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Research Article

EFFECT OF AQUEOUS EXTRACT OF *CYNODON DACTYLON* (DOOB GRASS) ON NORMAL AND IMPAIRED MEMORY IN MICE

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ABSTRACT

Objective: Memory impairment in any age affects the quality of life, though this problem is common in old age. The present study was carried out to study the effect of aqueous extract of *Cynodon dactylon* (AECD) on normal and impaired memory in mice.

Methods: The gum acacia suspension of AECD was administered by gavage at the dose of 200 and 400 mg/kg for 30 days to evaluate memory-enhancing effect on normal and scopolamine-induced impaired memory in albino mice. Escape latency (EL) in Morris water maze (MWM) and transfer latency (TL) in elevated plus maze were recorded. Mice were given four trial sessions per day to locate the platform for 5 days in MWM model. Scopolamine 1 mg/kg was injected i.p. to produce memory impairment in mice.

Results: AECD at the dose of 200 mg/kg ($p>0.05$) and 400 mg/kg ($p<0.05$) showed reduction of EL and TL as compared to control group in normal mice. AECD 200 mg/kg ($p>0.05$) and 400 mg/kg ($p<0.05$) showed reduction of EL and TL as compared to negative control group in impaired memory mice. AECD 400 mg/kg was comparable to that of piracetam at the dose of 200 mg/kg in normal and scopolamine-treated mice. However, AECD showed better memory-enhancing effect in scopolamine-induced impaired memory model than in normal memory.

Conclusion: The study revealed that the chronic administration of AECD exhibited significant memory-enhancing activity against both normal and scopolamine-treated impaired memory mice groups.

Keywords: *Cynodon dactylon*, Scopolamine, Morris water maze, Elevated plus maze, Piracetam.

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INTRODUCTION

Learning is the progression of acquiring information about the world and memory is the retention of the acquired knowledge [1]. Acquired learning is stored as memory to be recalled at a later stage. Some of this acquired information is lost due to spontaneous decay or interference by other stimuli. This extension of memory can be reinforced by repeated exposure to original stimuli. This registration (acquirement of learning), retention (memory storage), and retrieval (recall) constitute the process of memory.

Cognition refers to the process of acquiring, storing, and exercising intellectual knowledge. Impairment in memory, cognition, compaction, problem-solving, judgment, and learning is a normal consequence of aging and is seen in most types of mental illness. Several central depressants including alcohol, scopolamine, and hallucinogens can impair memory [2]. Cognitive enhancers such as piracetam (PCT), oxiracetam, and aniracetam act by increasing the brain's supply of acetylcholine, improving blood and oxygen supply to the brain, and rejuvenating the cells to normal functioning but their therapeutic effects are low and exert undesirable side effects such as nervousness, weight gain, dizziness, gastric discomfort, and insomnia [3]. Acetylcholinesterase inhibitors are the most common drugs used for Alzheimer's disease. However, these drugs may cause peripheral cholinergic side effects that may restrict their use [4].

For the treatment of Alzheimer's disease and memory deficit, herbal medicine could be a good source of drugs with fewer or no side effects. Several medicinal plants have been used for decades in different cultures for memory improvement such as *Valeriana officinalis*, *Punica granatum* L., *Salvia officinalis*, *Myristica fragrans*, *Bacopa monnieri* Linn., *Centella asiatica* Linn., and *Evolvulus alsinoides* Linn. [5,6].

Cynodon dactylon known as "Bermuda grass" in English and "Durva" in Hindi is perennial, creeping grass growing throughout the country. *C. dactylon* possesses a variety of biological activities such as antiviral, antibacterial, antidiabetic, immunomodulatory, anti-inflammatory, antidepressant, antioxidant, hypolipidemic, and wound healing properties [7,8]. It is traditionally used to treat hypertension, epilepsy, dropsy, cough, diarrhea, headache, cramps, hysteria, etc. [9]. Little information is available regarding the effect of *C. dactylon* on memory [10]. No information is available regarding the study of effect of aqueous extract of *C. dactylon* (AECD) on normal and impaired memory. Therefore, the present study was carried out to study the effect of **AECD** on normal and impaired memory in mice.

