Anti-amnesic effect of the ethanolic extract of *Helicanthus elasticus* in the scopolamine-induced memory impairment in Wistar rats

Rishabh Shukla, Dr. Sanjay Singh and Priti Khanduri*
Siddhartha Institute of Pharmacy, Near I.T. Park, Sahastradhara Road, Dehradun, India.
*Corresponding author:
Priti Khanduri
Siddhartha Institute of Pharmacy
Near I.T. Park, Sahastradhara Road,
Dehradun, India.
E-mail: pritiajay.nautiyal@gmail.com

Abstract
Neurodegenerative disorders are often associated with cognitive impairment, with nearly 57 million people living with it. Cognitive deficits, especially in learning and memory, are associated with the hippocampus; therefore, neuronal degeneration due to impaired cholinergic neurotransmission and oxidative stress in the hippocampus is primarily responsible for cognitive impairment. Therefore, modulating cholinergic neurotransmission and downregulating oxidative stress are key therapies for cognitive impairment. Medicinal plants rich in bioactive phytochemicals have gained considerable attention as potential therapeutic agents due to their ability to modulate multiple pathological pathways implicated in cognitive decline. Therefore, in the present study, we have explored the effect of the ethanolic extract of *Helicanthus elasticus* in scopolamine-induced memory impairment. The potential ameliorative effect of the extract was determined by behavioural studies, antioxidant levels, and histopathology of the rat hippocampus. The results showed significant improvements in behavioural studies in the treatment group at dose-dependent levels, and the dose-dependent downregulation of oxidative stress further supports the extract's potential therapeutic effect. Similarly, histopathological staining revealed protection against neuronal degeneration. Overall, the study established a potential therapeutic effect of the ethanolic extract in the scopolamine-induced memory impairment in rats.

Keywords: *Helicanthus elasticus*; cognitive impairment; scopolamine; oxidative stress; acetylcholinesterase; hippocampus; neuroprotection.
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Introduction

Memory impairment is a hallmark feature of several neurological and neurodegenerative disorders. Progressive neuronal deterioration contributes to memory dysfunction and places a substantial burden on patients and caregivers (Bartus et al., 1982; Livingston et al., 2020; Scheltens et al., 2021). The rapid growth of the ageing population, together with the increasing risk of cognitive impairment and dementia, represents a major global public health challenge. According to the World Health Organization (WHO), approximately 57 million people were living with dementia worldwide in 2021, more than 60% of whom resided in low- and middle-income countries; nearly 10 million new cases are reported each year (Livingston et al., 2020). Therefore, there is an urgent need to develop cost-effective therapeutics targeting memory dysfunction and cognitive decline.

Memory and cognitive impairment are frequently associated with dysfunction of the cholinergic system. Impaired cholinergic neurotransmission, particularly in the hippocampus and cerebral cortex, is a key feature of memory impairment and is commonly linked to neurodegenerative diseases. Current therapeutic options, including acetylcholinesterase inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, provide symptomatic benefits but are often limited by modest clinical efficacy and adverse effects (Bartus et al., 1982; Breijyeh and Karaman, 2020; Hampel et al., 2018). Therefore, there remains a continuous need to explore novel therapeutic agents capable of ameliorating cognitive and memory impairment.

Experimental studies have also reported antioxidant, hepatoprotective, immunomodulatory, anti-inflammatory, and antimicrobial properties of *H. elasticus*, suggesting its potential to protect against oxidative tissue injury (Jincy and Sunil, 2020). Since oxidative stress and cholinergic dysfunction are key contributors to scopolamine-induced cognitive impairment, phytochemicals that attenuate oxidative damage may be promising candidates for preserving neuronal function and cognitive performance. Despite its diverse pharmacological activities, the

neuropharmacological potential of H. elasticus for cognitive impairment has not been systematically investigated. Therefore, the present study evaluated the effect of the ethanolic extract of *Helicanthus elasticus* against scopolamine-induced cognitive impairment in Wistar rats.

1. Materials and methods

1.1 Materials

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), Acetylthiocholine iodide, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), phosphate buffer salts, formalin, paraffin wax, hematoxylin, and eosin were procured from CDH Fine Chemical, New Delhi, India. All chemicals used in the study were of analytical grade and used without further purification.

