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Molecular understanding of Epigallocatechin gallate (EGCG) in cardiovascular and metabolic diseases

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Abstract

Ethnopharmacological relevance: The compound epigallocatechin-3-gallate (EGCG), the major polyphenolic compound present in green tea [*Camellia sinensis* L. (Theaceae], has shown numerous cardiovascular health promoting activity through modulating various pathways. However, molecular understanding of the cardiovascular protective role of EGCG has not been reported.

Aim of the review: This review aims to compile the preclinical and clinical studies that had been done on EGCG to investigate its protective effect on cardiovascular and metabolic diseases in order to provide a systematic guidance for future research.

Materials and methods: Research papers related to EGCG were obtained from the major scientific databases, for example, Science direct, PubMed, NCBI, Springer and Google scholar, from 1995 to 2017.

Results: EGCG was found to exhibit a wide range of therapeutic properties including anti-atherosclerosis, anti-cardiac hypertrophy, anti-myocardial infarction, anti-diabetes, anti-inflammatory and antioxidant. These therapeutic effects are mainly associated with the inhibition of LDL cholesterol (anti-atherosclerosis), inhibition of NF-κB (anti-cardiac hypertrophy), inhibition of MPO activity (anti-myocardial infarction), reduction in plasma glucose and glycated hemoglobin level (anti-diabetes), reduction of inflammatory markers (anti-inflammatory) and the inhibition of ROS generation (antioxidant).

Conclusion: EGCG shows different biological activities and in this review, a compilation of how this bioactive molecule plays its role in treating cardiovascular and metabolic diseases was discussed.

Keywords: EGCG, diabetes, atherosclerosis, antioxidant, heart failure, anti-inflammatory

List of abbreviations

AAC: abdominal aortic constriction; ACC: acetyl-CoA carboxylase; ADR: adenosine receptor; AE: alveolar epithelial; AF, atrial fibrillation; AGEs: advanced glycation end products; ALP: alkaline phosphatase; ALT: alanine transaminase; AMPK: AMP-activated protein kinase; Ang II: angiotensin II; ANP: atrial natriuretic polypeptide; AP-1: activator protein-1; aP2: adipocyte fatty acid-binding protein-2; AR: aldose reductase; AST: aspartate transaminase; ATGL: adipose triglyceride lipase; BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; BNP: brain natriuretic polypeptide; CABG: coronary artery bypass grafting; CC: corpus cavernosum; CCL2: chemokine C-C motif ligand 2; CD: cyclodextrins; CD44: cell-surface glycoprotein; C/EBT-α: CCAAT enhancer-binding protein-α; CKMB: creatine kinase; CL-HPLC: chemiluminescence detection-high performance liquid chromatography; COL1A1: α-1 type 1 collagen; COX-2: cyclooxygenase-2; CPK: creatine phosphokinase; CPT-1: carnitine palmitoyl transferase-1; CRP: C-reactive protein; CTGF: connective tissue growth factor; cTn: cardiac troponin; CVD: cardiovascular disease; DDC: diethyldithiocarbamate; DM: diabetes mellitus; DPPH: 1,1-diphenyl-β-picrylhydrazyl; DOX: doxorubicin; eEf2: eukaryotic elongation factor-2, EGCG: epigallocatechin gallate; ERK: extracellular regulated kinase; EWP: egg white protein; FAS: fatty acid synthase; FasR: Fas receptor; FFA: free fatty acid; FN: fibronectin; GCA: germ cell apoptosis; GK: glucokinase; GSH: glutathione; GSK: glycogen synthase kinase; GPX: glutathione peroxidase; GTE: green tea extract; H/R: hypoxia/reoxygenation; HbA1C: glycosylated haemoglobin; HDL: high density lipoprotein; HFD: high-fat diet; HNE: hydroxynonenal; HO-1: heme oxygenase-1; HOMA-IR: homeostasis model assessment for insulin resistance; HR: heart rate; HREC: human retinal epithelial cell; HSC: hepatic stellate cell; HSL: hormone sensitive lipase; HSP 60: heat shock protein 60; HUVEC: human umbilical vein endothelial cell; hyp: hydroxyproline; ICAM-1: intercellular adhesion

molecule-1; IL: interleukin; iNOS: inducible nitric oxide synthase; I/R: ischemia reperfusion injury; IRS-1: insulin receptor substrate-1; ISO: isoprenaline; JNK: c-Jun N-terminal kinase; LDH: lactate dehydrogenase; LDL: low density lipoprotein; LPA: lysophoaphatidic acid; LPL: lipoprotein lipase; LOX-1: lectin-type oxidized LDL receptor-1; LV: left ventricular; MAPK: mitogen-activated protein kinase; Mb: myoglobin; MCP-1: monocyte chemoattractant protein-1; MDA: malondialdehyde; ME: malic enzyme; MGO: methylglyoxal; MI: myocardial infarction; MMP: matrix metallopeptidase; MnSOD: manganese superoxide dismutase; MPO: myeloperoxidase; NF-kB: nuclear factor kappa b; NOS-2: nitric oxide synthase-2; NOX-4: NADPH oxidase-4; Nppa: natriuretic peptidase type A; NQO-1: NADPH quinone oxidoreductase-1; Nrf-2: nuclear transcription factor NF-E2-related factor 2; NT: nitrotyrosine; 8-oxo-dG: 8-hydroxydeoxyguanosine; OPN: osteopontin; OPR: opioid receptor; p22phox: human neutrophil cytochrome b light chain; PARP: poly(ADP-ribose) polymerase; PCB: polychlorinated biphenyl; PD: peritoneal dialysis; PDGF: platelet-derived growth factor; PE: phenylephrine; PEPCK: phosphenolpyruvate carboxykinase; PPAR: peroxisome proliferator-activated receptor; PUFA: polyunsaturated fatty acid; RAS: renin-angiotensin-aldosterone system; ROS: reactive oxygen species; RQ: respiratory quotient; SAA: serum protein amyloid A; SCD-1: stearoyl-CoA desaturase-1; Sirt-1: NAD-dependent deacetylase sirtuin-1; SMA: smooth muscle actin expression; SREBP-1C: regulatory element-binding protein 1-C; STAT-1: signal transducer and activator of transcription-1; STZ: streptozotocin; SOD: superoxide dismutase; SULT: sulfotransferase; TAG: triacylglycerol; TC: total cholesterol; TG: triglyceride; TGF: transforming growth factor; TIMP: tissue inhibitor metallopeptidase; TLR: toll-like receptor; tMCAO: transient middle cerebral artery occlusion; TNF-α: tumor necrosis factor alpha; TnT: troponin T; TRF_2: telomere repeat-binding factor 2; TT: testicular torsion;

UCP-2: uncoupling protein-2; UGT: UDP-glucuronosyltransferase; VEGF: vascular endothelial growth factor

Table of content

1. Introduction

Cardiovascular disease (CVD), a kind of disease due to abnormal function of the heart as well as blood vessels, is known as the number one killer worldwide. It includes coronary heart disease, congenital heart disease, rheumatic heart disease, cerebrovascular disease and peripheral arterial disease. According to the 2013 Global Burden of Disease study, approximately 17.3 million deaths were caused by CVD, which represents 31.5% of all deaths. Among these, 7.4 million were having coronary heart disease and 6.7 million died due to stroke (European et al., 2016). Usually, a heart attack or stroke would be the first warning of underlying diseases. Both of them are acute events that are mainly caused by the presence of fatty deposits which block blood from flowing smoothly in the blood vessels and this event is known as atherosclerosis (American Heart Association, 2013; European et al., 2016) . However, stroke can also be caused by the formation of blood clots. Framingham Heart Study found that the risk factors that lead to CVD through metabolic syndrome (diabetes and obesity) includes poor glucose tolerance, physical inactivity, tobacco use, hypertension, and high level of blood cholesterol (American Heart Association, 2013).

Numerous clinical approaches had been done to alleviate and cure CVD but none of were proven to show perfect results. Side effects including nausea and vomiting, dizziness, angina and edema are the typical symptoms when patients are given CVD medication therapies (Nagle et al., 2006). Transplantation of heart may be a hope for those patients who fail to recover from the conventional therapies, but there is a chance of organ rejection and the number of donors are limited. Hence, herbal medicine became another useful alternative therapy as they do not show any side effects. In addition, they are relatively cheaper than

pharmaceutical drugs, easily acquired and can be used for multipurpose. One of the most valuable advantages of herbal medicine is that it helps in utilizing the body's natural healing process as it contains ingredients that are regularly produced by the body. Amongst numerous herbal medicines that have been discovered, green tea is found to be one of the therapeutic agents with most potential against CVD.

Green tea is the least processed tea from the buds of *Camellia sinensis* plant and it contains epigallocatechin gallate (EGCG), which is an ester that forms from the reaction of epigallogatechin and gallic acid (Zaveri, 2006). EGCG can be found abundantly in green tea leaves (7.1 g per 100 g green tea leaves), oolong tea (3.4 g per 100 g oolong tea), and black tea leaves (1.1 g per 100g black tea leaves) (Chacko et al., 2010). In Traditional Chinese Medicine and Ayurvedic practices, green tea has been used extensively as a stimulant, diuretic and astringent. Other traditional uses of green tea include promoting digestion, improving mental health and regulating blood sugar as well as body temperature (Cooper et al., 2005). Furthermore, due to the presence of catechin in EGCG, scientists believe that it may act as an antioxidant which plays a role in reducing the amount of free radicals involved in numerous diseases states including CVD (Lobo et al., 2010). This makes scientists believe that EGCG could be a potential therapeutic agent against CVD, which are mainly caused by oxidative stress. However, a review to understand the cardiovascular protective role of EGCG from molecular aspect has not been reported. This review aims to compile the information from *in vivo* as well as *in vitro* studies that had been done by collecting research journals published from the past 12 years (1995-2017) on this bioactive molecule.

