Original Article

Saraswatarishta Reverses Neuronal Injury in Brain Tissues of Scopolamine-induced Rat Model

Jai Prabhu, Jayakumari S, Prabhu K, Jyothi Ashok Kumar[1], Manickam Subramanian[1], Kavimani

Department of Anatomy, Sree Balaji Medical College, Chrompet, Chennai, [1]Department of Anatomy, Chettinad Academy of Research and Education, Kanchipuram, Tamil Nadu, India

Abstract

Introduction: Neuroinflammation has been implicated in the pathogenesis or the progression of the variety of acute and chronic neurological and neurodegenerative disorders, including Alzheimer's disease. The Saraswatarishta is an Ayurvedic medicine utilized in many health conditions such as anti-aging, to improve memory, immunity, and quality of semen and sperms to treat epilepsy and cosmetic use for skins. It is a liquid Ayurvedic medicine. Saraswatarishta contains 5%–10% of self-produced alcohol in it, which serves as a vehicle to deliver water- and alcohol-soluble active herbal components to the body. It is also called Sarasvatarishtam. The aim of this study was to find the possible neuroprotective role of Saraswatarishtam as a preventive Ayurveda and Siddha drug to hamper cholinergic dysfunctions and histopathological changes in scopolamine-treated rat model. **Material and Methods:** The compound Saraswatarishtam was obtained from standard Ayurvedic vendor at Chennai. Group 1 – normal control animals received normal saline for 8 continuous days. Group 2 – positive control treated with scopolamine (0.4 mg/kg). Group 3 – received 200 mg/kg of piracetam for 8 continuous days. Group 4 and Group 5 served as a test and received 200 mg/kg and 400 mg/kg of Saraswatarishtam, respectively, for 8 continuous days. On the 8th day, after 90 min of drug administration, Group 2, Group 3, Group 4 and Group 5, were treated with 0.4 mg/kg of scopolamine. Brain tissues were dissected out and analyzed for histopathological changes after sacrifice with high dose of halothane. **Results:** Administration of scopolamine produced marked focal gliosis with mononuclear infiltration. The hippocampal region showed neuronal degeneration with sclerosis. Piracetam treated group showed pyknotic nucleus in neurons of the cerebral cortex and mild edema. Low dose (200 mg/kg) treatment with Saraswatarishtam followed by scopolamine administration showed moderate histopathological changes such as mild infiltration of monocytes but normal neuronal architecture. High dose (400 mg/kg) treatment with Saraswatarishtam followed by scopolamine administration shows abnormal morphology of cerebrum, cerebellum, basal nuclei, and hippocampus. **Discussion and Conclusion:** The results of the present study suggested that Saraswatarishtam exhibits neuroprotective properties against scopolamine-induced neuronal damage.

Keywords: *Albino mice, ayurvedic, neuroinflammation, neuroprotective, saraswatarishta*

Introduction

Neuroinflammation is an inflammatory response characterized by gliosis in the central nervous system due to triggering of the immune system. There are various medicines to treat neuronal inflammation such as nonsteroidal anti-inflammatory drugs (NSAIDS), opioid antagonists, selective cyclooxygenase inhibitor, N-methyl-D-aspartate receptor antagonists, and occasionally antibiotics.[1,2] However, those allopathic drugs are efficient to relive analgesia and inflammation, but they create unwanted adverse effects. For example, NSAIDS causes gastric irritation, pain abdomen, nausea, cramping, ringing in ears, confusion[3] diminish the motility of the sperm.[4] While the opioid antagonist induces adverse effects such as loss of appetite, dizziness, and nervousness. To avoid these adverse effects, the researchers tried to explore the neuroprotective role of certain drugs in the Indian system of medicine, one such preparation found in the ancient Indian treatment is Saraswatarishta.

