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Short Communication

Acetylcholinesterase Inhibitory Activity of Green Tea Polyphenols

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Abstract

Inhibition of acetylcholinesterase activity is one of the most popular approaches for treatment of neurological disorders such as Alzheimer's disease and others. In the present study, we evaluated inhibition of acetylcholinesterase activity by different concentrations of green tea (*Camellia sinensis* L.) extract using acetylthiocholine as substrate. The green tea extract inhibited AChE activity dose dependently with an IC_{50} value of 42.05µg/ml. The observed inhibitory activity could be ascribed to the polyphenolic content of green tea extract. Consumption of green tea might provide protection against neurological disorders.

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INTRODUCTION

Acetylcholine is a neurotransmitter which plays a key role in memory and cognition. Acetylcholinesterase (AChE) is an enzyme that causes the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Hence, inhibition of AChE is an important strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia, myasthenia gravis and Parkinson's disease. Drugs such as tacrine, donepezil, and rivastigmine have been used to treat cognitive dysfunction and memory loss associated with Alzheimer's disease. These drugs have shown to slow down neurodegeneration process. However, these compounds have adverse effects including gastrointestinal disturbances and problems associated with bioavailability. This necessitates an immense interest in searching better AChE inhibitors from natural resources. Ethnopharmacological studies and bioassay-guided isolation have provided a lead in identifying novel and potent AChE inhibitors from plants (Mukherjee *et al*., 2007; Ohran *et al*., 2008; Lu *et al*., 2011).

Tea (*Camellia sinensis* L., family Theaceae) is one of the most popular beverages consumed all over the world. It is consumed as green, black, or Oolong tea. Among these, significant effects on human health have been observed with the consumption of green tea. Green tea is manufactured by drying fresh tea leaves. Green tea is non-fermented and the beneficial effects of green tea are mainly due to its polyphenols which may account for up to 30% of dry weight. Catechin is the one of the most important phenolic constituent of green tea. Green tea extract and its components have shown to exhibit activities such as anticancer, hepatoprotective, antimicrobial, antioxidant, neuroprotective and others (Cabrera *et al*., 2006; Chacko *et al*., 2010; Jo *et al*., 2012). In our previous study, we have undertaken an extraction and examination of chemical constituents of green tea for its *in vitro* antioxidant activity (Raghavendra *et al*., 2011). The present study focused on the AChE inhibitory activity of green tea extract.

MATERIALS AND METHODS

Chemicals

Electric eel AChE, Acetylthiocholine iodide and 5-5'-thiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (USA). Eserine was obtained from Merck (Germany). All other reagents were of analytical grade.

Preparation of Green Tea Extract

Green tea extract was prepared by following the methodology employed in our previous study (Raghavendra *et al*., 2011). The green tea extract at different concentrations (1-100µg/ml) was used to screen AChE inhibitory activity.

AChE Inhibition Assay

AChE inhibition assay of the green tea extract was carried out according to the method of Orhan *et al*. (2007) with some modifications. Here, 250µl of extract/standard of various concentrations in 200mM phosphate buffer (pH

7.7), 80µl of DTNB (3.96mg of DTNB and 1.5mg sodium bicarbonate dissolved in 10ml phosphate buffer pH 7.7) and 10µl of enzyme (2U/ml) was incubated at 25^{o}C for 5min. After incubation, 15µl of the substrate (acetylthiocholine iodide: 10.85mg in 5ml of phosphate buffer) was added and incubated again for 5min. The color so developed was measured in a microwell plate reader at 412nm (Versamax, Molecular Devices, Sunnyvale, USA). The percent inhibition was calculated using the formula:

AChE inhibitory activity (%) = (*Ac* - *At* / *Ac*) × 100, where *Ac* is absorbance of control and *At* is absorbance of test. Eserine hemisulphate was used as the standard drug.

Statistical Analysis

The extract and standard were assayed in triplicate and the result was recorded as Mean inhibition (M.I) ± Standard deviation (S.D). The IC_{50} (Inhibitory concentration) values were determined by log-probit analysis.

RESULTS

The results of the AChE inhibitory activity of green tea extract and standard were depicted in Table 1. Green tea extract showed 66.46±1.38 % of AChE inhibitory activity at 100µg/ml concentration and Eserine hemisulphate showed 93.87±0.96% of in inhibitory activity at 0.25µg/m. IC_{50} value of green tea extract and standard was 42.05 and 0.02µg/ml respectively.

Table 1: AChE inhibitory activity of Green tea extract and standard Eserine hemisulphate

Treatment	Concentration (µg/ml)	M.I±S.D	IC_{50} (µg/ml)
Green tea extract	1	06.41±1.29	**42.05**
	50	49.89±1.43	
	100	66.46±1.38	
Eserine hemisulphate	0.01	28.16±0.98	**0.02**
	0.025	57.01±0.77	
	0.05	73.04±1.03	
	0.10	87.91±0.82	
	0.25	93.87±0.96	

MI: Mean % inhibition; S.D: Standard Deviation

DISCUSSION

It is forecasted that 5% of the global population will be aged 85 years or over by 2034 and it inevitably lead to an increase in age-associated disorders such as Alzheimer's disease (Okello *et al*., 2012). Alzheimer's disease is one of the neurodegenerative disorders resulted by the loss of cholinergic neuromediators in the brain and enhanced AChE activity. This disease is the most common cause of dementia leading to the loss of intellectual and social abilities severe enough to interfere with daily functioning. The remarkable biochemical change which can be seen in neurodegenerative diseases is the reduction of ACh levels in the hippocampus and cortex of the brain. Therefore, inhibition of AChE is presently the most established approach to treating Alzheimer's disease (Ohran *et al*., 2008; Okello *et al*., 2012).

There is high evidence that green tea exhibits a number of health-promoting effects. It may beneficial potentially to those suffering from neurodegenerative diseases, cardiovascular disease and cancer. The beneficial effects of green tea are mainly attributed to the high polyphenol content in particular catechins (Okello *et al*., 2012). In the present study, we evaluated AchE inhibitory activity of green tea extract. AChE hydrolyses ACh to give thiocholine and acetate. The reaction between thiocholine and DNBT gives 2-nitro-5-mercaptobenzoate, a yellow compound which is measured at 412 nm. The green tea extract exhibited dose dependent inhibition of AchE activity with an IC_{50} value of 42.05µg/ml. It has been shown earlier that white and green tea extract and purified tea compounds exhibit AChE inhibitory activity (Okello *et al*., 2012). The seed and pericarp of tea were shown to possess AChE inhibitory activity (Jo *et al*., 2012).

CONCLUSION

The experimental data obtained from the present study showed that green tea extract exhibit potent *in vitro* AChE inhibition activity. The further *in vivo* research is undertaken in order to study the exact mechanism of action of green tea polyphenols.

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