METHODS

Plant materials

Whole plants of *C. dactylon* (without roots) were purchased from Aveek nursery situated at Gwalior in the month of February 2019. The plant was identified by Dr. Avinash Tiwari, Professor, Department of Botany, Jiwaji University, Gwalior, Madhya Pradesh (M.P.), and a voucher specimen SKM/19 has been retained in our department.

Preparation of extract

AECD was prepared by maceration. The collected plants were washed thoroughly with tap water and dried at room temperature in the absence of sunlight. The dried plants were powdered using the grinder. One kilogram of dry powder soaked into 5 L of distilled water for 3 days. Whatman filter paper is used for filtration of the mixture. The solution was finally evaporated to dryness using a water bath. Dry powder was stored in the amber color bottle for further use. The yield was 5% w/w.

Phytochemical screening

AECD was subjected to phytochemical tests for the presence of bioactive compounds by standard methods as described by Harborne [11].

Drugs and chemicals

PCT (Tab. Cerecetam 800 mg – Intas Pharmaceuticals Ltd.) and scopolamine butyl bromide (Inj. Buscogast – Sovereign Pharma Pvt. Ltd.) were bought from the medical store of Gwalior, M.P. All drugs were administered as 2% gum acacia suspension by gavage.

Animals

A 6–8-week-old male albino mice weighing 30–40 g used for the study were available in the animal house of the Department of Pharmacology, Gajra Raja Medical College Gwalior, M.P. All animals under experiment were kept in 12 h light-dark cycle and were provided with food and water *ad libitum*. The care and maintenance of animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals in India. The experimental protocol was approved by the institutional Animal Ethics Committee of Gajra Raja Medical College, Gwalior, registration number 846/PO/Re/S/04/CPCSEA.

Methods

Morris water maze test (MWM)

The MWM is a white, circular pool with an inner diameter of 110 cm and a height of walls is 20 cm. It was filled with normal tap water to 13 cm deep. The water was at room temperature (±22°C) and made opaque by adding a small amount of milk powder with no noticeable side effects to the animals. The entire pool was divided into four quadrants of the same size by two (imaginary) diagonal lines running through the center of the pool. A circular escape platform, which is removable (diameter: 10 cm) positioned at the middle of a quadrant. The pool was positioned at the far end of a rectangular room dimly lit by white light. The walls of the room were equipped with a variety of spatial cues which remained unchanged during the whole experiment [3].

Drugs were administered orally for 30 days in normal and impaired memory groups. On the 25th day, training of mice was started. Mice were transferred from their housing facility to the behavior room and kept in an area where they cannot see the pool or spatial cues to adjust to the new environment for at least half-hour before testing. During the 5 subsequent days, the mice were given four trial sessions per day with the platform in place. During each training day, the starting place was similar for all the mice and the mice had pointed at the sidewall. If mice located the platform, it was permitted to remain on it for 10 s. The escape latency (EL) in seconds to reach the platform was recorded. If the mice did not locate the platform within 2 min, it was placed on the platform for 10 s. The time interval between trial sessions was a ½ h. EL was noted after 45 min of administration of the last dose of AECD on the 30th day to know learning and again after 24 h, that is, on the 31st day in normal mice to know retention of learning (memory). To study the effect on impaired memory, scopolamine (1 mg/kg) was injected intraperitoneally after 45 min of administration of AECD or standard drugs or vehicle on the 30th day and EL was recorded after 45 min of injection of scopolamine on the 30th day and after 24 h, that is, on the 31st day [12].

Elevated plus maze test (EPM)

The plus maze was in the shape of a cross or plus with two enclosed arms each with roof open measuring 16 cm ×5 cm ×12 cm, extending from the central region (5 cm ×5 cm) running along a North-South axis and two open arms each measuring 16 cm ×5 cm running East-West. The wooden apparatus was elevated to a height of 25 cm from the floor in a dimly illuminated room [13]. Mice were placed individually at the end of either of the open arms facing away from the central platform. The time taken by each mouse to move from the open arm to either of the enclosed arms was recorded. This time duration was called transfer latency (TL). If mice do not enter into any of the enclosed arms within 2 min, it was gently pushed into any of the enclosed arms and TL was considered as 2 min. Later, the mouse was allowed to explore the plus maze for 5 min and send back to the home cage. TL measured on the 30th day indicates acquisition or learning, while TL on the 31st day indicates retention of learning (memory). An arm entry was defined as the presence of all four paws in the arm. EPM was cleaned with alcohol after each mouse was tested to remove any residue or odor.