2. Method

The information on EGCG (Fig. 1) relating to cardiac and metabolic diseases were collected from several databases such as Science direct, PubMed, NCBI, Springer and

Google scholar, limiting publications from 1995 to 2017. We have searched the work related to EGCG and cardiovascular disease in PubMed.gov today results in a listing of 283 with the earliest publication dated 1997. From these publications, we have narrowed down search criteria by limiting to the below mentioned categories presented in the current review. The keywords used as below:

i) Epigallocatechin gallate and atherosclerosis

ii) Epigallocatechin gallate and cardiac hypertrophy and heart failure

iii) Epigallocatechin gallate and myocardial infarction (MI)

iv) Epigallocatechin gallate and diabetes, metabolic syndrome, and obesity

v) Epigallocatechin gallate and anti-inflammatory

vi) Epigallocatechin gallate and antioxidant

vii) Metabolism and pharmacokinetics of Epigallocatechin gallate

viii) Toxicological studies

ix) Miscellaneous

Epigallocatechin gallate and cerebral ischemia reperfusion (I/R) injury

Epigallocatechin gallate and fibrosis

2.1 Epigallocatechin gallate

Camellia sinensis is a plant in which its leaves are used to make green tea or black tea. The difference between green tea and black tea is that the oxidation process does not take place in the production of green tea. Due to the lack of steaming process, green tea is found to have a relatively high polyphenol content compared to black tea (Hu et al., 2009). These polyphenols consist of a number of phenolic rings and EGCG is known as the most abundant (nearly 40% of the total polyphenol content) and the most active chemical component which

belongs to this family. According to studies, polyphenols are powerful antioxidants and cancer chemopreventive agents (Zaveri, 2006). They play a role in neutralizing free radicals, reducing inflammation and slowing down the growth of tumour.

2.2 Pharmacological action of Epigallocatechin gallate

2.2.1 *Epigallocatechin gallate and atherosclerosis*

Atherosclerosis is a disease of arteries that is due to endothelial dysfunction, inflammatory vascular cells and lipid accumulation (Hansson, 2009). Plaque which is usually made of fatty substances (cholesterol, triglycerides, lipoprotein etc.) is the main culprit that causes atherosclerosis, and it can partially or completely block the blood flow in the arteries. Stages of plaque development includes fatty streak formation, plaque progression and plaque disruption. Risk factors of atherosclerosis that could be modified are dyslipidemia, smoking, hypertension, and diabetes mellitus (DM). These factors can directly or indirectly promote the development of atherosclerotic lesions for example dyslipidemia causes the accumulation of low-density lipoprotein (LDL) in the arteries while hypertension enhances the retention of LDL by modifying the vessel wall. The modification of LDL through oxidation and advanced glycation end products by smoking and dyslipidemia linked to plaque progression. However, there are some risk factors, for instance older age, male gender and family history of coronary heart disease are not modifiable (Shah et al., 2011).

Effects of EGCG from in-vivo preclinical study

Lipid peroxidation is considered as a major factor that can lead to atherosclerosis. Xu et al., demonstrated the effectiveness of EGCG (100 mg/kg) in an atherosclerosis disease model induced by feeding atherogenic diet to Wistar rats for 30 days, and EGCG treatment was given for at least six days or maximum up to a maximum of 12 days. Results of the tissue morphometric analysis and lipid profile of the EGCG treated atherogenic diet fed rats showed that there was a reduction in total cholesterol (TC), triglycerides (TG), low-density and very low-density lipoprotein (LDL/VLDL) cholesterol fractions as compared to those untreated atherogenic diet fed rats. Additionally, there was an increase in high-density lipoprotein cholesterol (HDL) (Xu et al., 2014). Another study using atherosclerosis-susceptible C57BL/6J apoprotein (apo)E-deficient ($ApoE^{-/-}$) mice showed that the ingestion of EGCG (0.8 g/L) did not cause any changes in plasma cholesterol or TG levels. However, the EGCG-treated mice which had been given atherogenic diet for 14 weeks had a reduction of 23% in aortic weights and their aortic cholesterol and TG were 27% and 50% lower, respectively compared to the controls (Miura et al., 2001). Cai Y et al. studied that 0.02% EGCG solution can effectively reduce the production of CRP, monocyte chemoattractant protein-1 (MCP-1) and oxidized LDL/VLDL cholesterol level in the serum of $ApoE^{-/-}$ mice which had been treated with *Porphyromonas gingivalis*-induced atherosclerosis. There were also reductions in chemokine (C-C motif) ligand 2 (CCL2), matrix metallopeptidase (MMP-9), ICAM-1, heat shock protein 60 (HSP60), cell-surface glycoprotein (CD44), lectin-type oxidized LDL receptor 1 (LOX-1), NADPH oxidase 4 (NOX-4), human neutrophil cytochrome b light chain (p22phox) and inducible nitric oxide synthase (iNOS) gene expression levels in the aorta. The elevation of HO-mRNA suggested that EGCG can be used to treat atherosclerosis

through its anti-inflammatory and antioxidant effects (Cai et al., 2013). In addition, another study demonstrated that EGCG (100 mg/kg) caused the reduction of CRP and other inflammatory markers such as fibrinogen, sialic acid, serum protein amyloid A (SAA) in male albino Wistar rats subjected to atherogenic diet (Ramesh et al., 2010) (Table 1).

Effects of EGCG from in-vitro preclinical study

Jagged-1, a ligand for the activation of receptor notch 1, is an important protein in the EGCG-mediated protection against ox-LDL-induced apoptosis and ox-LDL-diminished cell adhesion in the human umbilical vein endothelial cells (HUVECs). According to Yin et al., treatment of 50 μM EGCG protects HUVECs against ox-LDL-induced endothelial dysfunction through the Jagged-1/Notch pathway (Yin et al., 2015).

Nanoformulation of drugs has been proved as an effective solution to overcome the solubility obstacles faced by poorly water-soluble drugs. The approach is based on the size reduction of drug to nanosize in order to alter their physical properties (Wais et al., 2016). Zhang et al., synthesized (-)-EGCG encapsulated nanostructured lipid carriers and chitosan-coated NLCE using natural lipids, surfactant, chitosan, and EGCG. *In vitro* study demonstrated that these nanoformulations were found to have the ability to inhibit the development of atherosclerotic lesions by reducing macrophage cholesteryl ester content and mRNA as well as protein expressions of MCP-1 in THP-1 derived macrophages (Zhang et al., 2013) in comparison with non-encapsulated EGCG at the same concentration (10 μM) (Table 2). In addition, these nanoformulations dramatically improved EGCG stability and were found to have the ability to increase its cellular

bioavailability, with enhancement of the sustained release effect (Zhang et al., 2013).

Effects of EGCG from clinical study

A double-blind, randomized trial was conducted with 82 patients having early atherosclerosis, and the results showed that the consumption of 30 ml EGCG supplemented with olive oil significantly improved endothelial function by reducing the number of leukocytes (Widmer et al., 2012) (Table 3). Momose et al., carried out systematic review on EGCG in reducing LDL-C levels in humans. The selected study design was a randomized, controlled trials (total of 17 trial; n=1356), using parallel design, involving the consumption of green tea EGCG at a dose varied from 107 to 857 mg/d in liquid form or capsules of green tea extract. Participants involved in all the trials were adults of aged 20 years or older without any diseases and study duration varied from 3-14 weeks. All the trials demonstrated significant reduction of LDL-C was observed in EGCG treated subjects compared to control; however, there were no significant changes in TG and HDL-C. A comparison of the effects of EGCG consumption showed a more significant reduction in the LDL-C levels in the subjects with a higher baseline LDL-C (>120 mg/dl) than those with lower baseline LDL-C (<120 mg/dl). Furthermore, subjects with higher baseline BMI (>25 kg/m2) showed a greater reduction in LDL-C than those with lower baseline BMI. These results suggest that EGCG at any dose level has potential to prevent the development of CVD and hypertension through significant reduction of LDL-C (Momose et al., 2016).

2.2.2 Epigallocatechin gallate and cardiac hypertrophy and heart failure

The maintenance of cardiac homeostasis includes hypertrophy, angiogenesis and metabolic plasticity. Increasing of the heart size and enhanced protein synthesis are the main symptoms of cardiac hypertrophy and they usually lead to a reduction of heart capacity to pump blood to the organs. Pathological cardiac hypertrophy is characterized by cardiac dysfunction and it is often a consequence of chronic mechanical pressure overloaded whereas physiological cardiac hypertrophy usually occurs due to excessive exercise (Frey et al., 2004; Lyon et al., 2015). In order to classify these two types of cardiac hypertrophy, we have to look into the chamber morphometrics, type of overloading stimuli, duration and intensity of the cardiac overload (Dorn, 2007; Katholi and Couri, 2011). According to the research that had been done, it was found that the cause of cardiac hypertrophy may not necessarily be due to pressure overload in the heart chamber; trophic effects may also be exerted on the myocytes and non-myocytes compartment in the heart by neurohormonal substances, for example, aldosterone, insulin, angiotensin II (Ang II), norepinephrine and some other growth factors. These trophic factors enhance the development of cardiac hypertrophy through the production of cytokines and growth factors which are an important process in elevating the systemic arterial pressure (Shimizu and Minamino, 2016).

Effect of EGCG from in vivo preclinical studies of hypertrophy and heart failure

Cai et al., found that the treatment of EGCG (50 mg/kg) inhibited nuclear factor- kappa B (NF-κB) activation and subsequent connective tissue growth factor (CTGF) overexpression. It also reduced collagen synthesis, fibronectin (FN) expression and proliferation of rat cardiac fibroblasts which are induced by Ang II or abdominal aortic constriction (AAC), suggesting the potential of EGCG in treating patients with pressure overload-induced hypertrophy (Cai et al., 2013).

