Chapter

Black Tea: Chemical and Pharmacological Appraisal

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Abstract

Medicinal plants are gaining popularity as folk medicine due to future demand to get rid of synthetic health promoting medicines. Nowadays, black tea is gaining interest as the most frequently consumed therapeutic drink after the water. The importance of black tea is due to existence of flavonoids such as (Thearubigins (TRs) and theaflavins (TFs) and catechins) that are the main therapeutic agents and are more bio-direct and stable compounds compared to those exist in other herbal plants alongside some other promising compounds which enhance is credentials as therapeutic drug. Numerous scientific explorations have elucidated the biological worth of these bioactive moieties against plethora of ailments with special reference to metabolic disorder. The mandate of current chapter is to discuss the black tea chemistry for elucidating its pharmacological worth.

Keywords: black tea, catechins, theaflavins, oxidative stress, metabolic syndromes

1. Chemical illustration of black tea

Black tea is manufactured by the fermentation of green tea involving two steps; oxidation and polymerization [1, 2]. The oxidation involves enzymatic catalysis of polyphenol while the polymerization, involves a nucleophilic addition and further oxidized by oxygen or hydrogen peroxide, Black tea polyphenols consist of 2–6% theaflavins, 12–18% thearubigins, 5–10% catechins, 6–9%flavonols, 10–12% phenolic acids, 12–14% proteins, 8–12% methylxanthines, 15–20% fiber, 2–5% alkaloids [3].

1.1 Theaflavins

Theaflavin is an important antioxidant and metal chelating polyphenol present in black tea due to the presence of hydroxy groups and gallic acid moiety [3]. The structures of theaflavins possess benzotropolone nucleus that are formed through the co-oxidation of selected catechin pairs, one with a vic-trihydroxyphenyl structure, and the other with an ortho-dihydroxyphenyl moiety [4, 5]. Theaflavins including theaflavin, theaflavin-3-gallate, theaflavin-3-gallate, and theaflavin-3,3-digallate,

consist of benzotropolone rings with dihydroxy or trihydroxy substitution groups [6]. Theoretically, there are eight or more theaflavins that can be produced proportionally.

1.2 Thearubigins

Black tea leaf catechin are oxidized to ortho-quinones that further react with water, a nucleophile resulting in the formation of oxygenated catechins that will further reacts with other catechins. In order to replace all the aromatic hydrogen by oxygen to create 90 different compounds of catechin diamers, the oxygenation of catechin is repeated several times. Further oxidation of highly oxygenated black tea polyphenol to form quinine and quinine-methide type derivates is done that in equilibrium with its counterpart are present within the black tea. To study the formation of chromatographically resolvable and unresolvable thearubigin like substance many experiments use an in vitro enzymatic model fermentation system that involves reverse phase HPLC [7–13]. Formation of TR's from catechin. Chromatographically resolved TR's are produced by polyphenoloxidase while peroxidase produce unresolved TR's [10]. Oxidation and reaction of two gallocatechins i.e. epigallocatechins and epigallocatechingallate are shown to produce TR's [14]. Recent studies have also demonstrated the production of TR's through oxidation of TF's in the presence of H_2O_2 with the help of tea peroxidase [13, 15].

1.3 Flavonols

Flavonoids are polyphenolic compounds that are biologically active [16]. Flavonoids are widely distributed in plants and are common component of our diet [17]. Flavonoids are divided into 6 subclasses; one of which is flavonols. Flavonols such as quercetin, kaempferol, myricitin, and their glycosides are found in black tea, and these possess a 4-oxo 3-hydroxy C ring. 2–3% of the water-soluble extract solids of tea consist of flavonol glycosides. Due to poor water solubility, the flavonol aglycones are not found in significant amounts in tea. Chemically, flavonols are aglycons with 3-hydroxyflavone backbone [3].