TL was recorded after 45 min of administration of the last dose of AECD on the 30th day and again after 24 h, that is, on the 31st day in normal mice. To study the effect on impaired memory, scopolamine (1 mg/kg) was injected intraperitoneally after 45 min of administration of AECD or standard drugs or vehicle on the 30th day and TL was recorded after 45 min of injection of scopolamine on the 30th day and after 24 h, that is, on the 31st day.

Study design

To study the effect on normal memory, MWM and EPM models were used. Mice were divided into four groups. Each group has six animals. Group1: Control received 2% GA at the dose of 10 ml/kg, Groups 2 and 3: Test drug-treated group received AECD 200 mg/kg and AECD 400 mg/kg, respectively, and Group 4: Standard drug-treated group received PCT 200 mg/kg.

To study the effect on impaired memory, MWM and EPM models were used. Mice were divided into five groups. Each group has six animals. Group 1: Normal control received 2% GA at the dose of 10 ml/kg, Group 2: Negative control received Scopolamine 1 mg/kg, Groups 3 and 4: Drug-treated group received scopolamine 1 mg/kg + AECD 200 mg/kg and scopolamine 1 mg/kg + AECD 400 mg/kg respectively, and Group 5: Standard drug-treated group received scopolamine 1 mg/kg + PCT 200 mg/kg.

Statistical analysis

The data collected after experiments were represented as mean±standard error of the mean and were analyzed using SPSS software version 20. One-way ANOVA followed by Tukey's multiple comparison tests was used for statistical evaluation. $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical study

Phytochemical screening of *C. dactylon* revealed the presence of phenols, flavonoids, tannins, alkaloids, phytosterols, saponins, glycosides, proteins, and carbohydrates.

Effect of AECD on normal memory

EL and TL on the 30th day of drugs treatment reflected learning behavior of animals, whereas EL and TL on the 31st day reflected retention of learned task as memory. Administration of AECD 200 decreased EL by 3% and TL by 6% in MWM and EPM, respectively, and was statistically not significant ($p>0.05$) as compared to control. AECD 400 mg/kg decreased EL by 19% and TL by 30% in MWM and EPM, respectively, and was statistically significant ($p<0.05$) as compared to control and AECD200 mg/kg suggesting memory-enhancing effect. PCT decreased **EL** by 26% and TL by 35% in MWM and EPM, respectively, and was statistically significant ($p<0.05$) as compared to control and AECD 200 mg/kg. Effect of AECD 400 mg/kg on TL and EL was comparable to PCT (Table 1 and Fig. 1).

Effect of AECD on impaired memory

Administration of scopolamine 1 mg/kg increased **EL** by 49.87% in MWM and TL by 50% in EPM as compared to normal control group and was statistically significant ($p<0.05$) demonstrating memory impairment. Administration of AECD at the dose of 200 mg/kg in scopolamine-treated animals decreased EL by 7% and TL by 8% in MWM and EPM, respectively, as compared to negative control SCM1 suggesting cognitive- enhancing effect and was statistically not significant ($p>0.05$). AECD at the dose of 400 mg/kg in scopolamine-

Table 1: Effect of AECD on escape latency using MWM in normal mice

Mean escape latency (s)		
Treatments	**On the 30th day**	**On the 31st day**
GA10	69.67±2.03	66.17±2.02
AECD200	66.67±2.82	64.17±1.70
AECD400	55.17±1.47[ab]	53.83±1.70[ab]
PCT200	53.33±1.89[ab]	49.00±1.18[ab]

GA10=2% gum acacia 10 ml/kg, AECD200=AECD 200 mg/kg, AECD 400=AECD 400 mg/kg. PCT200=Piracetam 200 mg/kg, n=6 animals in each group. Values are expressed as mean±SEM, [a]p<0.01 as compared to GA10, [b]p<0.01 as compared to AECD 200. SEM=Standard error of the mean

