ABSTRACT

Introduction: Alzheimer’s type sporadic dementia was induced in rats by intracerebroventricular (ICV) to the lateral ventricle of the brain using robotic stereotaxic apparatus.

Aims: The present study was aimed at evaluating the neuroprotective effect of extract of *Cleome viscosa* Linn. against colchicine-induced dementia in rats.

Methodology: For this experiment, male Wistar rats were used and were administered with ICV injection of colchicine and methanol extract of *Cleome viscosa* (100 or 200 mg/kg. p.o) for 3 weeks to determine the preventive effect of extract against colchicine toxicity. Exposure to colchicine produced oxidative stress in rats and model for memory deficit. The spatial memory of animals was evaluated using Morris water maze followed by biochemical estimation of acetylcholinesterase in the hippocampal and frontal cortex region of the brain.

Results: Exposure to colchicine produced oxidative stress in rats and model for the deficit in memory in rats. The level of acetylcholine esterase was not significantly changed in the hippocampal region and the frontal cortex region in the extract-treated groups. And also failed to exert any protective effect against colchicine induced dementia was evaluated using Morris Water Maze for parameters related to Path efficiency, Escape Latency, D quadrant latency followed by biochemical estimation of Acetylcholinesterase (ACHE) enzyme level in the frontal cortex and Hippocampal region of rat brain.

Conclusion: The current study suggests the inability of methanol extract of *Cleome viscosa* used at this dose to provide neuroprotective action in ICV injected Colchicine induced sporadic model of Alzheimer’s disease model in rats.

Key Words: Dementia, Morris Water Maze, *Cleome viscosa*, intracerebroventricular (ICV), Neuroprotection, Acetylcholinesterase inhibitor

INTRODUCTION

Alzheimer’s disease (AD) is a chronic neurodegenerative disorder in which there is memory loss which results in the poor societal and personal life of the patients. AD is a type of dementia involving the chronic reduction in the cognitive and intellectual functioning of a person. As the disease progresses, the nerve cells involved in thinking, learning becomes damaged followed by eventual damage of neurons associated with basic functions of the body like walking and swallowing. The late phase of AD comprises of loss of ability to think, plan and difficulty in problem-solving skills, difficulty in walking and swallowing leading to requirement of close care and bed-bound situation. It is chiefly caused by the production & accumulation of abnormal amyloid proteins also known as β amyloids outside the neurons leading to the formation of Senile plaques. Secondly, the hyperphosphorylation of Tau proteins and their accumulation inside the neurons result in neurofibrillary tangles thereby blocking the conduct of nutrients and essential molecules required for a healthy neuron. Hyperphosphorylated tau results in dissociation of tau protein from microtubules, misfolding and aggregation to form paired helical filaments, which enhance Aβ toxicity. A reduced level of neurotransmitters occurs in AD
The neuroprotective potential of extracts has been investigated. Memantine prevents the overstimulation of receptors and prevents neuronal damage. These drugs are primarily used for the symptomatic treatment of dementia. Plant-based natural drugs have immense potential to be used as an alternative for the treatment of AD. The natives and traditional healers of India use Cleome viscosa Linn. (CV) for various therapeutic purposes. In the traditional system of medicine, this plant is used to treat various disorders such as diarrhoea, fever, inflammation, liver diseases, bronchitis, skin diseases and malarial fever. The juice is useful in piles, lumbago, and earache. CV leaves possess high phenolic and flavonoid contents which show potential antioxidant activity, and free radical scavenging activity. Currently, in this study, we have evaluated the neuroprotective potential of CV extract in Colchicine induced sporadic dementia model of AD.

Colchicine has been proven to induce behavioural, biochemical and pathological changes as that of sporadic AD. Colchicine when given by the intracerebroventricular (ICV) route destroys hippocampal cells leading to loss of cholinergic neurons and loss of memory. It is cytotoxic that binds irreversibly to the dimer of tubulin and prevent the microtubule assembly. The neuroprotective potential of extracts has not been explored in the colchicine induced sporadic model of AD. Hence, in this current study, for the first time, the neuroprotective role of extracts has been investigated in the ICV colchicine induced cognitive deficit and oxidative stress model of AD in rats.

MATERIALS AND METHODS

Preparation of methanol extract of plant Cleome viscosa Linn.

