Black Tea

Antiviral Activity & Boosting Immunity

Dr. A.K. Barooah

Tea Research Association
Tocklai Tea Research Institute, Jorhat - 785008, Assam
www.tocklai.net

Dr. A.K. Barooah
Director, Tea Research Association,
Tocklai Tea Research Institute, Jorhat-785008, Assam
Email: director@tocklai.net; website: www.tocklai.net

Black Tea: Antiviral Activity & Boosting Immunity

Contents

SUMMARY

Tea, prepared from the leaves of *Camellia sinensis*, is the most popular beverage and has a number of bioactives with proven health benefits. Tea is rich in polyphenols which are well documented to have antioxidant, free radical scavenging, hypotensive, hypolipidemic, anti-radiation and anti-cancer properties, as well as other biological activities and pharmacological effects. About 75% of the total polyphenols predominantly exists as catechines and their gallates in green tea which are oxidized and polymerized to theaflavin and its gallates during processing in black tea. Both catechins (in green tea) and theaflavins (in black tea) are strong antioxidants and their effectiveness in prevention of several types of cancers, cardiovascular diseases, hypertension, diabetes, obesity and alleviation of metabolic syndromes, antiviral activity and modulation of immunity is well documented. Apart from polyphenols other tea bioactives like caffeine, theanine are also associated with health promoting activities.

About 78% of the tea consumed globally is black tea and India is the largest producer. Despite being the world's most popular beverage, the health benefits associated with tea are quite often ignored. While several studies have established the beneficial properties of green tea, the efficacy of the bioactive compounds present in black tea has been the subject of several recent studies. Extracts of black tea have been found effective in combating inflammatory diseases, cancers and neurological disorders, while being efficient in cardiovascular disease prevention. Black tea consumption has also been associated with weight loss and reduction in obesity. L-theanine, the most predominant amino acid present in tea boosts immunity by fighting infection by bacteria, viruses and fungi.

The theaflavins present in black tea have been reported to inhibit HIV, hepatitis C and influenza viruses. A recent study in China suggested that theaflavins, present in abundance in the black teas of Assam, could be used as a lead compound to inhibit the SARS-CoV2 virus. Further, in another study in Taiwan, black tea extracts were demonstrated to have inhibitory activities against 3C-like protease (3CLPro) enzyme of another SARS virus.

Thus, in view of the documented evidence on immunity and antiviral activity of black tea and its bioactives, regular consumption of black tea could very well fit in as a component of healthy diet for boosting immunity which is considered essential in this critical time of Global pandemic created by COVID-19.

Black Tea: Antiviral Activity & Boosting Immunity

A.K. Barooah

Tea, prepared from the leaves of *Camellia sinensis*, is the most popular beverage and has a number of bioactives with proven health benefits demonstrated through vitro cell culture, animal and clinical trials. In fact, all types of tea like black tea, green tea, oolong tea, white tea, yellow tea, etc., are considered as a nutraceuticals rather than a traditional beverage due to the presence of diverse pharmacologically active compounds. Tea is rich in polyphenols, favonoids, alkaloids, free amino acids, polysacharides and saponins which have been widely studied for their antioxidant and other beneficial properties. While initial studies were mostly confined to green tea, later studies clearly demonstrated that black tea and its bioactive compounds can prevent many infammatory diseases, carcinogenesis, cardiovascular diseases, diabetes and hypertension, promote weight loss, oral health and demonstrated anti-aging and antiviral activity against a number of viruses and immunomodulatory effects.

Tea Bioactives

Polyphenols are the most important the tea bioactive compounds known for its antioxidant properties. The four main polyphenol derivatives present in tea shoots and green tea are (-)-epicatechin (EC), 3(-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG) and (-)-epigallocatechin gallate (EGCG). These catechins undergo oxidation and polymerization during manufacture of black tea to theaflavins (TFs) which gives the characteristic bright orange-red colour of the black tea extract. The major TFs in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2a), theaflavin-3′-gallate (TF2b) and theaflavin-3,3′-digallate (TF3). Flavonols, including quercetin, kaempferol, myricitin and their glycosides are also present in tea. The total polyphenol contents in green and black tea may be similar but with different stages of polymerization due to the degree of oxidation as mentioned above during tea processing (Stangl et al. 2006). Both catechins and theaflavins have been documented as strong antioxidants and their effectiveness in prevention of cardiovascular diseases, hypertension, diabetes, obesity and alleviation of metabolic syndrome, inhibition of antiviral activity and modulation of immunity (Liu *et.al*, 2005; Clark *et al*., 1998,; Zu *et. al*., 2012, Khan and Mukhtar, 2013, Yang, 2018; Teng et al., 2019). The other tea bioactives like caffeine and theanine which are highly bioavailable are also reported to contribute to health benefits. The unique amino acid L-theanine has neuroprotective effects (Chung, 2018) and demonstrated to prime the immune system in fighting infection by bacteria, viruses and fungi in vitro (Bukowski *et. al.*, 1999) and human clinical trials (Kamath *et. al*., 2003).

Health benefits

The efficacy of green tea polyphenol to reduce blood cholesterol and lipid levels (Fekuyo *et al.,* 1986) as well as its anti altherosclerotic property (Muramatsu *et al.,* 1986) and anti tumour and anti mutagenic activities are well documented (Hara *et al.,* 1989; Okudu *et al.,* 1984). Inhibitory effects of green tea polyphenols on chemically or UV radiation induced carcinogenesis were also reported in early 1990s (Wang *et al*., 1991; Yamane *et al.,* 1991). Clinical efficacy of green tea in *diabetes mellitus* as well as the water soluble polysaccharide fraction of green tea has been reported to decrease the blood sugar level in *diabetes mellitus* patients and in rats in which hyperglycemia was induced by administration of Streptozotocin (Isigaki *et al.,* 1991). These early studies

indicating health benefits of tea were mostly reported from Japan for green tea (*Proceedings of the International Symposium on Tea Science, Shizuoka, Japan, 26-29 August 1991*).