2.2 Methodology

2.2.1 Experimental animals

Adult female Wistar rats (30) weighing approximately 200–250 g were used in this study. The animals were procured from Subharti University, Dehradun, and housed in the Central Animal Facility of Siddhartha Institute of Pharmacy, Dehradun, under standard laboratory conditions with a 12 h light/dark cycle. Rats were provided standard pellet feed and water ad libitum. All animal experiments were conducted in accordance with institutional guidelines and were approved by the Institutional Animal Ethics Committee under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The project approval number was SIP/IAEC/PCOL/13/2026.

2.3 Experimental design for the scopolamine-induced memory impairment and treatment group

The rats were divided into 5 groups of 6 rats each.

- Group 1. – **Control** – The rats received normal saline as a treatment
- Group 2 – **Toxic control** – Scopolamine (**Scop**) (1 mg/kg, IP)
- Group 3 – **Treatment 1** – Scopolamine + *Helicanthus elasticus* low dose (**Scop + LD@E_HE**) (100 mg/kg)
- Group 4 – **Treatment 2** – Scopolamine + *Helicanthus elasticus* high dose (**Scop + HD@E_HE**) (200 mg/kg)
- Group 5 – **Standard** – Scopolamine + Donepezil (**Scop + Std**) (1 mg/kg)

2.3.1 Procurement and authentication of *Helicanthus elasticus*

The ethanolic extract of *Helicanthus elasticus* was already prepared and used in a separate study. In brief, the whole plant was procured from Akathrthara village, Palakkad, Kerala, India, and the Botanical Survey of India, Northern Regional Centre, Kaulagarh Road, Dehradun, Uttarakhand, India, has authenticated it and provided accession number 1221.

2.3.2 Preparation of ethanolic extract of the whole plant of Helicanthus elasticus

The plant leaf was shade-dried and pulverized using a mechanical grinder. The Soxhlet apparatus was used for extraction with 99.9% ethanol at 80 ^{0}C. Finally, the extract was filtered, dried and stored in a desiccator for further use.

2.3.3 Cognitive assessment of the rats

2.3.3.1 Morris water maze test

Learning and memory in rats were assessed using the Morris water maze test according to a previously reported protocol (Morris, 1984). Briefly, a circular tank was filled with water maintained at 22–25 °C, and a hidden platform was placed approximately 1 cm below the water surface. Rats were trained to locate the hidden platform for four consecutive days. On test day, animals were treated in accordance with the experimental protocol and then individually placed into the tank from different starting positions. The escape latency time (ELT), defined as the time required to locate the hidden platform, was recorded. Rats that failed to locate the platform within 60 s were gently guided to it and assigned an ELT of 60 s.

2.3.3.2 Elevated plus maze test

The elevated plus maze apparatus (Itoh et al., 1990; Parle and Dhingra, 2003) consisted of two open arms and two closed arms arranged in a plus-shaped configuration and elevated approximately 50 cm above the floor. Each rat was placed at the distal end of one open arm, facing away from the central platform. The time taken by the rat to enter any closed arm with all four paws was recorded as transfer latency. Animals that failed to enter a closed arm within 60 s were gently guided into one of the closed arms and assigned the maximum transfer latency of 60 s.

2.3.3.3 Y maze test

The Y-maze apparatus (Kraeuter et al., 2019; Lalonde, 2002) consisted of three identical arms labelled A, B, and C. Following treatment, each rat was placed at one end of an arm and allowed to explore freely for 5 min. The sequence of arm entries was recorded, and the spontaneous alternation percentage was calculated.

2.3.4 Biochemical parameters

2.3.4.1 Collection and preparation of brain tissue

After completion of the study, rats were euthanized under mild ether anaesthesia followed by cardiac perfusion, and the brains were carefully isolated for further analysis.

For biochemical studies, the isolated brains were rinsed with ice-cold normal saline and dissected on an ice pack to collect the hippocampal region. The harvested hippocampal tissue was homogenized in 0.1% ice-cold KCl solution, and the homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C. The

supernatant was collected and stored at 4 °C until further analysis.

