

Can drinking black tea fight diabetes: literature review and theoretical indication

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Abstract

Obesity and diabetes, called in the past “rich men disease”, are one of the biggest public health challenges of the 21st century. Obesity, being a gateway to diabetes is a global problem now. Over 300 million people worldwide have diabetes and this number will most likely will rise to 500 million within one generation. Diabetes is at crisis levels; three out of four people with diabetes live in low- and middle-income countries. Many medicaments to treat diabetes are on the market, but these are out of reach for many people living in poverty. These people cannot afford buying insulin and/or other medicaments but they are instead using traditional folk remedies. We have reviewed and evaluated black tea to find out if there is any scientific merit to the claims to alleviate symptoms of diabetes. There are both studies showing anti-diabetic properties of black tea or its ingredients and some that do not. These differences may be related to lack of uniformity in the completed studies rather than absence of anti-diabetic activity. Nevertheless, the final conclusion could only be drawn after completion of well controlled clinical studies.

Key words: black tea, diabetes, theaflavins, plasminogen activator inhibitor.

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Obesity, and diabetes called in the past “rich men disease”, are one of the biggest public health challenges of the 21st century. Obesity, being the gateway to diabetes is a global problem now. Over 300 million people worldwide have diabetes and this probably will rise to 500 million within a one generation. Diabetes is at crisis levels; three out of four people with diabetes live in low- and middle-income countries. Prognosis shows that Africa will experience a 98% rise in the number of diabetes cases over the next 20 years, the greatest increase in the world. The highest incidence of this disease is in the Arabic Middle East (Bahrain, Egypt, Kuwait, Oman, Saudi Arabia, The United Arab Emirates) reaching in some cases 27% of the population. However, the largest numbers of diabetics are in China and India [1-5].

The number of people around the world suffering from diabetes has risen steeply in the last decades severely exhausting the ability of health care systems to deal with the pandemic. In the large population of people screened, some protective features related to type two diabetes have

been identified. They include: moderate alcohol consumption, caffeine, brown rice consumption instead of white rice, healthy eating and exercise habit. Contributory features have been identified as well and these include: high calorie diet, low physical activity, aging, sleep deprivation, maternal malnutrition, obesity, chronic physiological stress [6-13].

There is strong demand to identify more potent protective features to slow down the progression of obesity and diabetes. Many medicaments to treat diabetes are on the market, but these are out of reach for many people living in poverty for which we have to protect the fundamental right to health and right to live. As an example according to a 2005 World Bank estimate, 41.6% of the total Indian population falls below the international poverty line of US dollars 1.25 a day [14]. These people cannot afford 20-30 dollars for one vial of insulin and/or other medicaments [15]. Thus we have reviewed and evaluated traditional folk remedies to find any scientific merit to claims of alleviation symptoms of diabetes.

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Black tea consumption and diabetes

The real teas are produced from the plant *Camellia sinensis* and there are three major types of teas: unfermented green tea, fully fermented black tea, red tea which is partially fermented oolong tea. Green tea leaves are steamed, which prevents bioactive compounds, like many polyphenolic flavonoids, from being oxidized. Main catechins found in green tea are: catechin (C), galliccatechin (GC), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The tea fermentation process allows the leaves to undergo enzymatic oxidation when polyphenol oxidase causes polymerization of flavan-3-ols to catechin oligomers – bisflavanols, theaflavins, thearubigins and others. Oolong tea as partially fermented will contain compound of green and black tea [16].

A literature search on green tea and its abundant polyphenol EGCG provided evidence of the diverse pharmacological activities of green tea [17, 18] but there was no evidence of protective curable properties of green or oolong teas against diabetes [19-21]. Black tea was investigated less than green tea but showed anti-diabetic properties.