Sheng et al., demonstrated the effectiveness of EGCG (25, 50 and 100 mg/kg) in AAC model of cardiac hypertrophy and the result showed that there was a reduction in heart weight indices, atrial natriuretic polypeptide (ANP), plasma endothelin concentrations, hydroxyproline (hyp) concentrations and the expression of proliferating cell nuclear antigen which is present in the hypertrophic myocardium. In contrast, the concentration nitrite, which is the oxidation product of NO, increased in the serum as well as in the myocardium (Sheng et al., 2009). Another study demonstrated that the administration of EGCG in drinking water at different concentration (0.02, 0.04 and 0.08%) over a period of three weeks after AAC surgery suppressed the load-induced increase in heart to body weight ratio by 69% as compared to AAC group (34% increase in AAC group compared to sham group). It also attenuated the increase in myocyte size as well as fibrosis induced by AAC. In addition, systolic functional parameters were shown to be improved by EGCG (Hao et al., 2007). Furthermore, Sheng et al., demonstrated the effect of EGCG (50 mg/kg and 100 mg/kg) in AAC-induced hypertrophic model by showing that it significantly reduced heart weight indices and cardiomyocytes apoptosis by preventing telomere shortening and the loss of telomere repeat-binding factor 2 (TRF_2) while also reducing the malondialdehyde (MDA) content in AAC-induced cardiac hypertrophy rats (Sheng et al., 2013). In studies using animals with heart failure, the administration of 10 mg/L and 100 mg/L of EGCG in heart/muscle-specific manganese superoxide dismutase (MnSOD)-deficient mice for eight weeks showed a decrease in the levels of myocardial oxidative stress and free fatty acids (FFA) decreased. EGCG was also shown to improve dilated cardiac remodelling with reduced cardiac contractility. The increased expression of nitric oxide synthase 2 (NOS2), nitrotyrosine (NT), fatty acid synthase (FAS), Toll-like

receptor 4 (TLR4), and NAD-dependent deacetylase sirtuin-1 (Sirt1) was prevented by EGCG (Oyama et al., 2017) (Table 1). Pan et al., studied the effectiveness of EGCG on cTnI expression induced by histone acetylation in age-related cardiac diastolic dysfunction. The treatment of EGCG (50 mg/kg/day) for 8 weeks in aged mice significantly improved cardiac diastolic function through up-regulating cTnI by inhibiting histone deacetylase 1 and 3 expression. These results demonstrate new mechanical insights into histone acetylation mechanisms of EGCG that may contribute to the prevention of cardiac diastolic dysfunction (Pan et al., 2017) (Table 1).

Effect of EGCG from in vitro studies of hypertrophy, heart failure and atrial fibrilation

AMP-activated protein kinase (AMPK) has the ability to inhibit p70S6 kinase and eukaryotic elongation factor-2 (eEf2) signalling pathways that result in suppression of cardiac myocyte hypertrophy. In the study done by Cai et al., it was found that the consumption of 100 μM EGCG can activate AMPK in H9C2 cardiomyocytes by reducing natriuretic peptides type A (Nppa), brain natriuretic polypeptide (BNP) mRNA expression on phenylephrine (PE)-induced cardiac hypertrophy and decrease cell surface area of H9C2 cardiomyocytes (Cai et al., 2015). Another study carried out by Cui et al., investigated the potential of EGCG (10, 50 and 100 μg/mL) on cultured hypertrophic myocytes induced by Ang II and showed that there was a dose-dependently reduction of the protein contents, myocytes volume as well as the amount of fibrocasts in the EGCG treated hypertrophic myocytes compared to the untreated hypertrophic myocytes group (Cui et al., 2008).

Hypertrophic cardiomyopathy is the most common inherited cardiac disease with left ventricular (LV) hypertrophy, interstitial fibrosis and diastolic dysfunction. Since increased myofilament Ca^{2+} sensitivity could be one of the underlying causes of diastolic dysfunction, the effects of EGCG were tested on fractional sarcomere shortening and Ca^{2+} transients in intact ventricular myocytes as well as on force-Ca^{2+} relationship of skinned ventricular muscle strips isolated from Mybpc3-targeted knock-in and wild-type mice. In both types of mice, neither diastolic sarcomere length nor fractional sarcomere shortening were influenced by 1.8 μM EGCG treatment, but relaxation time was reduced to a greater extent in Mybpc3-taegeted knock in cells. In skinned cardiac muscle strips, EGCG (30 μM) decreased Ca^{2+} sensitivity in both groups of mice. These observed effects may be due to the Ca^{2+} desensitization of the myofilaments (Friedrich et al., 2016) (Table 2).

Effect of EGCG from in vitro studies of atrial fibrillation (AF)

Atrial fibrillation (AF), is characterized by sustained arrhythmia which increases the risk of stroke and heart failure. It has been reported that Ca^{2+} overload and oxidative stress are thought to be involved in the pathogenesis of AF. Chang et al., investigated the effects of EGCG on the modulation of electrophysiological characteristics and Ca^{2+} homeostatis of the left atrium. EGCG treatment at 0.01, 0.1, 1, 10 μm concentrations dose dependently decreased action potential duration. Furthermore, it decreased both intracellular Ca^{2+} transient and sarcoplasmic reticulum Ca^{2+} content in the left atrium cardiomyocytes as well as ISO (1 μM)-induced burst firing by inhibiting Ca^{2+}/calmodulin or GMP dependent protein kinases (Chang et al., 2017) (Table 2).

EGCG in hypertrophic and cardiomyopathic patients

A double-blind, placebo-controlled, crossover design study done by Michael et al., showed that EGCG (300 mg for initial dose and 150 mg twice daily for two weeks) improved endothelial function by increasing the brachial artery flow-mediated dilation in 42 subjects with cardiac hypertrophy (7.1 ± 4.1 to 8.6 ± 4.7% two hours after the first dose of 300 mg) (Widlansky et al., 2007). Another clinical study showed that 12 months of 600 mg EGCG consumption decreased left LV myocardial mass and TC of 25 male patients with wild-type transthyretin amyloid cardiomyopathy. However, the LV wall thickness and mitral annular plane systolic excursion remained unchanged (aus dem Siepen et al., 2015) (Table 3).

In conclusion, these data suggest that EGCG is a potential therapeutic agent against cardiac hypertrophy as well as heart failure in human.

2.2.3 Epigallocatechin gallate and myocardial infarction (MI)

MI, also known as heart attack, is an ischemic heart disease which involves myocardial cell death due to chronic ischaemia. This is caused by an imbalance between the myocardial metabolic demand and the myocardial blood flow that consequently results in the starvation of oxygen in the heart. In fact, there are many factors that can affect myocardial blood flow, for example, progressive atherosclerosis, elevated LDL levels, or some superimposed events including vasospasm, thrombosis, or circulatory changes that lead to hypoperfusion (Anversa et al., 1990; Nissen and Wolski, 2007). For the sake of searching different treatment approaches, MI is classified into five categories: spontaneous MI, MI secondary to an ischaemic imbalance, MI resulting in death when biomarker values are unavailable, MI related to percutaneous coronary intervention or related to stent

thrombosis, and lastly MI related to coronary artery bypass grafting (CABG) (Jaffe, 2013). The roles of biomarkers such as cardiac troponin (cTn) or MB fraction of creatine kinase (CKMB) are important in such a way that their increment in blood levels indicate the presence of myocardial injury (Thygesen et al., 2007).

Effect of EGCG from in vivo preclinical studies of MI

As mentioned previously, Ang II, a peptide hormone that enhances the expression of endoglin which contributes to heart diseases, was prevented from binding to activator protein-1 (AP-1) transcription factor by the administration of EGCG (50 mg/kg). The combination of EGCG and c-Jun N-terminal kinase (JNK) inhibitor markedly attenuated the expression of endoglin in cardiac fibroblasts from the thoracic aorta of adult Wistar rats (Lin et al., 2016). Aneja et al., proved that the treatment of EGCG (10 mg/kg) reduced IL-6 plasma concentration, myeloperoxidase activity (MPO), creatine phosphokinase (CPK) concentration, NF-κB and AP-1 DNA binding in rats with ischemia-reperfusion injury induced MI. (Aneja et al., 2004). Furthermore, administration of EGCG (10, 20, 30 mg/kg) for 21 days in rats with isoprenaline (ISO)-induced MI showed a dose-dependently reduction in terms of heart weight, activities of membrane-bound ATPases, levels of electrolytes and cardiac marker enzymes, such as lactate dehydrogenase (LDH), myoglobin (Mb), and aspartate transaminase (AST) (Devika and Prince, 2008). Apart from this, EGCG also showed its pre-treatment effect by inhibiting the changes of CKMB, LDH, alkaline phosphatase (ALP), alanine transaminase (ALT), troponin T (TnT) and inhibiting the stimulation of the pro-inflammatory cytokine TNF-α levels in the serum. EGCG also maintained redox balance by upregulating SOD and CAT activity while limiting the lipid peroxidation. In addition, treatment with EGCG significantly ameliorate apoptotic markers such as Bax, caspase 3 and

9, and this was accompanied by the protection of genomic integrity by inhibiting DNA fragmentation along with the downregulation of p53. These results suggest that EGCG protects the heart against ISO-induced myocardial damage by inhibiting apoptotic signaling molecules, oxidative stress and inflammation, thereby sustaining cardiac health (Othman et al., 2017) (Table 1).

Effect of EGCG from in vitro studies of MI

A preliminary study showed that 10 µM treatment of EGCG is able to attenuate MI through the reduction of infarct volume in Langendorff perfused rat hearts by a percentage of ischemic volume ($33.5 \pm 4.1\%$) through acting on adenosine receptor (ADR) and opioid receptor (OPR) (Lee et al., 2012). Isolated perfused rat heart is mainly used to scrutinize the effect of ischaemia or hypoxia on the myocardial cells and the main advantage of using isolated perfused rat heart is that it allows continuous experiments in the face of events, which is a considerably harmful procedure during in vivo experiments (de Leiris et al., 1984). In a study conducted by Townsend et al., EGCG was found to have potential in reducing both the signal transducer and activator of transcription 1 (STAT-1) phosphorylation by reducing the expression of Fas receptor (FasR), which is a STAT-1 pro-apoptotic target gene. EGCG infusion also decreased the infarct size and attenuated myocyte apoptosis in the isolated rat heart exposed to myocardial injury (Townsend et al., 2004). Kim et al., also subjected isolated rat heart to ischemia and reperfusion, then administered EGCG of different doses (1 µM and 10 µM). Results showed that 1 µM of EGCG reduced the infarct volume while 10 µM of EGCG strikingly improved the LV developed pressure (Kim et al., 2010). 1 µM of EGCG was able

to activate the mitochondrial K (ATP) channels and reduce the infarct volume significantly in isolated rat hearts (Song et al., 2010).

Moreover, Zeng and Tan., demonstrated that the combination of EGCG (10 μM) with Zn^{2+} (5 μM) enhanced the anti-apoptotic activity and protected H9c2 cells in hypoxia/reoxygenation (H/R) injury through the activation of PI_3K/Akt pathway via upregulated expression of p-p85, as well as p-Akt. In addition, downregulation of the expression levels of TNF-α, IL-6 and IL-8 was observed (Zeng and Tan, 2015) (Table 2). It was suggested that similar methods may be applied in the prevention of MI in clinical practices.

