Biomedicine & Pharmacotherapy 108 (2018) 374–390

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Neuroprotective effects of *Bergenia ciliata* on NMDA induced injury in SH-SY5Y cells and attenuation of cognitive deficits in scopolamine induced amnesia in rats

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ARTICLE INFO

Keywords:
Bergenia ciliate
SH-SY5Y
Scopolamine
Streptozotocin
NMDA
Cholinesterase
Alzheimer's disease

ABSTRACT

Bergenia ciliata (Haw) Sternb. possess immunomodulatory, anti-inflammatory, antioxidant, anti-urolithiatic, wound healing, anti-malarial, anti-diabetic and anti-cancer properties. Moreover, the methanolic extracts of the rhizomes of the plant were found to demonstrate beneficial neuroprotective effects in the intracerebroventricular streptozotocin-induced model in rats. Thus, the present study was undertaken to further explore the neuroprotective potential of the aqueous (BA) and methanolic extracts (BM) of *B. ciliata* through various *in-vitro* and *in-vivo* studies. Both the extracts at all tested concentrations i.e. 50–50,000 ng/mL did not cause any significant reduction of cell viability of SH-SY5Y cells when tested for 48 h when assessed through MTT and resazurin metabolism- based cell viability assays. The pre-treatment with the extracts could confer significant ($p < 0.001$) and dose-dependent protective effects against NMDA induced injury in SH-SY5Y cells. BM [IC_{50}: 5.7 and 5.19 µg/mL for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) respectively] led to more potent inhibition of both the enzymes as compared to BA (IC_{50}: 227.12 and 23.25 µg/mL for AChE and BuChE respectively). BM also proved to be a 1.85-fold better scavenger of the DPPH free radicals as compared to BA. Thus, BM was taken further for the evaluation of the beneficial effects of 14-day pre-treatment in rats in the scopolamine (2 mg/kg, i.p.) induced amnesia model at 125, 250 and 500 mg/kg, p.o. BM pre-treatment at 250 and 500 mg/kg could significantly ameliorate the cognitive impairment ($p < 0.001$), inhibit AChE ($p < 0.001$) and BuChE ($p < 0.05$) activity, restore GSH levels ($p < 0.05$) in serum and brain homogenates and recover the morphology of hippocampal neurons back to normal. Moreover, the BM administration at 500 mg/kg also showed beneficial effects through the significant ($p < 0.05$) reduction of $A\beta_{1\text{-}42}$, phosphorylated tau (p-tau) and GSK-3β immunoreactivity in the brain homogenates of the intracerebroventricularly streptozotocin (ICV STZ) injected rats as observed from the results of the ELISA assays. The outcomes of the study unveiled that BM exerts its beneficial effects through prevention of NMDA induced excitotoxic cell death, dual cholinesterase inhibition, antioxidant activity coupled with the reduction of the immunoreactivity for the $A\beta_{1\text{–}42}$, p-tau and GSK-3β indicating its potential to be screened further for various other models to determine the exact mechanism of action.

1. Introduction

Loss of cognitive function observed through impairment in learning and memory is one of the major manifestations of dementia [1]. The cholinergic neurons located in the hippocampus, basal forebrain and cerebral cortex are responsible for cognition and their destruction is responsible for the decrease in cholinergic activity and leads to the occuance of various cognitive deficits [2]. Targeting the acetylcholinesterase (AChE) enzyme which is responsible for hydrolysis of ACh into choline and acetate has proved of great importance in the management of dementia. Butyrylcholinesterase (BuChE) along with AChE helps in the maintenance of many non-cholinergic functions neurite outgrowth, cell proliferation and neurogenesis [3]. There are existing reports which state that manipulation of BuChE gene leads to the inflection of proliferation, differentiation, and cellular apoptosis. A study has also revealed that BuChE inhibitor leads to significant amelioration of scopolamine-induced reduction in neuroblast differentiation and cell proliferation in the dentate gyrus of rats without significantly affecting mature neurons [4]. Thus, cholinomimetics and cholinesterase inhibitors have drawn a great attention as they are

