

Nootropic Activity of dried Seed Kernels of *Caesalpinia crista* Linn against Scopolamine induced Amnesia in Mice

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Abstract: Dementia is one of the age related mental problem and characteristic symptoms of various neurodegenerative disorder including Alzheimer's disease. Stressful conditions are often associated with loss of memory and other cognitive functions. Various drugs like diazepam, alcohol, barbiturates disrupt learning and memory. Traditionally herbal drugs have been used to enhance cognitive function. Present work was undertaken to assess the potential of dried seed kernels of *Caesalpinia crista* extract as learning and memory enhancer. Aqueous extract of dried seed kernels of *Caesalpinia crista* linn. ameliorated the amnesic effect of scopolamine in mice. Radial arm maze and Morris water maze paradigm served as the exteroceptive behavioral models. Aqueous extract of dried seed kernels of *Caesalpinia crista* linn. is compared with standard drug piracetam in scopolamine induced amnesia in mice. Aqueous extract *Caesalpinia crista* linn. could be beneficial to improve cognition in disorders like dementia and various neurodegenerative disorders.

Key words: learning, Memory, *Caesalpinia crista*, Piracetam, Scopolamine.

INTRODUCTION

Memory is ability of an individual to record event, information and retains them over short or long periods of time and recalls the same whenever needed. Age, stress and emotion are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, to more ominous threat like schizophrenia and Alzheimer's diseases. Alzheimer's disease is progressive neurodegenerative disease that primarily affects the elderly population, and is estimated to account for 50-60% of dementia cases in persons over 65 years of age^{1, 2}. "Nootropics" are agents that enhance the cognitive skills, and "amnestics" are agent that disrupts the learning and memory processes. Learning and memory can be conceived as both a psychological process, as well as a change in synaptic neural connectivity^{3, 4}. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neuro-degenerative states⁵. Indian systems of medicine emphasize use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative

disorders. The yellowish white, bitter, fatty kernel of *Caesalpinia crista* is traditionally used as tonic and antipyretic. The leaves and seeds are used in external application for dispersing inflammatory swelling. The bark and leaves are considered emmenagogue, febrifuge and anthelmintic⁶. In various liver disorders tender leaves are used. In some part of Vidharbha region seeds kernels are traditionally used as crude learning and memory enhancer.

Realizing the fact, this research was carried out to evaluate the effect on learning and memory activity of aqueous extract at different dose of dried seed kernels of *Caesalpinia crista* Linn against scopolamine induced amnesia in mice.

MATERIALS AND METHODS

Plant material

Dried seeds kernels of *caesalpinia crista* linn. was collected from Yeotmal district of Maharashtra (India) in month of May and authenticated by Prof. Krishna Kadaskar, Dept. botany, P.N.College Pusad, Maharashtra.

Preparation of extract⁷

The authenticated dried seed kernels of *Caesalpinia crista* Linn was used for the preparation of the extract. The seed kernels of *Caesalpinia crista* Linn was collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for the extraction.

Aqueous Extract of dried seed kernels of *Caesalpinia crista* Linn

The powdered material was macerated with distilled water in a 2.5 liters round bottom flask, for 72 hrs. 10 ml of chloroform was added daily to avoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Brown color residue was obtained and it was stored in a dessicator.

Animals⁸

Swiss albino mice (20-25gm) of male sex and of approximately the same age, procured from listed suppliers of Yash Farms, Breeder/Supplier: Small Animals, Aundh Road, Pune-3 were used for the study. They were housed in polypropylene cages and fed with standard rodent pallet diet (Hindustan Level Limited, Bangalore) and water ad libitum. The animals are exposed to alternate cycle of 12hrs of darkness and 12hrs light. Before each test, the animals were fasted for at least 12 hrs and the experimental protocols were subjected to the scrutinization of the Institutional Animal Ethical Committee and were cleared by the same. All experiments were performed in the morning according to current guidelines for care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals. The standard organistic canula and syringe were used administration in experimental animals.

Acute Toxicity Studies⁹

The aim of performing acute toxicity studies is to establishing the Therapeutic index of a particular drug. Acute toxicity study is generally carried out by determination of LD₅₀ value in experimental animal.

Determination of LD₅₀ Value:**Requirements**

1. Animals : Swiss albino mice (20-25gm)
2. Drug: Aqueous extract of dried seed kernels of *Caesalpinia crista* Linn.

Methodology

Before starting LD₅₀ determination of pilot study was carried out in small group of animals to select the dose ranges for the subsequent study. The overnight fasted Swiss albino mice were weighed and divided into five

groups of six each. Group 1 to 5 received the various doses of Aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. (300mg/kg, 400mg/kg, 500mg/kg, 600mg/kg, 700mg/kg) by oral route. After administration of extract, all the mice were observed continuously for two hours and then occasionally for further four hours and finally overnight for death due to acute toxicity.