2.2.4 Epigallocatechin gallate and diabetes, metabolic syndrome and obesity

The term DM is a metabolic disease that is characterized by hyperglycaemia due to insulin malfunction. Some typical symptoms of DM include reduction in weight, blurred vision and polyuria. Chronic effects may cause blindness, renal failure, neuropathy with risk of foot ulcers and autonomic dysfunction. Also, diabetic patients have higher risk of exposure to CVD such as hypertension and atherosclerosis. DM can be classified into two categories: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). According to American Diabetes Association, T1DM is mainly due to deficiency of insulin production and this is commonly diagnosed in children as well as young adults (American Diabetes Association, 2010). For T2DM which is more prevalent, the disease is mainly caused by insulin malfunction, either disorder of insulin action or insulin secretion (Alberti and Zimmet, 1998; American Diabetes Association, 2010). However, the underlying mechanisms for these disorders are not known yet.

Effect of EGCG from in vivo preclinical studies of diabetes, obesity and metabolic

syndrome

Aldose reductase (AR), is an enzyme that generates secondary complications during diabetes. When HFD were introduced to male C57BL/6J mice, AR activity and total advanced glycation end products (AGEs) increased in a dose-dependent manner and cell viability was decreased due to high glucose-induced toxicity. The administration of EGCG (25, 75 mg/kg) significantly reduced blood glucose level, accumulation of AGEs as well as AR activity (Sampath et al., 2016). On the other hand, lipophilic EGCG derivative also showed its protective effect by reducing plasma glucose levels in streptozotocin (STZ)-induced diabetic rats' by 40.5±7.0 % and 17.0±2.8% at a dose of 50 mg/kg and 25 mg/kg, respectively. Moreover, this lipophilic derivative was able to decrease lipid metabolites including TC, TG and LDL cholesterol while maintaining HDL cholesterol levels in plasma (Li et al., 2007). Wolfram et al., studied that the administration of diet containing EGCG (2.5, 5, 10 g/kg of diet) decreased the levels of mRNA expression of phosphoenolpyruvate carboxykinase (PEPCK) in the adipose tissue as well as the plasma concentration of triacylglycerol (TAG). On the other hand, there was an increase in glucose-stimulated insulin secretion, and the levels of mRNA expression of glucokinase (GK) was upregulated dose-dependently in the liver of db/db mice and ZDF rats (Wolfram et al., 2005). These results suggest that dietary supplementation with EGCG could potentially contribute to the prevention of T2DM.

Othman et al., demonstrated that EGCG (2 mg/kg) showed remarkable antihyperglycemic and antidysdlipidemic activity in type 2 diabetic rats, evidenced by significant reduction in plasma glucose, HbA1c, HOMA-1R and lipid profile, along with an elevation in insulin level. In addition, EGCG treatment also

suppressed oxidative stress and apoptosis which is demonstrated through the increase in levels of antioxidant enzymes (SOD, GSH and CAT) and antiapoptotic marker (Bcl-2) and the decrease in protein carbonyl and BAX, Cas 3 as well as 9. Furthermore, it protects against diabetes through improving myocardial function by reducing the levels of TnT, LDH, AAT and proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in the serum. It also exhibited decreased level of DNA damage and morphological alterations in the myocardium. These results suggest that EGCG has a potential effect on the heart against T2DM (Othman et al., 2017).

According to Fu et al., the onset of T1DM was delayed in non-obese diabetic mice by administration of 0.05% EGCG in drinking water. When compared with the control group, the mice treated with EGCG showed significant increase in insulin and decrease in glycosylated haemoglobin (HbA1C) levels in the plasma thereby improving survival rate of animals. In addition, EGCG did not exert any obvious effects on food or water intake as well as the body weight in mice suggesting that the glucose-lowering effect was due to the elevation of circulating anti-inflammatory cytokine IL-10 level (Fu et al., 2011). As T1DM patients are predisposed to erectile dysfunction, owing to it's the structural and molecular alterations in the corpus cavernosum (CC) vessels, Lombo et al., carried out a study investigating the early treatment of EGCG in cavernous diabetes-induced vascular modifications. Results showed that the reduction in smooth muscle content in the CC of diabetic rats were significantly attenuated in the EGCG treated (2 g/L) group and there were no significant changes in VEGF expressions (Lombo et al., 2016). On the other hand, it was found that EGCG (500 mg/kg)_showed its fat-reducing effect by decreasing the expression of leptin and stearoyl-CoA desaturase-1, malic enzyme (ME), as well as GK in HFD-induced obese mice (Klaus et al., 2005).

When 0.5% or 1.0% w/w of EGCG were given as a short-term supplementation (4-7days) to HFD-induced obese mice, there was a reduction in terms of post-prandial TG, liver glycogen content, and incorporation of dietary lipids into fat tissues, liver and skeletal muscles. However, it showed an increase in the oxidation of dietary lipids (Friedrich et al., 2012). In another study using HFD-induced obese (C57BL/6J) mice, it was shown that the administration of 0.2% or 0.5% w/w of EGCG for 8 weeks significantly decreased the mRNA levels of adipogenic genes such as peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT enhancer-binding protein-α (C/EBP-α), sterol regulatory element-binding protein-1c (SREBP-1c), adipocyte fatty acid-binding protein (aP2), lipoprotein lipase (LPL) and FAS. On the other hand, the mRNA levels of carnitine palmitoyl transferase-1 (CPT-1) and uncoupling protein 2 (UCP2), as well as lipolytic genes such as hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), were significantly increased (Lee et al., 2009) (Table 1).

Effect of EGCG from in vitro studies

The investigation of EGCG on insulin signalling was demonstrated in high glucose treated insulin-responsive human HepG2 cells. Results showed that the administration of EGCG decreased Ser307 phosphorylation of insulin receptor substrate-1 (IRS-1) through activating AMPK pathway, thus promoting insulin-stimulated phosphorylation of PI3K signalling (Lin and Lin, 2008). Zhang et al., proved that when human retinal endothelial cell line was put under high glucose conditions, 20 and 40 mM of EGCG exerted its protective effects by regulating the inflammatory cytokines and decreasing the expression of phosphorylated p38-mitogen activated protein kinase (MAPK), extracellular regulated kinase (ERK),

and vascular endothelial growth factor (VEGF) (Zhang et al., 2016). In addition, the administration of 10 μM EGCG greatly increased the glycogen synthesis, phosphorylation of Ser9 glycogen synthase kinase (GSK) 3β and Ser641 glycogen synthase and inhibits lipogenesis, which was associated with an increased expression of phosphorylated AMPK and acetyl-CoA carboxylase (ACC) in HepG2 cells (Kim et al., 2013) (Table 2). In the case of non-insulin-dependent diabetes, cytokines are usually associated with malfunction of beta-cell in high glucose condition. *In vitro and in vivo* studies showed that EGCG exerted its antidiabetogenic effect by preventing cytokine-induced beta cells destruction in insulinoma cell line (RINm5F cell) due to downregulation of nitric oxide synthase (NOS) through inhibition of NF-kB activation (Song et al., 2003). To verify the antidiabetogenic effect of EGCG, its potential was investigated in multiple low doses of STZ-induced diabetes and it was shown that EGCG (100 mg/kg for 10 days) could significantly attenuate the increase of glucose concentrations in blood. Apart from this, further analysis of beta cells showed that EGCG could inhibit the onset of STZ-induced diabetes by protecting the pancreatic islets and thus the therapeutic potential of EGCG against the progression of DM was again confirmed (Song et al., 2003).

Effect of EGCG from clinical studies

There were some clinical studies investigating the potential of EGCG in reducing the risk of T2DM. A prospective cross-sectional study showed that 4 cups of green tea per day decreased the risk of T2DM by 30% in 38,018 women aged 45 years and older who are free from CVD, cancer and diabetes (Song et al., 2005). In

addition, a randomized, double-blind, placebo-controlled clinical trial showed that daily dose of 856 mg EGCG in 68 obese individuals, aged 20-65 with T2DM for 16 weeks caused significant reductions of homeostasis model assessment for insulin resistance (HOMA-IR) index, HbA1C, fasting insulin, fasting glucose, lipids, ALT, and creatinine or uric acid levels and a significant increase in the level of ghrelin (Hsu et al., 2011). This study results suggest that intake of decaffeinated green tea extract containing 856 mg EGCG is safe with devoid of any adverse effects in obese individuals with T2DM (Song et al., 2005).

In a pilot study where six overweight men were given 300 mg EGCG for 2 days, their respiratory quotient (RQ) values were found to be obviously lower than the placebo group (Boschmann and Thielecke, 2007). When 150 mg of EGCG capsules were given to 38 obese postmenopausal women, who exercised at moderate intensity for 12 weeks, it showed a great reduction in resting heart rate (HR) ($p < 0.01$) and plasma glucose in subjects with impaired glucose tolerance ($p < 0.05$). This study suggested that the loss of body fat may require a higher intake of EGCG or addition of other stimulants (Hill et al., 2007). Brown et al., found that when 100 obese males, aged between 40-65 were administered with 400 mg capsules of EGCG twice daily for 8 weeks, the diastolic blood pressure reduced in a modest manner which may contribute to some of the observed cardiovascular benefits. In addition, subjects in the EGCG group had a positive effect on mood compared to the placebo group (Brown et al., 2009), indicating that EGCG (or its metabolites) may also have anxiolytic effects in men. However, a randomized controlled trial showed that the supplementation of 300 mg/day EGCG to 83 obese pre-menopausal Caucasian women for 12 weeks did not alter their body weight, fat mass and fat

metabolism, HOMA-IR total or LDL cholesterol levels. These results suggest that dietary supplementation with EGCG did not alter adiposity content and did not improve weight loss-induced changes in cardiometabolic risk factors in women following energy-restricted diet interventi (Mielgo-Ayuso et al., 2014). In contrast, when 27 healthy male subjects were given 1200 mg EGCG accompanied by 240 mg caffeine per day for 7 days, the citric acid cycle activity, lipolysis, and fat oxidation were increased (Hodgson et al., 2013) (Table 3).