The studies on pharmacotherapeutics of black tea have been taken up later in view of the increasing global awareness on the influence of food and beverages on human health. These later studies in India and China have demonstrated that black teas are also equally effective as green teas with respect to antioxidant and other beneficial properties and hence could counter some of the old myths associated with this beverage (Leung *et al,* 2001). The hot water extract of tea leaves not only showed direct pharmacological properties, but also delayed effects which may be closely correlated with the prevention of several degenerative disorders. In a series of metabolic animal studies at Indian Institute of Chemical Biology (IICB), Kolkata showed that Indian black tea extracts could reduce blood cholesterol and blood glucose, facilitates muscular performance and reduced gastric ulcer induced by different ulcerogens in rats. The anti cancer activity of tea root extracts were also demonstrated (Ganguly, 1994). The antioxidant activity of black tea extract using erythrocyte hemolysis, plasma oxidation, and cellular antioxidant activity assays was studied in China that showed that black tea extract dose-dependently protected erythrocytes from 2, 20-azobis (2-amidinopropane)-induced oxidative hemolysis and copper-induced plasma oxidation, and the tea pigments, especially thearubigins and theabrownins mainly contributed to the antioxidant activity of black tea extract (Liu and Huang, 2015).

Tea and its bioactive components possess multiple health benefits including antioxidation, anti-inflammation, immuno-regulation, anticancer, cardiovascular-protection, anti-diabetes, anti-obesity, and hepato-protection (Khan and Mukhtar, 2013). In a recent review, Teng *et al.,* (2019) presented a comprehensive review of tea and its bioactive components, bioavailability, health functions and safety mainly based on in vitro, in vivo and clinical studies highlighting the molecular mechanisms of health functions for better utilization of tea as a beverage and functional foods to prevent and control certain chronic diseases.

Health Benefits of Black Tea

Anti-inflamatory

Black tea extracts suppresses inflammatory diseases by reducing lipopolysaccharide (LPS)-induced NO and O2 production as well as inducible nitric oxide synthase (iNOS) expression in murine macrophage (Sarkar and Bhaduri, 2001). It is also reported to help maintain skeletal health through reduction of active osteoclasts, inflammatory cytokines production and oxidative stress (Das *et. al.*,2009). In another study Karmakar *et. al.* (2011) reported that black tea extacts provided protection against high fat diet (HFD) fed non-alcoholic steatohepatitis (NASH)-induced bone skeletal dysfunctional changes in Wistar rats.

Anticancer

Black tea extracts inhibited 7,12-dimethylbenz(a) anthracene (DMBA)-induced skin tumorigenesis through activation of superoxide dismutase (SOD), catalase (CAT) as well as induced apoptosis in mouse skin tumours (Saha and Das, 2002), induced of apoptosis by tea polyphenols mediated through mitochondrial cell death pathway in mouse skin tumours (Roy *et. al.*, 2009), suppressed 1,2-dimethylhydrazine (DMH)-induced colonic tumorigenesis by inhibition of cyclin D1, c-myc and cyclooxygenase-2 (COX-2) gene expression through blockage of Wnt/β-catechin pathway

(Patel *et. al.* (2008) and provided protection against oxidative stress, interfered with the activity of carcinogen metabolizing enzymes and inhibited DMBA-induced tumour in hamster buccal pouch carcinogenesis model (Mohan *et. al.*, 2005). Polyphenols bind to biomolecules like proteins, lipids and nucleic acid through H-bonding via phenolic groups and this high affinity binding to many proteins has been proposed to be a key mechanism for anticancer activity of EGCG. In comparison the black tea polyphenols with more phenolic groups, may bind to biomolecules with even higher affinity than EGCG (Yang, 2018).

Cardiovascular disease prevention

Black tea extracts have been demonstrated to reduce the risk of coronary heart disease and myocardial infarction (Arts *et. al.,* 2001 ; Grassi *et. al.*, 2008; Geleijnse *et. al.,* 2002). Black tea extracts have been reported to prevent cardiovascular diseases by inhibitng the acetyltransferase and 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) biosynthesis that inhibited platelet aggregation (Sugatani *et. al*.; 2004) and protecting the tert-butyl hydroperoxide (t-BHP)-induced malodialdehyde (MDA) and dityrosine levels in human vein endothelial cells (HUVEC) as demonstrated by Kuczaj *et. al.* (2009). In another study, Widlansky *et. al.* (2005) reported that black tea extract reversed endothelial dysfunction in patients with coronary artery disease.

Anti-obesity and metabolic syndrome

Black tea consumption can help weight loss and reduce obesity. Metabolic syndrome is a set of conditions such as increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels that increases the risk of heart disease, stroke and type 2 diabetes. Black tea reduced HFD-induced body weight gain, improved hyperglycemia and glucose intolerance via increasing the level of glucose transporter type 4 (GLUT4) in male C57BL/6J mice (Nishiumi *et. al.*, 2010). In another study, Uchiyama *et. al.* (2011) also reported that black tea extracts suppressed HFD-induced body weight increase, adipose tissue mass and liver lipid content through inhibition of intestinal lipid absorption in C57BL/6N mice. The sucrose-rich diet-induced body weight gain and hypercholesterolemia was reported to reduce in male SD rats after consumption of black tea extracts (Yang *et. al.*, 2001) while Majumdar *et. al.* (2011) observed that black tea consumption reduced HFD-induced NASH through regulation of pro-oxidant and antioxidant status, protected hepatocellular damage and against apoptosis in Wistar rats.

Effects on neurological disorders

Scientific evidence are also available on the black tea consumption on a few neurological disorders. Gauci *et. al.* (2011) reported that black tea extracts inhibited lipid membrane destabilization induced by amyloid β peptide (Aβ) 42 peptides in vitro while Tan *et. al.* (2007) reported that black tea could reduce the risk of Parkinson's disease in the Singapore Chinese Health Study. In a study carried out in India, Chaturvedi *et. al.* (2006) showed that black tea extracts could protected 6-hydroxydopamine (6-OHDA)-induced dopaminergic neuron damage, improved motor and neurochemical deficits in Wistar rats.

