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Research Article

ACETYLCHOLINESTERASE INHIBITORY PROPERTIES OF BLACK TEA AND ITS POLYPHENOLIC COMPONENTS

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ABSTRACT

Both infusion and decoction of the four tea varieties were assayed for acetylcholinesterase (AChE) inhibitory property. Theaflavin and thearubigin, the two most exclusive polyphenols of black tea, were also studied for their AChE properties. The present study showed that infusion and decoction of each variety of tea inhibited AChE in a dose dependent manner. There was no significant difference in activity between the varieties. In general the activity of tea decoction was significantly higher than that of the infusion. Theaflavin and thearubigin also showed AChE inhibitory properties in a dose dependent manner. The individual tea extract had higher activity than either theaflavin or thearubigin. The present study suggests that AChE inhibitory activity of black tea could be due to theaflavin, thearubigin and other tea flavonols, phenols or some other constituents and perhaps a combination of some of the constituents acting synergistically.

Keywords: Black tea, Theaflavin, Thearubigin, Acetylcholinesterase inhibitor.

INTRODUCTION

According to the cholinergic hypothesis, the memory impairment in the patients of Alzheimer's disease (AD) results from a deficiency in cholinergic function in the brain. Thus attempts to restore cholinergic function have been a rational target for drugs used to treat the symptoms of AD. Approaches to enhance cholinergic function in AD have included simulation of cholinergic receptors or prolonging the availability of acetylcholine (ACh) released into the neuronal synaptic cleft by inhibiting ACh hydrolysis by acetylcholinesterase (AChE); the latter may be achieved through the use of AChE inhibitors [1]. Physostigmine and tacrine are the only AChE inhibitors reasonably evaluated in AD patients. But their use is limited by the short half-life and peripheral cholinergic side effects of physostigmine, and dose-dependent hepatotoxicity of tacrine [2,3]. The identification of alternative AChE inhibitor with fewer side effects in AD patients is required.

Tea (*Camellia sinensis*, Family Theaceae) is one of the most frequently consumed beverages in the world. Black tea represents approximately 78% of total consumed tea in the world, whereas green tea accounts for approximately 20% of tea consumed [4]. Tea contains a number of bioactive chemicals like caffeine and different polyphenolic compounds e.g. flavonoids. During the manufacture of black tea (BT), the green tea catechins undergo oxidation by polyphenol oxidase to form the complex condensation products theaflavins (TF) and thearubigins (TR), by a process commonly known as "fermentation" [5]. TF and TR are the most exclusive polyphenols of BT [6]. The contents of TF and TR vary with species of tea and the process of "fermentation". Neverthtless, TR is the most abundant polyphenolic fractions in black tea [5]. Black tea and polyphenolic compounds present in it have been found to be efficient antioxidants [7,8,9]. Tea prevents cancer [10], cellular DNA damage [11] and have chemopreventive properties [12,] anti-inflammatory [13], antidiarrhoeal activity [14], antimicrobial activity [15]. Tea may lower the risk of type 2 diabetes [16] and possesses neuroprotective properties under conditions like hypoxia, ischemia and Parkinson's disease [17]. The regular consumption of black and green tea may also provide some protection against hypertension in humans [18]. The plant *C. sinensis* is reported to have memory enhancing properties and demonstrated anxiolytic activity [19]. Black tea consumption reduces total and LDL cholesterol in mildly hypercholesterolemic adults [20]. Recently AChE inhibitory activity of green tea [21] and black tea [22] has been reported. But studies regarding variation of activity in different varieties, effect of mode of extraction on activity and a search for the components responsible for such activity have not been done.

In India black tea is consumed as infusion or as decoction. In this paper both infusion and decoction of four garden varieties of black tea were compared for their AChE inhibitory properties. Such activity of the major polyphenolic compounds of black tea, theaflavin and thearubigin is also reported. This study was carried out in continuation to our search for AChE inhibitors from plants [23-25].

MATERIALS AND METHODS

Plant materials

The garden varieties (Doors tea, Siliguri tea, Guwahati tea) of black tea (CTC, first flush) were collected in 2006 from a tea packaging company. Nilgiri tea (CTC) was collected in 2004.

Chemicals and reagent

Theaflavin [tea extract from black tea containing minimum 80% theaflavins (theaflavin and theaflavin gallates)] was purchased from Sigma. 5,5'-dithiobis (2-nitrobenzoic acid), acetylthiocholine iodide were obtained from Sisco Research Laboratories PVT. Ltd., India. All other reagents were of analytical grade.

Preparation of infusion and decoction of black tea (BT)

Infusion of tea was prepared by soaking BT (20 g) in boiling distilled water (170 ml) for five minutes. The aqueous filtrate was evaporated to dryness under reduced pressure. Decoction of BT was prepared by boiling tea leaves (20 g) in distilled water (170 ml) for 5 minutes. The aqueous extract was filtered and evaporated to dryness.