Kaempferol is a yellow colored flavonol with a low molecular weight and high boiling point [3, 27]. These are used in herbal medicines and are commonly found plant foods like broccoli, brussel sprout, grapefruit etc. [3, 27]. Kaempferol consist of a diphenylpropane structure [27] Human dietary intake of kaempferol is estimated to be 10 mg/day [18–20].

Myricetin (3,5,7,3,4,5 -hexahydroxyflavone, cannabiscetin) is a natural flavonol from fruits, vegetables, tea, berries, red wine and medical plants [21]. Myricetin consumption is higher than any other flavonol and its dietary intake ranges 0.98–1.1 mg per day [22]. It has a unique chemical structure. The antioxidant property of myricetin is enhanced by the presence of hydroxyl group at 3, 5 position and continuous hydroxyl groups at position 3, 4 and 5 but it's negative factor of hydrophobicity decreases due to attachment of 6 hydroxyl group [23, 24]. Myricetin tend to show antioxidative and cytoprotective effects. It also has anti-carcinogenic actions and anti-viral properties but recent studies have demonstrated its use as a hypoglycemic component of plant sources [25–30].

2. Health endorsing benefits of black tea with special emphasis on mechanism targets

2.1 Cancer

One of the leading causes of human mortality worldwide is cancer. Black tea has been proven to have anti-carcinogenic effect and is helpful in protecting against

cancer. It has been observed that women who tend to consume black tea daily have lower concentration of 17β-estradiol (E2) thus reducing the risk of hormone related disease [31] Regular black tea consumption is also very effective in reducing the risk of ovarian and bladder cancer in women [32–34]. Theaflavin in black tea is found to suppress Akt signaling, inhibiting Wnt/β-catenin signaling, cyclin D1 level and enhancing the FOXO1 and p27 level in human leukemic U937 and K562 cells thus helping to seize the cell in G0/G1 phase [35]. Breast cancer cells contain tumor suppressor gene p53 that is inactivated and therefore cause drug resistance in them. TF1 is found to cause apoptosis by inducing Fas death receptor/caspase-8 pathway and suppressing the pAkt/pBad survival pathway in these cells [36]. TF1 is also effective in suppressing the gene and protein expression of metalloproteinases (MMP)-2 in human melanoma cell line by reducing the epidermal growth factor receptor (EGFR) and inhibiting the NF-κB signaling pathways [37]. TF2 found in black tea are capable of activating apoptosis signaling that is imparted by the mitochondria in human colon cancer cell [38]. If combined with ascorbic acid, TF3 is actively involved in seizing human lung adenocarcinoma cells in G0/G1 phase [39].

Cancer initiation stage is greatly influenced by oncogene mutation and ROS. Oncogene mutation causes the activation of phase I enzymes such as cytochrome P450 that leads to the activation of procarcinogen while ROS is involved in the metabolic activation of procarcinogen. EGCG prevents the activation of cytochrome P450 thus suppressing the cancer initiation stage. Several studies indicate the importance of EGCG in protecting cell against the cancer activity. The galloyl or hydroxyl groups in the EGCG molecule is responsible for inhibitory effect on the microsomal enzyme system [40]. The strong ROS-scavenging effect of ECGC proves that it is a powerful antioxidants which further validated by its ability to bind with transition metal ion. This is due to presence of pyrogallol structure in its molecule that has a strong metal chelating effect [41–43]. In another study it was observed that EGCG inhibited the activity of NF-κB /p65 component of the NF-κB complex during apoptosis [44]. Irregular expression of CO-X and iNOS is one of the reasons of carcinogenesis in the cells. It is observed that EGCG by manipulating the activity of NF-kB suppressed the activation of CO-X and iNOS [45]. Hence it is concluded that EGCG exhibits its anti-cancer properties by aiming inhibition of NF-κB and its components. AP-1 is an important contributing factor in carcinogenesis especially during the tumor promotion stage. Don et al. observed the EGCG can suppress transcription activity mediated by AP-1 by inhibiting the JNK dependent pathway [46]. Cell cycle modulation is also an important factor in inducing carcinogenesis.