Modulation of immune system by tea constituents

The human immune system is complex and highly regulated internally. A healthy diet and lifestyle helps in normal functioning of the immune system, which may be altered by some diseases and

medications. Bioactives present in tea, viz., Catechins, Theaflavins, Quercetin, Caffeine and Theanine can positively affect the immune system to bolster the body's natural response to infection.

The most predominant amino acid present in tea is L-theanine, which primes the immune system in fighting infection by bacteria, viruses and fungi. In vitro research on γδ T cells (Bukowski *et. al.*, 1999) and human clinical trials (Kamath *et. al.*, 2003) showed that majority of the tea drinkers exhibited increased γ-interferon production, an important part of the body's immune defense within 2-4 weeks of tea consumption, in response to the secreted bacterial antigens isobutylamine and ethylamine. Consumption of same amount of coffee for same duration had no effect on interferon levels. Drinking tea, which contains L-theanine, a precursor of the non-peptide antigen ethylamine, primed peripheral blood γδ T cells to mediate a memory response on re-exposure to ethylamine and to secrete γ-interferon in response to bacteria. The results of this study suggested that regular intake of black tea provides the body's immune system with natural resistance to microbial infection.

Findings of another study on the protective role of tea drinking against upper respiratory tract infections suggested that tea act as a first line of defense by killing viruses in the upper respiratory tract. Iwata *et. al.* (1997) demonstrated that gargling with black tea could be a prophylactic agent against influenza infection. In this study, the test subjects gargled with 0.5% wt/vol of black tea extract twice daily. A significantly greater level of the influenza hemagglutinin antigen was demonstrated by the control group compared with the test group (49 % vs 35 %, respectively). In vitro, tea extract has been shown to inhibit absorption of the influenza viruses A and B but has no effect on the multiplication of the virus (Nakayama *et. al.*, 1990).

In-vitro studies conducted by Yan *et. al*, (1997) and Ciraj *et. al.*, (2001), has shown that infections caused by bacterial and viral species viz. *S. aureus* (tonsilitis), *H. influenzae* (pneumonia), *P.gingivalis* (periodontal disease) etc. can be denatured by tea extracts. There is also evidence from a cross-sectional study by Yee *et. al.* (2002) that high tea consumption was related to lower H. *pylori* infection (stomach ulcer) rate compared with low tea consumption. Effects of green tea against the *Herpes simplex* virus, rotavirus and enterovirus infections by tea extracts have also been reported by Mukoyama *et. al.* (1991) & Yama *et. al.*(1997). Furthermore, Weber *et al.* (2003) observed that adenovirus infection is inhibited in vitro by green tea catechins.

A review by Bukowski *et. al.* (2008) on the positive effect of tea drinking on immunity stated that the test subjects supplemented with capsules containing L-theanine and flavan-3-ols from tea exhibited lower incidence of cold and flu. Their recommendation from the study was to consume five cups of tea a day. In addition, tea also contains tannins that have the ability to fight viruses and hence keep the body protected from influenza, stomach flu and other such commonly found viruses in our everyday lives.

Bhat *et. al.* (2009) conducted a study to test the putative immune-enhancing effect of fortified black tea with five ayurvedic herbs (*Withania somnifera, Glycyrrhzia glabra, Zingiber officinale, Ocimum sanctum and Elettaria cardamomum*) on innate immunity. *Ex vivo* natural killer (NK) cell activity was assessed after consumption of fortified tea compared with regular tea in two independent double blind intervention studies. The study showed that drinking tea fortified with botanicals for at least two months enhanced the body's natural killer cell activity, and suggested that regular consumption of such a tea could potentially enhance immunity, especially in those who suffered recurring colds and flu.

As biomarkers of immune activation, circulating neopterin and the ratio of kynurenine (KYN)

to tryptophan (TRP) concentrations have been linked to cardiovascular and total mortality. A number of studies (in vitro) indicated that tea flavonoids can modulate tryptophan breakdown rates and neopterin production in immune cells. In a study supported by the National Health and Medical Research Council of Australia Gostner *et. al.* (2015) assessed the effects of regular black tea consumption on tryptophan and neopterin metabolisms in vivo. Healthy individuals having normal to mildly elevated systolic blood pressure were randomly selected to consume 3 cups/ day of either powdered black tea solids or a flavonoid-free caffeine-matched beverage as control and serum concentrations of tryptophan, kynurenine and neopterin were assessed periodically after daily ingestion of the respective beverages. Regular consumption of tea over 6 months, compared to control, did not significantly alter neopterin or tryptophan concentrations, but significantly increased the concentration of kynurenine ($p = 0.016$) and KYN/TRP ratio which the authors have attributed to enhanced tryptophan breakdown, possibly due to immune activation-induced tryptophan degrading enzyme indoleamine 2,3-dioxygenase. The influence of black tea consumption on biomarkers of immune system activation thus could relate to its general health benefits, though the net effect would strongly depend on the individual immune state (Gostner *et. al.,* 2015).

Investigations carried out by Kim *et. al.* (2011) on the effect of L-theanine on allergic response in animal model showed that in the L-theanine treated group, the histamine release from mast cells was decreased.

