κB regulated intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) play a pivotal role in the binding of leukocytes to the sites of inflammation [33-34]. Once adhered to the endothelium, the leukocytes penetrate into the intima. Chemoattractant molecules, such as monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) and E-selectin, are responsible for the direct migration of monocytes into the intima at the sites of lesion formation [32, 35]. Further, macrophage colony-stimulating factor (MC-SF) also contributes to the transmigration of monocytes into the intima [36]. Once resident in the arterial wall, the blood-derived inflammatory cells participate in, and perpetuate, a local inflammatory response. The potent pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α), plays a pathogenic role in chronic inflammation and atherosclerosis [37].

GTC prevent inflammation-mediated atherosclerosis by

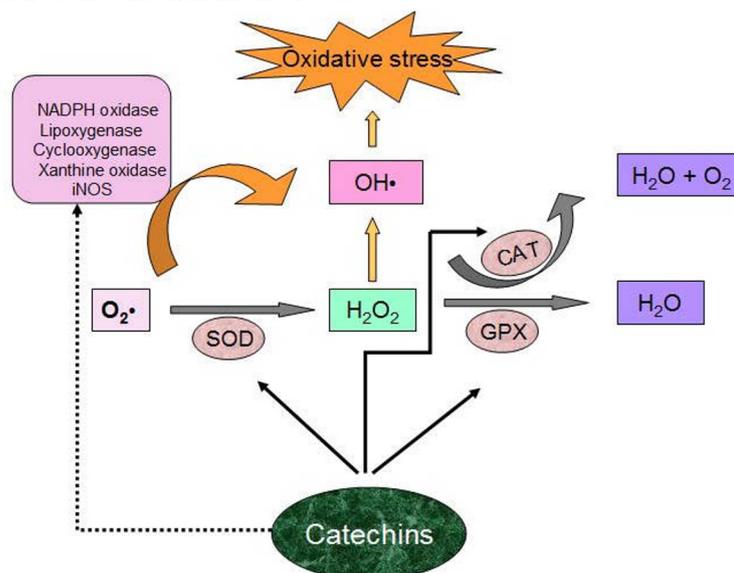suppressing leukocyte adhesion to the endothelium and its

Fig. 1   Anti-oxidant defense mechanism of catechins

Dotted arrow indicates inhibition, while the simple arrows indicate activation. Catechins activate antioxidant enzymes such as Superoxide dimutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPX), and inhibit the enzymes which are responsible for oxidative stress such as NADPH oxidase, lipoxygenase, cyclooxygenase, xanthine oxidase, and iNOS (inducible nitric oxide synthase)

subsequent transmigration[18]. Catechins induce an anti-inflammatory effect by the suppression of several inflammatory factors, including NF-κB, cytokines, and adhesion molecules [18, 38]. Suzuki *et al.* confirmed that GTC attenuated the development of the systemic inflammatory diseases with the suppression of the inflammatory factors [39]. Catechins reduced the expression of cytokines, NF-κB, ICAM-1, and TNF-*α* responsible for inflammation, and suppress myocardial inflammation in rats [40]. Further, GTC can suppress leukocyte adhesion to ECs. EGCG and ECG at different doses prevented the expression of VCAM-1, and thus reduced the leukocyte adhesion to ECs [41]. Liang *et al.* demonstrated that EGCG treatment could significantly reduce monocyte chemoattractant protein-1 (MCP-1) responsible for inflammation [42] (Fig. 2).   Thus GTC prevents inflammation-induced atherosclerosis.