2.2 Hyperglycemia

Diabetes is a chronic disease that occurs due to insulin absence or insufficient insulin production by pancreatic beta-cells (Type 1 diabetes) or alternatively, the inability of the body to effectively use insulin (Type 2 diabetes). Tea is one of the world's most widely consumed beverage. Black tea polyphenols (catechins) have strong antioxidant activities. In a recent study, the protective effect of tea catechins (Epigallocatechingallate(EGCG), Epigallocatechin (EGC), Epicatechingallate (ECG) and Epicatechin (EC)) on the indicators of oxidative stress (malondialdehyde, reduced glutathione and membrane -SH group) in Type 2 diabetic erythrocytes was evaluated. Normal and Type 2 diabetic erythrocytes were incubated with tert-butyl hydroperoxide (t-BHP). Diabetic erythrocytes have higher MDA, reduced GSH and membrane -SH group as compared to the normal erythrocytes. It was seen that tea catechins protected against t-BHP induced oxidative stress [47].

Glucose transporters (GLUTs) are important in controlling blood glucose concentrations. Black tea polyphenols improve translocation of GLUTs in skeletal

muscles where GLUTs uptake glucose and reduce postprandial hyperglycemia [48]. In the skeletal muscles, glucose uptake and glycogen synthesis is promoted by insulin. But, glucose uptake and glycogen synthesis abnormalities occurs in diabetes leading to hyperglycemia and other complications [48]. Black tea polyphenols translocate GLUTs in L6 myotubes through phosphatidylinositol 3-kinase (P13k) and AMPK dependent pathways. Theaflavins promote glucose uptake (**Figure 1**).

In a recent randomized, double-blind, placebo-controlled crossover study, the effect of black tea consumption on postprandial glucose elevations and insulin response was tested. A sample population consisting of 24 subjects (male and female aged 20–60 years, normal and pre-diabetic) consumed low dose sucrose diet (110 mg black tea polymerized polyphenols) and high dose black tea (220 mg BTPP) or a placebo drink (0 mg BTPP). Blood samples were taken at intervals of 0, 30, 60, 90 and 120 min. Results indicated a significant decrease in postprandial blood glucose elevations in low dose and high dose of BTPP after sucrose consumption as compared to placebo and prediabetic individuals [49]. Leptin has a role in the regulation of body weight. Increased level of leptin resulted in the insulin resistance and led to hyperinsulinemia [50]. Adiponectin regulate plasma glucose concentrations and also involved in fatty acid breakdown. Black tea polyphenols decreased leptin level by 75% and adiponectin level by 50% [51].

Glucose uptake in the intestinal epithelial cells is performed by SGLT1 and GLUT 2 and 5. Black tea components Epicatechin gallate (ECg) and Epigallocatechin gallate (EGCg) inhibits the activity of SGLT1 and GLUT 2 and 5 and contributed to blood glucose homeostasis. In an in vitro study, a 15-fold increase in insulin activity was examined by black tea polyphenols (theaflavins, thearubigins, EGCG and catechins). The possible mechanism for enhanced insulin secretion is stimulation of enteroinsular axis (EIA) in pancreas and increased activity of GIP and GLP-1 factors. Tea polyphenols also enhance glucose uptake by myocytes and glucose binding to adipocytes increasing the activity of glucose transporter in the myocytes [52].