Various polyphenolic components of tea have been shown to possess anti-inflammatory properties in animal and in vitro models. In particular, the green tea polyphenol epigallocatechin gallate (EGCG) has been shown to inhibit cyclooxygenase- 2 and nitric oxide synthase expression by blocking excessive nuclear factor–κB activation (Surh *et. al.*, 2001). It is likely that the antioxidant capacity of the tea polyphenols plays an important role in the anti-inflammatory actions. In another study conducted by Crouvezier *et. al.* 2001, the effect of four major tea-derived catechins and a black tea extract on the production of pro- and anti-inflammatory cytokines by human leukocytes in vitro, was investigated. Epicatechin gallate, epigallocatechin and epigallocatechin gallate decreased the production of interleukin-1β and enhanced the production of interleukin-10, but had no effect on the production of interleukin-6 or tumour necrosis factor- α. Although these effects suggests the anti-inflammatory properties of the tea-derived catechins, they were observed at concentrations which were unlikely to be achievable in plasma in vivo and are therefore unlikely to contribute to the protective effects of tea-derived flavonoids in inflammatory diseases.

Report on a randomized, double-blinded, placebo-controlled clinical trial by Shamekhi *et. al.* (2017) indicated that the daily consumption of green tea extracts (1000 mg, two capsules/day) for 12 weeks significantly improved the systemic lupus erythematosus (SLE) disease as well as the corresponding vitality and general health. The results from another clinical trial (Zhang *et. al.* 2016) suggested that green tea consumption (@12 g/day) decreased plasma levels of inflammatory factors, such as IL-6 and NF-kB, in soldiers with sleep deprivation. In a human study by Yusni *et. al.* (2015), green tea polyphenol administration (2x350 mg/day, for 14 days) has been reported to decrease the level of IgE in patients with allergic rhinitis compared with those in the control group, though not statistically significant.

In a study reported in 2012 sponsored by National Tea Research Foundation (NTRF), India the potential immunemodulatory and anti-inflammatory activities of black tea was studied in a rat model as well as in human peripheral mononuclear cells. The acute anti-inflammatory activity of black tea extract (10 and 20%) was evaluated using carrageenan and dextran whereas chronic

anti-inflammatory (Immunomodulatory) effects were evaluated in a complete Freunds' adjuvant-induced arthritis model. Immunostimulatory role was evaluated in cultured human (in vitro) peripheral mononuclear cells (T-lymphocytes) by using methyl thioazolyl tetrazolium (MTT) and Trypan blue assay. Results indicated that black tea decoction (10% and 20%) strength has shown significant anti-inflammatory effects (64.8% and 77% reduction, respectively), on carrageenan-induced acute inflammatory models (rat paw edema) which can be comparable with the standard drug indomethacin (89.1%). In a chronic anti-inflammatory model, black tea decoction (10% and 20%) has shown significant suppressive effects on rat paw edema (38.56% and 69.53%) observed on 21st day. Lymphoproliferative action of tea was evaluated on human peripheral mononuclear cells using an MTT assay where the number of living cells were expressed in terms of optical density at 570 nm. An experiment has shown that black tea increases the maximum number of T-lymphocytes at 72 h with a maximum strength of 20%. Maximum number of viable cells (T-lymphocytes) was observed with black tea at 20% strength at 72 h. These findings indicate that black tea has potential anti-inflammatory and immunomodulatory action and results corroborates with the current trend of tea being promoted as a 'health drink' (Chattopadhay et al, 2012).

Ding *et al.*, (2018) has extensively reviewed published studies on regulation of immune function by polyphenols and observed that immune dysfunction is caused by various factors, including changes in relevant immune regulators and environmental stress leading to a variety of diseases in humans. Nutrition may play an essential role in immunity by interfering with proinflammatory cytokine synthesis, immune cell regulation, and gene expression. Polyphenols, one of many categories of natural substances, promote immunity to foreign pathogens via various pathways. Different immune cells express multiple types of polyphenol receptors that recognise and allow cellular uptake of polyphenols, which subsequently activate signalling pathways to initiate immune responses as in case of polyphenols curcumin and epigallocatechin gallate (EGCG) was demonstrated to induce epigenetic changes in cells. Polyphenols can be used to regulate intestinal mucosal immune responses, allergic diseases, and antitumour immunity. Polyphenols are among the most abundant chemicals in the plant kingdom, which yields consumables such as vegetables, fruit, and tea. Polyphenols have been shown to enhance antitumour immune activity, as well as immunomodulatory processes and intestinal mucosal immunity.

Tea, antiviral activity and Corona viruses

Liu *et al.*,(2005) showed that the theaflavin derivatives had more potent anti-HIV-1 activity than catechin derivatives. These tea polyphenols could inhibit HIV-1 entry into target cells by blocking HIV-1 envelope glycoprotein-mediated membrane fusion. The fusion inhibitory activity of the tea polyphenols was correlated with their ability to block the formation of the gp41 six-helix bundle, a fusion-active core conformation. Computer-aided molecular docking analyses indicate that these tea polyphenols, theaflavin-3,3′-digallate (TF3) as an example, may bind to the highly conserved hydrophobic pocket on the surface of the central trimeric coiled coil formed by the N-terminal heptad repeats of gp41. These results indicate that tea, especially black tea, may be used as a source of anti-HIV agents and theaflavin derivatives may be applied as lead compounds for developing HIV-1 entry inhibitors targeting gp41.

EGCG present in green tea has been shown to inhibit Herpes simplex virus type-1 (HSV-1) (Oleviera, 2008; Issack *et al*., 2008) by possibly binding to the glycoproteins on the envelope of the virus, thereby preventing viral entry into the host cell. The increased stability of theaflavins compared to EGCG at neutral pH could make these black tea compounds a more feasible option for the design of an antiviral therapeutic agent than EGCG (Su et al, 2003). Black Tea Extract

consisting primarily of theaflavins is not cytotoxic and can reduce or block the production of infectious HSV-1 virions in cultured A549 and Vero cells, thus inhibiting the infectivity of the virus by interfering in the attachment, penetration and viral DNA replication of HSV-1 particles (Cantatore et al.,2013).