Various naturally available elements have been anticipated as a potential treatment for reduction or slow down the process of different types of acute and chronic neurological and neurodegenerative disorders, including Alzheimer's disease.[5,6] In the formulation of Ayurvedic medicines, many herbal and herbo-mineral elements are commonly used to treat various

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Address for correspondence:
Dr. Jayakumari S, Department of Anatomy, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research, Chromepet, Chennai - 600 044, Tamil Nadu, India.
E-mail: jayakumarinisha@gmail.com

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conditions these formulations are based on the concept that they provide synergistic therapeutic outcomes and aids in reducing the adverse effects of major drugs[7] (Bhattacharya SK 1994). One such multi-ingredient plant-based herbo-mineral formulation is "Saraswatarishta" which comprises 18 plants.[8] Some of which include Ashwagandha, Brahmi, and Shatavari which are Medhya rasayanas. Medhya rasayanas are used to improve memory and cognitive deficits.[9] Saraswatarishtais claimed to be helpful for the management of acute anxiety, fatigue, insomnia, partial loss of memory, low grasping power, and slurred speech, etc.,[8] In view of the central nervous system effects of Saraswatarishta described in Ayurveda, it was of interest to study whether it has a protective effect on neurons of the central nervous system.

Material and Methods

Preparation of saraswatarishta

Saraswatarishtam

The compound Saraswatarishtam was obtained from standard Ayurvedic vendor at Chennai. Integrands of this formulation are illustrated in Table 1.

Animals

Healthy adult Swiss Albino mice of both sexes weighing 25–30 g were selected as exterminate animals. Standard laboratory procedures were maintained for animals. The laboratory temperature was kept at 22°C ± 3°C, and the humidity was maintained at 45%–55%. 12 h dark/light cycles were maintained.

Table 1: List of ingredients used in the preparation of Saraswatarishta

Name	Botanical Name	Quantity
Water		4000 ml
Boil and Reduce to		1000 ml
Brahmi	Bacopamonnieri	320 gms
Shatavari	Asparagus racemosus	80 gms
Vidari	PuerariaTuberosa	80 gms
Haritaki	Terminalia chebula	80 gms
Vala	Vetiveriazizanioides	80 gms
Ardrak	Zingiber officinalis	80 gms
Mishi	Foeniculum vulgare	80 gms
Honey	Honey	160 gms
Khandasharkara	Candy sugar	400 gms
Dhataki	Woodfordiafruticosa	80 gms
Renukbee	Vitexnigundo	4 gms
Trivrit	operculinaterpenthum	4 gms
Pippali	Piper longum	4 gms
Lavang	Syzyziumaromaticum	4 gms
Vacha	Acoruscalomus	4 gms
Kushtha	Sausurrealappa	4 gms
Ashwagandha	Withaniasomnifera	4 gms
Bibhitaki	Terminalia bellerica	4 gms
Guduchi	Tinosporacordifolia	4 gms
Ela	Ellatariacardamomum	4 gms
Tvak	Cinnamomumzeylanicum	4 gms

All the animal experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Experimental protocol was approved by Institution of Animal Ethical Committee of KMCH governed by CPCSEA, Government of India. Proposal number: 685/po/02/a/DPCSE.[10]

Group 1 – normal control animals they received normal saline for 8 continuous days. Group 2 – negative control treated with scopolamine (0.4 mg/kg). Group 3 – received 200 mg/kg of piracetam for 8 continuous days. Group 4 and Group 5 served as test and received 200 mg/kg and 400 mg/kg of Saraswatarishtam, respectively, for 8 continuous days. Saraswatarishtam was dissolved in water and administered by oral feeding with a feeding needle. On the 8th day, after 90 min of test drug administration, Group 2, Group 3, Group 4 and Group 5 were treated with 0.4 mg/kg of scopolamine.

Histopathology

Brain tissue was dissected out after sacrifice with a high dose of halothane. Tissues were fixed in 10% buffered formalin, dehydrated with alcohol, cleared with xylene, impregnated with paraffin, embedded in paraffin wax, and sectioned with microtome to obtain 4–5 μm thick paraffin sections, Dewaxed sections were stained with hematoxylin and eosin and observed under microscope for scopolamine-induced changes and neuroprotective efficiency of Saraswatarishtam.