The result of LD₅₀ determination was done on mice by Karber's method and LD₅₀ of aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. was found to be 500mg/kg. and ED₅₀ value = 50 mg/kg

EVALUATION OF NOOTROPIC ACTIVITY OF DRIED SEED KERNELS OF CAESALPINIA CRISTA LINN¹⁰**Requirements**

- Animals** : Swiss albino mice (22-25 gm)
Adult male mice weighing between 22 to 25 gm were used.
- Drug** : 1. **Test drug**
Caesalpinia crista Linn dried seed kernel extract (Aqueous)
Dose: 50mg/kg and 150 mg/kg Oral
2. **Standard drug**
Piracetam in water for injection was prepared as per requirement.
Dose: 150 mg/kg i.p.

Selection and acclimatization of Animals:

Swiss albino mice weighing about 22-25 gm each were selected. The animals were kept under a conventional light regimen with a dark night at room temperature (about 25°C) and humidity. Animals were housed in plastic bottom cages and were allowed free access to standard laboratory feed and water. All the animals have been divided into seven groups and placed in separate cages, each consisting of 6 animals. The animals were acclimatized to the laboratory condition for one week before the onset of experiment.

Grouping and treatment protocol

Five groups of animals were made, each group consisting of six mice. The following were the groups.

- Group 1** Normal Control Mice received only vehicle
- Group 2** Amnesic Control (1mg/kg) i.p. Mice received only vehicle against scopolamine-induced amnesia.
- Group.3** Aqueous extract (50mg/kg) p.o. treated mice against scopolamine-induced amnesia
- Group 4** Aqueous extract (150mg/kg) p.o. treated mice against scopolamine-induced amnesia

Group 5 Standard drug Piracetam (150mg/kg) i.p. treated mice against Scopolamine-induced amnesia.

The animals were trained for maze task performance by conducting one daily training trial. It took 15 days to get the animal completely trained. During which they did not received any drug. The completely trained animals were chosen for the study. These animals were dosed once in a day with the respective drugs for ten days along with daily training trial.

In this group I served as normal control. Group II served as amnesia control which received the vehicle for ten days and scopolamine (1 mg/kg i.p.) on day eleven. Group three and four received the aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. (50 and 150 mg/kg p.o.) for ten days and scopolamine was given on day eleven.

Group five served as standard control which received Piracetam (150 mg/kg i.p.) for ten days and scopolamine was given on day eleven.

After one hour all animals were tested on Radial arm maze and Morris water maze task performance.

I) Evaluation of nootropic activity by Radial arm maze model^{9,10}

Construction of Radial arm maze:

A Radial arm maze is used to evaluate working memory in the animals. Each arm (50 x 12 cm.) of the eight-arm Radial maze extends from an octagonal shaped central hub of 30 cms. diameter.

The platform is elevated 40 cms above the floor, small black metal cups (3cm in diameter & 1 cm deep) are mounted at the end of each arm that serve as receptacles for reinforces food. The Radial arm maze

method serves as exteceptive behavioral model to evaluate learning and memory in mice. The procedure and end point applied in present study for testing learning and memory have been described below.

Mechanical screening for memory in radial arm maze:

The animals for the experiments were preselected by conducting at least one daily training trail. Five groups of animals were made, each group consisting of six mice. In this group one served as normal control, Group two as amnesic control and Group three, four, served as treatment control and Group five as standard control.

At the beginning of trail, a food pellet was placed in one receptacle. An overnight fasted mouse was placed in the central hub and allowed to choose the arm freely, to get the food. The trail was considered to be complete when mouse visited all eight arms.

Entry into an arm that the mouse had not previously visited was recorded as a correct response & re-entry was counted as an error. A trail in which animals made no error or only one error at the eight choices was recorded as a "successful" trail. The percentage of successful mice was calculated as the index of radial maze task performance. On the 11th day, 60 minutes after the last dose, animals of respective groups were subjected to scopolamine (1mg/kg i.p.) treatment for inducing amnesia. After 30 minutes each mouse was placed on central hub & tested again for successful trail. Results of Radial arm maze method were shown in Table No.1 & 2.

