Amelioration of Cognitive Deficits, Oxidative Damage, Neurochemical Alteration by *Bauhinia purpurea* (stem bark) on Scopolamine Induced Amnesia

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ABSTRACT

**Aim:** To evaluate the anti-amnesia effect of *Bauhinia purpurea* in Scopolamine induced amnesia in rats. **Materials and Methods:** A total of 30 rats were divided into 5 groups 6 rats in each. Group I considered as normal control. Group II served as negative control. Group III, IV and V were treated with Donepezil (3 mg/kg), ethanolic extract of *Bauhinia purpurea* 200mg/kg and 400 mg/kg respectively for 14 consecutive days followed by single administration of Scopolamine (3 mg/kg) to all the groups except group I. Cognitive performance was assessed by the Morris water maze, elevated plus maze and passive avoidance paradigm. Acetyl cholinesterase enzyme level, biochemical markers such as lipid peroxidation, reduced glutathione and β amyloid 1 42 level, Neurotransmitters including dopamine and serotonin and histopathological study of rat brain were estimated. **Results:** *Bauhinia purpurea* and Donepezil rats showed significant increase in escape latency, step-through latency and decreased transfer latency in respective cognitive models of the Morris water maze, passive avoidance test and elevated plus maze. Additionally, *Bauhinia purpurea* extract remarkably promoted the cholinergic neurotransmission, decreasing β amyloid protein and protected against the oxidative stress damage as indicated by, increasing reduced glutathione level, lowering the level of lipid peroxidation, restored dopamine and serotonin level in the brain. Furthermore, histopathological studies revealed the reversal of neuronal damage in the treatment group compared to Scopolamine treated rats. **Conclusion:** *Bauhinia purpurea* extract showed promising antiamnesia activity against scopolamine induced amnesia in rats. This could be attributed to its brain acetyl cholinesterase level, β amyloid level inhibition, antioxidant activity and alteration in neurotransmitters level. **Key words:** *Bauhinia purpurea*, Neuroprotective, Acetylcholinesterase, β amyloid protein, Scopolamine.

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DOI: 10.5530/jyp.2020.12.66

INTRODUCTION

Alzheimer's disease (AD) is a widespread neurodegenerative condition that causes memory loss and dementia and the aging population is a major health concern. This is characterized by the development of senile plaques which are rich in insoluble aggregates of β amyloid and neurofibrillary tangles in the brain, neurotransmission deficiency, neuroinflammation, neuronal death and free radical formation.3 Thirty million people worldwide are affected by dementia and 4.6 million cases annually are well documented according to AD society analysis.3 The primary cause of AD such as decrease in the level of acetyl choline, oxidative stress followed by impaired memory. Memory is one of an individual's important roles in remembering events, storing and maintaining information over short and long periods.3 Induction of cognitive deficiencies by cholinergic antagonism that is similar to Alzheimer's cognitive symptomology.4 Scopolamine (Scop) is cholinergic antagonist and can induce cognitive dysfunction in rodents as well as to humans by decreasing the CNS efficacy of ACh in animals and humans cause impairment in learning and memory.4 Currently, AD can be treating with acetyl cholinesterase inhibitors with fewer benefits and also accompanied by adverse effects including hepatotoxicity and nausea with short half-lives.4 According to the estimation World Health Organization (WHO) that majority of the population presently uses herbal drugs for many health issues such as neurodegenerative disease, diabetes and cancer etc., Herbal drugs may provide a new source of beneficial neuropsychotropic drugs.7-9

*Bauhinia purpurea* L. belonging to the family Leguminosae, grows small to medium sized deciduous tree upto 17 m tall. Parts of this plant have been used in the field of medicines traditionally to treat various kinds of disease including body pain, rheumatism, fever, dropsy, stomach tumour, skin diseases, septicemia and diarrhea.5,10 Analgesic, anti-inflammatory, antipyretic, antidiabetic, free radical scavenging and thyroid hormone-stimulating activities were scientifically documented.11-13 Several plant constituents including foliar flavonoids, flavones, flavonone glycosides were reported.14,15 However, its antiamnesic activity has yet to be investigated. Hence, the present study was conducted to evaluate the antiamnesic effect of *Bauhinia purpurea* (stem bark) in scopolamine induced amnesia in rats.