2.3   *Effect of GTC on vascular endothelial dysfunction*

The vascular endothelium is a simple monolayer of inner blood vessels that separates blood and peripheral tissues, and maintains homeostasis by regulating vascular tone [43]. The ECs maintain vascular tone by balancing vasoconstricting substances, such as endothelin-1 (ET-1), prostaglandins, angiotensin II (Ang-II) and vasodilating substances, such as nitric oxide (NO), prostacyclin and various endothelium-derived hyperpolarizing factors (EDHFs) [44-45]. Vascular endothelial dysfunction (VED) is a systemic pathological state of the endothelium, and can be broadly defined as an imbalance between these vasodilators and vasoconstrictors. VED is involved in the pathogenesis of various cardiovascular disorders such as hypertension, atherosclerosis, coronary artery diseases, diabetes mellitus and nephropathy [46].

Nitric oxide (NO), an endothelium-derived relaxing factor (EDRF), is generated during the conversion of L-arginine to L-citrulline by endothelial NO synthase (eNOS) in the presence of various substrates and co-factors [47]. NO is a fundamental determinant for maintaining the vascular functions. The phosphatidylinositol 3-kinase (PI3-K) pathway plays a key role in maintaining vascular function by activating serine/threonine protein kinase (protein kinase B/Akt), which subsequently enhances eNOS phosphorylation/activation and NO production [48-49]. VED occurs as a result of high oxidative stress, down regulation and inactivation of eNOS, and diminished production and bioavailability of NO [50].

NO, produced by green tea catechins through the activation of eNOS enzyme, has the ability to improve vascular endothelial dysfunction. EGCG is capable of modulating ROS production and eNOS activation, thereby increasing the production of NO [18]. EGCG produced vasorelaxation in rat aortic rings by activating eNOS through the PI3K/Akt pathway in ECs [51]. Further, EGCG was shown to produce NO from endothelium using PI3-kinase-dependent pathway in spontaneously hypertensive rats [52]. Catechins also stimulated the production of prostacyclin in bovine aortic ECs [53]. Asymmetric dimethylarginine (ADMA) competes with L-arginine and, by inhibiting nitric oxide synthase, reduces NO production in the vascular wall [54]. EGCG preserves endothelial function by reducing the endogenous ADMA

level [55]. Consumption of green tea in drinking water atte-   nuated blood pressure in stroke-prone spontaneously hyper-
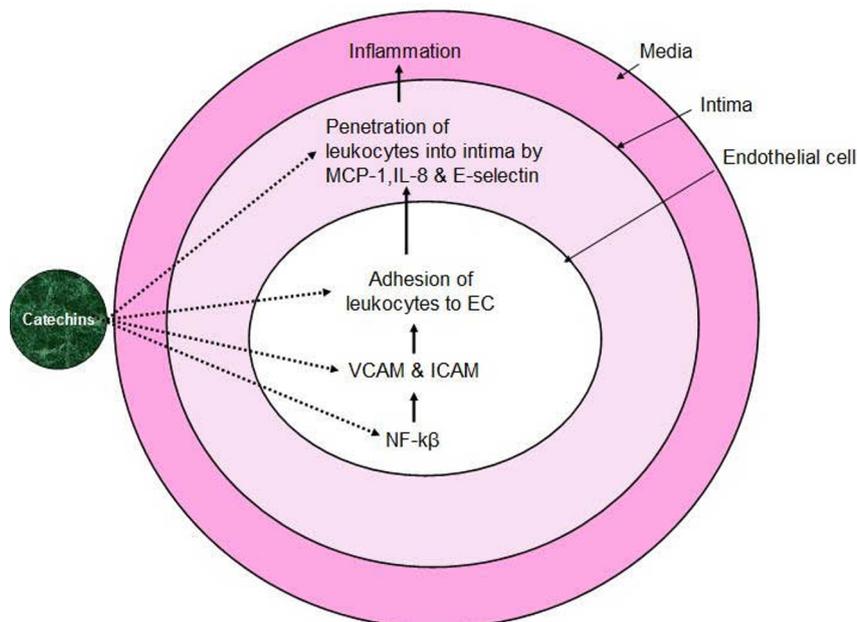

Fig. 2   Effect of catechins against inflammatory factors

Dotted arrows indicate inhibition, while simple arrows indicate activation. NF-κB regulated intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) play a pivotal role in binding of leukocytes to the endothelial cell (EC) which are further penetrated into intima by monocyte chemo-attractant protein-1 (MCP-1), interleukin-8 (IL-8) and E-selectin, and lead to inflammation. Catechins could inhibit all these steps of inflammation

tensive rats [56]. (Fig. 3) Improvement in vascular endothelial dysfunction might potentially contribute to the beneficial effects of GTC in the treatment of patients with hypertension.