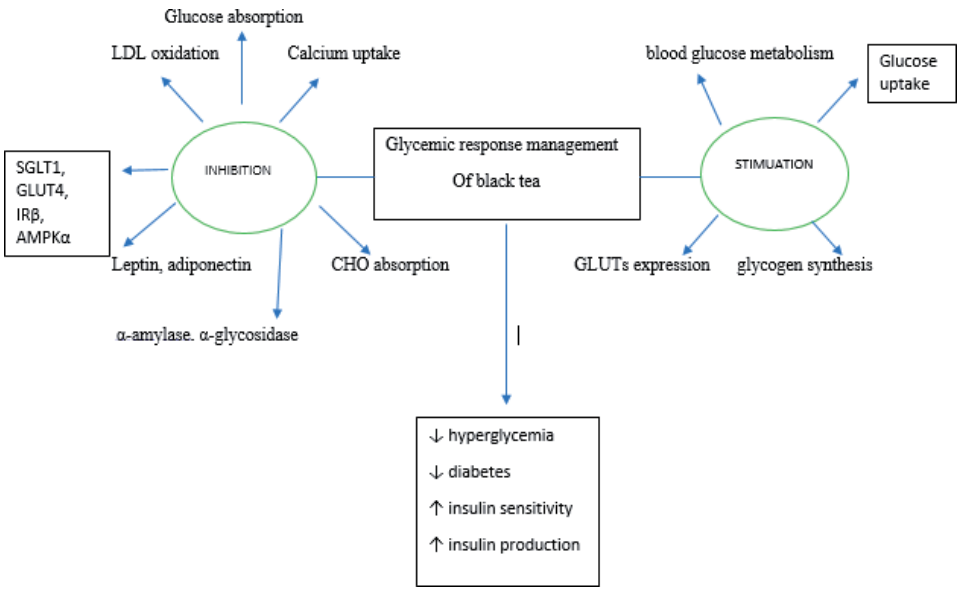

Figure 1.
Mechanistic routes associated with tea as glycemic management drug.

2.3 Hyperlipidemia and hypercholesteremia related complication

Cholesterol is one of the necessary compounds that is carried in the blood around the body. Black tea is a functional beverage that can tackle oxidative stress related disorders like hypercholesterolemia, obesity, diabetes and cancer owing to the presence of polyphenols in it. Bioactive components of black tea, especially theaflavins, thearubigins, theasinensin and catechins provide protection against plenty of oxidative stress related diseases. Theaflavins and thearubigins have singlet oxygen quenching ability and protect against oxidative stress [53]. LDL oxidation can lead to atherosclerosis and other related complications. Black tea polyphenols prevent LDL oxidation due to their antioxidant activity, free radical scavenging activity and chelating properties [53, 54]. Black tea flavonoids may act as chain-breaking antioxidants, resulting in the scavenging of some radical species [55]. Some flavonoids can chelate divalent metal-ions such as transition metals copper and iron, preventing free radical formation [55]. These polyphenols also stimulate antioxidant enzymes like glutathione-S-transferase and catalase that helps to maintain antioxidant levels in the body [56]. In the liver, tea polyphenols increase fat oxidation by increasing adipocyte differentiation and fatty acid uptake in the adipose tissues [57]. In a recent study, 5% black tea polyphenol extract (BTPE) consumption lead to an increase in fecal triglyceride level in mice given high-fat diet. This indicated the inhibitory effect of BTPE on lipid absorption [58].

The cholesterol absorption in the body occurs in different steps including formation of emulsions, hydrolysis of ester bond, micellar solubilization, esterification and chylomicron mediated transport into the lumen [53, 59]. TSA and TRs also have cholesterol metabolism effects. TSA reduces hepatic cholesterol concentration. This is due the increased fecal steroid excretion, which diminishes the amount of cholesterol returned to liver via entero-hepatic circulatory system. On the other hand, it has been found that TSA inhibits squalene epioxidase, a rate-limiting enzyme of cholesterol synthesis in rats (Abe et al., 2000). It inhibits biogenesis of cholesterol in liver [60]. TRs also plays role in hepatic cholesterol reduction. They increase fecal bile acid excretion that results in the decrease in micellar solubilization of cholesterol. This leads to decreases cholesterol absorption and hence decreased cholesterol levels in the liver [60].