The anti-influenza virus and anti-inflammatory activities of theaflavin derivatives have been reported by Zu *at. el.*,(2012). The theaflavins fraction (TF80%, with a purity of 80%) and three theaflavin (TF) derivatives from black tea have been found to exhibit potent inhibitory effects against influenza virus in vitro. The authors have used assays for neuraminidase (NA) activity, hemagglutination (HA) inhibition, a real-time quantitative PCR (qPCR) for gene expression of hemagglutinin (HA) and a cytopathic effect (CPE) reduction assay for studying the activity of TFs. The results showed that the TFs exerted significant inhibitory effects on the NA of three different subtypes of influenza virus strains and also on HA through two major mechanisms. The TF derivatives might have a direct effect on viral particle infectivity affecting replication of the viral HA gene during early stage of infection. In addition, TFs decreased the expression level of the inflammatory cytokine IL-6 during viral infection, expression of which may result in serious tissue injury and apoptosis. Thus, the results indicated that TF derivatives are potential compounds with anti-influenza viral replication and anti-inflammatory properties.

A study of antiviral activity of theaflavins (extracted from black tea) against Hepatitis C virus (HCV) using human hepatoma Huh-7 cells showed significant decrease of infectivity of the virus in the presence of each of the three theaflavins, with a clear dose-dependent inhibitory effect. The antiviral activity of the theaflavins was confirmed by quantification of viral RNA. TF3 was found to be more active and the HCV pseudotyped virions confirmed their activity on HCV entry and demonstrated their pan-genotypic action by directly acting on the virus particle and inhibited cell-to-cell spread. Further, TFs in combination with Sofosbuvir and Daclatasvir which are FDA approved drugs for HCV, enhanced the antiviral activity of both drugs (additive effect) demonstrating that it could be used in combination with direct acting antivirals (DAA) used in hepatitis C therapy. Thus, theaflavins, that are present in high quantity in black tea, hold promise for therapeutic use against HCV infection and also as neutraceutical as it inhibit cell-to-cell entry of the virus (Chowdhury, *et al*., 2018).

In a study reported by Clark *et al.* (1998) it was demonstrated that theaflavins extracted from black tea were able to neutralize bovine coronavirus and rotavirus infections. The crude black tea extract and the various fractions of theaflavins extracted from black tea were tested individually and in combination for antirotaviral activity. The combination of theaflavin fractions (TF1 + TF2a + TF2b + TF3) was more active than the sum of the activities of these four fractions individually, indicating synergism amongst the TF components. The results of this study showed that theaflavin and theaflavin gallate derivatives have inactivation activity (in vitro) against both rotavirus and coronavirus. The crude black tea extract was also able to neutralize the coronavirus.

In view of the current pandemic created by the novel corona virus COVID-19, lot of efforts are on globally to develop suitable vaccine and to relook the existing drugs and molecules for effectiveness against the causative agent SARS-CoV-2 (Tang *et al.,* 2020). Coronaviruses are enveloped positive-stranded RNA viruses that replicate in the cytoplasm (Belouzard *et al.* 2012). To deliver their nucleocapsid into the host cell they rely on the fusion of their envelope with the host cell membrane. The spike glycoprotein (S) mediates virus entry and is a primary determinant of cell tropism and pathogenesis. The RNA dependent RNA polymerase (RdRp) is known to be an important enzyme that catalyzes the replication of RNA from RNA templates.

In a recent study published in the ***Journal of Medical Virology,*** 83 compounds used in Chinese medicine system were screened for their potential efficacy against SARS-CoV-2 by assessing their binding efficiency onto this RNA dependent RNA polymerase (RdRp) of the COVID-19 virus (Lung *et. al.,* 2020). The authors have generated three dimensional model structures of RdRp of SARS-CoV-2 (2019 Pandemic), SARS-CoV (2002 epidemic) and MERS-CoV (2012 epidemic) using Modeller UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) and SWISS-MODEL (https://swissmodel. expasy.org/) to test the efficacy of the compounds. This virtual screening in this bioinformatics study revealed that out of the 83 compounds screened, theaflavin was the best compound on the basis of idock score (prediction of binding affinity), hydrophobic interactions and additional hydrogen bonds between theaflavin and amino acid near the active site of RdRp. This was further confirmed by lower binding energy when it docks the catalytic pocket of SARS-CoV-2 RdRp. These finding suggested that theaflavins could be used as a lead compound for developing a SARS-CoV-2 inhibitor that targets the RdRp. Theaflavins are present in black tea and the highest theaflavin contents are present in the black teas of Assam.

Though further in vivo, animal and clinical trials would be required to carry forward this research finding, it is quite interesting to note that an earlier study from Taiwan has in fact convincingly demonstrated the inhibition of SARS-CoV 3C-like protease activity by Theaflavin-3,3'-digallate (TF3) published in the journal ***Evidence based Complementary and Alternative Medicine*** (Chen *et.al*.,2005). The authors have reported that the extracts from Puer and Black tea were more potent than the green or oolong tea extracts in their inhibitory activities against a chymotrypsin-like (3CLPro) protease. In this study 3CL protease was a target and the virus of interest was SARS-CoV (2002 epidemic). This study also used docking approach to screen out the best inhibitory compounds using a natural product library consisting of 720 compounds. Two compounds, tannic acid and TF2b (Theaflavin 3-gallate) were found to be active against 3CL Protease. Since many other related to tannic acid and TF2b are also present in various kinds of teas, the authors further examined the inhibition of activity by various tea extracts and several well known pure ingredients present in teas. The water extracts of TF2b (theaflavin-3-gallate), TF3 (theaflavin diggallate) and tannic acid were found to be the best effective 3CLPro inhibitors with inhibitory concentration (IC50) of less than 10 μM. The results from this study showed that Puer and Black tea extracts were more potent than the green or oolong tea extracts in inhibitory activities against 3C-like protease (3CLPro) of severe acute respiratory syndrome coronavirus (SARS-CoV), notably the active constituents viz. Theaflavin-3-gallate (TF2b) theaflavin-3,3'-digallate (TF3) and tannic acid were effective 3CLPro inhibitors.