Preparation of thearubigin

TR was isolated from BT following the method of Misra et al. [5]. BT (6 g) was boiled in 50 ml sodium acetate (10 mM, pH 5.0) for 10 min, cooled and filtered. The filtrate was extracted successively with equal volumes of chloroform, methyl isobutyl ketone and ethyl acetate. The organic layers were discarded and the aqueous layer was extracted with butanol followed by lyophilization. The residual dark orange powder constituted the TR.

Animals

Brains from male mice were obtained from the Central Drugs Laboratory, Kolkata, India.

Acetylcholinesterase inhibitory activity

Acetylcholinesterase inhibitory activity was assayed according to Nag and De [23] modifying the method of Ellman et al. [26] following Oh et al. [27] and Siqueira et al. [28]. Brains from 3-4 weeks old mice were

washed with cold sodium phosphate buffer (0.2M, pH 8), homogenized in buffer and centrifuged at 10,000 RPM at 4 ^{0}C. The supernatant was used as the enzyme source (AChE). The BT extracts were dissolved in distilled water. AChE (1.4 ml) and BT extract solution (100 µl) were added to 1.44 ml buffer. The reaction was started by adding 30 µl 0.5 mM 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) and 30 µl 0.6mM acetylthiocholine iodide solution. The reaction mixture was incubated at 37 ^{0}C for 20 min. The optical density was measured at 412 nm immediately. The percentage inhibition of AChE activity by plant extract was calculated.

Determination of total Phenol content

Total phenol content was determined by Folin-Ciocalteau reagent in alkaline medium [29] and was expressed as thearubigin equivalent (TRE). Total phenol content was calculated from the regression equation prepared from a range of concentrations of thearubigin and optical densities of the concentrations.

Statistical analysis

Each experiment was repeated 3-5 times. Percentage inhibition in activity is presented as mean ± standard deviation. Results have also been analyzed by ANOVA (one way) and Welch's t test.

RESULTS AND DISCUSSION

The four garden varieties studied during the present experiment were Doors tea, Siliguri tea, Guwahati tea and Nilgiri tea. Both infusion (Fig. 1) and decoction (Fig. 2) of these tea varieties showed AChE inhibitory properties. In all the extracts the activity, as determined by the percentage inhibition of AChE activity, were significantly proportional to the concentration (correlation coefficient being > 0.9). Regression equations were prepared from the concentrations of the extracts and percentage inhibition AChE activity. IC_{50} values (concentration of sample required to inhibit 50% AChE activity) were calculated from these regression equations. IC_{50} value is inversely related to the activity.

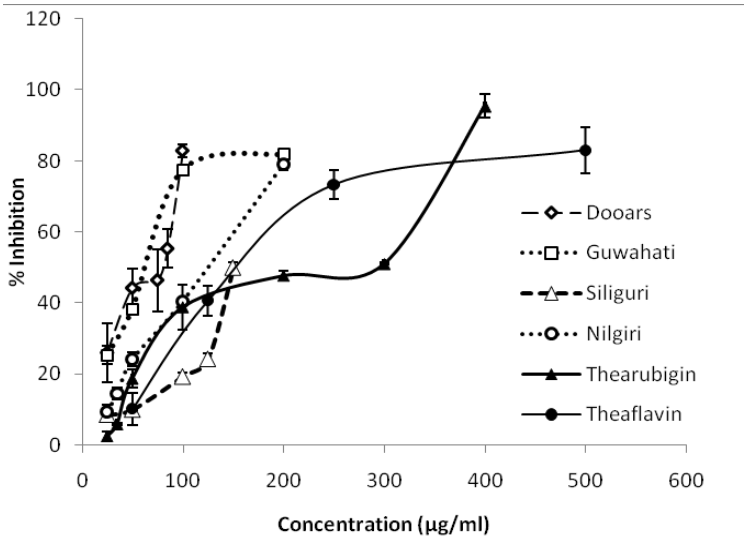

Fig. 1: Acetylcholinesterase inhibitory properties of BT infusions, thearubigin, theaflavin

Decoction of all the varieties showed higher activity than the infusion as evident from the IC_{50} values (Table 1). Welch's t-test also showed that in general the activity of tea decoction was significantly higher than that of the tea infusion (Z > 0.05). Highest activity was observed in Nilgiri decoction. Among the infusions, highest activity was observed in Guwahati tea and the lowest in Siliguri tea. ANOVA (one way) does not show significant differences in activity between the varieties.

