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Protective effect of black tea extract against aluminium chloride-induced Alzheimer's disease in rats: A behavioural, biochemical and molecular approach

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ABSTRACT

Aluminium is reported to play an important role in the aetiology, pathogenesis and development of Alzheimer's disease (AD). Black tea (BT, *Camellia sinensis*, family – Theaceae) represents approximately 78% of total consumed tea in the world and possesses neuroprotective properties under conditions like hypoxia, ischaemia and Parkinson's disease. This research aimed to evaluate neuroprotective effect of black tea extract (BTE) on the cognitive deficits, activity of acetylcholinesterase (AChE), levels and activities of oxidant–antioxidant indices (thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx)), expressions of β amyloid 1–42 ($A\beta_{1-42}$) synthesis related (amyloid precursor protein (APP), β and γ secretases) and apoptotic markers (Bax, Bcl-2, cyto c, caspases 3, 8 and 9) in hippocampus and cortex of aluminium chloride ($AlCl_3$) induced AD rats. Chronic $AlCl_3$ administration (100 mg/kg body weight *i.p.*) in Wistar rats for 60 days significantly enhanced the AChE activity, memory impairment, oxidative damage, Aβ burden and apoptosis markers. Co-administration of BTE to $AlCl_3$ rats for 60 days significantly ameliorated the aluminium induced pathological changes. Thus, it is suggested that the anti-Alzheimer role of BTE may be attributed mainly to the active components present in black tea.

1. Introduction

Alzheimer's disease (AD) is an age related progressive neurodegenerative ailment characterized by the presence of intracellular amyloid aggregates and extracellular neurofibrillary tangles. The earliest stage in AD is characterized mainly by short term memory loss and in advanced stages; it is manifested by confusion, aggression, mood changes, long term memory loss and social withdrawal (Waldemar et al., 2007). The cholinergic system, especially the basal forebrain projections to hippocampus and cortex, is responsible for memory and learning (Cain, 1998). Aluminium is a potent cholinotoxin (Gulya, Rakonczay, & Kasa, 1990) that causes apoptotic neuronal loss in hippocampus and degeneration of cholinergic terminals in the cortical areas. In addition, aluminium promotes the

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formation and aggregation of amyloid-β plaques and tau, (microtubule-associated proteins of neurons that are involved in the assembly and stabilization of microtubules) tangles (Walton & Wang, 2009), which are the major hallmarks of AD.

Aluminium is one of the most abundant, non-essential metals existing in our environment and is a constituent of cooking utensil, medicine and drinking water, that gain easy access into the body via air, food and water (Ochmanski & Barabasz, 2000; Yokel, 2000). Tea is one of the most widely consumed beverage in the world that represents a major dietary source of aluminium and its concentration in tea infusions ranges between 0.035 and 16.82 mg/l (Karak & Bhagat, 2010). Studies by Yokel and Florence (2008) showed that the oral bioavailability of aluminium from the tea beverage was 0.37%. Powell, Greenfield, Parkes, and Thompson (1993) attributed this low bioavailability to the action of polyphenols in tea, which binds strongly to aluminium, thus preventing its intestinal absorption. Flaten (2002) has reported that both black and green tea consumption leads to measurable, but moderate increase in urinary aluminium excretion and that aluminium present in tea is not much more bioavailable than that from any other sources (Gardner & Gunn, 1995). Mehra and Baker (2007) reported that the moderate intake of tea is unlikely to have any harmful effects on healthy individuals.