Conclusion and Suggestions

The tea plant has been cultivated for thousands of years and its leaves used for medicinal purposes. The health promoting benefit of drinking tea has been known since ancient times. Modern research has only provided scientific basis for this belief (Khan and Mukhtar, 2013). Evidence supporting the health benefits of tea drinking is growing stronger with each new scientific publications. The forgoing review of the various research findings clearly indicate the health benefits and antiviral activity of black tea and theaflavins against a number of viruses including the SARS-CoV coronavirus (Chen *et.al*.,2005).

It is well known that the pathogenesis of many human diseases involve immune functions. The immune dysfunction's influence on health and the scope of relying on functional foods, defined as those providing specific nutrition or targeting multiple functional components are considered a form of preventive medicine (Cornò *et al*., 2014). Nutrition plays an essential role in immunity by

interfering with proinflammatory cytokine synthesis, immune cell regulation, and gene expression. Polyphenols promote immunity to foreign pathogens via various pathways and different immune cells expresses multiple types of polyphenol receptors that recognise and allow cellular uptake of polyphenols, which subsequently activate signalling pathways to initiate immune responses (Ding *et al.*, 2018). Tea is rich in polyphenols and since it contains the unique amino acid L-theanine, its consumption provides the body's immune system with natural resistance to microbial infection.

Thus, in view of the available evidence of antiviral activity of black tea and its theaflavins against the SARS-CoV coronavirus (Chen *et.al*., 2005) and the recent demonstration of the binding efficiency of theaflavins onto the RNA dependent RNA polymerase (RdRp) of the COVID-19 virus (Lung *et. al.,* 2020), the black tea could very well fit in as a component of healthy diet for boosting immunity which is considered essential in this critical time of Global pandemic created by COVID-19.

References:

Arts, I. C., Jacobs, Jr., D. R., Harnack, L. J., Gross, M. And Folsom, A. R. (2001). Dietary catechins in relation to coronary heart disease death among postmenopausal women. *Epidemiology* 12(6):668-75.

Bhat, J., Damle, A., Vaishnav, P.P., Albers, R., Joshi, M., Banerjee, G. (2010). In vivo enhancement of natural killer cell activity through tea fortified with ayurvedic herbs. *Phytother. Res.* ; 24: 129–135.

Bukowski, J. F., & Percival, S. S. (2008). L-Theanine intervention enhances human γδT lymphocyte function. *Nutrition Reviews*; 66, 96–102.

Bukowski, J. F., Morita, C. T., and Brenner, M. B. (1999). Human gamma-delta T cells recognize alkylamines derived from microbes, edible plants, and tea: Implications for innate immunity. *Immunity*; 11:57–65.

Cantatore, A.; Randall, S.D.; Traum, D. and Adams, S.D. (2013). Effect of black tea extract on herpes simplex virus-1 infection of cultured cells. *BMC Complementary and Alternative Medicine* 13: 139 (http://www.biomedcentral.com/1472-6882/13/139)

Chen, Chia-Nan.; Coney, P. C.; Lin, Kuo-Kuei; Huang,Wei-Cheng; Chen, Hsin-Pang, Hsieh, Po-Huang Liang and John T.-A. Hsu. (2005). Inhibition of SARS-CoV 3C-like Protease Activity by Theaflavin-3,3_-digallate (TF3); *eCAM* ;2(2)209–215.

Chowdhury, P.; Sahuc, M-E; Rouille´, Y.; Rivière, C.; Bonneau, N; Vandeputte, A.; Brodin, P.; Goswami, M.; Bandyopadhyay, T.; Dubuisson, J. and Se´ron, K. (2018). Theaflavins, polyphenols of black tea, inhibit entry of hepatitis C virus in cell culture. *PLoS ONE* 13(11): e0198226. https://doi.org/10.1371/journal.pone.0198226

Ciraj AM, Sulaim J, Mamatha B, Gopalkrishna BK, Shivananda PG. (2001). Antibacterial activity of black tea (Camellia sinensis) extract against Salmonella serotypes causing enteric fever. *Indian J Med Sci.*; 55:376-81.

Chaturvedi, R., Shukla, S., Seth, K., et. al. (2006). Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Neurobiol. Dis*. 22(2):421-34.

Chattopadhyay, C., Chakrabarti, N., Chatterjee, M., Mukherjee, S., Sarkar, K. and Roy Chaudhuri, A. (2012). Black tea (Camellia sinensis) decoction shows immunomodulatory properties on an experimental animal model and in human peripheral mononuclear cells. *Pharmacognosy Res*. 4(1): 15–21.

Clark KJ, Grant PG, Sarr AB, Belakere JR, Swaggerty CL, Phillips TD. (1998). An *in vitro* study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovine coronavirus infections. *Vet Microbiol*; 63:147–57.

Cornò, M.; Scazzocchio, B.; Masella, R. and Gessani, S. (2014). Regulation of Dendritic Cell Function by Dietary Polyphenols. *Critical reviews in food science and nutrition*. 56. 10.1080/10408398.2012.713046.

Crouvezier S, Powell B, Keir D, Yaqoob P. (2001). The effects of phenolic components of tea on the production of pro- and anti-inflammatory cytokines by human leukocytes in vitro. *Cytokine*; 13:280-6.

Das, A.S., Mukherjee, M., Das, D. and Mitra, C.(2009). Protective action of aqueous black tea (*Camellia sinensis*) extract(BTE) against ovariectomy-induced oxidative stress of mono-nuclear cells and its associated progression of bone loss. *Phytother Res*.23(9):1287-94

Ding, S; Jiang, H; and Fang, J. (2018). Regulation of immune function by polyphenols. *J. Immunol. Res*. Article ID 1264074, doi.org/10.1155/2018/1264 4074.

Fukuyo, M, Hara, Y. and Muramatsu, K. (1986) *J. Jpn. Soc. Nutra. Food Sci*., 39, 495-500.