MATERIALS AND METHODS

Plant Material and Extraction

*Bauhinia purpurea* is an accepted plant name and in the listed of plants (www.theplantlist.org). The plant material was obtained from Tirunelveli...
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Schematic description of experimental design: After 7 days acclimatization period, animals were divided into 5 Groups: Control group; Scop group (3mg/kg, i.p.); Scop + Donepezil group (3mg/kg) and Scop + EEBP (200 and 400 mg/kg, p.o.). Rats were pretreated with EEBP and DNP for 14 days followed by single challenge of Scop. Memory evaluation parameter such as Morris water maze, elevated plus maze and passive avoidance were assessed. Following the behavioral tests all rats were sacrificed and brain were harvested for further biochemical studies.

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**Animals**

Adult male wistar rats (weighing 180-200g: 30 rats) were procured from CI. Baid Metha College of Pharmacy, Chennai and divided into five groups of six in each. Rats were housed in cages in groups of six per cage and maintained under natural light and dark cycle and standard laboratory conditions. Before going to conduct the experiment rats should be acclimatized for a week with *ad libitum* free access to food and water. Behavioral tests were performed in a quiet environment from 9.00 am to 11.00 am to prevent circadian variability. All the experimental procedures were carried out in accordance with the CPCSEA guidelines.

**Drugs and Chemicals**

Scopolamine (Sigma Aldrich, New Delhi), Donepezil (Eisai Pharmaceuticals Ltd., Mumbai). ELISA kit for rat Aβ (YH Bioresearch Laboratory, Shanghai, China). All other chemicals and reagents unless specified were of analytical grade. All the drug solutions were freshly prepared and use.

**Experimental design**

Three behavioral models were used to assess learning and memory, namely Morris water maze (MWM), elevated plus maze (EPM) and modified passive avoidance study. Rats were randomly divided into five groups (*n=6* group); Group 1– Normal control (Vehicle 0.9% Nacl i.p.), Group II – Negative control (Vehicle + scopolamine 3 mg/kg, i.p.), Group III – Standard drug (Donepezil 3 mg/kg, i.p. + scopolamine 3 mg/kg, i.p.), Group IV – Low dose Ethanolic extract of *Bauhinia purpurea* (EEBP 200 mg/kg, p.o. + Scopolamine 3 mg/kg, i.p.) and Group V – High dose of ethanolic extract of *Bauhinia purpurea* (EEBP 400 mg/kg, p.o. + Scopolamine 3 mg/kg, i.p.). The rats were administered every 24th interval with respective drugs for 14 consecutive days. Memory evaluation parameter such as MWM, EPM and passive avoidance acquisition trail was carried out on the 14th day, intraperitoneal administration of Scopolamine 3 mg/kg was administered to all the groups except normal control group after completion of acquisition trail. The timeline scheme of the experiment is shown in Figure 1.

**Screening Methods for Amnesia**

**Elevated plus-maze test**

Memory enhancement index screening was conducted in rats using elevated plus maze. Every rat was placed in the elevated plus maze apparatus at the end of an open arm. The time required for each rat to enter to the closed arm was deemed to be transfer latency (TL). Each rat allotted cutoff time is 180 s and the retention trial was performed 24 hr after the first trial, transfer latency was reported in a manner similar to that previously described. Shortened transfer latency was used as an indicator of memory improvement.

**Morris water maze**

Spatial learning and memory were evaluated by the Morris water maze. The procedure included two steps. The first step was the place navigation test from day 1 to 4, in which the escape latency (EL) (the time required to escape onto the hidden platform) was used to evaluate learning and memory function. Mice that found the platform were allowed to remain on the platform for 20 s and were then returned to the home cage. If mice did not reach the platform within 120 s, it was gently guided to the platform by the experimenter, where it remained for 20 s. The last trial was regarded as the probe test. The second step was the spatial probe test on day 5 after removal of the platform and after the space navigation test, which was performed to test the ability of mice to find the removed platform by memory.