In a search for possible effects on insulin function, Broadhurst *et al.* investigated 49 herbs, spices, and medicinal plant extracts for the insulin-dependent utilization of glucose in a rat epididymal adipocyte assay. They found that cinnamon was the most bioactive product followed by black tea. The glucose oxidation enhancing bioactivity was lost by poly(vinylpyrrolidone) treatment, indicating that the active phytochemicals are to be phenolic [22].

Gomes *et al.* evaluated the effect of the hot water extract of black tea on streptozotocin induced diabetes in rats. They observed significant reduction of the blood glucose level and they found preventive and curative effects of extract on diabetes in rats [23]. Abeywickrama *et al.* describes that Sri Lankan traditional practitioners recommend drinking of black tea for regulation of hyperglycemia. They evaluated the blood glucose lowering potential of Sri Lankan grade tea and its anti-diabetic potency using streptozotocin induced diabetic rats. Different concentrations of warm black tea infusion (60, 120 and 480 mg/ml) were orally administered to different groups of rats, and hypoglycemic activity was detected. The black tea infusion (BTI) exhibited significant ($P < 0.05$), dose-dependent reduction of hypoglycemic activity. They did not find any toxicity of BTI in concentrations used [24].

Odegaard *et al.* reported that coffee consumption was inversely associated with the risk of type 2 diabetes in populations of European descent. Using this observation as a lead they investigated associations between intakes of coffee, black tea, and green tea with the risk of type 2 diabetes in Singaporean Chinese men and women. They analyzed data from 36,908 females and males in the Singapore. In multivariate models participants reporting four or

more cups of coffee per person they found a 30% reduction in risk of diabetes type 2 in comparison with nondaily consumers. Participants reporting one or more cups of black tea per day had a 14% reduction in risk of diabetes and no reduction of diabetes risk was observed in the group that drink green tea [25].

Boggs *et al.* analyzed coffee, tea, and alcohol consumption on diabetes risk in African American women (46,906 participants). During 12 years of follow-up relative risks were estimated using Cox proportional hazards models adjusted for diabetes risk factors. Results suggest that African American women who drink moderate amounts of caffeinated coffee or alcohol have a reduced risk of type 2 diabetes but intakes of decaffeinated coffee and tea were not associated with decreasing risk of diabetes [26].

Ankolekar *et al.* explored the influence of extraction time typically used in daily consumption of tea, as a therapeutic dietary supplement for management of early stage type 2 diabetes. They compared black tea extraction times of 2 and 5 minutes. The 5-minute extraction time produced significantly higher total of phenolic compounds believed to be active constituents of black tea. Also, they observed significant differences in amount of total phenolic between different brands of tea; 5 minutes extraction of Choice Darjeeling yielded 300 mg/g, while Bigelow Green 2-minute extraction had the lowest total phenolic content 55 mg/g. Thus it is no surprise that 1,1-Diphenyl-2-picrylhydrazyl scavenging-linked antioxidant activity was influenced by the extraction time. Consequently they have concluded that the 5-minute extraction time has more relevance for potential benefits for managing hyperglycemia than the 2-minute procedure [27]. It seems that variances in tea preparation, and in brands of teas might be an explanation of differences in findings of tea curable properties.

Mechanism of anti-diabetic properties of black tea

The majority of studies have demonstrated that tea prevents and sometime treats various chronic diseases including diabetes and obesity, but its basic mechanism is unclear [28]. Chronic inflammation is related to visceral obesity that causes insulin resistance in the liver. It induces production of adipokines and cytokines such as: tumor necrosis factor α (TNF- α), free fatty acids (FFA), interleukin 1 (IL-1), interleukin 6 (IL-6), leptin and resistin that inhibits insulin signaling. Consequently other proteins are activated, such as: c-Jun N-terminal kinases (JNK), inhibitor of NF- κ B kinase (IKK- β) and protein kinase C (PKC) and protein tyrosine phosphatases such as protein-tyrosine phosphatase 1B (PTP1B) and phosphatase and tensin homolog (PTEN), which impair insulin signaling at insulin receptor and insulin receptor substrate level. Insulin resistance further stimulates the production of C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1) [29]. Two of

these proteins (PTP1B and PAI-1) are inhibited by black tea extracts or its components.