For histopathological examination, the isolated hippocampal region was fixed in 10% formalin. Paraffin-embedded tissue sections were prepared using a tissue microtome for subsequent histological evaluation.

2.3.4.2 Estimation of malondialdehyde level

Malondialdehyde (MDA) levels were estimated using a previously reported method (Ohkawa et al., 1979). Briefly, the supernatant obtained from brain tissue homogenate was mixed with equal volumes of trichloroacetic acid (TCA) and thiobarbituric acid (TBA), heated in a water bath, and then centrifuged. The resulting supernatant was collected, and absorbance was measured at 535 nm using a UV–vis spectrophotometer. MDA levels were calculated using the appropriate extinction coefficient and expressed as nmol MDA/g tissue.

2.3.4.3 Estimation of tissue catalase

Catalase activity was estimated using a previously reported method (Aebi, 1984). Briefly, the brain tissue homogenate supernatant was added to a reaction mixture containing phosphate buffer and hydrogen peroxide. The decomposition of hydrogen peroxide was monitored by measuring the decrease in absorbance at 240 nm for 1–3 min using a UV–vis spectrophotometer. Catalase activity was calculated from the rate of absorbance decrease and expressed as units/mg protein.

2.3.4.4 Estimation of tissue acetylcholinesterase activity

Acetylcholinesterase activity was estimated using a previously reported method (Ellman et al., 1961). Briefly, acetylthiocholine iodide was added to a reaction mixture containing brain tissue homogenate supernatant, phosphate buffer, and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The formation of the yellow-colored 5-thio-2-nitrobenzoate anion was monitored at 412 nm for 5 min using a UV–vis spectrophotometer. Enzyme activity was calculated from the rate of increase in absorbance and expressed as µmol of acetylthiocholine hydrolysed/min/mg protein.

2.3.5 Histopathological examination of the hippocampus

Tissue sections were processed for hematoxylin and eosin (H&E) staining (*Bancroft's Theory and Practice of Histological Techniques*, 2019). Briefly, the slides were rehydrated by passing them through a graded series of ethanol solutions and finally rinsed in water before staining. After staining, the slides were dehydrated through increasing concentrations of ethanol, cleared in xylene to remove residual water, and mounted using DPX. The stained sections were examined under a light microscope to assess histopathological changes associated with different treatments.

2.3.6 Statistical analysis

Data are expressed as mean ± SD. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Behavioral data recorded across training days were analyzed using two-way repeated-measures ANOVA followed by Bonferroni's post hoc test. Other parameters were analyzed using one-way ANOVA followed by Bonferroni's post hoc test for group comparisons. A p-value < 0.05 was considered statistically significant.

2. Results and discussion

3.1. Effect of ethanolic extract of *Helicanthus elasticus* on behavioural parameters

Careful behavioural assessment provides an important functional measure of brain integrity and cognitive performance in experimental models of neurological disorders. Behavioural paradigms are widely used to evaluate learning, memory, and cognitive function and are essential for assessing the efficacy of therapeutic interventions in neuroscience research (Crawley, 2008; Cryan and Holmes, 2005). Therefore, behavioural studies were initially performed to determine the effect of the ethanolic extract of *Helicanthus elasticus* on memory and learning in scopolamine-induced cognitive impairment.

The Morris water maze test was first used to assess hippocampus-dependent spatial learning and memory (**Figure 1a**). Scopolamine administration significantly impaired spatial learning, as indicated by increased escape latency compared with the normal control group, consistent with previously reported disruption of hippocampal cholinergic neurotransmission (Klinkenberg and Blokland, 2010a; Vorhees and Williams, 2006). Treatment with the ethanolic extract of *H. elasticus* at both low and high doses significantly reduced escape latency compared with the scopolamine-treated group in a dose-dependent manner, suggesting improved spatial learning and memory performance. This improvement may be associated with the antioxidant phytoconstituents present in the extract, including phenolic compounds and flavonoids, which have been reported to attenuate oxidative stress and support neuronal function.