Fatty acid synthase (FAS) is a key enzyme that catalyzes palmitate synthesis from acetyl CoA, malonyl-CoA and NADPH into long-chain saturated fatty acids. In humans it is encoded by FASN gene. FAS action may be suppressed by the downregulation of EGF-receptor/P13K/Akt/sp-1 signal transduction pathway. This leads to the inhibition of cellular lipogenesis and tissue growth. Epidermal growth factor receptor(EGFR) is a transmembrane protein that is a member for Epidermal growth factor family (EGF) of extracellular protein ligands. Black tea polyphenols EGCG and TF-3 inhibit EGF binding to EGFR, inhibiting the activation of P13K/Akt signal pathway and blocks the binding of sp-1 to its target site resulting in the downregulation of FAS gene [61, 62].

Adiponectin is protein hormone and it is present in the circulation. It is encoded by ADIPOQ gene and produced by adipose tissues. Its function is to regulate glucose levels as well as fatty acid breakdown. Plasma adiponectin level reduces in type 2 diabetes and coronary artery diseases [55]. Studies have shown that LDL particle size is significantly reduced in CAD patients. LDL particle size less than 25.5 nm is known as small dense LDL [63]. Increased levels of small dense LDL lead to increased oxidation, decreased bonding to LDL receptors and increased binding to arterial walls. Tea polyphenols influences LDL, HDL, TC, TGs and glucose levels in CAD patients. Tea polyphenols are found to increase adiponectin levels in plasma

and also LDL particle size by increasing fat metabolism, oxidation and energy consumption [63]. They increase coronary flow in CAD patients.

Black tea polyphenols also play role in the suppression of hepatic cholesterol synthesis [64]. Cholesterol synthesis starts with acetyl-CoA, which synthesizes Hydroxymethylglutaryl-CoA (HMG-CoA). HMG-CoA reductase, the third enzyme in the pathway, reduces HMG-CoA to mevalonate. AMP-kinase is involved in the phosphorylation- mediated HMG-CoA reductase inactivation. A recent study showed that black tea extracts act both directly and indirectly to decrease HMG-CoA reductase activity and increases AMP-kinase levels in the cells, resulting in the suppression of cholesterol synthesis. 100 μg/ml of black tea extract resulted in 78% decrease in cholesterol synthesis [64, 65]. AMPK is the key enzyme involved in cellular energy homeostasis. AMPK phosphorylates acetyl-CoA carboxylase 1 (ACC1) or sterol regulatory element-binding protein 1c (SREBP 1c), it inhibits synthesis of fatty acids, cholesterol, triglycerides and activates fatty acid uptake and β-oxidation. Theaflavins inhibits ACC1 by stimulating AMPK through LKB1 and reactive oxygen species pathways [66].

2.4 Obesity

Obesity is a medical condition associated with the accumulation of excess of triglycerides in the body that has adverse health effects. WHO defines obesity as body mass index (BMI) equal to or greater than 30 kg/m^2. Theaflavins and EGCG are FAS inhibitors and they reduce food intake and body weight and triglyceride blood levels [67]. Theaflavin suppresses visceral fat accumulation and inhibits high-fat diet induced body weight gain in mouse model [68]. The results of tissue dissection indicated that perirenal fat, peri gluteal fat, total fat mass, mesenteric fat, epididymal fat and periscapular fat were significantly reduced by theaflavins [68]. Black tea polyphenols and polysaccharides promotes fat breakdown and prevents obesity [69].

A recent research showed the effect of tea catechins on high fat diet-induced obesity in model mice (C57BL/6) [70]. Daily intake of black tea (500–600 mg) reduced body weight and body fat (especially abdominal fat) in overweight or obese subjects. The underlying mechanism for anti-obesity effects of tea catechins suggested was that the tea catechins activate β-oxidation of fatty acid in the liver [70, 71]. PPARα is expressed in the liver and muscles and it is a regulator of lipid metabolism. PPARγ is present in muscles and adipocytes and it stimulates lipid uptake and lipogenesis by adipose tissues. PPARα transcriptionally regulates lipid metabolizing enzymes, including Acetyl-CoA and Acyl-CoA dehydrogenase. Tea catechins (EGCG) inhibits the activation of nuclear transcription factor Kb (NF-kB), thus preventing NF-kB from inhibiting PPARα to regulate lipid metabolizing enzymes and increasing fatty acid oxidation. EGCG also inhibits the adipogenic transcription factor PPARγ that leads to weight reduction [70, 72–75].