2.2.5 Epigallocatechin gallate and anti-inflammatory

Inflammation is considered as an important part of the body immune response and it is often characterized by redness, swelling, pain or sometimes immobility. It prevents the body from infection which is mainly due to foreign invaders, for example bacteria and viruses ("S3.1 - From Acute to Chronic Inflammation," 2009). Usually, there are three stages of inflammation, namely irritation, suppuration, and lastly granulation. Acute inflammation is a type of inflammation that shows rapid onset and may last for few days or few weeks. On the other hand, chronic inflammation can persist for few months or even years and it has the potential of developing diseases such as CVD, cancers, diabetes, and hay fever (Stankov, 2012). Inflammatory biomarkers can be proteins or enzymes that provide diagnostic as well as prognostic information by demonstrating an underlying disease state. They are usually measurable in the serum, plasma or blood (Zakynthinos and Pappa, 2009).

Anti-inflammatory effect of EGCG from in-vivo studies

The effect of EGCG on decreasing MPO activity was demonstrated in an *in vivo* study that was carried out by Khalatbary and Ahmadvand. This study also showed that when the rats with spinal cord injuries were administered with 50 mg/kg EGCG, the expressions of TNF-α, IL-1β, NT, iNOS, COX-2, and poly(ADP-ribose) polymerase (PARP) could be attenuated and hence protect their spinal cord through the modulations of inflammatory reaction (Khalatbary and Ahmadvand, 2011). Another study using high-fat/high-sucrose diet fed-C57/BL6 male mice showed that 0.9 mg/kg EGCG treatment effectively suppressed TLR4 expression through E3 ubiquitin-protein ring finger protein 216 (RNF216) upregulation (Kumazoe et al., 2017). (Table 1). Yu et al., studied the protective effect of EGCG against arsenic-induced inflammation. The EGCG treatment at 10 mg/kg/day for 30 days in BALB/c mice significantly decreased oxidative stress (evidenced by upregulation of antioxidant enzymes), levels of inflammatory cytokines and apoptosis. These results suggest that EGCG attenuates not only arsenic-induced immunosuppression but also inflammation and apoptosis (Yu et al., 2017).

Anti-inflammatory effect of EGCG from in-vitro studies

EGCG has also been reported to inhibit the activation of NF-κB. NF-κB is a transcription factor that can be activated by ROS, leading to activation of a series of molecules including transcription of genes encoding pro-inflammatory cytokines. A study carried out by Liu et al., showed that the pre-treatment with EGCG (15, 30 μM) successfully prevented the increase of vascular inflammatory mediator expressions, which includes IL-6, CRP, ICAM-1, VCAM-1 and IL-1 α/β. This process leads to the protection against polychlorinated biphenyl (PCB)-induced endothelial inflammation in human endothelial cells (Liu et al., 2016). In addition,

a study using human corneal epithelial cells suggested that a treatment of EGCG at 3-30 μM inhibited the release of cytokines, phosphorylation of MAPK p38 and c-JNK, as well as the transcriptional activities of AP-1 in a dose-dependent manner (Cavet et al., 2011). Further, a recent work published in 2017 discussed about attenuation of inflammatory response in the immediate early stage of EGCG treatment by shuttling off Notch signalling pathway. This study utilized human monocyte cell line (THP-1) in which infection induced by Notch 1 or Notch 2 short hairpin RNA Lentiviral particles. The treatment was carried out by pre-treating the infected cell lines with 50 μg/mL EGCG for 30 minutes and the downregulation of transcription of the Notch target gene was observed. The downregulation of inflammatory response by EGCG was prevented by knockdown of Notch 1/2 expression by RNA interference. Furthermore, the study showed that EGCG inhibited lipopolysaccharide-induced inflammation and turned off Notch signaling in human primary macrophages. These results suggest that EGCG target Notch to regulate inflammatory response (Wang et al., 2017) (Table 2).

Topical anti-inflammatory effect of EGCG

According to Santosh et al., a topical pre-treatment of EGCG (3 mg/2.5 cm^2) effectively blocked UV-B induced infiltration of leukocytes which is the main source of ROS generation and thereby reduced the activity of MPO. This proved that EGCG may be a useful topical therapeutic agent for UV-B induced ROS-associated inflammatory dermatoses (Katiyar et al., 1999).

Anti-inflammatory effect of EGCG from clinical study

In an 8-week randomized, split-face clinical trial, 35 subjects (17 men and 18 women, mean age 22.1) with acne were treated with 1% or 5% EGCG solution topically twice a day on the affected areas. The results showed that EGCG significantly reduced inflammation by inhibiting NF-kB and AP-1 pathways, induced cytotoxicity of human sebocytes via apoptosis and decreased the viability of *P. acnes* (Yoon et al., 2013) (Table 3).

2.2.6 Epigallocatechin gallate and antioxidant

A series of ROS including superoxide anion radicals and hydroxyl radicals are generated during the consumption of oxygen inherent in cell growth. They are important for energy generation, phagocytosis and other biological processes. Those ROS are continuously generated and removed by antioxidant defence mechanisms during normal physiological events. However, they can be damaging as they are able to attack tissues by reacting with polyunsaturated fatty acids (PUFA) that are present on the cellular membranes, DNA nucleotides as well as the sulfhydryl bonds present in some protein, through oxidation process which will eventually lead to the development of different types of diseases (Sharma et al., 2012). In order to prevent the harmful consequences, the utilization of natural antioxidants is getting much attention compared to the synthetic antioxidants as synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been found to be carcinogenic (Machlin and Bendich, 1987). It was also found that the effectiveness of antioxidants increases as the number of hydroxyl group present in their aromatic ring(s) increases (Brewer,

2011). Hence, EGCG becomes one of the most potential natural antioxidant agents, thus scientists have shown more interest on this molecule nowadays.

In-vivo antioxidant activity of EGCG

A study done by using adult Sprague-Dawley rats with sodium iodate-induced retinal degeneration showed that the consumption of EGCG effectively reduced the thickness of the outer nuclear layer. Also, this protective effect was found to be associated with decreased expression of caspase-3, increased expression of SOD and GPX, as well as inhibition of 8-iso-Prostanglandin F2α generation (Yang et al., 2016).

According to Yoshino et al., EGCG was found to be unstable in authentic intestinal juice and mouse plasma (alkaline solutions over pH 7.4) and it generally undergoes oxidative dimerization through dehydrogenation and decarboxylation to produce three types of products, P-1, P-2, and P-3. These three dimerization products showed relatively higher Fe^{2+} chelating activities as well as superoxide anion radical-scavenging activity, and P-2 exerted a better pharmacokinetic profile compared to free EGCG (Yoshino et al., 1999) (Table 1).

Yao et al., demonstrated to show protective effect of EGCG (25 mg/kg) against DOX (2 mg/kg)-induced cardiotoxicity in sarcoma 180 tumor-bearing mice by inhibiting LDH release and apoptosis in cardiomyocyte. Further, EGCG exhibit cardioprotection was in association with the increase of mitochondrial membrane potential and MnSOD expression. It was also found to attenuate myocardial Ca^{2+} overload and ROS generation in sarcoma 180 tumor-bearing mice subjected to DOX. These results suggest that concomitant administration of EGCG along with DOX have potential to mitigate DOX-induced cardiotoxicity without compromising its chemotherapeutic value (Yao et al., 2017) (Table 1).

In-vitro antioxidant activity of EGCG and its bioavailability

An *in vitro* study using a phospholipid liposome model showed that the administration of EGCG (0.1 and 1.0 μg/mL) suppressed the initiation rate, sustained the lag phase of peroxyl radical-induced oxidation to a greater extent compared to both Trolox and α-tocopherol and also contributed to the protective effect on peroxyl radical and hydroxyl radical-induced supercoiled DNA nicking. The rate constant which characterized the EGCG against hydroxyl radical was 4.22 ± 0.07 × 10^{10} M^{-1}·sec^{-1}. In a multiphasic system, EGCG enhanced LDL oxidation by converting Cu^{2+} to Cu^{+}, which explained the pro-oxidant activity of EGCG (Hu and Kitts, 2001).

In addition, 10 μM administration of EGCG, the stable free radical, 1,1-diphenyl-β-picrylhydrazyl (DPPH) can be scavenged, leading to inhibition of H_2O_2-induced DNA damage in the Jurkat T-lymphocytes. However, significant DNA damage was induced when 10-fold higher concentrations of EGCG was administered (Johnson and Loo, 2000) (Table 2)

According to Yin et al., phosphorylation and conjugation of EGCG with egg white protein (EWP) significantly improved the antioxidant activity by enhancing the $ABTS^{+}$ free-radical scavenging capacity, oxygen radical antioxidant capacity, reducing power, chelating capacity, and superoxide anion scavenging activity of EWP (Yin et al., 2014). Some other improvements had also been done on EGCG such as encapsulation in cyclodextrins (CDs) whereby its thermodynamic stability and antioxidant properties was found to be better than EGCG alone through the deduction of DPPH assay (Aree and Jongrungruangchok, 2016). Apart from this, EGCG also can also be encapsulated in niosomes that

consist of Tween-60 and cholesterol which might be a promising technique to enhance the oral bioavailability of EGCG in the human body (Liang et al., 2016).

2.2.7 Metabolism and pharmacokinetics of Epigallocatechin gallate

Preclinical studies

A few metabolism and pharmacokinetics studies on EGCG had been carried out to identify the metabolites as well as the extent of absorption in rats. Nakagawa and Miyazawa employed chemiluminescence-detection high-performance liquid chromatography (CL-HPLC) to determine the concentrations of EGCG in various parts of the rats. It was found that before the treatment of EGCG, the EGCG concentrations were below the detection limit (<0.002 nmol/ml, 0.002 nmol/g), but it increased in 1 hour after a single oral administration of EGCG (500 mg/kg body weight) in plasma (12.3 nmol/ml), liver (48.4 nmol/g), brain (0.5nmol/g), small intestinal mucosa (565 nmol/g), and colon mucosa (68.6 nmol/g) indicating that EGCG was absorbed from the digestive tract (Nakagawa and Miyazawa, 1997). However, it was found that EGCG disappeared from the plasma within four hours after the administration (Unno and Takeo, 1995).