Learning and memory were further evaluated using the elevated plus maze (**Figure 1b**), a well-established behavioural paradigm for assessing memory retention based on transfer latency (Itoh et al., 1990). During the acquisition phase, rats learn to locate the enclosed arm and subsequently show reduced transfer latency during the retention trial. In the present study, scopolamine significantly increased transfer latency compared with the normal control group, indicating impaired memory retention. In contrast, treatment with the ethanolic

extract of *H. elasticus* significantly reduced transfer latency in a dose-dependent manner, suggesting improved retention of the learned task and partial restoration of cognitive function.

Spatial working memory was further assessed using the Y-maze spontaneous alternation test (Kraeuter et al., 2019) (**Figure 1c**). This paradigm relies on the natural exploratory behaviour of rodents and their tendency to enter a less recently visited arm, thereby providing a measure of hippocampus-dependent working memory. Scopolamine administration significantly reduced the percentage of spontaneous alternation, indicating impaired working memory. However, treatment with the ethanolic extract of *H. elasticus* significantly increased spontaneous alternation behaviour in a dose-dependent manner, indicating improvement in spatial working memory.

Collectively, these behavioural findings show that the ethanolic extract of *H. elasticus* attenuated scopolamine-induced cognitive deficits by improving spatial learning, memory retention, and working memory. The consistent improvement observed across three behavioural paradigms suggests that the extract possesses anti-amnesic potential. Although the precise molecular mechanisms remain to be clarified, these beneficial effects may be associated with the phytochemical composition of *H. elasticus*, particularly its phenolic and flavonoid constituents, which have been reported to exhibit antioxidant and neuroprotective properties (Amol A. Joshi*, 2018). Further mechanistic studies are required to identify the bioactive constituents responsible for these cognitive benefits.

Figure 1. Effect of the ethanolic extract of *Helicanthus elasticus* on scopolamine-induced learning and memory impairment in Wistar rats. (a) Escape latency comparison of different groups after scopolamine treatment in groups treated with the ethanolic extract of *Helicanthus elasticus* in the Morris water maze test. **(b)** Effect of the ethanolic extract of *Helicanthus elasticus* on transfer latency in the elevated plus maze test. **(c)** Effect of the ethanolic extract of *Helicanthus elasticus* on spontaneous alternation behaviour in the Y-maze test. Data are presented as mean ± SD (n = 6 rats/group). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Differences were considered statistically significant at. #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$ versus the scopolamine treated group; *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the normal control group.

3.2. Effect of the ethanolic extract of *Helicanthus elasticus* on antioxidant activity in the hippocampus

Oxidative stress is recognized as a major pathological mechanism contributing to neuronal dysfunction and cognitive impairment (Butterfield and Halliwell, 2019; Sies et al., 2017). Because of its high oxygen demand, abundant polyunsaturated fatty acids, and relatively limited antioxidant defence capacity, the brain is particularly vulnerable to oxidative damage (Cobley et al., 2018). Malondialdehyde (MDA), a major end product of lipid peroxidation, is widely used as a biomarker of oxidative stress (Ayala et al., 2014; Sies et al., 2017). Increased MDA levels indicate enhanced reactive oxygen species (ROS)-mediated lipid peroxidation, which can disrupt membrane integrity, impair mitochondrial function, promote oxidative modification of proteins and nucleic acids, and contribute to neuronal degeneration (Ayala et al., 2014; Butterfield and Halliwell, 2019; Cobley et al., 2018).

Scopolamine has been reported to induce oxidative stress in the hippocampus by increasing ROS generation and lipid peroxidation while impairing endogenous antioxidant defence mechanisms (Butterfield and Halliwell, 2019; Klinkenberg and Blokland, 2010b; Uttara et al., 2009). Such oxidative injury can disrupt synaptic transmission and neuronal function in the hippocampus, a brain region essential for learning and memory. Consistent with these reports, the present study showed a significant increase in hippocampal MDA levels (**Figure 2a**) in the scopolamine-treated group compared with the normal control group, indicating increased lipid peroxidation. Treatment with the ethanolic extract of *H. elasticus* reduced hippocampal MDA levels, with the high-dose group showing a significant reduction compared with the scopolamine-treated group. This reduction in lipid peroxidation may be associated with phenolic compounds and flavonoids reported in *H. elasticus*, which are known for their free radical-scavenging and antioxidant activities, potentially limiting ROS-mediated neuronal damage.