Table no. 1: Effect of aqueous extract of dried seed kernels of *Caesalpinia crista* Linn on memory retention against scopolamine induced amnesia by Radial arm maze model.

| Group No | Sex | Average Body weight in grams | Dose of drug (mg/kg) | Radial arm maze performance | | | Percentage Memory retention |
|-------------------------------|-----|------------------------------|----------------------|------------------------------|--------------------|--|-----------------------------|
| | | | | Before scopolamine | | After scopolamine | |
| | | | | Average time taken (Seconds) | Average days taken | Average time taken (Seconds) (On Eleventh day) | |
| I Normal Control | M | 23.50 | 20 | 127.21 | 7.33 | 124.33 ± 0.2650 | 97.73% |
| II Amnesic Control | M | 25.50 | 20 | 113.10 | 7.66 | 196.66 ± 0.0950 | 26.11% |
| III Test (Aqueous Ex.) | M | 24.83 | 50 | 65.10 | 6.50 | 108.66 ± 0.1800 | 33.09% |
| IV Test (Aqueous Ex.) | M | 24.66 | 150 | 67.55 | 6.00 | 104.50 ± 0.3400 | 45.29% |
| V Test (Piracetam) | M | 25.00 | 150 | 27.78 | 3.50 | 35.00 ± 0.3350 | 74.02% |

Values are mean SEM, n= 6 when compared with amnesic control.

*P< 0.05, **P<0.01, ***P<0.001, Onaway ANOVA followed by Dunnett's multiple comparison tests.

II) Evaluation of nootropic activity by Morris water maze model^{9,10}

Construction of Morris water maze

The Morris water maze consists of large circular tank made of black opaque PVC or hard board coated with fiberglass and resin and then surface painted white (1.8-2.0m in diameter and 0.4-0.6m height). The pool is filled with water (20-22°C) to a depth of 0.3-0.4m, and rendered opaque by the addition of small quantity of milk or non-toxic white colour. The pool is fixed with filling and draining facilities and mounted on a frame so that the water is at waist level. The floor of circular tank is marked off in to four equal quadrants arbitrarily designed north, south, east or west. And escape platform is made of plexiglass with a 13 cm

square platform attached to a 34 cm long clear plexiglass cylindrical pedestal (3cm. Diameter) mounted on a 1sq. m (5mm thick) plexiglass base. The top of the platform is covered with a coarse material that provides a good grip for the rat when climbing on a platform. For the hidden platform task, water is added to circular tank to a level 2cm above the top of the platform. Water maze represents a versatile tool in which a number of distinct tasks can be measured. The simplest measure of performance is the Latency to escape from the water on to the hidden platform. The procedure and end point applied in present study for testing learning and memory have been described below.

Table no.2: Effect of aqueous extract of dried seed kernels of *Caesalpinia crista* Linn on learning performance by Radial arm maze model.

| Group | Treatment design | Average time required for three successful trail |
|-----------|----------------------------|--|
| Group I | Normal Control | 113.11 ± 1.412 |
| Group II | Test Aqueous Ex. 50 mg/kg | 65.107 ± 1.390** |
| Group III | Test Aqueous Ex. 150 mg/kg | 57.552 ± 5.768*** |
| Group IV | Test Piracetam 150 mg/kg | 27.385 ± 2.036*** |

Values are mean SEM, n= 6 when compared with amnesic control.

*P< 0.05, **P<0.01, ***P<0.001,

Onaway ANOVA followed by Dunnett's multiple comparison tests

Table no.3: Effect of aqueous extract of dried seed kernels of *Caesalpinia crista* Linn on memory retention against scopolamine induced amnesia by Morris water maze model.

| Group No. | Sex | Average Body weight in grams | Dose of drug (mg) | Morris water maze performance | | | Percentage memory retention |
|------------------------|-----|------------------------------|-------------------|-------------------------------|--------------------|--|-----------------------------|
| | | | | Before scopolamine | | After scopolamine | |
| | | | | Average time taken (Seconds) | Average days taken | Average time taken (Seconds) (On Eleventh day) | |
| I Normal Control | M | 23.50 | 20 | 37.16 | 6.50 | 36.00 ± 0.5774 | 98.87% |
| II Amnesic Control | M | 25.50 | 20 | 37.55 | 6.66 | 69.66 ± 0.6991 | 14.49% |
| III Test (Aqueous Ex.) | M | 24.83 | 50 | 32.55 | 6.33 | 52.50 ± 0.4653 | 38.71% |
| IV Test (Aqueous Ex.) | M | 24.66 | 150 | 22.49 | 4.66 | 33.33 ± 0.5918 | 51.81% |
| V Test (Piracetam) | M | 25.00 | 150 | 21.05 | 3.33 | 25.83 ± 1.317 | 77.30% |

Values are mean SEM, n= 6 when compared with amnesic control.

*P< 0.05, **P<0.01, ***P<0.001, Onaway ANOVA followed by Dunnett's multiple comparison tests

Mechanical screening for memory in Morris water maze

The animals for the experiments were preselected by conducting at least one daily training trail. Five groups of animals were made, each group consisting of six mice. In this group one severed as normal control, Group two as amnesic control and Group three, four, severed as treatment control and Group five as standard control. At the beginning of trail animals are place in the Morris water maze and allowed to swim freely and to seat on hidden platform. The trial was considered to be successful when mouse set on the hidden platform within three minutes. Time spent more than three minutes to find hidden platform recorded as error. The percentage of successful mice was calculation as the index of Morris water maze task

performance. On the 11th day 60 minutes after the last dose, animals of respective groups were subjected to scopolamine (1mg/kg i.p) treatment for inducing amnesia. After 30 minutes each mouse was subjects to Morris water maze task performance.