The whole plant of Cleome viscosa Linn. belonging to the family Capparidaceae was collected from the district of Udupi, Karnataka in October. The plant was authenticated by Dr K. Gopalakrishna Bhat, Head, Department of Botany, Poornaprajna College, Udupi, Karnataka. The authenticated sample was submitted to the Manipal College of pharmaceutical sciences herbarium. The plant material (2kg) was shade dried, powdered coarsely was extracted using soxhlet apparatus for 24h by methanol. The crude methanol extract was concentrated in a rotary evaporator under reduced pressure for solvent recovery and the collected concentrated extract was dried and preserved in a desiccator for later use. The yield was 4.3% for crude methanol extract.

Animals

The male Wistar rats of 150-200 g used in the study were obtained from the Central Animal Research Facility, Manipal Academy of Higher Education and housed in propylene cages (3 animals per cage). The temperature and humidity were maintained at 23 ± 3°C and 55 ± 5% respectively. The animal handling and care were carried out according to the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the study was approved by the Institutional Animal Ethics committee, Manipal. (IAEC/KMC/104/2018).

Preparation of vehicle Carboxymethyl cellulose (CMC)

0.25g of CMC was weighed accurately into a 200ml beaker and 40ml of distilled water was added. This solution was sonicated for about 45 minutes and volume was made up to 100ml with distilled water.

Preparation of Cleome viscosa extract

The methanol extract of CV was weighed accurately and triturated with Tween80 until a clicking sound was heard. The resulting mixture was suspended in 0.25% CMC. The animals were given doses according to their body weight. [100mg/kg (Test treatment 1), 200mg/kg (Test treatment 2)]

Treatment regimen

The animals were divided into six groups with six animals in each group namely, Normal control, Sham control with artificial cerebrospinal fluid 5 μL, Disease control where intracerebroventricular injection of colchicine in artificial cerebrospinal fluid was done. The treatment of various drugs, surgery and the conduct of the experiment was done as per the regimen shown in Table 1 and Figure 1. (Schematic diagram for representing drug/Extract treatment plan, disease induction and evaluation of parameters).

Intracerebroventricular administration of Colchicine

Before the beginning of the surgical procedure, the frame of the apparatus was cleaned properly using 70% ethanol. Thiopental sodium (45mg/kg) was administered to anaesthetize the rats. The animal’s head was then fixed properly into the frame of the apparatus and an incision was carefully made along the midline of the scalp. The administration of colchicine was done in the lateral ventricle at the coordinates 0.8 mm posterior to bregma (AP), 1.8 mm lateral to sagittal suture (ML) and 3.6 mm below the cortical surface (DV).
The rat was infused with colchicine 15μg in 5 μL of artificial cerebrospinal fluid (in mM; 147 NaCl, 2.9 KCl, 1.6 MgCl₂, 1.7 CaCl₂, and 2.2 dextrose) using a quintessential stereotaxic injector and the microsyringe was left there for 2 minutes to avoid backflow. In the Sham group, only ACSF was administered without Colchicine. The scalp was closed by suturing and the antibacterial betadine was applied on the area where the incision was made using sterile cotton to prevent any infection.

**Evaluation of spatial memory using Morris water maze**

Spatial memory was evaluated with Morris water maze consisting of a circular pool of 150 cm diameter and height of 40 cm divided into four North-east, North-west, South-east, South-west quadrants 15. The animals were placed in either of the quadrants alternating for each trial. The water in the pool was made opaque with non-fat milk and a platform of 10 cm diameter was placed 2 cm immersed, hidden in the water. Animal use external/extra-maze cues to find the platform. 16 A tracking software system with a camera was placed to capture the behaviour and movements of the animal. Water maintained at a temperature of 26 ± 1°C was changed every day. The experiment was performed in the dim light intensity was kept constant throughout the trial.

**Acquisition trial:** After 16 days of colchicine administration (15μg, ICV) training was provided for four consecutive days to all animals. For 60 seconds, animals were allowed to swim freely to find the platform. If the animal does not find a platform, they are directed to the platform manually and held on it for 30 seconds. This training was conducted as four trials a day with a 5-minutes intertrial interval. The path efficiency, time spent in each quadrant, escape latencies, were recorded.

**Probe trial:** After the training phase, the retention trial was done on the 5th day for the 60s Post-acquisition trial. The platform was removed in this trial, without altering other external/extra-maze conditions. The animals were kept with the head facing to the wall in the quadrant opposite to the one containing platform and were allowed to explore the pool wherein parameters like escape latency, path efficiency, time in all zones, and total distance were considered and recorded using ANY-maze software with the camera fixed above the pool.