Excess lipids stored in adipocytes results in obesity due to an increase in size and number of adipocytes. Theaflavins have been reported to inhibit the differentiation and proliferation of preadipocytes by down-regulating the gene expression of adipose differentiation-related protein (ADRP). Theaflavins exhibit an inhibitory effect in the differentiation of mesenchymal stem cell into adipocytes [76] **Figure 2**.

Theaflavins suppresses the intestinal cholesterol absorption and micelle formation by inhibiting the incorporation of cholesterol in mixed micelles (Vemeer, Mulder and Molhuizen., 2008). Black tea extract causes malabsorption of ingested carbohydrates by 25% thus, reducing the caloric availability [77]. Alpha-amylase gene expression in liver and serum were found higher in obese patients [78]. Black tea theaflavins are inhibitors of α-amylase and are beneficial in weight control [77, 79].

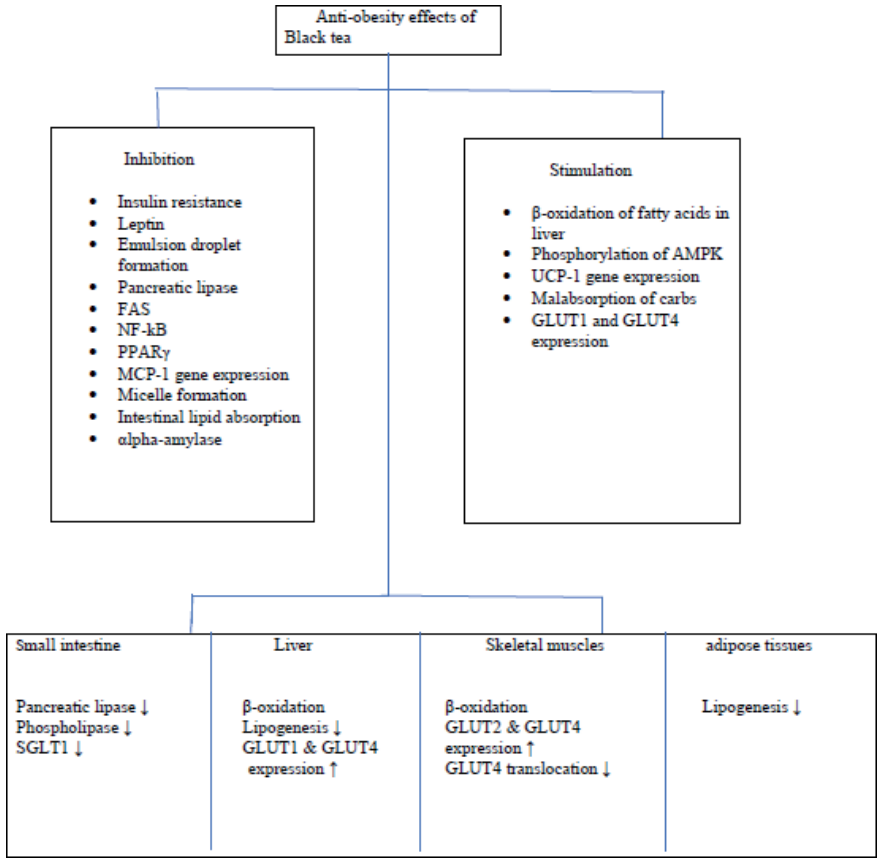

Figure 2.
Mechanistic route associated with tea as weight management drug.

2.5 Oxidative stress

Loss of balance between the production of reactive oxygen species (free radicals) and antioxidant defense, resulting in tissue injury is known as Oxidative stress [80]. Free radicals are produced as result of splitting of water forming hydroxyl ion which may occur as response to electromagnetic radiations. The presence of unpaired electrons is the reason of instability of free radical consequently making them more reactive. Radical molecules react with non- radical molecule resulting in free radical chain reaction. Lipid peroxidation is an important demonstration of such reactions.