Catalase is a major endogenous antioxidant enzyme that protects cells from oxidative stress by catalyzing the decomposition of hydrogen peroxide into water and molecular oxygen, thereby contributing to cellular redox homeostasis (Aebi, 1984; Sies et al., 2017). Reduced catalase activity weakens endogenous antioxidant defence and increases neuronal susceptibility to oxidative injury. Previous studies

have reported that scopolamine suppresses hippocampal catalase activity, thereby promoting oxidative damage and neuronal dysfunction (Butterfield and Halliwell, 2019; Cobley et al., 2018). The present study showed a marked reduction in catalase activity following scopolamine administration (**Figure 2b**). Treatment with the ethanolic extract of *H. elasticus* restored catalase activity, with the high-dose group showing a significant improvement compared with the scopolamine-treated group. These findings suggest that the extract may support endogenous antioxidant defence mechanisms and protect hippocampal tissue against oxidative damage. The restoration of catalase activity may be related to antioxidant phytoconstituents in H. elasticus, including phenolic compounds, flavonoids, gallic acid, and ethyl gallate, which have been reported to exhibit antioxidant activity.

Acetylcholine is a key neurotransmitter of the central cholinergic system and plays an important role in learning, memory, and cognitive function. Acetylcholinesterase (AChE) is the principal enzyme responsible for the rapid hydrolysis of acetylcholine in the synaptic cleft, thereby terminating cholinergic neurotransmission. Increased AChE activity accelerates acetylcholine degradation, leading to impaired cholinergic signalling and cognitive dysfunction. Accordingly, AChE inhibition remains a major therapeutic strategy for the symptomatic management of cognitive disorders (Bartus et al., 1982; H. Ferreira-Vieira et al., 2016; Hampel et al., 2018; Scheltens et al., 2021).

In the present study, scopolamine significantly increased hippocampal AChE activity (**Figure 2c**) relative to the normal control group, indicating a disruption of cholinergic neurotransmission. Administration of the ethanolic extract of *H. elasticus* reduced hippocampal AChE activity, with the high-dose treatment producing a significant reduction compared with the scopolamine-treated group. Although the precise mechanism underlying this effect remains unclear, it may be associated with the diverse phytochemicals present in *H. elasticus*. Several phenolic compounds and flavonoids have been reported to inhibit AChE activity and support cholinergic neurotransmission, potentially contributing to improved cognitive performance.

Collectively, these biochemical findings show that the ethanolic extract of *Helicanthus elasticus* attenuated scopolamine-induced oxidative stress and cholinergic dysfunction in the hippocampus. The extract reduced lipid peroxidation, improved endogenous antioxidant defence by restoring catalase activity, and decreased AChE activity, thereby supporting cholinergic neurotransmission. These biochemical changes are consistent with the behavioural findings and suggest that the ethanolic extract of *H. elasticus* could be a potential therapeutic option for memory impairment. Nevertheless, further studies are required to clarify the precise molecular mechanisms and identify the specific bioactive constituents responsible for these effects.

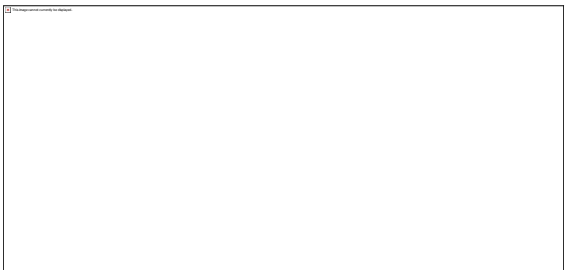

Figure 2. **Antioxidant levels in the rat hippocampus after treatment with the ethanolic extract of *Helicanthus elasticus*. (a)** MDA, **(b)** Catalase, and **(c)** Acetylcholinesterase levels were quantified in the hippocampus of the rats after the various treatments. Data is represented here as mean ± SD. ANOVA, followed by a Bonferroni post hoc test, was performed to calculate the significance. The notation "*" (*$p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$) was used to represent the comparison between the control group and the scopolamine-treated group. The notation "#" ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ was used to represent the comparison between the scopolamine-treated group and the other treated group.