**Collection of Brain Sample**

The brain were removed carefully from the skull by cervical dislocation and weighed. Ten percent w/v brain homogenate was then prepared by homogenizing it in ice chilled phosphate buffer (pH 8, 0.1M). The homogenate was subsequently centrifuged using a refrigerated centrifuge at 3000 rpm for 10 min and the supernatant was separated and used for the biochemical estimations.

**Antioxidant Estimation**

**Estimation of Lipid Peroxidation**

About 0.2 mL of brain homogenate, 0.2 mL of sodium dodecyl sulfate, 1.5 mL of acetic acid, and 1.5 mL of thiobarbituric acid were added. The mixture was made up to 4 mL with water and then heated in a water bath
Results

Effect of Bauhinia purpurea extract on transfer latency in elevated plus maze

The memory function was tested on long-term spatial memory using the EPM test. Time required for each rat to enter from the open arm into the closed arm was known as the transfer latency period. Scopolamine treated rats produced significantly increased (P<0.001) in transfer latency on retention trial compared to control rats which indicates learning and memory impairment. Pretreated with BP extract in both doses (P<0.01, P<0.001) and Donepezil rats were produced significantly decreased (P<0.001) the transfer latency when compared to scopolamine treated rats. (Table 1)

Effect of Bauhinia purpurea extract on escape latency in morris water maze

The escape latency time (ELT) is the time taken by the rats to locate the secret platform in the labyrinth of water that was used as a measure to determine the performance of the treated rats. The scopolamine-treated rats showed significantly prolonged the ELT compared to normal rats on fourth day of the acquisition period. On 5th day, time spent in the particular quadrant was significantly reduced compared to control rats (P<0.001). In acquisition trial, pretreatment with BP extract was found significantly decrease the ELT when compared to Scopolamine treated rats. On day five, BP extracts (P<0.01, P<0.001) and Donepezil (P<0.001) pretreatment groups exhibited significantly increased time spent in the target quadrant compared with scopolamine rats (Table 1).

Effect of Bauhinia purpurea extract on latency time in passive avoidance test

On Day 1, Scopolamine treated rats resulted in a significantly (P<0.001) shorter latency time compared with the control group. The shorter retention latency suggests impairment in learning and memory. Pretreated with BP extracts (P<0.01, P<0.001) and Donepezil rats significantly (P<0.001) increased the latency time compared with the scopolamine rats. (Table 1).

Effect of Bauhinia purpurea extract on scoplamine induced oxidative stress

Scopolamine treated rats induced oxidative damage indicated by the significant decreased (P<0.001) GSH content and increased (P<0.001) the level of MDA in the brain compared to control rats. Pretreated with BP extract and Donepezil treated rats produced significantly decreased the MDA level (P<0.01, P<0.001 and P<0.001) and increased the GSH level (P<0.001) compared to Scopolamine treated rats (Figure 2a, b).

Effect of Bauhinia purpurea extract on scoplamine induced Acetylcholinestrase level

Rats were treated with single administration of Scopolamine rats significantly increased (P<0.001) the AChE activity compared to normal control rats. Treatment with Bauhinia purpurea extracts and Donepezil rats significantly (P<0.01, P<0.001 and P<0.001) reverse the Scopolamine induced increase the AChE activity (Figure 3a).

Effect of Bauhinia purpurea extract on scoplamine induced β amyloid level in brain homogenate

The concentration of β amyloid level increased significantly in the treatment of scopolamine treated compared to control rats (P<0.001). Pretreatment with Bauhinia purpurea extract and Donepezil rats

Statistical Analysis

The results are reported as the mean ± SEM of the mean analysis of variance followed by the Tukey’s multiple comparison tests were used for comparison. Differences were considered significantly at P<0.05.
significantly decreased the concentration of Aβ1-42 as compared to scopolamine treated group (Figure 3b).

