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Effects of Ethanolic Extract of Dried Seed Kernels of *Caesalpinia crista* Linn. On Learning and Memory in 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 Ceasalpinia crista extract as learning and memory enhancer. Ethanolic extract of dried seed kernels of Ceasalpinia crista linn.ameliortated the amnesic effect of scopolamine in mice. Morris water maze task and Passive avoidance task paradigm served as the exteroceptive behavioral models. Ethanolic extract of dried seed kernels of *Caesalpinia crista* linn. Was compared with standard drug piracetam in scopolamine induced amnesia in mice. Moreover, acetylcholinesterase activity and the levels of thiobarbituric acid-reactive substance in the serum and brain of animals treated with ethanolic extract of dried seed kernels of *Caesalpinia crista* linn were lower than those of the control group.

Keywords - Acetyl choline esterase, learning Memory, *Ceasalpinia crista*, Piracetam, Morris water maze.

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INTRODUCTION

Plants used in traditional medicine contain wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illness may be expected to have accumulated in areas where the use of plants is still of great importance [1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The important constitutent of these plants are alkaloids, Flavonoids, tannins and phenolic compounds [2].

Caesalpinia crista (Linn.) synonym: Caesalpinia bonduc (L.) Roxb.), Caesalpinia bonducella belongs to family Caesalpiniaceae and is commonly known as kat-takaranja in Hindi and sagargota in Marathi. It is prickly shrub found throughout the hotter regions of India and srilanka [3]. The seeds of plant are almost globular in shape, grey, hard with a smooth shiny surface [4]. The seeds of the plant contain bonducin, proteins, saponin, starch, sucrose, an enzyme, two phytosterols namely sitosterol and hepatsane, fattyacids such as palmitic acid, stearic acid, lognoceric, oleic, linolenic acid. The seed kernals of the plant contain furanoditerpenes- ά-caesalpin, ß-caesalpin, γ-caesalpin, δ-caesalpin, ε-caesalpin,and F-caesalpin [5]. The leaves of *Caesalpinia crista* (Lin) are tradionally used for tumors, inflammation and liver disorders.

In India, various parts of this plant has been used in various therapeutic uses like adaptogenic [6], antimicrobial [7], antiproliferatve [8], antidiabetic [9], anti-filarial [10], contractility on uterus [11], hepatprotective [12], antitumor and antioxidant activities [13]. Because of their various pharmacological activities, present work was undertaken to study the learning and memory enhancing activity of ethanolic extract of seed kernels of *Caesalpinia crista* (Linn.) in albino mice.

MATERIALS AND METHODS

Plant Material:

The *Caesalpinia crista* (Linn.) seeds were collected from the local market Yogesh Pharmacy Nanded Maharastra. It was authenticated by Dr. Mr. Krishna G. Kadaskar, Department of Botany P. N. College, Pusad (Maharastra).

Preparation of Extract:

The seeds kernels of *Caesalpinia crista* (Linn.) were shade dried at room temperature. Then the shade dried seeds kernels were powdered to get a coarse powder. 100g of coarse powder was defatted with petroleum ether and extracted exhaustively with 95% ethanol at Temperature 60^{o}C, in a soxhlet extractor. The extract was concentrated in a rotary flash evaporator residue was dried in desiccator over sodium sulfite. This procedure was repeated for 5-6 times to receive sufficient quantity of ethanolic extract [14].

Phytochemical Investigation:

Ethanolic extract of *Caesalpinia crista* (Linn.) seeds kernels were subjected to further preliminary qualitative phytochemical investigation [15].

Experimental Animals:

Healthy adult Albino mice of either sex weighing 24-32 gm acclimatized for 15 days. The animals were housed under standard conditions and room temperature (25±2^{o}C). During the acclimatization period of 15 days, animals were observed for general condition every day and weighed on the next day of arrival and on the last day of acclimatization. The animals were fed with balanced pellet and water ad libitum. The experimental protocol of these studies was reviewed and approved by the Institutional Animal Ethical Committee of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and Chemicals

Drug: Test drug

Caesalpinia crista Linn dried seed kernel extract (Ethanolic)
Dose: 200mg/kg, and 400mg/kg body weight given orally.

Standard drug

Piracetam in water for injection was prepared as per requirement.
Dose: 150 mg/kg given I.P.

Amnesic drug

Dose: Scopolamine1mg/kg given I.P.