Results of Radial arm maze method were shown in Table No.3 & 4

Statistical analysis

The data were statically analyzed by student's t-test all the value were expressed as mean \pm SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values $p < 0.05$ were considered significant.

Table no.4: Effect of aqueous extract of dried seed kernels of *Caesalpinia crista* Linn on learning performance by Morris water maze

| Group | Treatment design | Average time required for three successful trail |
|-----------|----------------------------|--|
| Group I | Normal Control | 37.550 \pm 2.238 |
| Group II | Test Aqueous Ex. 50 mg/kg | 32.553 \pm 1.290** |
| Group III | Test Aqueous Ex. 150 mg/kg | 22.495 \pm 1.138*** |
| Group IV | Test Piracetam 150 mg/kg | 21.052 \pm 1.418*** |

Values are mean SEM, n= 6 when compared with amnesic control.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, One way ANOVA followed by Dunnett'S multiple comparison tests.

RESULTS AND DISCUSSION

Drugs are the potential tools in the study of behavioral and neurobiological basis of learning and memory which may provide critical data for understanding and treating disorders of cognitive dysfunctions.

Radial arm maze model for learning performance.

Radial arm maze task performance for learning of Group I Animals treated with vehicle was found to take 113.11 \pm 1.412 seconds averagely for three successful trials. Group II Animals treated with 50mg/kg, p.o. aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. was found to took 65.107 \pm 1.390 seconds averagely for three successful trials. Group III Animals treated with 150mg/kg, p.o. aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. was found to took 57.552 \pm 5.768 seconds averagely for three successful trials. Group VI

Animals treated with 150mg/kg, p.o. standard drug Piracetam was found to take 27.385 \pm 2.036 seconds averagely for three successful trials.

Among the test extract, tested at 150mg/kg p.o. showed the value near to standard drug Piracetam. This clearly indicates aqueous extract significantly

increased the learning memory performance. The result shown in the table 2

Radial arm maze model for memory retention.

100% of mice were trained for successful trial as criterion in the Paradigm Radial Maze task performance. The Memory retention in mice Group I was found to be 97.73% in Radial arm maze task performance. The Memory retention in mice Group II, treated with 1mg/kg i.p. scopolamine induced amnesia was found to be 26.11 % in Radial arm maze task performance. The Memory retention in mice Group III, treated with 50mg/kg aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. against scopolamine induced amnesia was found to be 33.09 % in radial arm maze task performance was compared to scopolamine treated control animal. The Memory retention in mice Group IV, treated with 150mg/kg aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. against scopolamine induced amnesia was found to be 45.29 % in radial arm maze task performance was compared to scopolamine treated control animal. The memory retention in mice, Group VI treated with 150mg/kg standard Piracetam injection i.p. against scopolamine induced amnesia was found to

be 74.02 % in radial arm maze task performance as compared to control group mice. The result shown in the table 1

Morris water maze model for learning performance.

Morris water maze task performance for learning of Group I Animals treated with vehicle was found to take 37.550 ± 2.238 seconds averagely for three successful trials. Group II. Animals treated with 50mg/kg, p.o. aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. was found to took 32.553 ± 1.290 seconds averagely for three successful trials. Group III. Animals treated with 150mg/kg, p.o. aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. was found to took 22.495 ± 1.138 seconds averagely for three successful trials. Group VI. Animals treated with 150mg/kg, p.o. standard drug Piracetam was found to take 21.052 ± 1.418 seconds averagely for three successful trials. Among the test extract, tested aqueous extract at 150mg/kg p.o. showed the value near to standard drug Piracetam. This clearly indicates aqueous extract significantly increased the learning

memory performance. The result are shown in the table 4

Morris water maze model for memory retention.

The Memory retention in mice Group I was found to be 98.87 % in Morris water maze task performance. The Memory retention in mice Group II treated with 1mg/kg i.p. scopolamine induced amnesia was found to be 14.49 % in Morris water maze task performance. The Memory retention in mice Group III, treated with 50mg/kg aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. against scopolamine induced amnesia was found to be 38.71% in Morris water maze task performance and was compared to scopolamine treated control animal. The Memory retention in mice Group IV, treated with 150mg/kg aqueous extract of dried seed kernels of *Caesalpinia crista* Linn. against scopolamine induced amnesia was found to be 51.81% in Morris water maze task performance and was compared to scopolamine treated control The memory retention in mice, Group VII treated with 150mg/kg standard Piracetam injection i.p. against scopolamine induced amnesia was found to be 77.30 % in Morris water maze task performance and was compared to control group mice. The result are shown in the table 3

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