**Locomotor activity evaluation by Actophotometer**

The locomotor activity of each animal was evaluated on days 3, 7, 14 after colchicine administration (15μg, ICV), using a digital actophotometer. The animals were placed on the actophotometer that contains light-sensitive infrared photocells to monitor the movement of animals. The animals were acclimatized for 3 minutes and then were kept in the instrument for 10 minutes to record their locomotor activity.

**Brain isolation and homogenization of hippocampus and frontal cortex**

After the end of the probe trial, the rats were euthanized by administering a high dose of thiopental. Perfusion was done by injecting 20mL of ice-cold normal saline. The brain was removed from the cranial vault and then two lobes of the frontal cortex and hippocampus were isolated, stored at -20°C. 10% w/v of the tissue homogenate was prepared in 0.1M ice-cold Phosphate buffer (pH 7.4) using an UltraTurrax T25 homogenizer at the speed of 6000 rpm for 15 minutes and the tissue homogenates were measured for the following biochemical parameters.

**Estimation of acetylcholinesterase enzyme (AChE) activity**

Ellman’s method was followed for the estimation of acetylcholinesterase activity, which is a cognitive marker that indicates loss of cholinergic neurons. To the 5 μL acetylthiocholine iodide 25 μL of 5,5’-dithiobis-2-nitrobenzoic acid (DTNB) was added followed by 650 μL of 0.1M phosphate buffer and finally 100 μL of homogenate was added. The absorbance was then checked for 180 secs at 412 nm in UV spectrophotometer.

**Statistical Analysis**

Graph Pad Prism software was used to analyse the results. Two Way ANOVA followed by Tukey’s multiple compartment test was used to analyse locomotor activity. All of the other tests were analysed using One Way ANOVA followed by Tukey’s multiple compartment test and expressed as Mean±SEM. Experimental results after analysis were considered significant at the p value less than 0.05.

**RESULTS**

**Impact of extract of Cleome viscosa on the locomotor activity:**

The locomotor activity of rats was evaluated using actophotometer after ICV injection of Colchicine. The mean counts of locomotor activity per 10 minutes were normal and stable without significant variation as shown in Figure 2 (Effect of extract of Cleome viscosa on locomotors activity). This indicates that there was no detrimental effect on locomotion due to surgery. The locomotive operation had no significant difference in all groups compared to normal and disease control.
Effect of extract of Cleome viscosa on AChE levels of the brain:
Injection of colchicine ICV in the brain showed a significant rise in the level of AChE compared to the normal group and sham control group. A significant reduction in AChE activity in the frontal cortex and no significant reduction in the hippocampal AChE activity was observed in the Donepezil treated groups compared to the Colchicine only treated disease group. The AChE activity in the hippocampal region was significantly elevated in the Donepezil treated group as compared to the normal and sham control rats. There is no significant reduction in AChE activity in the frontal cortex and hippocampal was observed in the extract of CV (100 and 200 mg/kg) treated groups compared to the Colchicine only treated disease group. (Figure 3: Effect of extract of Cleome viscosa on acetylcholinesterase).

Effect of extract of Cleome viscosa in the spatial memory:
Escape latency refers to the time taken to find the hidden island located in D-quadrant. ICV Colchicine treatment increased the escape latency compared to the vehicle control. Treatment with the standard Donepezil showed a significant decreased latency period as compared to the disease group. The dose of an extract of CV (100 and 200mg/kg) did not show a reduction in the Escape latency period when compared with rats of the disease group. Administration of ICV colchicine significantly decreased the path efficiency in disease control animals subjected to the Morris water maze test. There was no significant change in the path efficiency among all groups. Similarly, rats treated with ICV Colchicine took a longer time to cross the D quadrant as compared to normal and Sham control rats. A significant reduction in the Latency to D quadrant was observed in Donepezil treated group and insignificant reduction for the extract of CV (100 and 200mg/kg) treated groups (Figure 4: Effect of extract of Cleome viscosa on Path efficiency, Escape Latency, D quadrant latency).