Results

Group 1 animals showed normal histoarchitecture of the cerebral cortex and cerebellar cortex [Figure 1a], no changes were seen in the molecular and Purkinje cell layer of the cerebral cortex [Figure 1b]. Brain sections of scopolamine treated rats (Group 2) showed focal gliosis with marked monocyte infiltration suggestive of inflammation in the cerebral cortex, and the neurons exhibited pyknotic nucleus suggestive of chromatolysis [Figure 2a and b]. Brain sections of Group 3 animals showed tissue edema in the cerebral cortex [Figure 3b] in addition to the Pyknotic nucleus [Figure 3a].

In Group 4 animals, the white matter of cerebrum did not show any degenerative changes [Figure 3a]. Group 4 animals were treated with low dose of Saraswatarishtam which showed normal cyto-architecture of the cerebral cortex [Figure 4a] but mild infiltration of monocytes [Figure 4b]. The hippocampus of Group 5 animals showed normal architecture with normal polymorphic, pyramidal, and granular layers [Figure 5a], the cerebellum of this group showed normal morphology [Figure 5b].

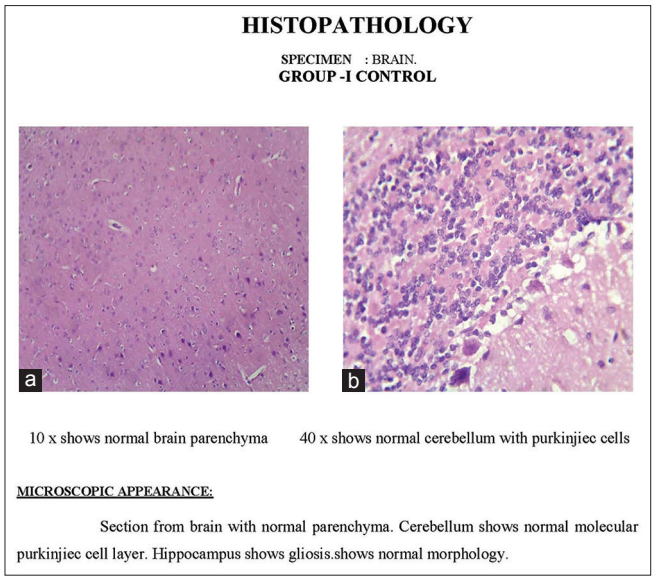

Figure 1: (a) The cerebrum of group showing normal histological features. H and E, ×10. (b) Photomicrographs of the cerebellum of Group 2 showing a normal cerebellum with Purkinje cells. H and E, ×40

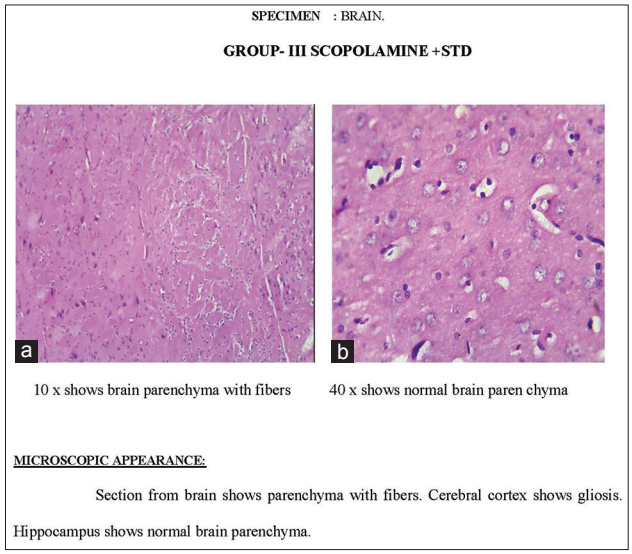

Figure 3: (a) Photomicrographs of the cerebrum of Group 3 showing the brain parenchyma with Swollen neurons (arrow). H and E, ×10. (b) Photomicrographs of the cerebrum of Group 3 showing neurons with pyknotic nucleus (arrow) and edema. H and E, ×40