Kohri et al., reported that, the intravenous administration of EGCG to Wistar male rats produced the metabolites such as (−)-epicatechin gallate, 3‘-O-methyl-(−)-epicatechin gallate, 4‘-O-methyl-(−)-epicatechin gallate, 4‘-O-methyl-(−)-epicatechin gallate, and 3‘,4‘-di-O-methyl-(−)-epicatechin gallate. Furthermore, the most abundant metabolite found in the plasma and urine was the conjugated form of pyrogallol (Kohri et al., 2003). In the rat intestine, some microorganisms including *Enterobacter aerogenes, Raoultella planticola, Klebsiella pneumoniae*

susp. pneumoniae, and *Bifidobacterium longum subsp. infantis* were found to be responsible for EGCG hydrolysis (Takagaki and Nanjo, 2010).

Clinical studies

When human subjects were given 2 mg/kg of EGCG administration, it was found that the maximum plasma concentrations of EGCG in three repeated experiments with green tea was 77.9±22.2 ng/ml. The time required to reach the peak concentrations lied between 1.3 to 1.6 hours and the elimination half-life was found to be 3.4 ± 0.3 hours (Pastore and Fratellone, 2006). However, the bioavailability of EGCG is poor as it was only found to be 0.2 to 2% of the total ingestion in people with healthy conditions. This phenomenon is particularly obvious when administration of EGCG by ingestion is given. Most of the EGCG ingested is absorbed in the gastrointestinal system and subsequently degraded in the large intestine by microorganisms, hence the amount of EGCG that gets into the blood is relatively low (Mereles and Hunstein, 2011; Nakagawa et al., 1997). In addition, they reported that the following factors, namely cool and dry storage, fasting conditions, albumin, soft water, vitamin C, fish oil and piperine enhance the bioavailabilty of EGCG (Mereles and Hunstein, 2011). Naumovski et al., reported that the ECGC was best absorbed when consumed in capsule form on empty stomach and it is the most appropriate method for ECGC oral delivery (Naumovsk et al., 2015).

As EGCG is the only polyphenol that exists in its free form in the plasma, it undergoes biotransformation in the liver as well as the intestine. EGCG could be methylated by catechol-O-methyltransferase, glucuronidated by UDP-

glucuronosyltransferases (UGT) and sulfonated by sulfotransferases (SULT). In the colon, it was also proved to be degraded by microorganisms to form valerolactones, phenolic and benzoic acids which is excreted out through faeces (Li et al., 2000). Even though EGCG is able to produce metabolites after administration into the body, only a limited number of metabolites were proven to exert similar or higher activity than the parent compound (Feng, 2006). Therefore, further study are required to investigate the effects of these metabolites on the human.

2.2.8 Toxicological studies

A single oral dose of EGCG (1.5 g/kg) reported to cause hepatotoxic effect in male CF-1 mice by increasing plasma ALT levels by 138 fold and reduced survival rate by 85%. Further, one daily repeated dose of EGCG (500-750 mg/kg) for 2-7 days increased plasma ALT level by 184 fold. This hepatotoxicity caused by increased hepatic lipid peroxidation, plasma 8-isoprostane, hepatic metalothionin and γ-histone 2AX protein expression (Lambert et al., 2010). The study conducted by Isbrucker et al., showed that the oral administration of EGCG (500 mg/kg/day) for 13 weeks increased bilirubin and decreased fibrinogen in rats, while a single dose of 2 g/kg EGCG by the oral route was toxic (Isbrucker et al., 2006). In addition, the study conducted in beagle dogs showed that the administration of 500 mg/kg Teavigo^{TM} for 9 or 13 weeks caused proximal tube necrosis. Interestingly, more than half of the male Beagle dogs were found to be having elevated AST levels while more than half of the female Beagle dogs developed liver necrosis, which was not observed in male Beagle dogs. (Isbrucker et al., 2006).

A daily dose of 800 mg caffeine free EGCG for 4 weeks was found to be a safe dosage regimen for healthy human(Chow et al., 2003). Daily administration of EGCG produced only minor gastrointestinal effects (Nagle et al., 2006). The bioavailability of orally administered EGCG is normally low. However, under specific conditions such as fasting as well as repeated administration, increases its plasma concentration and it may reach toxic levels, which in turn cause hepatotoxicity by inducing oxidative stress in liver (Mazzanti et al., 2009).

2.2.9 Miscellaneous

2.2.9.1 Epigallocatechin gallate and cerebral ischemia reperfusion (I/R) injury

I/R injury is a type of disease mainly caused by the restoration of blood flow to tissues that previously experienced insufficient blood flow, which may eventually lead to a systemic inflammatory response. This is because the reperfusion process proceeds with a sudden increase of oxygen radicals which may overwhelm the cells' usual defences leading to uncontrolled oxidation of vital cell components (“Ischemia/Reperfusion Injury: R&D Systems,”). The common characteristics of I/R injury include oxidant production, complement activation, leucocyte–endothelial cell adhesion, platelet–leucocyte aggregation, increased microvascular permeability and decreased endothelium-dependent relaxation (Eltzschig and Collard, 2004).

There are a few studies that had been conducted on how EGCG can treat or prevent cerebral I/R injury. Zhang et al., conducted a study on transient middle cerebral artery occlusion (tMCAO) in male Sprague-Dawley rats and treated them with 50 mg/kg of EGCG at a dose of. The results showed

that EGCG ameliorated TNF-α, IL-1β, and IL-6 levels. Also, the upregulation of NF-κB/p65, and induction of cyclooxygenase-2 (COX-2) and iNOS were inhibited (Zhang et al., 2015). Lee et al., investigated the effect of EGCG (25 or 50 mg/kg at 30 mins before and immediately after I/R injury) on brain edema caused by cerebral I/R and found that EGCG reduced excitotoxin-induced MDA production and neuronal damage (Lee et al., 2004). Apart from this, it was found that 50 mg/kg of EGCG significantly reduced gelatinase levels and suppressed MMP-9 activation which eventually reduced the development of delayed neuronal death after transient cerebral ischemia in mouse brain (Park et al., 2009).

2.2.9.2 Epigallocatechin gallate and fibrosis

Fibrosis is a term that is used to describe the event where fibrous connective tissue is established in response to injury. It can be a part of body healing process or pathological process if there is an excess of tissue deposition ("What is Fibrosis?,"). When tissue is injured due to virus infection, heavy alcohol consumption or other factors, the body immune system is activated in order to heal the tissue. During the healing process, chemical messengers such as cytokines and growth factors are released by the immune system to produce collagen, glycoproteins and other substances help to build extracellular matrix. When the process of synthesizing collagen is faster or the process of degrading collagen is impaired, fibrosis occurs (Wynn, 2008). This disease can be developed in many organs, including liver (hepatic fibrosis), lungs (pulmonary fibrosis, idiopathy pulmonary fibrosis and cystic fibrosis),

heart (atrial fibrosis, endomyocardial fibrosis), and some other parts of the body.

An in silico molecular docking analysis showed that EGCG inhibits active MMP-9 activities in pulmonary artery smooth muscle cell culture supernatant and galloyl group was found to be responsible for enhanced interaction. Through the inhibition of MMP-9 activities, pulmonary hypertension, cardiac and neurological disorders can be prevented (Chowdhury et al., 2017).

In hepatic fibrosis, the major cell type involved is hepatic stellate cell (HSC). An *in vitro* study showed that EGCG reduced the number of HSC in normal rat liver by inhibiting the phosphorylation of tyrosine in platelet-derived growth factor (PDGF)-β receptor in a dose-dependent manner (20, 50, and 100 μM). Also, it inhibited the activation of AP-1 and NF-κB which were required for transcription of activated HSC (Chen and Zhang, 2003). 50 μM of EGCG was also found to significantly inhibit collagen synthesis and collagenase activity in rat primary HSC (Nakamuta et al., 2005).

Apart from this, three mRNA miR-181a, miR-10b, and miR-221 had been identified to be able to inhibit the expression of osteopontin (OPN) which promotes hepatic fibrosis. EGCG was able to upregulate these three mRNA expression in HepG2 cells dose-dependently (0.02-20 μg/ml) and at the same time inhibit the effect of thioacetamide which is important for OPN activation (Arffa et al., 2016). Another study using activated human HSC-derived TWNT-4 cells showed that the administration of EGCG at a dose of 100 μmol/L inhibited lysophosphatidic (LPA), an activator of Rho (a small GTPase), and suppressed phosphorylation of focal adhesion kinase to

eventually downregulate the proliferation of HSC. The same dose of EGCG also strongly induced phosphorylation of MAPK and caused apoptosis in half of the total HSC (Higashi et al., 2005). Yu et al., demonstrated a hepatic fibrosis model by using transforming growth factor-β1 (TGF-β1)-stimulated human hepatic stellate LX-2 cells and concluded that the administration of EGCG (10, 25 μ mol/L) dose-dependently suppressed TGF-β1-stimulated expression of α-1 type 1 collagen (COL1A1), MMP-2, MMP-9, TGF-β1, tissue inhibitor metallopeptidase 1 (TIMP1), and α-smooth muscle actin expression (α-SMA). In addition, EGCG was able to successfully suppress the phosphorylation of Smad2/3 and Akt (Yu et al., 2015).

Oxidative stress plays a role in the pathogenesis of pulmonary fibrosis. Supplementation of EGCG (20 mg/kg) to bleomycin-induced pulmonary fibrosis animals resulted in significantly improved body weight, enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase), and non-enzymatic antioxidants (reduced GSH). Whereas, the lung's wet weight to dry weight ratio and hyp level were lowered (Sriram et al., 2008). Another study using a rat model of irradiation-induced pulmonary fibrosis with 25 mg/kg administration of EGCG for 30 days showed that there were reductions in mortality rates, lung index scores, collagen deposition and MDA contents. SOD activity was enhanced, alveolar epithelial type II (AE2) cells were protected, protein levels of nuclear transcription factor NF-E2-related factor 2 (Nrf-2) and its associated antioxidant enzymes heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO-1) were activated (You et al., 2014). In diethyldithiocarbamate (DDC)-induced pancreatic fibrosis in rats, different doses of EGCG (50, 100 and 200 mg/kg

daily for 8 weeks) markedly suppressed collagen deposition and inhibited overexpression of TGF-β and α-SMA. On the other hand, expression of Smad3 can be inhibited and expression of Smad7 can be enhanced (Meng et al., 2007). The effect of an anti-fibrotic property of EGCG had also been investigated by pre-administrating 25 μM EGCG on the oxalate-induced epithelial mesenchymal transition of renal tubular cells. The results showed that EGCG noticeably inhibited the increasing expressions of vimentin and FN, and levels of epithelial markers (E-cadherin, occluding, cytokeratin and Zonula occludens-1) were increased. This was due to the reduced production of intracellular ROS through activation of Nrf2 signalling and increased catalase (Kanlaya et al., 2016).

