Effect of ethanolic seed extract of *Bauhinia purpurea* linn on cognition in scopolamine induced Alzheimer’s disease rat’s model

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ABSTRACT

**Aim:** The present study was taken to investigate the effect of Ethanol Seed extract of *Bauhinia purpurea* Linn (ESEBP) in Scopolamine induced Alzheimer’s disease rat model.

**Materials and methods:** The ESEBP was administered orally at two doses (200 and 400mg/kg) for a period of 14 successive days followed by Scopolamine (1 mg/kg, i.p) was administered after 30 min of last dose (on day 14). Rivastigmine (1.5mg/kg) was used as standard drug. Cognitive functions are estimated by using elevated plus maze (EPM), Y-maze, and Rota rod apparatus.

**Results:** ESEBP extract has shown a significant memory enhancing activity at the selected doses by decrease in the transfer latency in EPM, significant increase in the percentage of spontaneous alteration on acquisition of the short term memory of the scopolamine treated rats within Y-maze task and there was absolute cognitive learning improvement is related to central cognitive mechanism’s not the motor coordination paradigms by Rot rod test.

**Conclusion:** In the present study ESEBP may prove to be a useful medicine on account of its, memory improving property, and it would be worthwhile to explore the potential of this plant in the management of Alzheimer’s patients.

**Key words:** Alzheimer’s diseases, memory enhancing, Scopolamine

INTRODUCTION

Alzheimer’s diseases (AD), is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behaviour, personality changes and ultimately death [1]. The primary cause of AD remains unclear; it may be considered that the amyloid and tau protein aggregation, reduced acetylcholine (ACh), and glutamatergic deficit are regarded as principal pathogenesis of AD. According to WHO, 5% of men and 6% of women aged above 65 years suffers from dementia of AD worldwide.

Formation of memory is the most complex process and involves multiple neuronal pathways and neurotransmitters. It is well known that the cholinergic neuronal system plays an important role in learning and memory in humans and animals. This is the therapeutic rational behind the use of acetyl cholinesterase inhibitor agents such as rivastigmine, glutamine, donepezil. But there are many side effects like loss of appetite, nausea, vomiting, diarrhoea, stomach cramps, headache, dizziness, fatigue and insomnia etc, are associated with these agents have limited their use.
So, there is growing recognition for newer products, one among them are medicinal herbs due to their non-toxic nature with less side-effects and available at low price.

The plant Bauhinia purpurea belongs to the family Leguminaceae is used extensively in traditional system of medicine to cure dropsy, pain, rheumatism, convulsions, delirium, Septicaemia, etc. the bark of the plant is used as an astringent in the treatment of diarrhoea [2]. Considering the beneficial effects of herbs over allopathic treatment, there was an outlook for herbs which has a medicinal use in treating the memory loss. Due to lack of scientific evidence and also a proper drug to treat AD, an attempt was made to study the beneficial effects of the plant Bauhinia purpurea against scopolamine induced Alzheimer’s disease rat model. Thus the present study was aimed to investigate the effect of Ethanolic Seed extract of Bauhinia purpurea Linn (ESEBP).

MATERIALS AND METHODS

Collection of Seed: The seeds of Bauhinia purpurea Linn. was been collected during the month of January 2015 and dried under the shade. The dried seeds were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using ethyl alcohol. The solvent was removed by distillation under reduced pressure, which produces a dark brown sticky residue.

Acute Toxicity Study

Acute toxicity study of ESEBP was carried out in rats according to OECD-423 guidelines. Different doses of ESEBP were administered up to 2000 mg/kg b.w. (p.o) and the rats were observed for a period of 72hr for behavioural changes, toxic symptoms and mortality.

Experimental Design

The animals were divided into 5 groups of 6 rats each. Doses of ESEBP (200 & 400mg/kg), selected on the basis of acute toxicity studies, and were administered orally for 14 days. The standard drug rivastigmine (1.5mg/kg, p.o) administered for 14 days. After 30 min of administration of last dose (on day 14) Scopolamine (1mg/kg, i.p) was used to induce AD in all groups except normal. Thereafter, neurobehavioral cognitive were performed on day 14. Animals are provided with food and water as usual before experiment.

Behavioural Parameters

Elevated plus maze (EPM):

Elevated plus maze served as exteroceptive behavioural model to evaluate learning and memory in rats [3]. The apparatus consisted of two open arms (50×10 cm each), two enclosed arms (50 × 10 ×20 cm each) and a central platform (10×10 cm), arranged in such a way that the two arms are opposite to each other. The elevation of the maze was 100 cm above the floor. On the day 14, approximately 1hr after the administration of the Bauhinia purpurea Linn extract, each animal was placed at the centre of the maze facing one of the enclosed arms. During the 5 min test period, the number of open and enclosed arm entries plus the time spent in the open and enclosed arms were recorded. Entry into an arm was defined as the point when the animals place all paws onto the arm. Animal behaviour was recorded manually. After the test the maze was carefully cleaned with a wet tissue paper (10% ethanol solution) [4].