Antioxidant therapy is one of the promising therapeutic strategies for AD that prevents the onset of the disease by sequestering the primary targets and reduces the secondary pathologies, slows disease progression or delay onset of disease, leads to the termination or even the repair of neuronal damage after onset of disease and eventually prevents the development of AD. Tea contains antioxidants such as flavonoids, carotenoids, tocopherols and ascorbic acid, among others. There are three types of tea, classified by the degree of fermentation: green (unfermented), oolong (partially-fermented) and black (fully fermented) tea (Haslam, 2003). Among three types of tea, black tea (BT) is the most popular tea produced and consumed worldwide which is 78%, followed by 20% for green tea, and less than 2% for oolong tea (Liu & Huang, 2015). The most potent group of tea components, which influences human health benefits is polyphenols, in particular the catechins (Ferruzzi & Green, 2006). In BT, catechins such as [-]-epicatechin and its gallate derivatives are observed to be present in small amounts which is supposed to be the result of the formation of theaflavins and thearubigins (Balentine, Wiseman, & Bouwens, 1997). Other related compounds observed to be present in tea are myricetin, conjugates of quercetin and kaempferol, gallic acid, quinic esters of gallic, coumaric, and caffeic acids, purine alkaloids such as theobromine and caffeine, proanthocyanidins and traces of flavones (Senanayake, 2013).

The constituents of BT offered neuroprotection against 6-hydroxydopamine induced in *in vitro* and *in vivo* models of Parkinson's disease (Chaturvedi et al., 2006; Levites, Youdim, Maor, & Mandel, 2002), stroke (Larsson, Virtamo, & Wolk, 2013) and cerebral ischaemia (Vlasov, 2012). BT extract (BTE) displayed neuroprotective effect against Aβ-induced cytotoxicity in primary culture of rat hippocampal cell (Bastianetto, Yao, Papadopoulos, & Quirion, 2006). Although therapeutic activities of green tea on experimental AD (Jelenković et al., 2014) and *in vitro* acetylcholinesterase inhibitory effect of anthocyanin-rich red leaf tea have been studied (Maeda-Yamamoto et al., 2012), no detailed mechanistic studies have been carried out to shed light on anti-alzheimeric role of BT. Therefore, the present study aimed to evaluate the activity of AChE, oxidative–antioxidative indices and expressions of Aβ biosynthesis related and apoptotic markers in hippocampus and cortex of aluminium induced AD rats.

2. Materials and methods

2.1. Chemicals

Aluminium chloride, anti-β-Amyloid, anti-γ-secretase, anti-β-secretase, anti-amyloid precursor protein (rabbit) and anti-β-actin (mouse), horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG were purchased from Sigma-Aldrich, Bangalore, India and used in this study. Anti-rabbit Bax, Bcl-2, cytochrome c (cytosol and mitochondria) caspase 3, 8, 9 antibodies were obtained from Cell Signaling. Dry BT leaves were purchased from Assam (India). All other chemicals used were of analytical grade.

2.2. Characterization of black tea

2.2.1. Analysis of total polyphenols

Total phenolics of BTE were estimated spectrophotometrically using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). The extract (125 μl) was mixed with 125 μl of Folin–Ciocalteu reagent along with 500 μl of distilled water and allowed to stand for 5 min at 22 °C. Then, 4.5 ml of sodium bicarbonate solution (7%) were added to the mixture. After 90 min, absorbance was measured at 765 nm against the control. Total polyphenols were calculated and expressed as gallic acid equivalents (mg gallic acid equivalents/100 g).

2.2.2. Estimation of theaflavin and thearubigins

Estimation of theaflavin (TF) and thearubigins (TR) of the BTE was carried out following the method of Angayarkanni, Palaniswamy, Murugesan, and Swaminathan (2002). Briefly, in a separatory funnel equals amount of extract and iso-butyl methyl ketone were added. After separation, the resultant organic layer was diluted with 9 ml of ethanol; absorbance (380 nm) was read and considered as A. In the next step, 10 ml of organic phase were diluted by adding 10 ml of Na_2HPO_4 (2.5%). The separated layer was again estimated and termed as B. Lastly, butanol treated aqueous phase was eluted with 9 ml of ethanol and the absorbance at 380 nm, named as C.

$$TF\ (\%) = 4.313 \times C$$

$$TR\ (\%) = 13.643 \times (A + C - B)$$

2.2.3. Determination of catechins

Total catechins in the extracts were estimated through vanillin–HCl method using UV/vis Spectrophotometer at 500 nm (Ayumiko, Sumikotsuji, & Tonogai, 2003).