Table 2: Effect of AECD on escape latency using MWM in scopolamine-treated mice

Mean escape latency (s)		
Treatments	**On the 30th day**	**On the 31st day**
GA10	69.67±2.03	66.17±2.02
SCM1	96.17±2.76[a]	99.17±2.46[a]
AECD200+SCM 1	94.00±0.89	92.67±2.95
AECD400+SCM 1	72.17±1.49[bc]	66.83±1.35[bc]
PCT200+SCM 1	68.50±2.43[bc]	64.33±1.99[bc]

GA10=2% gum acacia 10 ml/kg, AECD200=AECD 200 mg/kg, AECD 400=AECD 400 mg/kg. PCT200=Piracetam 200 mg/kg, n=6 animals in each group. Values are expressed as mean±SEM, [a]p<0.01 as compared to GA10, [b]p<0.01 as compared to SCM1. [c]p<0.01 as compared to AECD200+SCM1, SEM: Standard error of the mean

treated group decreased **EL** by 33% and TL by 35% in MWM and EPM, respectively, and was statistically significant (p<0.05) as compared to negative control suggesting improvement in memory. Effect of AECD 400 mg/kg was dose dependent and significant as compared to AECD 200 mg/kg. Administration of PCT in scopolamine-treated animals decreased EL by 36% and TL by 41% in MWM and EPM, respectively, and was significant (p<0.05) as compared to negative control and AECD 200 mg/kg. Effect of AECD 400 mg/kg was comparable to PCT (Table 2 and Fig. 2).

DISCUSSION

Alzheimer's disease is a neurodegenerative disorder of elderly population characterized by memory loss, language deterioration, poor judgment, impaired visuospatial skills, etc. [14]. The hippocampus is associated with spatial and episodic memory. In Alzheimer's disease, it is damaged early and it is very sensitive to hypoxia [15]. Alzheimer's disease is characterized by cholinergic neurons loss in the forebrain, responsible for attention and memory status, and in the cortex and the hippocampus. Cognitive restoration is also required in ischemic brain damage, schizophrenia, chronic alcoholism, and mental retardation in children.

In Alzheimer's disease, the activity of choline acetyltransferase, the enzyme responsible for acetylcholine synthesis is decreased while acetylcholinesterase activity is increased which breaks down acetylcholine. Hence, the current approach to improve memory impairment is the inhibition of acetylcholinesterase activity.

Oxidative stress is also associated with major pathological processes underlying Alzheimer's disease and correlates with the severity of neurodegenerative changes. It is believed that preventing reactive oxygen species formation could slow both the onset and progression of Alzheimer's disease. Therefore, antioxidant agents may represent a successful approach in the prevention and treatment of Alzheimer's disease [16].

In the present study, effect of chronic administration of AECD was studied on learning and memory of normal and scopolamine-treated mice.

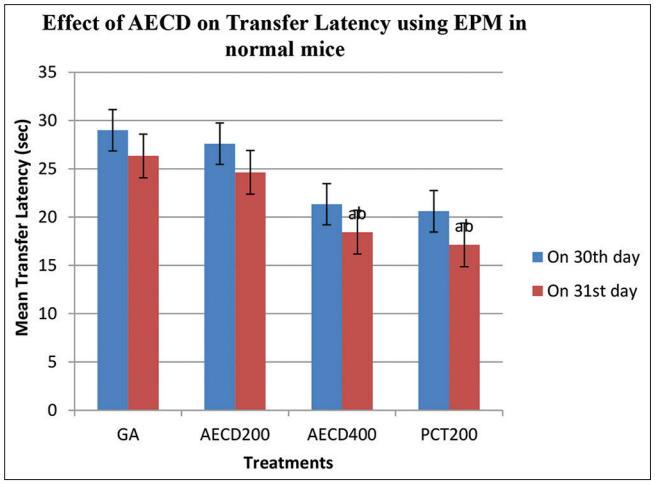

Fig. 1: Bar diagram showing effect of AECD (200 and 400 mg/kg) and piracetam on transfer latency in normal mice using elevated plus maze. Each column represents mean±standard error of the mean (SEM), n=6 mice in each group. [a]p<0.05 as compared to gum acacia 10 (normal control) and [b]p<0.05 as compared to AECD 200 mg/kg treated group