Y-maze:

The Y-maze in the present study consisted of three arms (35cm long, 25cm high and 10cm wide) and an equilateral triangle central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. The sequence of each arm entry recorded manually (i.e., ACBABACACBCA etc.). Spontaneous alternation behaviour was defined as entry into all three arms on consecutive choices in overlapping triplet sites (i.e., ACB, ABA, CAC, BCA) [5].

Animals performing less than five alternations in the 8 min period were excluded from the analysis of results. On the day 14, approximately 1hr after the administration of the Bauhinia purpurea Linn extract, each animal was placed at the end of one arm, allowed to move freely. The number of maximum spontaneous alternation behaviours = total number of arms entered minus 2 and % spontaneous alternation = actual alternations / maximum alternations × 100 [6].

Rota rod test

First, animals were trained by placing them on a rolling bar and had been to walk on it. Then, the animals were conducted to rota rod test with an appropriate speed (20-25rpm is ideal). Place the animal one by one on the rotating rod and fall off time i.e., when the animal fall from the rotating rod was recorded, which was taken as motor integrity and coordination.

Statistical analysis

In the present study, all the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.
RESULTS

Effect of ESEBP on Elevated plus maze test

Behaviour in the elevated plus-maze is mainly used to assess exploration and anxiety status.

I. Time spent in open arm and closed arm

The time spent in the open arm were decreased (23.0 ±1.39) and a significant increase of time spent were observed in the closed arm (289.2±1.07) in scopolamine induced animals on day 14 indicating anxiogenic response (p<0.001). Rivastigmine pre-treated group showed significant increase in the time spent in the open arm (145.3±1.58) and subsequent decrease of time spent in the closed arm were observed when compared to control group (p<0.001).

The groups pre-treated with ESEBP (200 & 400mg/kg) showed significant increase in the time spent in open arm (33.6±1.58 & 72.5 ±3.25) and a significant decrease in the open arm (249.5±1.33 & 216.5±3.93) in scopolamine induced animals on day 14 indicating anxiolytic response. However, time spent in high dose of extract group was more significant compared to control group (p<0.001). The results were shown in Graph no: 1.

II. No. of entries in open arm and closed arm

The no. of entries in the open arm were decreased (1.6 ±0.21) and a significant increase of entries were observed in the closed arm (6.6±0.49) in scopolamine induced animals on day 14 indicating anxiogenic response (p<0.001).

Rivastigmine pre-treated group showed significant increase in the no. of entries in the open arm (5.5±0.42) and subsequent decrease of entries in the closed arm were observed when compared to control group (p<0.001). The groups pre-treated with ESEBP (200 & 400mg/kg) showed significant increase in the no. of entries in open arm (3.1±0.30 & 4.5 ±0.22) and a significant decrease in the open arm (5.1±0.30 & 2.3±0.21) in scopolamine induced animals on day 14 indicating anxiolytic response. However, time spent in high dose of extract group was more significant compared to control group (p<0.001).

These results support the hypothesis that ESEBP affected the processes associated with initiation or maintenance of behavioural responses to novel and/or exploration situations. The results were shown in Graph no: 2.

Graph no: 1 Effect of ESEBP in time spent on Elevated plus maze test

| Treatment groups | Normal | Control | Standard | Test 1 | Test 2 |
|------------------|--------|---------|----------|--------|--------|
| Open arm         | 200    | 250     | 300      | 350    | 400    |
| Closed arm       | 300    | 400     | 450      | 500    | 550    |

Normal = vehicle treated; Control = Scopolamine (1mg/kg, i.p); Standard = Scopolamine (1mg/kg, i.p) + Rivastigmine (1.5 mg/kg, p.o); Test-1 = Scopolamine (1mg/kg, i.p) + ESEBP (200mg/kg, p.o); Test-2 = Scopolamine (1mg/kg, i.p) + ESEBP (400mg/kg, p.o)
Graph no: 2 Effect of ESEBP in no. of arm entries on Elevated plus maze test

![Graph showing the effect of ESEBP on the number of arm entries in Elevated plus maze test.](image)

**Treatment groups**

Normal = vehicle treated; Control = Scopolamine (1mg/kg, i.p); Standard = Scopolamine (1mg/kg, i.p) + Rivastigmine (1.5 mg/kg, p.o); Test-1 = Scopolamine (1mg/kg, i.p) + ESEBP (200mg/kg, p.o); Test-2 = Scopolamine (1mg/kg, i.p) + ESEBP (400mg/kg, p.o).