Protein tyrosine phosphatases (PTPs) constitute a large, structurally diverse family and is defined as a target to treat diabetes and obesity. Studies have demonstrated that PTP1B a member of the PTP superfamily is a negative regulator of insulin and leptin signaling pathways. Overexpression of PTP1B contributes to diabetes and obesity. Ma *et al.* searched for PTP1B inhibitors and found that black teas strongly inhibit PTP1B. Less potent was oolong tea and then green tea. The average IC_{50} values were observed for five brands of black teas around 0.4 g of tea leaves in one liter of water, corresponding to a dilution of the usually consumed tea by 100-fold. They concluded that components of tea corresponding to oxidized polyphenolic compounds were responsible for inactivation but did not identify specific chemicals accountable for inhibition [28].

Plasminogen activator inhibitor-1 belongs to plasminogen activator system that contains a number of enzymes. Plasminogen is a pro-enzyme that is cleaved by urokinase plasminogen activator (uPA) or tissue plasminogen activator (tPA) to its active form called plasmin that is able to digest proteins of connective tissue and basement membranes. Plasmin is a key enzyme in the mechanism responsible for tissue remodeling, blood clot lysis, tumor invasion, and development of distant metastasis and angiogenesis.

Urokinase plasminogen activator and tPA activators are weak proteolytic enzymes that activate plasminogen to plasmin by proteolytic cleavage. Urokinase plasminogen activator is mostly involved in pericellular proteolysis while tPA mainly mediates intravascular thrombolysis.

There are four known protein inhibitors of uPA/tPA: PAI-1, PAI-2, PAI-3 and a protein called nexin. All are regulatory proteins mediating proteolysis on the activation level. Plasminogen activator inhibitor-1 has a dual function; first, it plays an important role as a direct inhibitor of the plasminogen activation system, and second, its interaction with the adhesive glycoprotein vitronectin plays a role in tissue remodeling and metastasis and possibly other function, which is independent of its proteinase inhibitory properties [30].

Plasminogen activator inhibitor-1 is overexpressed in type 2 diabetes, and elevation correlates with complications of diabetes, unfortunately the link between insulin and up-regulation of PAI-1 regardless of some preliminary work done still remains unclear [31]. For example elevated levels of very low density lipoproteins (VLDL) and triglycerides are biochemical markers of diabetes and other diseases. It was reported that VLDL from diabetic patients increased the generation of PAI-1 from cultured vascular endothelial cells. Authors postulated that heat shock factor-1 (HSF1) is responsible for the transcriptional regulation of PAI-1 in cultured vascular EC or fibroblasts [32]. Furthermore, Ho *et al.* demonstrated that overexpression of Forkhead-related transcription factor FoxO3a augment insulin's

ability to activate the PAI-1 promoter. Using small interfering RNA to reduce the Fox transcription factors tested, they confirmed that reduction of FoxO3a inhibits insulin-increased PAI-1 expression [31, 33].

In addition, elevated glucose and consequent up-regulation of transforming growth factor β (TGF- β 1) mediate PAI-1 overexpression in renal cells. Park *et al.* found that high glucose and TGF- β 1 significantly increased PAI-1 in these cells [34]. It has been shown also that PAI-1 can regulate TGF- β expression by binding to uPAR and activating the extracellular-regulated signal kinase (ERK)/MAPK pathway. Since TGF- β 1 stimulates the PAI-1 promoter, authors suggest that TGF- β 1 and PAI-1 together constitute a positive feedback loop in diabetes [35, 36]. Lassila *et al.* reported that silencing of the PAI-1 gene protects mice against diabetic nephropathy and PAI-1 $-/-$ mice escapes obesity and insulin resistance [37].

