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Research report

β-glucan attenuated scopolamine induced cognitive impairment *via* hippocampal acetylcholinesterase inhibition in rats

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ABSTRACT

β-glucan (polysaccharide) rich diet has been reported to enhance cognition in humans but the mechanism remained elusive. Keeping this in mind, the present study was designed to investigate the interaction of β-glucan with central cholinergic system. Briefly, *in-silico* analysis revealed promising interactions of β-glucan with the catalytic residues of acetylcholinesterase (AChE) enzyme. In line with this outcome, the *in vitro* assay (Ellman's method) also exhibited inhibition of AChE by β-glucan (IC_{50} $=0.68 \pm 0.08$ μg/μl). Furthermore, the *in vivo* study (Morris water maze) showed significant dose dependent reversal of the amnesic effect of scopolamine (2 mg/kg *i.p.*) by β-glucan treatment (5, 25, 50 and 100 mg/kg, *i.p.*). Finally, the hippocampi of aforementioned treated animals also revealed dose dependent inhibition of AChE enzyme. Hence, it can be deduced that β-glucan possesses potential to enhance central cholinergic tone *via* inhibiting AChE enzyme. In conclusion, the present study provides mechanistic insight to the cognition enhancing potential of β-glucan. Keeping in mind its dietary use and abundance in nature, it can be considered as economic therapeutic option against cognitive ailments associated with decline in cholinergic neurotransmission.

1. Introduction

Cognition is affected in number of disorders such as Alzheimer's disease (AD). Learning and memory are the important components of the cognitive system. The former is the process of acquiring new information while later is the phenomenon of storing that information for future use. Central cholinergic system has been shown to play an important role in cognitive functions. In this regard, the degeneration of cholinergic neurons have been attributed to the cognitive impairment observed in AD subjects (Schliebs and Arendt, 2011). Furthermore, the antagonism of cholinergic system by scopolamine (a non-selective muscarinic antagonist) was shown to induce deficits in acquisition, retention, consolidation and retrieval of memory (Deiana et al., 2011; Stevens, 1981). Hence, enhancement of the cholinergic tone can presumably revert the cognitive impairment (Anand et al., 2014; Dumas and Newhouse, 2011; Haense et al., 2012).

Keeping in view this hypothesis, several strategies were devised to compensate the cholinergic deficit. This includes the use of ACh precursors and agonists of nicotinic and muscarinic receptors. Unfortunately, none of these showed efficacy because of bioavailability, safety and selectivity issues (Fisher, 2000; Francis et al., 1999; Mangialasche et al., 2010). However, the inhibitors of AChE enzyme appeared to be effective in attaining the desired therapeutic objectives. As a consequence, few inhibitors are developed and being used clinically such as donepezil, rivastigmine, galantamine and tacrine (Hasselmo, 2006; Takada-Takatori et al., 2006). Based on the cost and safety profile of these limited AChE inhibitors, there is a need to identify better candidate molecules.

Morris water maze (MWM) is a behavioural paradigm commonly used to assess spatial learning in rodents (Morris, 1984). Spatial memory is a sub-class of episodic memory, which helps in navigation and stores information in spatiotemporal frame (Burgess et al., 2002). Cognitive map theory proposed that there is a direct relation of spatial memory with the hippocampus (O'Keefe and Nadel, 2011). It is a brain structure, which is considered as cradle of cognition and critical for organization, formation and retrieval of new information (Graves et al., 2012).

The β-glucan is a polysaccharide (*i.e.* a chain of glucose molecules) found abundantly in nature such as oat, barley, rye, wheat, mushroom, fungi and yeast. It was reported to possess

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; MWM, Morris water maze; SIL, scopolamine induced locomotion; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus

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neuroprotective and immunomodulatory actions and reduces oxidative stress (Alp et al., 2012; Chan et al., 2009; Goodridge et al., 2009; Kulicke et al., 1997). It is also reported to improve spatial memory deficits in Sprague Dawley rats (Han et al., 2010; Nelson et al., 2012) and preserved memory in a mouse model of vascular dementia (Han et al., 2010). However, the mechanism underlying aforementioned cognition enhancing actions remain elusive. Keeping this in mind, the present study was designed to investigate the interaction of β-glucan with central cholinergic system.

2. Results

2.1. In-silico analysis

The docking results revealed that hydroxyl groups of middle glucose unit of β-glucan formed hydrogen bonding with SER125, THR83, TYR337 and TYR341 amino acid of active site of AChE. Furthermore, one of the glucose of β-glucan at the terminal end formed a vital interaction with important catalytic residue (SER203) of active site through hydrogen bonding. Thus, β-glucan was found to have very high affinity for active site of AChE enzyme as shown by interaction in Fig. 1(A) and (B).

2.2. In-vitro analysis

Donepezil treatment caused significant reduction in AChE enzyme action as compared to control as shown in Fig. 2(A) ($F(1, 4)=2080.546$, $p<0.001$). In similarity, β-glucan exhibited dose dependent inhibition of AChE enzyme as shown by the continuous decrease in the absorbance ($F(6, 14)=3394.258$, $p<0.001$). The IC_{50} value of β-glucan was found to be 0.68 ± 0.08 μg/μl (Fig. 2(B)).