Graph no: 3 Effect of ESEBP on spatial cognition deficit in Y-maze test

![Graph showing the effect of ESEBP on spatial cognition in Y-maze test.](image)

**Treatment groups**

Normal = vehicle treated; Control = Scopolamine (1mg/kg, i.p); Standard = Scopolamine (1mg/kg, i.p) + Rivastigmine (1.5 mg/kg, p.o); Test-1 = Scopolamine (1mg/kg, i.p) + ESEBP (200mg/kg, p.o); Test-2 = Scopolamine (1mg/kg, i.p) + ESEBP (400mg/kg, p.o).
Effect of EFECL on spatial cognition deficit in Y-maze test

Scopolamine induced group markedly decrease the score of spontaneous alternation behaviour (39.8 ± 2.58; P<0.001) in control animals compared to normal group (94.9±1.6) on day 14. Rivastigmine pre-treated group showed significant increase in the spontaneous alternation behaviour compared to control group (108.1±3.13; p<0.001) on day 14 after scopolamine induced.

The Scopolamine induced groups on day 14 in pre-treated rats with ESEBP in a dose of 200mg/kg rats significantly increase (65.2 ± 2.59; P<0.001) spontaneous alternation behaviour. However, the effect of ESEBP in dose of 400 mg/kg on spontaneous alternation behaviour is more significant (81.2±1.04; p<0.001), mild alleviation effect on scopolamine induced spatial cognition deficits. The results were shown in Graph no: 3

Effect of EFECL on Rota rod test

Motor integrity and coordination were assessed by the time latency from placement of the animal on the rotating drum until it fell. One-way ANOVA analysis showed significant differences among groups in the time latency to fall. Control group received scopolamine on day 14, showed (15.8±0.94) significant (p<0.001) difference from normal group (41.5±0.99).

The rivastigmine-received group (1.5mg/kg, p.o) showed significant difference (p<0.001) from control group (scopolamine 1mg/kg, i.p) (47.8±1.01) on day 14.

The group pre-treated with low dose of ESEBP (200mg/kg, p.o) showed 22.1 ± 1.35 (p<0.01) increased time latency when compared to control group, but the group pre-treated with low dose of ESEBP (400 mg/kg, p.o), showed 43.0 ± 0.90 increased time latency which was more significant (p<0.001) when compared to control group. The results were shown in Graph no: 4

DISCUSSION

AD is a progressive neurodegenerative disorder associated with a decline in cognitive abilities. Despite the severity and high prevalence of this disease, the allopathic system of medicine is yet not to provide a satisfactory antidote. Hence, the present study focuses on investigate the effect of Ethanolic Seed extract of Bauhinia purpurea Linn (ESEBP) in Scopolamine induced Alzheimer’s disease rat model.

Anxiety and depression are common in AD patients, and is related with duration of dementia, greater severity of dementia and lower education levels [7]. The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli [8]. An anxiolytic agent increases the time spent in open arms and the frequency of entries into the open arms in this test. In the present study, high and low doses of Bauhinia purpurea extract significantly reversed the average time spent in the open arms to closed arms ratio induced by scopolamine and showed anxiolytic effect. In similar to extract pre-treated rivastigmine showed anxiolytic effect.
The Y-maze task is a specific and sensitive test of spatial recognition memory in rodents. The test relies on an innate tendency of rats to explore a novel environment [9]. The Y-maze used in this study involves no aversive stimuli and was considered suitable for evaluating memory.

However, this result suggests that Bauhinia purpurea extract (400mg/kg) used in this study shows more significant increase the percentage of spontaneous alternation on acquisition of the short term-memory of the scopolamine treated rats within Y-maze task. The standard drug rivastigmine also showed significant increase in percentage of spontaneous alternations.

Motor integrity and coordination data obtained from animal groups showed a significant difference between scopolamine, rivastigmine, and Bauhinia purpurea extract pre-treated groups with respect to control. However, the motor integrity and coordination data between scopolamine, rivastigmine and Bauhinia purpurea extract pre-treated groups could explain that absolute cognitive learning improvement by Bauhinia purpurea is related to central cognitive mechanism’s not the motor coordination paradigms.

CONCLUSION

Hence, from this study it was concluded that supplementation of Bauhinia purpurea extract and inducing scopolamine, a remarkable resurgence was observed in learning and memory. These results represented good therapeutic approaches for intervention against progressive neurological damage associated with AD.

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Conflict of Interest

We declare that we have no conflict of interest.