The best evidence of the causative role of PAI-1 in diabetes complication were proposed by Nagi *et al.* [38]. They found that *Pima Indians* with very high rate of obesity, insulin-resistant have high rates of diabetes but a low risk of diabetes complications. In contrast to other ethnic groups, PAI-1 activity is similar between non diabetic and diabetic *Pima Indians* [38]. Consequently it has been proposed that PAI-1 may be a therapeutic target in diabetic nephropathy [35]. It was postulated that PAI-1 inhibition might be therapeutic agent in other clinical conditions, such as obesity, diabetes and possibly fibrotic diseases [35, 39].

Loktionov *et al.* found that tea caused a significant decrease of PAI-1 activity but only in the subjects with E2/E3 genotype (mean placebo 7.21 U/ml vs. mean for tea users 5.88 U/ml, $P = 0.007$). Apolipoprotein E (ApoE) has six common isoforms: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4 [40]. We have discovered that some theaflavins of black tea inhibit PAI-1 which might provide some explanation of lower PAI-1 activity in tea lovers [41, 42]. We have searched nutraceutical inhibitors of PAI-1 and unexpectedly found that among four tested theaflavins theaflavin-3'-gallate and theaflavin-3,3'-gallate inhibit PAI-1 while theaflavin and theaflavin-3-gallate do not. Theaflavin-3'-gallate was most potent while theaflavin-3,3'-gallate showed lesser inhibitory activity [41]. Assuming good bioavailability of theaflavins and conversion of other components of black tea (for instance thearubigins) to theaflavins in the acidic environment of the stomach, it is possible to reach their PAI-1 inhibitory concentration in the blood of the black tea heavy drinkers. Based on presented data it is plausible that theaflavins can reduce PAI-1 activity and in that way alleviate symptoms of diabetes.

Mechanism of PAI-1 inhibition remains controversial. Plasminogen activator inhibitor-1 can be inhibited by antibody or small molecule inhibitors. As we reported in our paper monoclonal antibodies can inactivate PAI-1 by different mechanisms: a) by inducing turnover of the PAI-1 protease complex as a substrate; b) by blocking formation