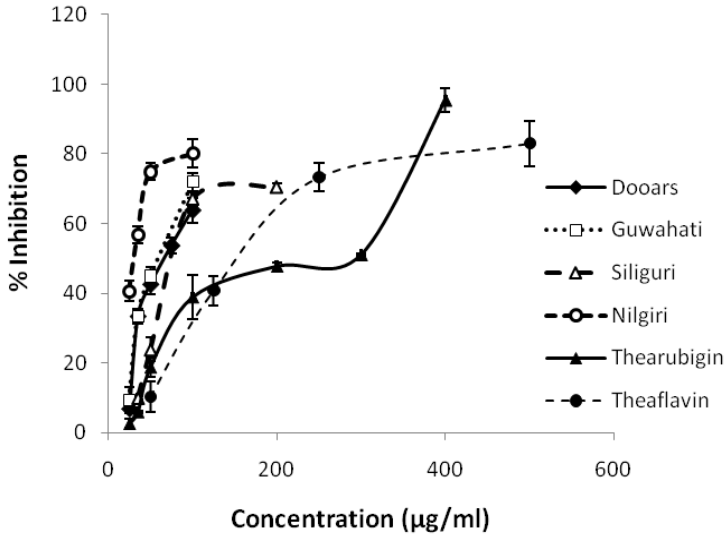

Fig. 2: Acetylcholineterase inhibitory properties of BT decoction, thearubigin, theaflavin

TF and TR are the most exclusive polyphenols of BT [6]. So the AChE activities of these two compounds were also studied. Both TF and TR showed AChE inhibitory properties in a dose dependent manner (Fig. 1 and Fig. 2). Activity of theaflavin was significantly higher than thearubigin. Welch's t-test also showed that the activity of either thearubigin or theaflavin was significantly lower than tea infusions and decoctions (except Siliguri tea). The major TFs in black tea are theaflavin, theaflavin-3-gallate, theaflavin-3`-gallate and theaflavin-3,3`-digallate[30]. TF comprises about 3-5% (wt/wt) of extract solids [31]. However, unlike the TFs, TRs have not yet been characterized [32] and comprise about 20% (wt/wt) of extracted solids [31]. TF exerted anticancer activity by inducing apoptotic signals [33]. TFs prevented cellular DNA damage by inhibiting oxidative stress and suppressing cytochrome P450 1A1 in cell cultures [11]. The antioxidant and anti-mutagenic activities of TFs have been demonstrated [34]. Antimutagenic and anticlastogenic effects of TF and TR in vivo and in vitro in multiple test systems have been reported [35,36]. Both TF and TR could exert inhibition of A431 (human epidermoid carcinoma) and A375 (human malignant melanoma) cell proliferation without adversely affecting normal human epidermal keratinocyte cells [6]. TFs, especially theaflavin-3-gallate, reduced the incorporation of cholesterol into mixed micelles [37]. TFs down-regulated the expression of the androgen receptor in LNCaP prostate cancer cells [38]. TF derivatives had potent anti-HIV-1 activity [39]. TFs were shown to exert potent anti-proliferative and cytotoxic effects on the leukemia WEHI-3B JCS cells in a dose –dependent manner [40]. TR, the major polyphenol of BT, ameliorates mucosal injury in trinitribenzene sulfonic acid induced colitis [41]. In this paper we report AChE inhibitory properties of TF and TR.

Table 1: AChE inhibitory activity and total phenol content of BT extracts

BT samples	Extract	IC_{50} value (µg/ml ± sd)	Total phenol content (µg TRE / µg extract)
Dooars	Infusion	70.43 ± 4.20	5.23±2.7
	Decoction	60.28 ± 2.18	4.18±1.8
Guwahati	Infuion	57.97 ± 1.47	6.33±2.3
	Decoction	52.33 ± 2.19	4.86±0.9
Siliguri	Infuion	153.69 ± 0.78	7.10±1.9
	Decoction	76.07 ± 2.18	4.89±2.0
Nilgiri	Infusion	124.52 ± 1.67	7.10±1.9
	Decoction	30.49 ± 0.86	4.81±0.2
Thearubigin		219.94±4.99	
Theaflavin		154.27±7.75	

Total phenol content (TRE) in each tea variety was measured (Table 1). But there was no correlation between total phenol content and AChE inhibitory properties of different varieties of tea. It was observed that the individual tea extracts had significant higher activity than either TF or TR (except Siliguri tea). Caffeine did not inhibit AChE. This finding suggests that the AChE inhibitory property of BT could be due to TF, TR and other tea flavonols, phenols or some other constituents and perhaps a combination of some of the constituents acting synergistically. The activity of TF and TR on animal model remains to be investigated.

CONCLUSION

The present study with four garden varieties of black tea indicated that both infusion and decoction of these tea varieties showed acetylcholinesterase inhibitory properties in a dose dependent manner. Theaflavin and thearibigin, the two most exclusive polyphenols of black tea, also showed acetylcholinesterase inhibitory properties in a dose dependent manner.

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