3.3. Ethanolic extract of *Helicanthus elasticus* mediated histological improvement in the hippocampus

Hematoxylin and eosin (H&E) staining (**Figure 3**) was performed to assess scopolamine-induced alterations in hippocampal neuronal morphology and to evaluate the effect of the ethanolic extract of *Helicanthus elasticus* on neuronal integrity. The hippocampus is particularly susceptible to oxidative stress and cholinergic dysfunction because of its high metabolic activity and central role in learning and memory. Oxidative injury and impaired cholinergic neurotransmission can disrupt synaptic transmission, synaptic plasticity, and neuronal viability, ultimately contributing to cognitive impairment (Hampel et al., 2018; Martin et al., 2000; Neves et al., 2008). Previous studies have reported that scopolamine induces marked histopathological changes in the hippocampus, including neuronal shrinkage, cellular disorganization, and reduced neuronal density are associated with learning and memory deficits (Butterfield and Halliwell, 2019; Klinkenberg and Blokland, 2010a; Uttara et al., 2009).

In the present study, the hippocampus of the normal control group showed well-organised neuronal architecture, intact pyramidal neurons, and normal cellular density. In contrast, the scopolamine-treated group exhibited marked neuronal shrinkage, reduced neuronal density, and disorganized hippocampal architecture, indicating substantial neuronal damage. Treatment with the ethanolic extract of *H. elasticus* improved hippocampal morphology, with significant improvement observed in the high-dose group. Rats receiving the higher dose of the extract showed better preservation of neuronal architecture, reduced neuronal shrinkage, and improved neuronal density compared with the scopolamine-treated group. The histopathological appearance of the high-dose treatment group was closer to that of the normal control group, suggesting protection against scopolamine-induced hippocampal injury.

The preservation of hippocampal neuronal morphology was consistent with the behavioural improvements and biochemical findings observed in the present study. Reduced lipid peroxidation, improved endogenous antioxidant defence, and decreased acetylcholinesterase activity may have contributed to the maintenance of neuronal integrity in the hippocampus. Furthermore, phenolic compounds and flavonoids reported in *H. elasticus* are known to possess antioxidant properties that may help limit oxidative damage and support neuronal function. Although the precise molecular mechanisms remain to be elucidated, the present findings suggest that the ethanolic extract of H. elasticus ameliorates scopolamine-induced alterations in hippocampal neurons, thereby contributing to improved learning, memory retention, and cognitive performance.

Figure 3. Histopathological changes in the rat hippocampus due to various treatments. Various groups are represented here as **(a)** Control, **(b)** Scop, **(c)** Scop + LD@E-HE, **(d)** Scop + HD@E-HE and **(e)** Scop + Std. The black arrow showed neurons in the various groups. Neuronal degeneration was evident in the scopolamine-treated group; however, treatment restored neuronal function in a dose-dependent manner. Images were taken at 20x magnification, and the scale bar is 100 µm.

3. Conclusion

The present study demonstrates that the ethanolic extract of *Helicanthus elasticus* attenuated scopolamine-induced cognitive impairment by improving spatial learning and memory retention, while also preserving hippocampal neuronal integrity. These behavioural improvements were accompanied by reduced lipid peroxidation, improved endogenous antioxidant levels, and decreased acetylcholinesterase activity, suggesting that the extract may ameliorate oxidative stress and cholinergic dysfunction associated with cognitive impairment. Collectively, these findings indicate that the ethanolic extract of *H. elasticus* possesses promising anti-amnesic activity and may serve as a potential therapeutic candidate for managing cognitive impairment. Nevertheless, the present study provides preliminary evidence of its efficacy. Further investigations are required to identify the bioactive phytoconstituents responsible for these effects, clarify the underlying molecular mechanisms, evaluate long-term safety, and establish their translational potential in models of chronic neurodegenerative disorders.

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