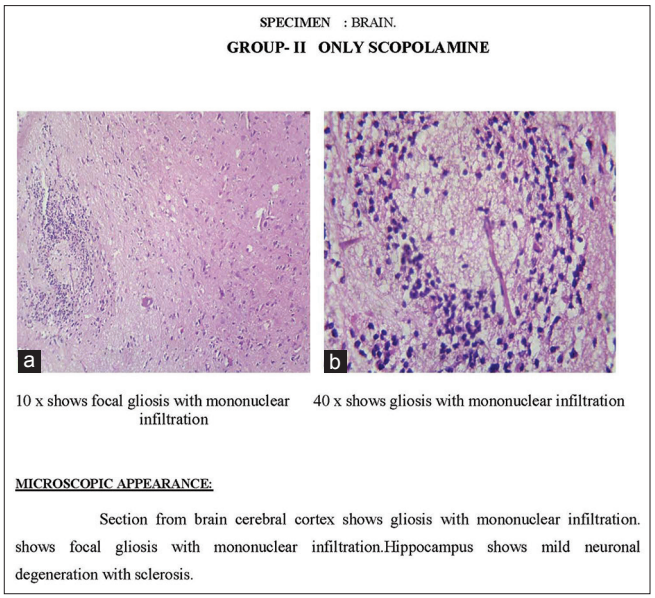

Figure 2: (a) Photomicrographs of the cerebrum of Group 2 showing focal gliosis with mononuclear infiltration (arrow). H and E, ×10. (b) Photomicrographs of the cerebrum of Group 2 showing pyknotic nucleus (arrow). H and E, ×40

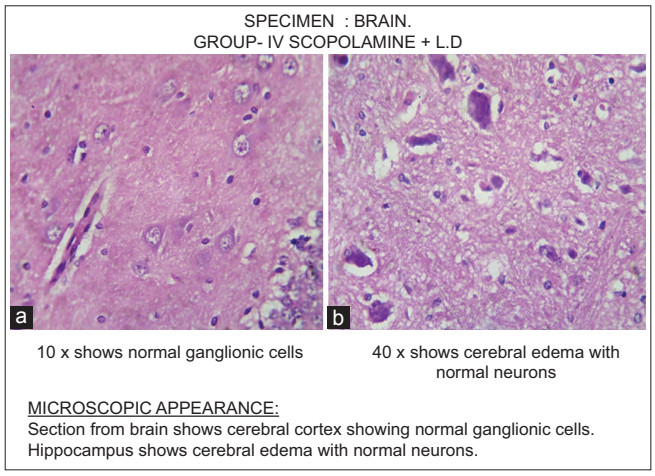

Figure 4: (a) Photomicrographs of cerebrum of group 4 showing normal white matter ×10 H and E. (b) Photomicrographs of cerebrum of group 4 showing mild infiltration of mononuclear cells ×40 H and E

Discussion

The findings of the current study suggest that Saraswatarishtam rescues the neurons from the scopolamine-induced neuroinflammation. Scopolamine, a nonselective muscarinic antagonist, stops cholinergic signaling and induces memory and cognitive impairment.[11] Administration of scopolamine has been extensively and successfully utilized as a model for Alzheimer's disease in rats.[12,13] Various studies have suggested that oxidative stress might be associated with the pathophysiology of many neurological disorders and brain dysfunction.[14] Therefore, neuronal cell death by oxidative stress and progression of neurodegenerative disorders can be attenuated by the supplementation of antioxidants and free radical scavengers.[15] Administration of scopolamine is associated with increased brain lipid peroxides and reduced brain anti-oxidant levels.[16] Histopathological examination of brain sections of control rats demonstrated the normal morphology of the cerebral cortex and hippocampus. While scopolamine induced at brain sections illustrated severe congestion in the capillaries with perivascular edema in the cortex. In the meantime, the hippocampus revealed encephalomalacia and edema in the tissue matrix with demyelination and neuronal degeneration.[17] Thus, previous studies concluded that scopolamine administration leads to neuronal inflammation, neuronal degeneration,