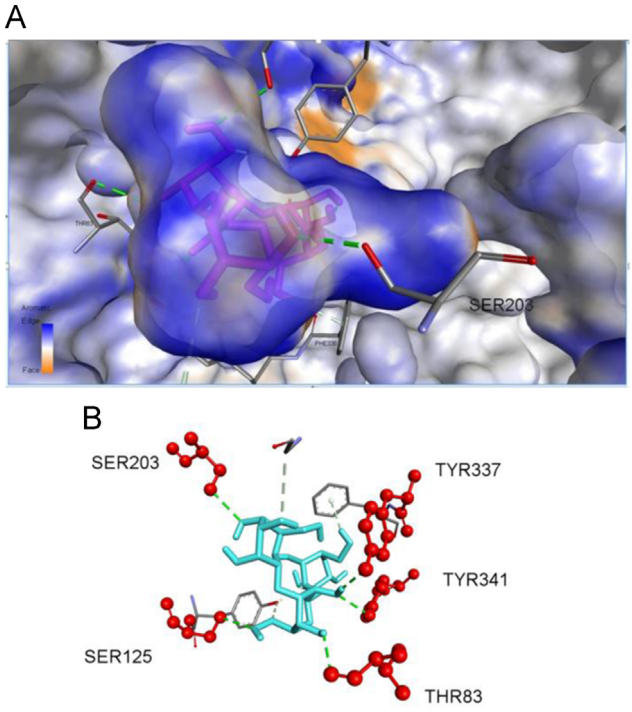

Fig. 1. (A) Representation of β-glucan in the active site of the AChE enzyme: Oxygen atom (red) of glucose subunit forms hydrogen bonding (green dotted line) with hydroxyl group (red) of Ser203. (B) Diagram showing chemical interaction of binding residues of β-glucan with AChE enzyme: Middle glucose unit hydroxyl groups of β-glucan made hydrogen bonding with SER 125, THR83, TYR337 and TYR341 of active site, while terminal glucose make interaction with catalytic residue (SER203).

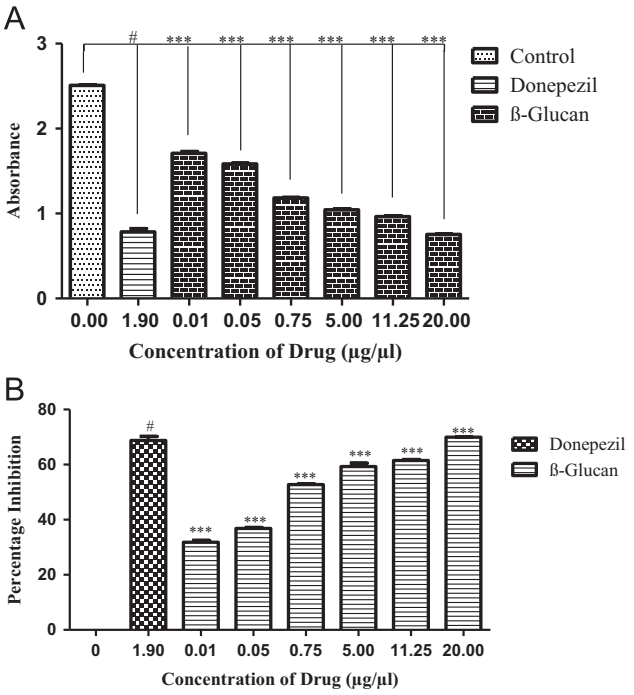

Fig. 2. (A) The Bar graph showing effect of β-glucan and donepezil on the absorbance in *in-vitro* AChE assay. The experiments were performed on different concentrations of β-glucan (0.01, 0.05, 0.75, 5.0, 11.25 and 20.0 μg/μl) and donepezil (1.90 μg/μl). The bars represent mean ± SEM of absorbance. Asterisks (#) and (***) suggest $p<0.001$ as compared to control. (B) The Bar graph showing effect of β-glucan and donepezil on the percentage inhibition of AChE enzyme *in-vitro*. The experiments were performed on different concentrations of β-glucan (0.01, 0.05, 0.75, 5.0, 11.25 and 20.0 μg/μl) and donepezil (1.90 μg/μl). The bars represent mean ± SEM of percent inhibition. Asterisks (#) and (***) suggest $p<0.001$ as compared to control.

2.3. In-vivo analysis

2.3.1. Training/acquisition trial

All the groups showed significant decline in the escape latencies (time to find platform position) on day 5 as compared to respective day 1, as shown in Fig. 3.1(A)–(G).

2.3.2. Probe trial

2.3.2.1. Time spent in platform quadrant. The rats treated with scopolamine showed significant impairment in time spent in the platform quadrant as compared to the normal saline treated rats as shown in Fig. 3.2 ($p<0.001$, ($F(1, 14)=7.889$)). The donepezil treatment has significantly reversed this effect by increasing the time spent in platform quadrant as compared to scopolamine treated rats ($p<0.001$, $F(2, 21)=4.405$). In similar manner, the β-glucan treatment also caused dose dependent increase in the time spent in the platform quadrant ($F(5, 42)=2.648$).

2.3.2.2. Latency to find previous platform position. The scopolamine treated rats showed significant impairment in latency to find previous platform position as compared to the rats treated with normal saline as shown in Fig. 3.3 ($p<0.001$, ($F(1, 14)=13.081$)). The treatment with donepezil significantly antagonized this effect by decreasing the latency time as compared to scopolamine treated rats ($p<0.001$, ($F(2, 21)=9.627$)). In similarity with standard, the β-glucan treatment also caused dose dependent decrease in latency time to find previous platform quadrant ($F(5, 42)=6.514$).

2.3.2.3. Number of crossing through platform position. Rats treated with scopolamine showed significant impairment in number of crossings through platform position as compared to rats treated