Ganguly, D K (1994). Pharmacotherapeutics of Black Tea. **In** Proceeding of 32nd Tocklai Conference, TRA Pub. p336-347.

Gauci, A. J., Caruana, M., Giese, A., Scerri, C. and Vassallo, N. (2011). Identification of polyphenolic compounds and black tea extract as potent inhibitors of lipid membrane destabilization by Aβ42 aggregates. *J. Alzheimers Dis*. 27(4):767-79.

Geleijnse, J. M., Launer, L. J., van der Kuip, D. A., Hofman, A and Wittemah, J. C. (2002). Inverse association of tea and flavonoid intakes with incident myocardial infarction: The Rotterdam Study. *Am. J. Clin. Nutr*. 75(5):880-6

Gostner, J .M., Becker, K., Croft, K.D., Woodman, R.J., Puddey, I.B., Fuchs, D., Hodgson, J. M. (2015). Regular consumption of black tea increases circulating kynurenine concentrations: A randomized controlled trial. *BBA Clinical*; 3, 31–35.

Hara, Y et al. (1989). J. Jpn. Soc. Nutr. FoodSci., 42, 39-45.

Hara, Y et al. (1991). Proc. Int. Symp. on Tea Science, Shizuoka, Japan, pp.22-26.

Iwata M, Toda M, Nakayama M. (1997). Prophylactic effect of black tea extract as gargle against influenza. *Kansenshogaku Zasshi*; 71: 487-94.

Isaacs CE, Wen GY, Xu W, Jia JH, Rohan L, Corbo C, DiMaggio V, Jenkins EC, Hillier S.(2008). Epigallocatechin gallate inactivates clinical isolates of herpes simplex virus. *Antimicrob Agents Chemother*. 52: 962-970.

Isagaki, J., Takakuwa, T. and Takeo, T. (1991). Proc. of the Int. Symp. on Tea Science, Shizuoka, Japan, pp. 240-242.

Kamath, A., Wang, L., Das, H., Li, L., Reinhold, V. N., and Bukowski, J. F. (2003). Antigens in tea-beverage prime human Vγ2Vδ2 T cells in vitro and in vivo for memory and non memory antibacterial cytokine response. *Proc. Natl. Acad. Sci. U.S.A.*; 100:6009–14.

Karmakar, S., Majumdar, S., Maiti, A., et. al. (2011). Protective role of black tea extract against nonalcoholic steatohepatitis-induced skeletal dysfunction. *J. Osteoporosis*. 2011(426863), 1-12. doi: 10.4061/2011/426863.

Kim, N. H., Jeong, H. J., & Kim, H. M. (2011). Theanine is a candidate amino acid for pharmacological stabilization of mast cells. *Amino Acids*; 42(5), 1609–1618.

Khan, N. and Mukhtar, H. (2013). Tea and Health: Studies in Humans. *Curr Pharm Des*. 19(34): 6141–6147.

Kuczaj, W., Zapora, E., Szczepanski, M., Wnuczko, K. And Skrzydlewska, E. (2009). Polyphenols action against oxidative stress formation in endothelial cells. *Acta Pol. Pharm*. 66(6):617-24.

Leung, L. K.; Su, Y.; Chen, R.; Zhang, Z.; Huang, Y. and Chen, Z.Y. (2001). Theaflavins in Black Tea and Catechins in Green Tea Are Equally Effective Antioxidants. Biochemical and Molecular Action of Nutrients Research Communication. Pp 2248-2251. (https://academic.oup.com/jn/article-abstract/131/9/2248/4687692, accessed 07.04.2020)

Liu, S.M. and Huang, H.H. (2015). Assessments of antioxidant effect of black tea extract and its rationals by erythrocyte haemolysis assay, plasma oxidation assay and cellular antioxidant activity (CAA) assay. *J. Funct. Foods*. 18: 1095–1105.

Liu S, Lu H, Zhao Q, He Y, Niu J, Debnath AK, Wu S, Jiang S. (2005). Theaflavin derivatives in black tea and catechin derivatives in green tea inhibit HIV-1 entry by targeting gp41. *Biochim Biophys Acta*.,1723:270-281.

Lung, J.; Lin, Y.S.;Yang, Y.H.,Chou, Y.L.; Shu, L.H.; Cheng, Y.C.; Liu, H.T. and Wu, C.Y. (2020). The potential chemical structure of anti-SARS-CoV-2 RNA-dependent RNA polymerase. *Journal of Medical Virology*., 1-5. DOI: 10.1002/jmv.25761.

Majumdar, S., Karmakar, S., Maiti, A., et. al. (2011). Arsenic-induced hepatic mitochondrial toxicity in rats and its amelioration by dietary phosphate. *Environ.Toxicol.Pharmacol.* 31(1):107-18.

Mohan, K. C., Hara, Y., Abraham, S. And Nagini, S. (2005). Comparative evaluation of the chemopreventive efficacy of green and black tea polyphenols in the hamster buccal pouch carcinogenesis model. *Clin. Biochem*. 38(10):879-86.

Mukoyama A, Ushijima H, Nishimura S, Koike H, Toda M, Hara Y, Shimamura T. (1991). Inhibition of rotavirus and enterovirus infections by tea extracts. *Jpn J Med Sci Biol*; 44:181-186.

Muromatsu, K., Fukuyo, M. and Hara, Y (1986) *J Neutrition Sci, Vitaminol.* 32: 613-622.

Nakayama M, Toda M, Okubo S, Shimamura T.(1990). Inhibition of influenza virus by tea. *Lett App Microbiol*; 11:38-40.

Nishiumi, S., Bessyo, H., Kubo, M., et. al. (2010). Green and black tea suppress hyperglycemia and insulin resistance by retaining the expression of glucose transporter 4 in muscle of high-fat diet-fed C57BL/6J mice. *J. Agric. Food Chem.* 58(24):12916-23.