2.4 *Anti-proliferative activity*

In addition to inflammation, a key process of atherosclerosis involves the proliferation of vascular smooth muscle cells (VSMCs) [57]. In early atherosclerosis, VSMCs may contribute to the development of the atheroma through the production of pro-inflammatory mediators, such as monocyte chemoattractant protein 1 (MCP-1), vascular cell adhesion molecule (VCAM) and matrix metalloproteinases (MMPs) [58-59]. Fibroblast growth factor, released from dying vascular cells, can initiate proliferation [60], while platelet-derived growth factor (PDGF) may induce subsequent migration, as well as proliferation, of VSMCs toward the intima [61]. Ang II is highly involved in the proliferation of VSMC that result in atherosclerosis [62].

GTC produce an anti-proliferative effect which may be associated with the reduced risk of cardiovascular diseases. Catechins inhibited thrombin-induced VSMCs invasion by preventing matrix metalloproteinases-2 (MMP-2) expression and contributed to a protective atherosclerotic effect [63]. EGCG treatment has been shown to arrest VSMCs in the G_1 phase of the cell cycle by down-regulating important cell cycle regulators, such as cyclins/cyclin-dependent kinases [64]. Ahn *et al*. demonstrated that 80% of the proliferative effect of PDGF-BB (homodimeric form of PDGF) of vascular smooth

muscle cells was eliminated after EGCG treatment [65]. Proliferation induced by advanced glycation end products (AGEs) in VSMCs are reverted by GTC [66-67]. EGCG is capable of suppressing glucose accelerated VSMCs proliferation by up-regulating protein kinase-C (PKC) [68-69]. Further, catechins can also inhibit VSMCs proliferation via the inhibition of the Ang II-stimulated activation of the mitogen-activated protein kinase pathway [62]. Collectively, the above studies suggest that GTC can modulate the factors responsible for VSMCs proliferation.

2.5 *Anti-platelet and anti-thrombotic activity*

Platelet activation and aggregation are hallmarks of cardiovascular diseases, such as myocardial infarction and stroke [70]. Plaque rupture or damage to the vascular endothelial layer by any injury causes adhesion of platelets to the sub-endothelial matrix; platelets become activated, and then rapidly aggregate to form a prothrombotic surface that promotes clot formation and subsequently vascular occlusion. Multiple pathways, including those activated by adenosine diphosphate (ADP), thromboxane A2 (TXA2), epinephrine, serotonin, collagen, and thrombin are involved in platelet activation [71-72]. Evidential records suggest that GTC are anti-thrombotic in action. Anti-platelet activity mediated by EGCG in a dose-dependent manner prevents death caused by thrombosis in mice [73]. Yang *et al* also concluded their study with anti-platelet and anti-thrombotic effects of catechins in diabetic rats [74]. Arachidonic acid and TXA2 synthase