In a study with 55 patients given 50mg/kg/day (45 with and 10 without endometriosis) of EGCG, treatment of fibrosis in endometriosis was found to be effective. Results showed that EGCG inhibited cell proliferation, migration and invasion of endometrial and endometriotic stromal cells. TGF-β1-stimulated activation of MAPK and Smad signalling pathways were inhibited. Both endometriotic, as well as the endometrial stromal cell-mediated contraction of collagen gels, were also attenuated (Matsuzaki and Darcha, 2014).

3. Conclusion

As discussed in this review, EGCG has been showed to exert its beneficial effects on human especially on treating CVDs including atherosclerosis, cardiac hypertrophy and MI. It also exhibit anti-diabetic, anti-inflammatory, antioxidant, and antiapoptotic activities through modulating

various signalling pathway demonstrated in Fig. 2. The major pathway that EGCG to treat CVD and DM is through the reduction of NF-kB expression and its downstream marker molecules.

This review attempts to enhance our understanding about ECGC role in modulating various cellular, molecular mechanisms and its therapeutic potential in treating cardiovascular and metabolic diseases.

4. Future directions

This review summarized the preclinical and clinical studies as solid evidences to prove that EGCG as a potential therapeutic agent to mitigate CVD diseases through different pathways. However, the inconsistent results within preclinical and clinical studies of EGCG is a key factor for future research and modification in bioavailability, potential side effects as well as dose frequency for human should be investigated carefully as they are important parameters to be addressed in order for this bioactive molecule to be applied in human for therapeutic purposes.

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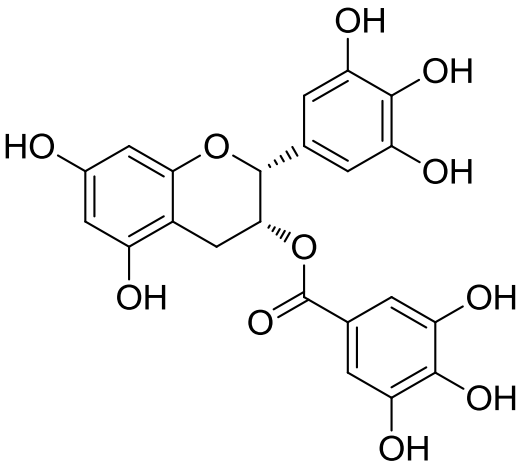
Figure 1. Summary of Epigallocatechin gallate (EGCG).



Formula: $C_{22}H1_8O_{11}$
IUPAC Name: (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate
Molecular weight: 458.375 g/mol
ALogP: 2.89
Polar surface area:197.37
Rotatable bond: 12
Hbond donors: 8
Hbond acceptors:11

Route of administration: Oral
Indication: Cardiovascular disease, metabolic diseases

Figure 2. Depiction of mechanistic profile of EGCG: Schematic diagram of the mechanisms by which EGCG exerts its protective effects. Increased oxidative stress and inflammatory markers are mainly observed in atherosclerosis and diabetes. The administration of EGCG is able to inhibit the lipogenesis pathway responsible for atherosclerosis and diabetes by reducing LDL cholesterol. Besides, cardiac hypertrophy, heart failure and myocardial infarction can be treated with the reduction in NF-κB & its downstream target molecules and ANP, which play a role in causing the diseases, respectively.

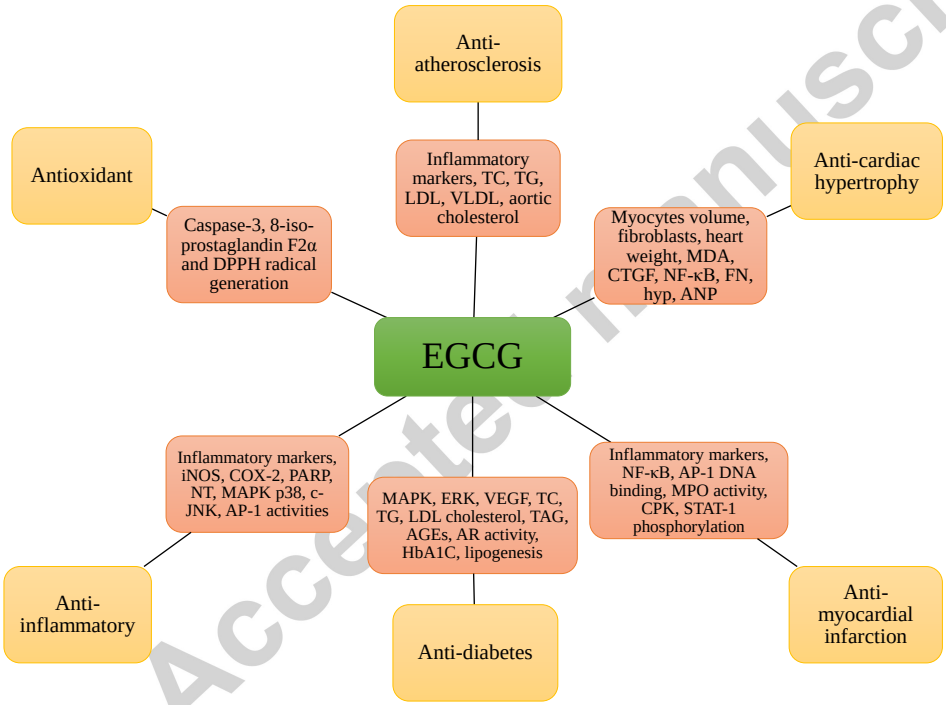


Table 1. Summary of *in vivo* studies of protective effect of Epigallocatechin gallate (EGCG) on cardiovascular and metabolic diseases

Disease model	Dose	Effect	References
Atherosclerosis (Hypercholesterolemic diet Wistar rats)	100 mg/kg	Reduction of TC, TG, LDL and VLDL cholesterol level	(Xu et al., 2014)
Atherosclerosis (apoE-deficient mice)	0.8 g/L	Reduction of aortic weights, aortic cholesterol and aortic TG	(Miura et al., 2001)
Atherosclerosis (*Porphyromonas gingivalis*-induced $apoE^{-/-}$ mice)	0.02% solution	Reduction of CRP, MCP-1, CCL2, MMP-9, ICAM-1, HSP60, CD44, LOX-1, NOX-4, p22phox and iNOS gene expression levels Increased expression of HO-1 mRNA	(Cai et al., 2013)
Atherosclerosis (Atherogenic-diet fed Wistar rats)	100 mg/kg	Reduction of CRP and other inflammatory markers (fibrinogen, sialic acid, SAA)	(Ramesh et al., 2010)
Cardiac hypertrophy (AAC-induced rats)	25, 50, 100 mg/kg	Inhibition of telomere shortening and loss of TRF_2. Reduction of MDA contents, heart weight indices, apoptosis, and ANP, plasma endothelin and hyp levels. Increment of nitrite concentrations were observed	(Sheng et al., 2013, 2009)
Cardiac hypertrophy (Ang II and AAC-induced Sprague-Dawley rats)	50 mg/kg	Inhibition of NF-κB and CTGF overexpression. Reduction of collagen synthesis, cardiac fibroblasts proliferation and FN expression	(Cai et al., 2013)
Cardiac hypertrophy (AAC-induced Sprague-Dawley rats)	0.02, 0.04 and 0.08%	Suppress load-induced increase in heart weight by 69%. Attenuation of the increase in myocyte cell size and fibrosis	(Hao et al., 2007)
Heart failure (H/M-$SOD2^{-/-}$ mice)	10, 100 mg/L	Reduction of myocardial oxidative stress and FFA	(Oyama et al., 2017)

		Inhibition of NOS2, NT, fatty acid synthase, TLR4, and Sirt1 expressions	
Heart failure (SPF class c57Bi/6 mice)	50 mg/kg/day	Improved cardiac diastolic function by upregulating cTnI through histone acetylation modification	(Pan et al., 2017)
MI (Ang II-induced thoracic aorta of adult Wistar rats)	50 mg/kg	Attenuation of endoglin expression from binding to AP-1 transcription in the cardiac fibroblasts	(Lin et al., 2016)
MI (Male Wistar rats)	10 mg/kg	Reduction of plasma IL-6, MPO activity, CPK levels, NF-κB and AP-1 DNA binding	(Aneja et al., 2004)
MI (ISO-induced male Wistar rats)	10,20,30 mg/kg	Reduction of heart weight, activities of membrane bound ATPases and cardiac marker enzymes, LDH concentrations	(Devika and Prince, 2008)
MI (ISO-induced albino Wistar rats)	15 mg/kg (pre-treatment before ISO)	Inhibition of CK-MB, LDH, ALP, ALT, cTnT and TNF-α changes in the serum and also upregulate SOD and CAT activity Reduced apoptotic markers and protect genomic integrity by inhibiting DNA fragmentation	(Othman et al., 2017)
Diabetes (HFD-induced C57BL/6J mice)	25 and 75 mg/kg	Reduction of blood sugar level, accumulation of AGEs and AR activity	(Sampath et al., 2016)
Diabetes (STZ-induced rats)	25 and 50 mg/kg	Reduction of TC, TG, LDL cholesterol, glucose levels. No significant changes in plasma HDL cholesterol	(Li et al., 2007)
Diabetes (db/db mice and ZDF rats)	2.5, 5, 10 g/kg of diet	Reduction of plasma TAG levels, mRNA expressions of PEPCK in adipose tissue. Increase in insulin secretion and mRNA expression level of GK	(Wolfram et al., 2005)
Type 2 diabetes	2 mg/kg	Significantly reduced	Othman et al.,