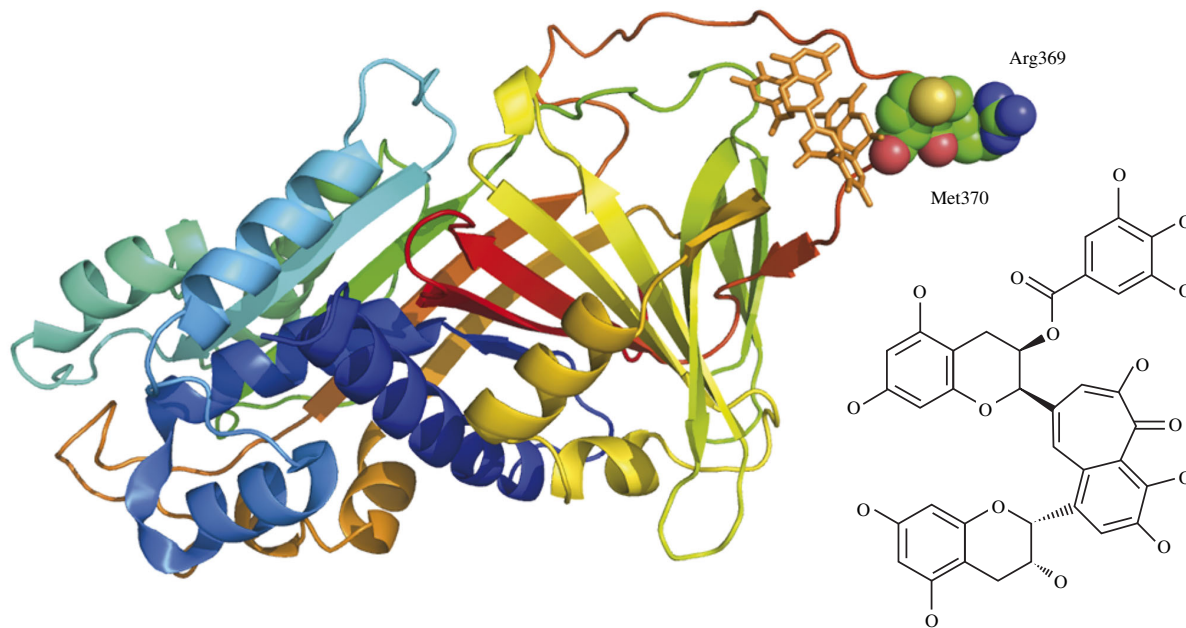


Fig. 1. Ribbon model of PAI-1 molecule (3r4l [51]) colored by rainbow (N terminal – blue; C terminal – red). Active site on reactive center loop is showed as spheres (green – carbon; red – oxygen; blue – nitrogen; yellow – sulfur). The theaflavin-3'-gallate molecule structure is shown in orange sticks in the proximity of PAI-1 active site close to Arg369 and Met 370 of the RCL complexed with PAI-1 and as a schematic drawing in the right lower corner (hydrogens are not shown)

of the Michaelis complex between uPA or tPA and PAI-1; c) by speeding up the transition into the latent form [43, 44]. Also, several small molecule inhibitors have been reported to inhibit PAI-1. For example PAI039 inhibitor binds to the hydrophobic cleft region around α -helices D and E and β -strand 1A of the PAI-1 molecule [45]. The authors hypothesize that inhibition of PAI-1 happens not by prevention of the interaction between PAI-1 and the substrate, but by inhibiting the formation of a stable covalent complex, that has to be formed with uPA or tPA to act on. Gardell *et al.* postulated the different binding site where PAI-749, other small molecule inhibitor blocks formation of the initial, reversible Michaelis complex between PAI-1 and its target protease. This indicates that binding in the proximity of Arg369 and Met370 in the reactive center loop (RCL) of PAI-1 possibly altering the RCL conformation to disable the insertion of Arg369 into the specificity pocket of uPA or tPA [44, 46]. Using molecular modeling methods Izuhara *et al.* [47] found different binding sites for PAI-1 inhibitors. By docking small chemicals into the gap between A3 and A5 β -sheet A of PAI-1 they found several organochemicals that bind to this gap. These novel chemicals were later found to inhibit PAI-1. Nevertheless, the PAI-1 activity neutralizing mechanism in this case remains elusive and difficult to comprehend [41, 44].

To clarify which is the binding site of theaflavin-3'-gallate we have performed molecular dynamics using an open-source program for drug discovery, molecular dock-

ing and virtual screening: AutoDock Vina developed at Molecular Graphics Lab at The Scripps Research Institute following default setup [48]. As it is shown in Fig. 1 theaflavin-3'-gallate molecule binds in the proximity of the active site of PAI-1 molecule including Arg369 and Met370 in such fashion that could restrict interaction of PAI-1 with its substrates (uPA and tPA). Thus we conclude that inactivation of PAI-1 by theaflavin-3'-gallate is caused by its attachment to the reactive center loop.

Summary

It must be stressed that it is challenging to compare the effects of black tea consumption in the absence of basic information on the concentration of theaflavins in the different brands of black tea and their concentration in the cup of tea or even ambiguity of that cup definition (150 ml vs. 250 ml). Also, diverse habits of tea drinking can make some changes in bioavailability of active components such as: tea with lemon (changes in pH) or tea with milk (high in Ca^{2+}) vs. without any of them. There are well documented interactions of theaflavins with Al^{3+} and Fe^{3+} especially in low pH. Other metals can react with theaflavins affecting their bioavailability [49, 50]. Thus water quality could be critical for health effects of black tea. These facts make it difficult to draw conclusions from presented human studies of tea drinkers in different parts of the world. Nevertheless, it is plausible that black tea could have protective or curable

properties against diabetes. However the final conclusion can only be drawn after completion of better controlled clinical studies.

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