generated thromboxane A2 was inhibited by green tea cate-

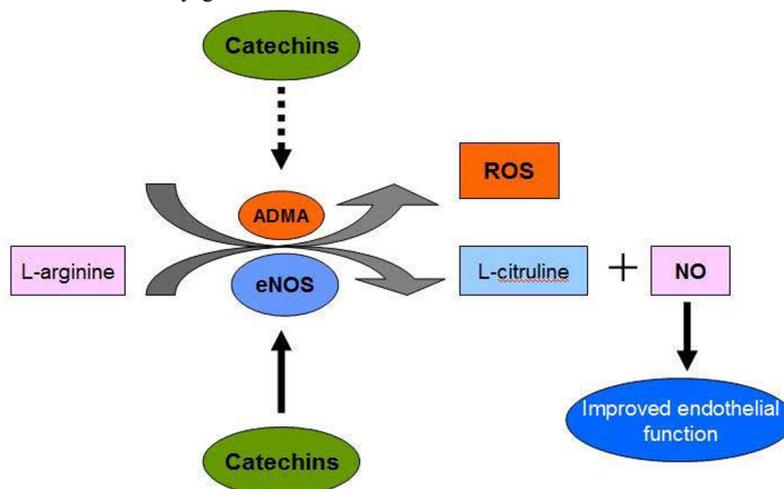chins [75]. Intracellular calcium concentration promotes

Fig. 3   Improved NO production by catechins through eNOS activation
Nitric oxide (NO) is generated during the conversion of L-arginine to L-citrulline by endothelial NO synthase (eNOS). Asymmetric dimethylargi-nine (ADMA) competes with L-arginine and, by inhibiting eNOS, produces reactive oxygen species (ROS) in the vascular wall. Catechins in-crease NO production by activating eNOS and inhibiting ADMA

fibrinogen binding to human platelet surface glycoprotein IIb/IIIa   (GP IIb/IIIa) complex via increasing inositol 1, 4, 5-triphosphate (IP(3)) formation and depleting Ca^{2+}-ATPase. GTC supplementation inhibited the cytoplasmic calcium increase, thereby showing their anti-platelet effect by revers-ing the above process [76].

3   Clinical Studies Pertaining to the Potential of GTC in Cardiovascular Disorders

Strong data from *in vitro* and *in vivo* studies, demonstrate the benefits of green tea, rich in catechins, on several me-chanisms contributing to cardiovascular health. This conclu-sion is substantiated by human studies, which highlights the contribution of GTC in supporting a healthy cardiovascular system. A number of potential outcomes addressed in various clinical studies revealed that green tea consumption benefits the cardiovascular system. The recent Ohsaki study, done on 40 530 Japanese individuals, revealed that those Japanese consuming five or more cups of green tea per day showed 12% reduction in total and a 26% reduction in cardiovascular mortality when compared to those who were consuming less than 1 cup per day [15]. The main outcome of clinical studies conducted on five healthy non-smokers was that green tea is efficient in protecting low density lipoprotein from oxidation driven by peroxyl and ferryl radicals, respectively [77]. Green tea consumption in a concentration of > 800 mL over a 4-month period improved endothelial function and other cardiovascular risk factors in a Japanese population [78]. Kim *et al*. reported that endothelial function significantly im-proved with improvement of flow mediated dilatation in 20 young smokers who consumed 8 g green tea per day (3.2%

EGCG) over a period of two weeks [79]. GTC also produced anti-atherosclerotic effects on dysfunctional vessels in smokers through increasing the level of NO [80]. Recently, Wang *et al*. reported that consuming more than 1 cup/day of green tea reduces the risk of developing coronary artery dis-ease by 10% [81]. Clinical use of green tea indicates that its consumption significantly decreased the urinary concentra-tion of 8-iso-prostaglandin-F2α, an index of oxidative stress and reversed endothelial dysfunction in healthy smokers [82]. *Ex vivo* study demonstrated that LDL oxidation, a risk factor for atherosclerosis, was inhibited by 3.9% and by 98% after 12 h incubation of human LDL and aortic endothelial cells with 0.08 and 5 ppm green tea extracts, respectively [83]. Oxi-dized LDL levels were significantly reduced in subjects con-suming 600 mL green tea, containing 5.2 g tea solids daily for 4 weeks [84]. Evidential studies suggest that consumption of four or more cups of green tea a day exhibited an inverse association with coronary atherosclerosis among Japanese men and women [16]. EGC reduced IL-8 production in human ECs, and thus can reduce inflammation induced atherosclero-sis [85].