**Effect of Bauhinia purpurea extract on scopolamine induced dopamine and serotonin level in brain homogenate**

Scopolamine treated rats significantly altered the levels of neurochemicals such as DA and 5-HT in the rat's brain hippocampus region. Specifically, the level of DA and 5-HT increased significantly ($P<0.001$) compared to control rats. Nevertheless, high dose of *Bauhinia purpurea* ($P<0.05$ and $P<0.01$) and Donepezil rats ($P<0.01$) significantly reversed the both the neurochemicals level such as DA and 5-HT produced by scopolamine treated rats (Table 2).

**Effect of Bauhinia purpurea extract on scopolamine induced neuronal damage in hippocampal region**

Scopolamine treated rats showed degeneration and decrease in number of neuronal cells along with edema and pyknotic nuclei. Pretreated with *Bauhinia purpurea* extract significantly recovered the neurodegeneration, edematous nuclei and congestion of neurons by dose dependently and Donepezil treated rats significantly ameliorate the neurodegeneration and decrease in neuronal cells compared to Scopolamine treated rats (Figure 4).

**DISCUSSION**

In the Indian medical system, *bauhinia purpurea* was prescribed for various nervous-related disorders, including convulsions, delirium, asthma and anti-inflammatory agents, but no neuroprotective research has been investigated till date. Our current study reveals the first evidence that *Bauhinia purpurea* (stem bark) has antiamnesic effect on scopolamine induced amnesia in rats. The effect BP extract was assessed by screening methods including Morris water maze, elevated plus maze, passive avoidance were performed. Additionally, using the homogenates of brain tissues levels of neurochemicals, oxidative markers, histopathological studies were also performed. In the present study, to evaluate the effect of BP extract on Scop-induced amnesia in rats, three types of cognitive memory tasks such as EPM, MWM and Passive Avoidance Task were used. For the time, BP extract treatment was found to strongly improve the impaired memory in the Scop-treated rats in the EPM task which indicates the long-term animal spatial memory enhancing effects. Scopolamine-treated rats increased the latency duration of escape during the acquisition process and decreased in the time spent in the probe trail in the target quadrant. Treatment with BP extract reverse the Scop-induced memory deficits in acquisition trail as well as on probe trail. The rat goes into the dark chamber, gets a painful electric shock in the first trial. Indeed, the animal’s ability to learn and memorize was demonstrated by avoiding entry into the dark chamber.

The results of the current study reveal that Scop- treated rats significantly reduced the latency time in the passive avoidance test and learning and memory function deficits. It is consistent with previous results that systemic administration of Scop-produced impaired in cognitive function in rodents and humans. Scop-induced memory deficits were reversed by pretreatment with *Bauhinia purpurea* extract proven the antiamnesic effect based on the behavioural study. In our in vivo study as mentioned earlier studies could reverse scopolamine-induced memory impairment.

Scop-induced amnesia has also been linked with enhanced oxidative stress damage in the brain particularly in the region of hippocampus it is
Table 1: Effect of *Bauhinia purpurea* and Donepezil on Elevated Plus maze, Morris water maze and Passive avoidance test in Scop-induced amnesia in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Transfer Latency (s)</th>
<th>Time spent in target quadrant (s)</th>
<th>Escape Latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>10.28± 4.23</td>
<td>5.28 ± 2.24</td>
<td>15.16 ± 4.56</td>
</tr>
<tr>
<td>Scopolamine control</td>
<td>28.15± 6.27</td>
<td>1.26± 0.98</td>
<td>4.67± 1.78</td>
</tr>
<tr>
<td>Donepezil (3mg/kg) + Scop (3mg/kg)</td>
<td>13.27± 2.18</td>
<td>4.98± 1.90</td>
<td>11.05± 3.68</td>
</tr>
<tr>
<td>EEBP (200mg/kg)+ Scop (3mg/kg)</td>
<td>16.04± 4.78</td>
<td>2.62 ± 3.26</td>
<td>7.98± 4.26</td>
</tr>
<tr>
<td>EEBP (400mg/kg)+ Scop (3mg/kg)</td>
<td>14.94± 6.20</td>
<td>3.96± 2.46</td>
<td>10.98± 2.29</td>
</tr>
</tbody>
</table>

Values represent in mean ± SEM (n=6): 1.P<0.05, 2.P<0.01 and 3.P<0.001 vs control rats. 4.P<0.05, 5.P<0.01 and 6.P<0.001 vs Veh+ Scopolamine rats, one way ANOVA followed by Tukey's post hoc test.