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 Mice received vehicle and scopolamine.
Group 3 Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn (200mg/kg) p.o. Treated mice
Group 4 Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn (400mg/kg) p.o. Treated mice
Group 5 Standard drug piracetam (150mg/kg) i.p. treated mice.

Passive Avoidance Task

The passive avoidance task was carried out in two identical light and dark compartment shuttle chambers equipped with a grid floor and shock generator. For the acquisition trials, each mouse was placed in the lighted compartment, and the door between the compartments was opened 10 seconds later. When the mice entered the dark compartment, the door was immediately closed, and an electrical foot shock (0.3 mA) 3 seconds in duration was delivered through the stainless steel rods. The latency period before entering the dark compartment was measured. The next day, for induction of amnesia, mice were injected with scopolamine (1 mg/kg of body weight, i.p.) 45 minutes before the avoidance trial. The mouse was again placed in the lighted compartment, and the time until it returned to the dark compartment was recorded as the step-through latency (with a maximum latency of 300 seconds).

Morris Water Maze Task

The water maze was slightly modified from the Morris water maze task. The experimental apparatus consisted of a circular water tank (diameter, 100 cm; height, 35 cm) containing water at 23^0C to a depth of 15 cm, and the water was rendered opaque by the addition of powdered milk. A platform was positioned inside the tank with its top submerged 2 cm below the water surface in the target quadrant of the maze. Each mouse was given three trials per day for 2 consecutive days for finding the hidden platform. On the first and second days of the training trial, the mice underwent the trials for finding the platform. Once a mouse located the platform, it was placed on the platform for 10 seconds. If the mouse could not locate the platform within 120 seconds, it was led to the platform by the experimenter. Twenty-four hours after the last training trial, amnesia was induced in the mice using scopolamine. Scopolamine dissolved in saline was injected intraperitoneally (1mg/kg of body weight) into the mice 45 minutes prior to the water maze test. In each trial, the time required to escape onto the hidden platform was recorded.

Measurement of Acetylcholine Esterase Activity

The mice were decapitated 120 minutes after injection with scopolamine, and the serum and the brains were removed. Acetylcholine esterase activity was measured using the method of Ellman et al [16]. with slight modification. The whole brain was rapidly homogenized with 10 volumes of sodium phosphate buffer (0.1mM, pH 7.4). A reaction mixture containing 2.9ml of sodium phosphate buffer (0.1mM, pH 8.0), 100ml of 0.01 M 5,5'-dithiobis-(2-nitrobenzoic acid), 20 ml of 0.075 M acetylcholine iodide, and 100 ml of homogenate was used for the acetylcholine esterase assay, and changes in absorbance at 412 nm were recorded. Protein concentration was determined by the method of Lowry et al. [17]

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Measurement of Thiobarbituric Acid Reactive Substance

The brains were homogenized in 10 volumes of sodium phosphate buffer (0.1mM, pH 7.4) with a Teflon coated homogenizer and centrifuged for 10 minutes. The supernatant was used to determine brain lipid peroxide content. Lipid peroxidation in brain was estimated by the production of thiobarbituric acid reactive substance according to the method of Ohkawa et al.[18] In the homogenates, the compound malondialdehyde, which has been identified as the product of lipid peroxidation, reacted with thiobarbituric acid, and the absorbance was determined at 532 nm.

Statistics

The results of the experiments were expressed as mean ± SE values. Statistical analysis was performed using oneway analysis of variance and Duncan's multiple range tests. P<.05 was considered statistically significant.

RESULTS

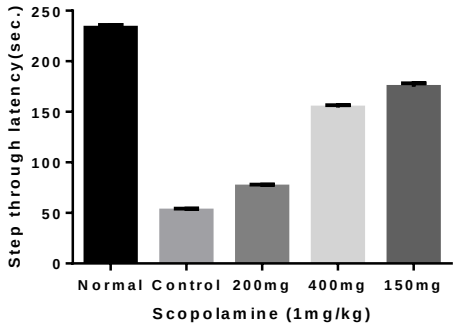

Fig 1

Effects of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn.200mg and 400mg and 150mg Piracetam as standard drug was compared on scopolamine-induced memory impairment of mice, using the passive avoidance task. All mice were given 1 day of training. Twenty-four hours after the training trial, amnesia was induced in mice by scopolamine (1 mg/kg I.P.). After 45 minutes, all mice were again placed in the light compartment. The latency to enter the dark compartment was measured. Data are mean ± SE values (n = 6).