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https://doi.org/10.1016/j.biopha.2018.09.011
Received 9 June 2018; Received in revised form 2 September 2018; Accepted 3 September 2018

capable of increasing the compromised ACh levels, recovering cholinergic neurotransmission and thereby provide symptomatic benefits through improved cognition [5]. The persistent cholinergic deficit has been reported to be associated closely to the amyloid β and tau pathology [6]. The amyloid plaques and NFTs are distributed majorly in the cortical and few subcortical regions. $A\beta_{1\text{-}42,}$ the main component of senile plaques, occurs in the form of both diffuse or scattered and cored or condensed plaques. The compact ones coexist with neurites containing tau and mutilate the brain tissues by induction if microglia and activation of astrocytes whereas the disseminated ones exist intermixed with the neuronal processes without affecting their normal function. NFTs formed by deposition of paired helical filaments (PHFs) exist in the neuritic processes and the cytoplasm of neuronal cell bodies. Oxidative stress also plays a central role in the pathogenesis of dementia causing eventual neurodegeneration and addressing this issue becomes essential to attain symptomatic relief in the observed cognitive deficits [7].

N-methyl-D-aspartate receptors (NMDARs) have been implicated in brain development, brain function, learning, and memory by playing an integral role in synaptogenesis, long-term potentiation, long-term depression, synaptic remodeling *etc*. via Ca^{2+} influx through glutamate-gated ion channels. The excessive stimulation of the NMDA receptors portrays enhanced permeability to various ions like Ca^{2+}, $Na^{+,}$ and K^{+} ions but the neuronal cell death is directly proportional to the amount of Ca^{2+} influx [8]. Intracellular Ca^{2+-}dependent cascades are activated along with upregulation of various detrimental signaling pathways, production of excessive reactive oxygen/nitrogen species (ROS/RNS) occurs in such conditions leading to the cell demise [9,10]. The abnormal expression of NMDARs is linked with neurodegenerative disorders adversely affecting cognition and they are also identified as an inducer of neuronal apoptosis and necrosis because of excitotoxic effects [11]. Memantine, a USFDA approved NMDAR antagonist, has been proven to be effective in dementia and cognitive dysfunction [12]. Thus, finding agents which could act as an NMDAR antagonist and reduce Ca^{2+}-mediated excitotoxicity induced by glutamate is imminent [13].

The various studies have undergone in pursuit of drugs for management of dementia since long but only a few have been approved for the same. Donepezil (DPZ), rivastigmine and galantamine are the acetylcholinesterase (AChE) inhibitors approved for providing symptomatic relief in dementia related to Alzheimer's Disease (AD). DPZ and galantamine are responsible for reversible inhibition of cholinesterases but rivastigmine leads to pseudo-irreversible inhibition of AChE and BuChE by transferring a carbamate moiety to the serine residue in their active sites which is succeeded by gradual hydrolysis [14]. These agents have vomiting, diarrhea, hepatotoxicity, bradycardia, *etc*. manifested as side effects owing to their cholinergic provocation [15,16]. The antioxidant therapy is also being explored widely in the recent years as the oxidative stress plays a fundamental role in the pathogenesis of various neurodegenerative disorders [17]. These data are suggestive that the agents with good antioxidant properties and contemporaneously targeting both the cholinergic neurotransmission and glutamate-induced excitotoxicity can afford beneficial effects in dementia and associated neurodegenerative conditions [14].

The accumulated experience regarding the benefits of the natural products has lately evoked interest in the search of plant-based remedies for the management of neurodegeneration [18–20]. Due to the existence of numerous interrelated mechanisms in various neurological disorders, the use of single molecule which can target only a specific aspect in these cascades doesn't suffice the need of producing an acceptable therapeutic response as they can only lead to the symptomatic relief of only some aspects of the disease, but the disease progression stays unhampered. The concoctions prepared using various drugs also pose complications while determining the proportions of the mixtures to be used. Moreover, the threat of drug-drug interactions, drug resistance and the presence of adverse effects is undeniable. Therefore, the use of crude extracts offers an added advantage of having multiple bioactives acting through various mechanisms which are desirable for the treatment of such diseases which involve multiple interdependent pathways and different targets [10,21,22].