DISCUSSION
Alzheimer’s disease is a neurodegenerative disorder identified by a reduction in cognition abilities. It is observed that colchicine injection into the brain precipitates the condition like Alzheimer’s disease. Colchicine is a compound that binds to tubulin proteins resulting in dephosphorylation and destabilization. This leads to the accumulation of amyloid beta (Aβ) and generates tangles similar to neurofibrillary tangles. Consequently, it triggers excessive oxidative stress and neuro-inflammation. Neuro-inflammation has been considered as one of the pathological cause for AD.

In the current study, extract of Cleome viscosa (100 and 200mg/kg) is evaluated for its neuroprotective activity against colchicine-induced dementia in rats. The Cholinergic hypothesis signifies the role of Acetycholine in memory and has led to the identification and use of acetylcholinesterase inhibitors for enhancing memory and cognition. Increased AChE activity has been observed in the plasma, human liver, red blood cells, cerebrospinal fluid and brain from Alzheimer’s patients. Donepezil, a cholinesterase inhibitor was selected as a standard drug for the current study. In this study, the increased level of AChE was found in the frontal cortex and Hippocampus of the rat brain after ICV Colchicine administration. An insignificant reduction in the level of AChE was seen after treatment with dementia in rats as compared to the disease control rats. Cleome viscosa has been reported to have antioxidant property showing its potential to scavenge free radicals. In this study, extract of CV treatment in the ICV Colchicine induced cognition impaired rats, the AChE level of the brain had no significant AChE inhibitor effect in the frontal cortex and the hippocampal region of the brain.

The ICV administration of Colchicine leads to the memory impairment indicated by elevated D-quadrant latency and escape latency and significantly decreased the path efficiency in disease control animals in the Morris water maze test. Donepezil, a standard drug showed a reduction in the latency time. Both the dose of the extract (100 and 200 mg/kg) was not capable to reduce the latency period effectively. There was no significant reduction in the latency period in both of the treatment groups. There was no change in the locomotor activity as observed in the results of actophotometer among the rats of different groups. This removes the possibility of Colchicine toxicity and experimental errors affecting locomotion in the rats.

CONCLUSION
In the current study, the neuroprotective activity of extract of Cleome viscosa (100 and 200 mg/kg) was evaluated against ICV colchicine induced dementia in the rat model. The extract was reported as antioxidant property but the extract showed insignificant activity for AChE level. Treatment with extract at 100 and 200 mg/kg failed to exert any protective effect against colchicine induced dementia in rats.

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Table 1: Treatment regimen

| Sl. No | Groups (n=6) | Dose |
|-------|-------------|------|
| 1     | Normal Control | Vehicle |
| 2     | Sham Control | Intracerebroventricular (5 μL) Injection of Artificial Cerebrospinal fluid (ACSF) |
| 3     | Disease Control | Intracerebroventricular Injection of Colchicine + artificial Cerebrospinal fluid (15 μg Colchicine/5 μL ACSF) |
| 4     | Standard Treatment | Donepezil 2 mg/kg, p.o (Given for 25 days) + Intracerebroventricular Injection of Colchicine + artificial Cerebrospinal fluid (15 μg Colchicine/5 μL ACSF) |
| 5     | Extract Treatment 1 | Extract of Cleome viscosa 100 mg/kg, p.o (Given for 25 days) + Intracerebroventricular administration of Colchicine + artificial Cerebrospinal fluid (15 μg Colchicine/5 μL ACSF) |
| 6     | Extract Treatment 2 | Extract of Cleome viscosa 200 mg/kg, p.o (Given for 25 days) + Intracerebroventricular administration of Colchicine + artificial Cerebrospinal fluid (15 μg Colchicine/5 μL ACSF) |
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Figure 1: Schematic diagram for representing drug/Extract treatment plan, disease induction and evaluation of parameters.

Figure 2: Effect of extract of Cleome viscosa on locomotor activity on Day 3, 7 and 14th in cognitively impaired rats. Values are expressed as Mean ± SEM, n=6.

Figure 3: Effect of extract of Cleome viscosa on 3A) Hippocampus and 3B) Frontal cortex acetylcholinesterase (AChE). Values are expressed as Mean ± SEM, n=6. ** represents p< 0.01, *** p<0.001, when compared with vehicle, and ## p< 0.01 as compared to disease control.

Figure 4: Effect of extract of Cleome viscosa on 4A) Path efficiency, 4B) Escape Latency, 4C) D quadrant latency. Values are illustrated as Mean ± SEM, n=6. ** represents p< 0.01 as compared with vehicle, # # represents p< 0.01 as compared with Disease control.