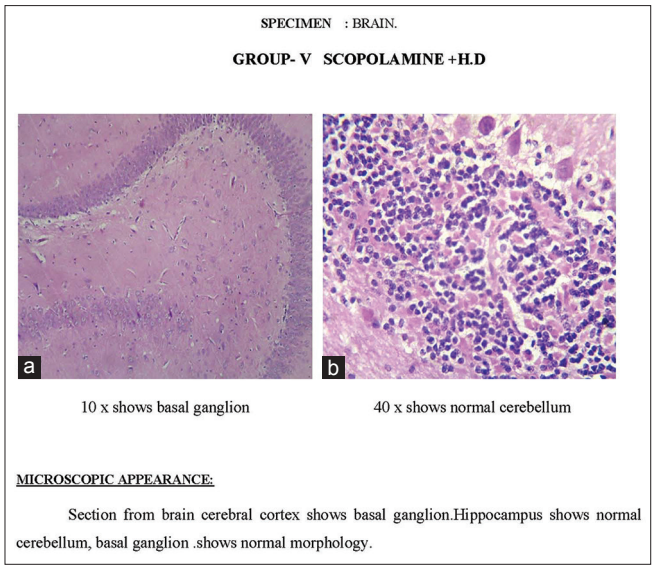

Figure 5: (a) Photomicrographs of hippocampal region Group 5 showing normal neuro architecture of hippocampus with three distinguish layers (arrow = polymorphic layer, arrowhead = pyramidal layer, star = ganglion layer). H and E, ×10. (b) Photomicrographs of cerebellum of Group 5 showing normal neuronal architecture (arrow = Purkinje cell layer, arrowhead = granular cell layer, star = molecular layer). H and E, ×40

encephalomalacia, cerebral edema, and demyelination. In the present study, the administration of scopolamine induced neuroinflammation in the brain tissue of the rat model.

The piracetam is a standard drug to treat Alzheimer's disease, dementia, memory dysfunction, alcoholism, Raynaud's phenomenon, deep-vein thrombosis, stroke, tardive dyskinesia, dyslexia, convulsions, brain injury, and vertigo. Piracetam helps by enhancing cell membrane permeability and boost oxygen consumption and serves as an enhancer of acetylcholine action through muscarinic cholinergic receptors, and these receptors give an essential contribution in memory and learning.[18,19] Piracetam can induce neuroplasticity which improves memory and learning process, thereby preventing lesions and ischemic damages. Even it increases microcirculation and will be administered as treatment of cerebral ischemia. However, piracetam administration can cause obesity, insomnia, depression, muscle spasm, hyperactivity, and skin rashes.

Group 3 animals were treated with this drug as a standard or negative control to compare the efficience of Saraswatarishtam. Ayurveda believes in treating complex diseases with a complex combination of natural products, including animals, plants, and minerals.[20] Saraswatharishta is a capable agent in prophylaxis and management of neuropsychiatric and neurodegenerative conditions. Earlier studies stated that Sarawatharishtam has anxiolytic and antidepressant activities, but there is a shortage of data concerning neuroprotective activity. Thus, the current study evaluates its role in neuroprotection.

Histopathological sections of scopolamine-induced animal brain tissue show abnormal cellular morphology with inflammatory changes such as focal gliosis with mononuclear infiltration and hippocampal neuronal degeneration with sclerosis. In piracetam-treated animals, few neurons with the pyknotic nucleus and mild edema of tissue fluid were observed. On the other hand, the animal receiving 200 mg/kg of Saraswatarishtam showed similar structure as piracetam-treated animals except mild gliosis in the cerebral cortex.

The brain sections of Saraswatharishtam treated animals at a dose of 400 mg/kg were similar to control animals. When compared with sections of with piracetam-induced animals, the Saraswatharishtam fairly inverted the scopolamine-induced neurodegeneration, and the reports were almost similar to piracetam treatment. Further, dose regulation and experimental modification are required to substitute Saraswatharishtam as a neuroprotective agent against neuroinflammation.

Conclusion

The findings of the present study conclude that Saraswatarishtam has a protective role against neuronal inflammation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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