Effects of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn.200mg and 400mg and 150mg Piracetam as standard drug was compared on scopolamine-induced memory impairment of mice, using the Morris water maze task. All mice were given 2 consecutive days of training trials for finding the platform. Twenty-four hours after the last training trial, amnesia was induced in mice by scopolamine (1mg/kg I.P.). All mice were tested for spatial memory 45 minutes after the injection of scopolamine. The latency for finding the platform was measured. Data are mean ± SE values (n = 6).

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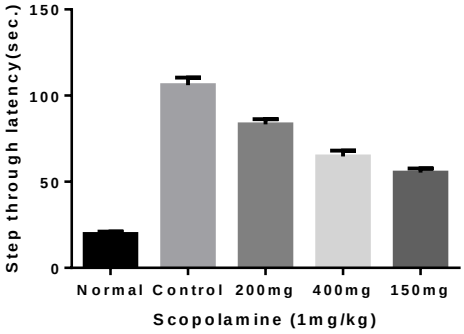
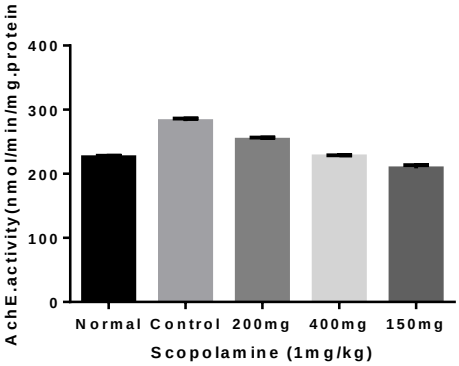
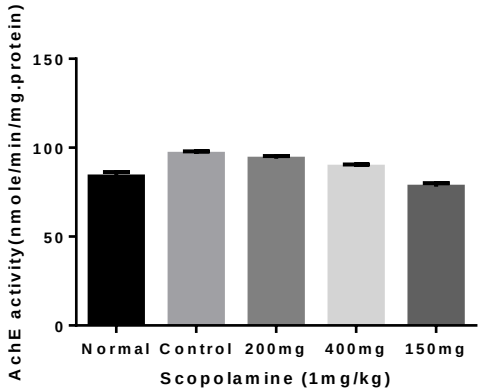


Fig 2



Serum



Brain

Fig 3

Effects of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn.200mg and 400mg and 150mg Piracetam as standard drug was compared on scopolamine-induced memory impairment of mice, on AChE activities in (A) serum and (B) brain. AChE activities are expressed

as amount of 5-thio-2- nitrobenzoic acid produced by hydrolysis of substrate. Data are mean ± SE values (n = 6).

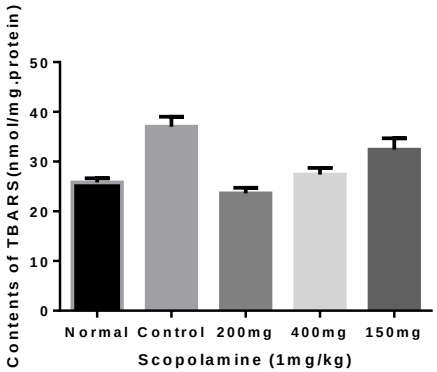


Fig 4

Effects of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn.200mg and 400mg and 150mg Piracetam as standard drug was compared on scopolamine-induced memory impairment of mice, on the contents of Thiobarbituric acid reactive substance(TBARS) in brain. Data are mean ± SE values (n = 6).

Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn improve cognitive deficit in the passive avoidance task:

The changes in the step-through latency in the passive avoidance task are shown in Figure 1. The step-through latency of the scopolamine treated control group-2 was significantly shorter than that of the saline treated normal group-1. Lower latency time indicates the impairment of memory retention in the passive avoidance task. Step-through latencies of experimental mice on the training day were not significantly different from each other. However, treatment with ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. significantly reversed the shorter step-through latency induced by scopolamine in a dose-dependent manner. The latency of the 400mg group-4 was increased dramatically to a level close to that of the 150mg piracetam as standard drug group-5. Step-through latency in the 200mg group-3 showed a tendency to increase compared to the control group-2.

Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn protect against cognitive deficits in the Morris water maze task:

The effect of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn on spatial memory was investigated using the Morris water maze task. In this test, we used the latency of finding the platform in the water maze as a measure for evaluating performance in the tested mice. As shown in Figure 2, scopolamine treated mice of control group-2 exhibited significantly longer escape latencies compared to vehicle treated mice (normal, 27.20 ± 9.98 seconds; control, 107.39 ±12.36 seconds; P<.01). The prolonged latency induced by scopolamine was

significantly reversed by 400mg ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. was compared with standard drug Piracetam, and the change was dose-dependent.

Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn decreased acetylcholine esterase activity:

The effect of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn on the acetylcholinesterase activity of serum and brain in the experimental mice is shown in Figure 3. The acetylcholinesterase activity of both serum and brain in the scopolamine treated control group was significantly increased compared to those of the normal group, but the increase in acetylcholinesterase activity induced by scopolamine was decreased by ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. Particularly, the acetylcholinesterase activity was significantly inhibited in the serum of the 400mg treated group-4 (standard drug Piracetam, 229.50 ±16.8 nmol/minute/mg of protein; 400 mg *Caesalpinia crista*, 205.32 ±14.70 nmol/minute/mg of protein; P<.05).

Ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. decreased Thiobarbituric acid reactive substance production:

To investigate the effect of ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. on scopolamine-induced lipid peroxidation, the levels of thiobarbituric acid reactive substance in the brains of the mice were measured. As shown in Figure 4, treatment with scopolamine increased the formation of thiobarbituric acid reactive substance in the brain. 400mg ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn decrease significantly thiobarbituric acid reactive substance level in the brains of the mice as compared with standard drug Piracetam150mg treated animals.

DISCUSSION

In the present study, we examined the effects of ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. on scopolamine-induced memory impairments in mice using the passive avoidance task and the Morris water maze task. Scopolamine is one of the classical antagonists of muscarinic acetylcholine receptors. It was able to pass through the brain–blood barrier and block muscarinic receptors throughout the brain, including the hippocampus and cerebral cortex, [19] The results showed that step-through latency of the scopolamine treated control group was significantly reduced up to 72.1% compared to the normal group in the passive avoidance task, This indicates that in mice, treatment with scopolamine impairs learning and memory. Effect of ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. prolonged the step-through latency that was shortened by scopolamine in the passive avoidance task in a dose dependent manner; however, during the acquisition trial, no differences in step-through latency were observed between any of the groups, suggesting that ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. has no effect on general behavior. To assess hippocampal-dependent spatial learning ability, we performed the Morris

water maze task. Ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. reversed the escape latency, which was prolonged by scopolamine. This suggests that ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn. attenuates the long term memory impairment induced by scopolamine injection in mice. Cholinergic neuronal loss in the hippocampal area is the major feature of Alzheimer Disease and enhancement of central cholinergic activity through the use of cholinesterase inhibitor is presently the mainstay of pharmacotherapy for senile dementia of the Alzheimer type. [20, 21] Therefore, to investigate the possibility of increasing cholinergic transmission by ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. mediated inhibition of acetylcholinesterase activity, we assessed the acetylcholinesterase activity in serum and brain. The acetylcholinesterase activity was increased in scopolamine-treated mice by 35.5% and 22% in brain and serum, respectively, compared to vehicle treated normal mice. It is well known that the anti-amnestic effect of tacrine is due to the inhibition of acetylcholinesterase activity in the brain. [22] Considered together, this evidence suggests that the anti-amnestic effect of ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. might be mediated in part by the inhibition of acetylcholinesterase activity. Scopolamine has been reported to trigger reactive oxygen species [23, 24] and elevate rat brain oxidative status by affecting thiobarbituric acid reactive substance levels. The increase of lipid peroxidation in mice hippocampus has been significantly correlated with a decline in neuronal electrical activity. [25, 26] In the present study, ethanolic extract of dried seed kernel of *Caesalpinia crista* Linn.prevented scopolamineinduced lipid peroxidation in the brains of mice.

CONCLUSION

This study proposes that ethanolic extract of dried seed kernel of *Caesalpinia crista* linn. have learning and memory enhancing activity and these effects are mediated in part by inhibition of acetylcholinesterase activity and thiobarbituric acid reactive substance accumulation in the brain. However, the mechanism of the memory enhancing activity of ethanolic extract of dried seed kernel of *Caesalpinia crista* linn completely not known and should be studied further.

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