Clinical studies also demonstrated that a population suf-fering from hypertension benefited from a high intake of green tea. Green tea is beneficial for lowering aortic stiffness and wave reflection, the parameters which lead to hyperten-sion. In non-habitual tea drinkers, the risk of developing hypertension decreased by 46% for those people who drank 120–599 mL/day, and this was further reduced by 65% for those who drank 600 ml/day or more [86]. Studies conducted by Sano *et al*. [87] and Sasazuki *et al*. [16] demonstrated that green tea intake lowers the incidence of coronary artery dis-

eases. Furthermore, daily consumption of more than four cups of green tea significantly reduced total cholesterol, LDL-cholesterol and triglycerides, and improved protective HDL cholesterol [87, 16].

4 Bioavailability of GTC

How much green tea does a person need to drink to reap its health benefits? The issue is the poor bioavailability of GTC, unfortunately, which is correlated with their therapeutic effects, and is the major concern for researchers. All of the catechins are rapidly absorbed and widely distributed after ingesting a cup of green tea [88]. As the catechins are metabolized through methylation (EGCG) [89], or conjugation with glucuronide and/or sulfate groups (all catechins except EGCG) [90], the ability of free catechins to produce their biological actions is reduced. Other reasons may be the variation of contents from the actual claim mentioned on the marketed products [91]. It has been reported in a clinical study that the plasma concentration of catechins in healthy individuals accounts for only 0.2% to 2% of the ingested amount after around 90 min, which is relatively very low [92]. This bioavailability issue must be overcome to optimize its benefits. In this regard, Chow *et al.* reported that the plasma concentration of catechins can be increased 3 to 4 times by ingesting the drug after an overnight fasting [93], which suggests that the bioavailability of catechins can be improved.

5 Conclusions and Future Direction

Overall, it is concluded that the intake of green tea which provides sufficient catechins can have beneficial effects against cardiovascular disorders in animals and humans. Green tea catechins exert a variety of beneficial metabolic effects by influencing the markers such as oxidative stress, inflammation, proliferation, and platelet aggregation. Although, GTC is beneficial in improving endothelial dysfunction, the positive effects of green tea catechins on cardiovascular system need further attention. Studies should be carried out on the catechin metabolites which are reported to be biologically active, but whose vascular effects are unknown. In addition, the structural aspects, as well as the bioavailability criteria of GTC, should be further explored, which may lead to clinically relevant strategies to prevent and treat vascular diseases.

Abbreviations

ADMA, Asymmetric dimethylarginine; AGEs, Advanced glycation end products; Ang-II, Angiotensin II; CAT, Catalase; EC, (−)-Epicatechin; ECG, (−)-Epicatechin-3-gallate; ECs, Endothelial cells; EDHFs, Endothelium-derived hyperpolarizing factors; EDRF, Endothelium-derived relaxaing factor; EGC, (−)-Epigallocatechin; EGCG, (−)-Epigallocatechin-3-gallate; eNOS, Endothelial NO synthase; ERK1/2, Extracellular regulatory kinase 1/2; ET-1, Endothelin-1; GPX, Glutathione peroxidase; GSR, Glutathione reductase; GTC, Green tea catechins; ICAM-1, Intercellular adhesion molecule-1; IL-8, Interleukin-8; iNOS, Inducible nitric oxide synthase; MCP-1, Monocyte chemoattractant protein-1; MC-SF, Macrophage colony-stimulating factor; MMPs, Matrix metalloproteinases; NADPH, Nicotinamide adenine dinucleotide phosphate; NF-κB, Nuclear factor-kappa B; NO, Nitric oxide; PDGF, Platelet-derived growth factor; PI3-K, Phosphatidylinositol 3-kinase; PKC, Protein kinase-C; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TNF-alpha, Tumor necrosis factor-alpha; VCAM-1, Vascular cell adhesion molecule-1; VED, Vascular endothelial dysfunction; VSMCs, Vascular smooth muscle cells.

Acknowledgment

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