Oliveira A, Adams SD, Lee L, Murray SR, Hsu SD, Hammond JR, Dickinson D, Chen P, Chu TC (2012). Inhibition of herpes simplex virus type 1 with the modified green tea polyphenol

palmitoyl-epigallocatechin gallate. *Food Chem Toxicol*. 52: 207-215.

Okudu et al. (1984). Chem. Pharm. Bull. 32, 3755-3758.

Patel, R., Ingle, A. And Maru, G. B. (2008). Polymeric black tea polyphenols inhibit 1, 2-dimethylhydrazine induced colorectal carcinogenesis by inhibiting cell proliferation via Wnt/β-catenin pathway. *Toxicol. Appl. Pharmacol*. 227(1):136-46.

Roy, P., Nigam, N., George, J., Srivastava, S. And Shukla, Y. (2009). Induction of apoptosis by tea polyphenols mediated through mitochondrial cell death pathway in mouse skin tumors. *Cancer Biol Ther*. 8(13):1281-7.

Saha, P. And Das, S. (2002). Elimination of deleterious effects of free radicals in murine skin carcinogenesis by black tea infusion, theaflavins & epigallocatechin gallate. *Asian Pac. J. Cancer Prev*. 3(3):225-30.

Sarkar, A. and Bhaduri, A. (2001). Black tea is a powerful chemopreventor of reactive oxygen and nitrogen species : Comparison with its individual catechin constituents and green tea. Biochem. Biophys. Res. Comm. 284(1):173-8.

Shamekhi, Z.; Amani, R.; Habibagahi, Z.; Namjoyan, F.; Ghadiri, A.; Malehi, A.S. (2017). Arandomized, double-blind, placebo-controlled clinical trial examining the effects of green tea extract on systemic lupus erythematosus disease activity and quality of life. *Phytother. Res.*; 31, 1063–1071.

Skrzypczak-Jankun, E and Jankun, J. (2010). Theaflavin digallate inactivates plasminogen activator inhibitor: Could tea help in Alzheimer's disease and obesity? *Int. J. Mol. Med*. 26(1):45-50.

Sugatani, J., Fukazawa, N., Ujihara, K., et. al. (2004). Tea polyphenols inhibit acetyl-CoA : 1-alkyl-sn-glycero-3-phosphocholine acetyltransferase (a key enzyme in platelet-activity factor biosynthesis) and platelet-activating factor-induced platelet aggregation. *Int. Arch. Allergy Immunol*. 134(1):17-28.

Surh, Y.J., Chun, K.S., Cha, H.H., Han, S.S., Keum. Y.S., and Park, K.K. (2001). Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kappa B activation. *Mutat Res*; 480- 81:243-68.

Tang, G.; Meng, X.; Gan, R.; Zhao, C.; Liu, Q.; Feng, Y.; Li, S.; Wei, X.; Atanasov, A.G.; Corke, H. and Li, H. (2019). Health Functions and Related Molecular Mechanisms of Tea Components: An Update Review. *Int .J. Mol. Sci*. 20: 6196; doi:10.3390/ijms20246196.

Uchiyama, S., Taniguchi, Y., Saka, A., Yoshida, A. And Yajima, H. (2011). Prevention of diet-induced obesity by dietary black tea polyphenols extract in vitro and in vivo. *Nutr*. 27(3):287-92.

Widlansky, M. E., Duffy, S. J., Hamburg, N. M., et. al. (2005). Effects of black tea consumption on plasma catechins and markers of oxidative stress and inflammation in patients with coronary artery disease. *Free Radical. Biol. Med.* 38(4):499-506.

Weber J.M., Ruzindana-Umunyana A., Imbeault, L. and Sircar, S. (2003). Inhibition of adenovirus infection and adenain by green tea catechins. *Antiviral Res*; 58:167-173.

Yam TS, Shah S, Hamilton-Miller JM. (1997). Microbiological activity of whole and fractionated

crude extracts of tea (Camellia sinensis), and of tea components. *FEMS Microbiol Lett*. 152:169-74.

Yang C.S. (2018). The Potential role of tea in combating chronic diseases. **In** Global Tea Science Current status and future needs (V S Sharma and M T K Gunasekhare eds.). Burleigh Dodds Sci. Pub. pp 427-451.

Yang, M. H., Wang, C. H. And Chen, H. L. (2001). Green, Oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet. *J. Nutr. Biochem.* 12(1):14-20.

Yama TS, Shaha S, Hamilton-Millera JMT. (1997). Microbiological activity of whole and fractionated crude extracts of tea (*Camellia sinensis*), and of tea components. *FEMS Microbiol Lett*, 152:169-174.

Yee YK, KooMW, Szeto ML. (2002). Chinese tea consumption and lower risk of Helicobacter infection. *J. Gastroenterol Hepatol*; 17:552-555.

Yusni Husni, T.T.; Achmad, T.H.2015. Aktivitas activity of green tea (Camellia sinensis (L) o. Kuntze) polyphenols as immunomodulator through response of suppression immunoglobulin e (IgE) in allergic rhinitis. *Majalah Kedokteran Bandung-Mkb-Bandung Med.*; 47, 160–166.

Yamane et al (1991). Jap. J. Can. Res., 82, 1336-1339.

Wang et al. (1991). Proc. Am. Asso. Can. Res., 32, 125-129.

Zhang, T.; Li, L.; Liu, Y.H.; Zhong, D.Q.; Tao, Y.; Jiang, X.J.; Xu, Z.Q. (2016). Effect of coffee and green tea on executive ability and plasma levels of inflammatory factors in soldiers with 48-hour total sleep deprivation. *Int. J. Clin. Exp. Med.*; 9, 19354–19362.

Zu M, Yang F, Zhou W, Liu A, Du G, Zheng L (2012). In vitro anti-influenza virus and anti-inflammatory activities of theaflavin derivatives. *Antiviral Res*. 94: 217-224.

Tea Research Association
Tocklai Tea Research Institute, Jorhat - 785008, Assam
www.tocklai.net