Table 2: Effect of *Bauhinia purpurea* and Donepezil on brain homogenates neurotransmitters content in Scop-induced amnesia in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dopamine(ng/mgprotein)</th>
<th>Serotonin(ng/mgprotein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>328.12± 8.96</td>
<td>596.12± 8.78</td>
</tr>
<tr>
<td>Scopolamine control</td>
<td>120.96±12.10</td>
<td>185.10± 5.45</td>
</tr>
<tr>
<td>Donepezil (3mg/kg) + Scop (3mg/kg)</td>
<td>252.10± 6.46</td>
<td>306.05± 12.67</td>
</tr>
<tr>
<td>EEBP (200mg/kg)+ Scop (3mg/kg)</td>
<td>140.18± 13.26</td>
<td>214.16± 9.75</td>
</tr>
<tr>
<td>EEBP (400mg/kg)+ Scop (3mg/kg)</td>
<td>210.46± 8.12</td>
<td>290.48± 13.67</td>
</tr>
</tbody>
</table>

Values represent in mean ± SEM (n=6): 1.P<0.001 compared to control rats. 2.P<0.01 and 3.P<0.001 compared to Scopolamine rats using one way ANOVA followed by Tukey's post hoc test.

In this study EEBP was assessed for inhibition of AChE activity, it is one of the key targets for AD therapy. Increased AChE activity contributes to a decreased level of ACh and thus neurological disorders associated with cholinergic deficiencies as seen in AD patients. Previous research suggested that scopolamine increases the production of AChE in both the hippocampus and cortex. Pretreated with BP extract administration significantly decreased the AChE level compared to Scopolamine treated rats. Previous report support for our present study flavonoids such as Quercetin, Kaempferol, luteolin and apigenin inhibited AChE inhibition and proved to be effective anti-AD and had neuroprotective effects. Plaque deposition in extracellular spaces by aggregation of β amyloid protein in the brain tissue is characterized by cognitive impairment. Consistent with previous findings supports for our results showed that treatment with scopolamine increase the β amyloid level in the brain tissue as compared with the control rats. Pretreated with BP extract attenuated the scopolamine induced elevated levels Aβ. Earlier studies found that phytoconstituents such as alkaloids have beneficial effects on learning and memory through stimulation of cholinergic neuronal transmission and research suggested that scopolamine increases the production of AChE in both the hippocampus and cortex.

Several studies were reported that neurochemicals such as DA, 5-HT, ACh and NE affects the learning and memory. Previous research suggested that scopolamine increases the production of AChE, DA and 5-HT in both the hippocampus and cortex. Current research reveals that pretreated with BP extract alter the brain neurotransmitter levels compared with scopolamine group. The neuronal damage, hippocampal edema, pyknotic cells and neuro fibrillary tangles were observed in scopolamine treated rats while groups pretreated with donepezil, BP extracts protected neurons by reversing the damage induced by scopolamine.

**CONCLUSION**

The observed neuroprotective and enhanced the improved in cognitive performance due to the decreased in the levels of AChE, β amyloid, restored the neurotransmitters and prevent the oxidative damage in rat brain homogenates. These findings thus provide evidence for the potential of BP extract as a natural alternative treatment for amnesia.

**ACKNOWLEDGEMENT**

I wish to acknowledge the management of Crescent School of Pharmacy, B. S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai.

**CONFLICT OF INTEREST**

All authors declare no conflict of interest.

**ABBREVIATIONS**

- **Scop:** Scopolamine; **AD:** Alzheimer’s Disease; **EEBP:** Ethanolic extract of *Bauhinia purpurea*; **LPO:** Lipid peroxidation.

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