The crude extracts of *Bergenia ciliata* (Haw) Sternb. belonging to family Saxifragaceae have been proven to possess anti-oxidant, anti-inflammatory, anti-urolithiatic, wound healing, anti-malarial, anti-diabetic and anti-cancer properties [23,24]. The plant consists of a concoction of bioactives like bergenin, gallic acid, catechin, tannic acid, β-sitosterol, stigmasterol, *etc*. belonging to the various classes of compounds like isocoumarins, polyphenols, phenolic acids, phytosterols, glycosides, *etc*. In Ayurvedic texts, the plant has been mentioned to be used for lithiasis and in the renal calculus disease [25]. The folk in the Chitwan region of Nepal use this plant to alleviate the symptoms associated with Parkinson's disease and other CNS disorders owing to its anti-inflammatory, analgesic and stimulant effects [25]. Traditional use of the plant indicates its application in digestive disorders, gastrointestinal complaints, diabetes and pyrexia and mentions it to be consumed with ghee [26]. *B. ciliata* crude extracts and bioactives derived from the plant have been reported to possess antidiabetic and hypoglycemic properties in various animal models [27,28]. Moreover, our previous studies have demonstrated beneficial neuroprotective effects of the methanolic extracts of *B. ciliata* rhizomes in the intracerebroventricular streptozotocin (ICV STZ) induced model in rats through significant improvement in cognitive function, cholinesterase inhibition, assuagement of oxidative stress and improvement in histopathological parameters [24].

Thus, the present study was undertaken to explore the neuroprotective potential of the aqueous (BA) and methanolic extracts (BM) of *B. ciliata* rhizomes through various *in-vitro* and *in-vivo* studies. The SH-SY5Y neuroblastoma cells were selected for preliminary screening of the cytotoxic effects of the extracts and to assess the preventive effects of extracts against the NMDA induced excitotoxic cell death because these cells closely mimic the high calcium influx induced cell demise [9]. Scopolamine hinders cholinergic neurotransmission being an antagonist for the muscarinic ACh receptors. It inhibits the depolarization of these receptors on being injected intraperitoneally and helps in simulating the phenomenon of cognitive impairment occurring in the humans [6,29]. Extracts of *Gladiolus dalenii* [1], *Quassia undulata*, *Tetrapleura tetraptera* [30], *etc*. and many polyherbal formulations [31] have been proven for their beneficial effects in the scopolamine-induced amnesia in rats. Hence, this model was selected being a well-established and widely used prototype to explore the potential therapeutic applications of the drugs in dementia [1]. Further, ICV STZ induced model was selected to check the effects of *B. ciliata* extract on the immunoreactivity to $A\beta_{42}$, hyperphosphorylated tau protein (p-tau) and GSK-3β to further illuminate its neuroprotective effects. ICV STZ impairs metabolism of insulin in the brain by entering the hippocampal and dentate gyrus neurons through glucose transporter 2 (GLUT2) [32]. Along with replicating the features of cholinergic discrepancies, oxidative stress, cognitive deficits, *etc*. The ICV STZ also demonstrates the appearance of senile plaques of $A\beta_{1\text{-}42}$, p-tau, and upregulation of GSK-3β in rodent brains rendering it a widely explored model for preliminary screening of effects of various drugs on these targets [33].

2. Materials and methods

2.1. Drugs and chemicals

The methanolic extracts of *B. ciliata* were administered orally in animals as a suspension of 0.3% sodium carboxymethylcellulose (NaCMC). (-) Scopolamine hydrobromide trihydrate (henceforth denoted as scopolamine), AChE enzyme (electric eel), BuChE enzyme (equine serum), butyrylthiocholine iodide (BuTCI), 2,2-Diphenyl-2-picrylhydrazyl (DPPH) and resazurin sodium (also known as Alamar blue) were acquired from Sigma Aldrich, Germany. Torrent Pharmaceuticals