(Nicotinamide - 100 mg/kg and STZ-55 mg/kg)		plasma glucose, HbA1c, HOMA-1R and lipid profile Increased plasma insulin level Increased antioxidant enzymes (SOD, CAT and GSH) and decreased apoptotic markers BAX, Cas 3, 9. Improved myocardial function by reducing inflammatory markers	2017)
Diabetes (non-obese rats)	0.05% solution	Delayed onset of T1DM. Enhanced plasma insulin and IL-10 levels. Reduction of HbA1C levels.	(Fu et al., 2011)
Diabetes (T1DM Wistar rats)	2 g/L	Reduction in smooth muscle content in the CC of diabetic rats. No significant changes in VEGF expressions	(Lombo et al., 2016)
Diabetes (MLD-STZ induced RINm5F cells)	100 mg/day/kg for 10 days	Inhibit onset of T1DM. Reduction of plasma glucose concentrations. Protection of pancreatic islets. Inhibit activation of NF-κB	(Song et al., 2003)
Obesity (HFD-induced obese New Zealand black mice)	500 mg/kg	Decreased expression of leptin, SCD1, ME, GK	(Klaus et al., 2005)
Obesity (HFD-induced obese mice)	0.5%, 1.0% w/w	Reduction of post-prandial TG, liver glycogen, incorporation of dietary lipids into fat tissues, liver and skeletal muscles. Increase in dietary lipid oxidation	(Friedrich et al., 2012)
Obesity (HFD-induced obese male C57BL/6J mice)	0.2%, 0.5% w/w for 8 weeks	Decreased mRNA levels of PPAR-γ, C/EBP-α, SREBP-1c, aP2, LPL and FAS. Increased mRNA levels of CPT-1, UCP2, HSL, and ATGL	(Lee et al., 2009)
Inflammation (UV-B induced	3 mg/2.5	Blocked ROS generation and	(Katiyar et al.,

infiltration of leukocytes)	cm^2	MPO activity.	1999)
Inflammation (Spinal cord injured rats)	50 mg/kg	Decreased expressions of TNF-α, IL-1β, iNOS, COX-2, PARP and NT	(Khalatbary and Ahmadvand, 2011)
Inflammation (HF/HS diet fed-C57/BL6 male mice)	0.9 mg/kg	Suppressed TLR4 expression through E3 ubiquitin-protein ring finger protein 216 (RNF216) upregulation	(Kumazoe et al.,2017)
Inflammation (arsenic-induced male BALB/c mice)	10 mg/kg/day for 30 days	Decreased oxidative stress, inflammatory cytokines and apoptosis	(Yu et al., 2017)
Oxidative stress (Sodium iodate-induced retinal degeneration in Sprague-Dawley rats)	550 mg/kg of GTE Theaphenon E	Reduction of outer nuclear layer thickness and expression of caspase-3. Increased expression of SOD and GPX Inhibition of 8-iso-Prostanglandin F2α generation	(Yang et al., 2016)
Oxidative stress (Anaethsia male ddY mice)	100 mg/kg	Enhanced Fe^{2+} chelating and superoxide anion-radical scavenging activities. Demonstrated better pharmacokinetic profile compared to free EGCG	(Yoshino et al., 1999)
Cardiotoxicity (DOX-induced in sarcoma 180 tumor-bearing mice	25 mg/kg	Increased mitochondrial membrane potential and MnSOD expression Inhibit LDH release and apoptosis of cardiomyocytes Attenuate myocardial Ca^{2+} overload and ROS generation	(Yao et al., 2017)

Table 2. Summary of *in vitro* studies of protective effect of Epigallocatechin gallate (EGCG) on cardiovascular and metabolic diseases

Disease model	Dose	Effect	References
Atherosclerosis (HUVECs with ox-LDL)	50 µM	Protection against ox-LDL-induced endothelial dysfunction	(Yin et al., 2015)
Atherosclerosis (THP-1 derived macrophages)	10 µM	Inhibit atherosclerotic lesions by reducing macrophage cholesteryl ester content and MCP-1 mRNA	(Zheng et al., 2013)
Cardiac hypertrophy (PE-induced H9C2 cardiomyocytes)	100 µM	Activation of AMPK by reducing Nppa, BNP mRNA	(Cai et al., 2015)
Cardiac hypertrophy (Ang II-induced neonatal rat heart myocyte)	10,50,100 µg/ml	No significant changes in number of myocytes. Dose dependent reduction of the protein contents, myocytes volume and number of fibrocasts	(Cui et al., 2008)
Heart failure (Ventricular muscle strips from Mybpc-3 targeted knock in and WT mice)	1.8 µM 30 µM	Diastolic sarcomere length and fractional sarcomere shortening were not affected in both mice Reduced relaxation time in knock in mice Decreased Ca^{2+} sensitivity in both mice due to Ca^{2+} desensitization of myofilaments	(Friedrich et al., 2016)
Cardiac arrhymogenic activity (ISO-induced mice cardiomyocytes)	0.01, 0.1, 1, 10 µM	Regulated electrophysical characteristics of left atrium Dose dependent reduction of action potential duration Suppressed ISO-induced atrial arrhymogenesis through inhibition of Ca^{2+}/calmodulin or cGMP-dependent protein kinase	(Chang et al., 2017)
MI (Langerdorff perfused rat heart)	10 µM	Reduction of infarct volume through acting on ADR and OPR	(Lee et al., 2012)
MI (Isolated perfused rat heart)	1 and 10 µM	Reduction of STAT-1 phosphorylation and infarct size. Attenuation of myocyte apoptosis. Improved LV developed pressure.	(Kim et al., 2010; Townsend et al., 2004)
MI (Isolated perfused rat heart)	1 µM	Activated mitochondrial K(ATP) channels	(Song et al., 2010)

		Reduction of infarct size	
MI (H/R injury)	10 µM EGCG combined with 5 µM Zn^{2+}	Protected H9c2 cells through activating PI3K/Akt pathway. Down-regulated TNF-α, IL-6 and IL-8 levels. Up-regulated p-p85 and p-Akt levels.	(Zeng and Tan 2015)
Diabetes (high glucose treated HepG2 cells)	1,5,10,20 µM	Decreased Ser307 phosphorylation of IRS-1	(Lin and Lin 2008)
Diabetes (HRC cells)	20 and 40 mM	Decreased the expressions of MAPK, ERK and VEGF	(Zhang et al., 2016)
Diabetes (HepG2 cells)	10 µM	Increased glycogen synthesis, phosphorylation of Ser9 GSK 3β and Ser641 glycogen synthase and inhibit lipogenesis	(Kim et al., 2013)
Inflammation (PCB-induced in human endothelial cells)	15, 30 µM	Prevented the increase of IL-6, C-reactive protein, ICAM-1, VCAM-1 and IL-1 α/β	(Liu et al., 2016)
Inflammation (Human corneal epithelial cells)	3-30 µM	Inhibition of cytokines, MAPK p38, c-JNK, AP-1 activities dose-dependently	(Cavet et al. 2011)
Inflammation (Human monocyte THP-1 cell line)	50 µg/ml	Shutting of Notch signaling pathway, downregulated transcription of Notch target gene Inhibited lipopolysaccharide-induced inflammation	(Wang et al. 2017)
Oxidative stress (H_2O_2-induced DNA damage in Jurkat T-lymphocytes)	10 µM	Scavenged DPPH radical	(Johnson and Loo 2000)
Oxidative stress (Phospholipid liposome model)	0.1 and 1.0 µg/ml	Suppressed initiation rate and prolonged the lag phase of peroxyl radical-induced oxidation. Protection on peroxyl radical and hydroxyl radical-induced supercoiled DNA nicking	(Hu and Kitts 2001)

Table 3. Efficacy of Epigallocatechin gallate (EGCG) on cardiovascular and metabolic diseases in clinical trials

Disease	Study population	Dose	Effect	References
Atherosclerosis (double-blind, randomized trial)	82 patients with early atherosclerosis	30 ml supplemented with olive oil	Improved endothelial function by reducing the number of leukocytes.	(Widmer et al., 2012)
Cardiac hypertrophy (double-blind, placebo-controlled, crossover design)	42 subjects	300 mg for initial dose and 150 mg twice daily for two weeks	Improved endothelial function by increasing the brachial artery flow-mediated dilation.	(Widlansky et al., 2007)
Cardiac hypertrophy	25 male patients with wild-type transthyretin amyloid cardiomyopathy	600 mg for 12 months	Decreased LV myocardial mass and TC. LV wall thickness and mitral annular plane systolic excursion remained unchanged.	(aus dem Siepen et al., 2015)
T2DM (prospective cross-sectional study)	38,018 women aged > or = 45 y and free of cardiovascular disease, cancer and diabetes	4 cups of green tea per day	Decreased the risk of T2DM by 30% in women aged 45 years and older	(Song et al., 2005)
T2DM (randomized, double-blind, placebo-controlled clinical trial)	68 subjects, aged 20-65 years with T2DM for more than one year	856 mg for daily dose	Reductions of HOMA-IR index, HbA1C, and fasting insulin levels	(Hsu et al., 2011)
Obesity (pilot study)	6 overweight men	300 mg for 2 days	Reductions of RQ value	(Boschmann and Thielecke, 2007)
Obesity	38 overweight or obese postmenopausal women exercised at moderate	150 mg for 12 weeks	Reductions of HR and plasma glucose levels.	(Hill et al., 2007)

	intensity			
Obesity (double-blind, randomized and parallel design study)	100 overweight or obese male subjects, aged 40–65 years	400 mg twice daily for 8 weeks	Reduction of diastolic blood pressure. More positive mood than controlled group.	(Brown et al., 2009)
Obesity (randomized, double-blind, placebo-controlled study)	83 obese pre-menopausal Caucasian women	200 mg for 12 weeks	No significant changes in body weight, fat metabolism, total or LDL cholesterol levels.	(Mielgo-Ayuso et al., 2014)
Obesity (randomized, double-blind, placebo-controlled, parallel study)	27 healthy physically active males	1200 mg accompanied by 240 mg caffeine per day for 7 days	Increased citric acid cycle activity, lipolysis and fat oxidation.	(Hodgson et al., 2013)
Inflammation (randomized, split-face trial)	35 (17 men and 18 women, mean age: 22.1)	1% or 5% topical solution for twice a day	Significantly reduced inflammation by inhibiting NF-Kb and AP-1 pathways. Induced cytotoxity of human sebocytes via apoptosis and decreased the viability of *P. acnes*	(Yoon et al., 2013)

Graphical abstract