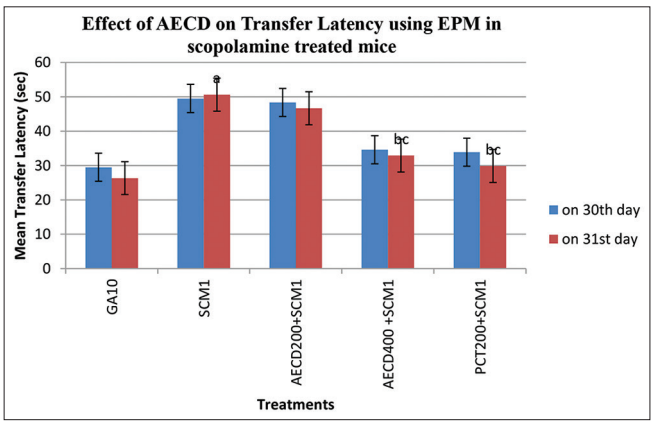

Fig. 2: Bar diagram showing effect of AECD (200 and 400 mg/kg) and piracetam 200 mg/kg on transfer latency following scopolamine using elevated plus maze. Each column represents mean±standard error of the mean (SEM), n=6 mice in each group. [a]p<0.01 as compared to gum acacia 10 (normal control), [b]p<0.05 as compared to SCM1 (negative control), and [c]p<0.05 as compared to AECD 200+SCM1 group

MWM test has proven to be the most useful and versatile assay of learning and memory function in rodents and a reliable and valid measure of hippocampal function [17]. Performance in MWM depends on the coordinated action of distinct regions of brain and neurotransmitter systems constituting a functionally integrated neural network. EPM served as the exteroceptive behavioral model to evaluate memory in rats. The spontaneous alteration in behavior in the EPM is considered to reflect working memory [3].

In the present study, chronic administration of *C. dactylon* in normal mice significantly decreased EL and TL in MWM and EPM suggesting enhancement in memory. This may be because of the presence of flavonoids in the AECD that protects against anoxic cellular damage [18]. Flavonoids also have a neuroprotective effect and it enhances cognitive function [19].

Scopolamine interferes with memory and cognitive function in human beings and experimental animals by blocking muscarinic receptors.

This experimental animal model of scopolamine- induced amnesia has been extensively used in research to screen for drugs with potential therapeutic value in dementia [20].

In the second part of the present study, chronic administration of *C. dactylon* significantly reversed scopolamine-induced increased EL in MWM and TL in EPM, respectively, suggesting spatial memory-enhancing effect. Memory-enhancing effect of *C. dactylon* on scopolamine-induced impaired memory in the present study was more marked than the effect on normal memory. This effect may be due to their ability to exert neuroprotective effects through inhibition of AChE as reported earlier [21]. Furthermore, memory impairment in the scopolamine-induced animal model is associated with increased oxidative stress. Another mechanism contributing to the improvement in the performance of memory due to *C. dactylon* aqueous extract administration may be due to the presence of antioxidant compounds, it decreased lipid peroxidation and increased superoxide dismutase and catalase activity [10,22].

CONCLUSION

Thus, the data of the present study support the cognitive-enhancing effect of chronic *C. dactylon* intake in normal and memory-impaired mice, however, the memory-enhancing effect of *C. dactylon* is more marked in scopolamine-induced memory impairment as compared to normal memory function. Long-term interventional studies are required to know the exact mechanism of the cognitive-enhancing effect of *C. dactylon.*

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AUTHORS' CONTRIBUTIONS

Dr. Saroj Kothari: Concept and design of study preparation, draft of the manuscript, and revision of the manuscript.

Dr. Monika Sahu: Review of literature, analysis of data, interpretation of data, statistical analysis, and review of the manuscript.

CONFLICTS OF INTEREST

None.

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None.

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