The antioxidative properties of polyphenols in black tea are exhibited by their abilities to inhibit free radical generation, scavenge free radicals and chelate transition metal ions, mainly Fe and Cu. Inhibition of enzymes involved in free radical generation is the key mechanism in preventing their production.

Inflammatory process is a response induced by any injurious stimulant in the form of various toxic agents such as infections, antibodies or physical injury. Inflammation is a normal protective response to tissue injury. Inhibition of NF-B is the key mechanism in promoting anti-inflammatory effect in black tea [81]. NF-B has an important role in inflammation as it is a transcription factor that can induce transcription of pro inflammatory genes. Delayed onset muscle soreness (DOMS) is common in athlete after they are indulged in high intensity exercise. The muscle damage triggers inflammatory and oxidative responses that may worsen muscle

injury and extend the time to regeneration [82]. In a human trial study involving healthy athlete that were indulged in high intensity anaerobic exercise and made to consume black tea. It was found that TF2-enriched black tea extract significantly reduced DOMS, increased glutathione (GSH)/oxidized glutathione (GSSG) ratio and performance [82]. Hence this study provides evidence about the anti-inflammatory action of black tea polyphenols that is achieved by the activation of Nrf2, which attenuates the NF-κB mediated inflammatory response [83].

2.6 Cardiovascular diseases

CVD's are the leading cause of death worldwide, that include coronary heart disease, stroke, Rheumatic heart disease and cardiomyopathy [84]. It is observed that inflammation is associated with progression or development of CHD. Chronic inflammation is an important factor in atherogenesis: inflammation of the vessel wall, activation of the vascular endothelium, increased adhesion of mononuclear cells to the injured endothelial layer, and their subsequent extravasation into the vessel wall, are initial events in this process. Therefore any reduction in markers that mediate the inflammatory process was assessed as suggestive finding that flavanoid reduces inflammation. Catechins are responsible for the inhibition of neutrophil adhesion and migration through endothelial layer. ECGC act on the neutrophils and are involved in the suppression of chemokines production at the site of inflammation [85]. The influence of EGCG on adhesion molecule expression has been studied. High sensitive C-reactive protein that is a major contributor in development of CVD is an inflammatory marker [86]. High relative risk is defined as >3.0 mg/L, average relative risk as 1.0–3.0 mg/L.

Smooth muscle cell proliferate and migrate leading to development and progression of atherosclerosis and may even cause restenosis after interventional vascular procedures [87, 88]. Tea catechin play an important role in suppressing SMC proliferation and migration [89–91]. Hence it can be deduced from the above studies that black tea consumption tends to exhibit anti-inflammatory effects reducing the development of cardiovascular disease.

It is proven that events leading to ischemic cardiovascular condition involve platelet aggregation and therefore anti-platelet agents are used to reduce the risk of cardiovascular diseases. Epidemiological studies show that tea is beneficial in reducing the platelet activation [92]. Duffy and colleagues also observed that consumption of black tea had no effect on ex vivo platelet aggregation in patients with coronary artery disease [93]. From the above observations it can be concluded that studies in in-vitro platelet activation show positive anti-platelet effect whereas no effect in seen in ex-vitro platelet aggregation. Platelet inhibitory effects support the observed relations between tea consumption and reduced cardiovascular risk.

3. Conclusion

The promising ethno medicinal health benefits of black tea and proven cherished herbal therapy contribute toward beneficial effects regarding health related maladies. It could be advisable to encourage its regular consumption exhibits maximum magnitude of health effects, however, still mechanism behind several of these properties in different species are not entirely known. This opens future doors for the scientist to more debate on these demanding areas to find and document responsible biomarkers and molecular markers which are responsible for a vast array of black tea benefits.

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