Evaluation of Antiamnesic Potentials of *Calotropis Procera* in Mice

C. C. Gavimath, V. Havannavar, P. Hulekal, P. Pattar and *Hanumanthachar Joshi

Dept of Biotechnology,
K.L.E.S’s College of Engineering, Belgaum, Karnataka

* Dept. of Postgraduate Studies and Research,
SET’s College of Pharmacy, Dharwad-580002, Karnataka.

Author for Correspondence : amanjoshi17@yahoo.com

Summary

Alzheimer’s disease (AD) is the most common cause of a medical condition known as dementia, which effects the brain and hence memory. The National Institute of Health predicts, if the current trend continues, there will be more than 8.5 million AD patients by the year 2030 in USA alone. Although there is no cure for dementia if AD type at present alternative pharmacologic treatment modalities can reduce the symptoms of cognitive improvement and slow disease progression. Nootropic agents like, piracetam and cholinesterase inhibitors like, Donepezil® are commonly used for improving memory, mood and behavior. However, the resulting adverse effects of these drugs have limited their use and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. The present work was undertaken to assess the potential of latex of *Calotropis procera* as a nootropic agent in mice. Elevated plus maze was employed to assess the memory of mice. Whole brain Ache activity was also measured. Diazepam (1 mg/kg, i.p.) and Scopolamine (0.4 mg/kg,i.p.) were used to induce amnesia in mice. *C. procera* (100 and 200 mg/Kg, p.o.) was administered for 3 successive days to both young and old aged mice. *C. procera* deceased transfer latencies indicating improvement in learning and memory and it also reversed amnesia induced by Scopolamine, diazepam and natural ageing. Hence *C. procera* can be employed as a memory restoration agent in patients suffering from amnesia.

Key words: Acetylcholinestrase activity, Memory, *Calotropis procera*.

Introduction

Alzheimer’s disease [AD] the most common form of dementia in the elderly population is characterized by an insidious onset with memory impairment and an inexorable progression of cognitive decline. Neuropathological examination of AD brain reveals extensive atrophy, accumulation of neurofibrillary tangles [1] and β-amyloid [Aβ] fibrillar deposits (Aβ Plaques) [2]. Aging is accompanied by an overall reduction in brain volume [3-5]. However there is considerable diversity in rates of decline for specific sub regions. Likewise, there are variable rates of decline across different cognitive domains with some functions remaining relatively intact and others showing unambiguous impairment [5-9]. According to the frontal aging hypothesis age related cognitive decline is driven by notably the prefrontal cortex [10, 11]. More general age related changes in the brain also include reductions in synapse density, grey and white matter and cerebral blood flow [12]. In healthy older adults white matter lesions are associated with speed of information processing [13, 14]. Along with rapid declines observed in PFC volume and function, moderate declines have also been found to develop gradually across the adult life span in the striatum, a region that is responsible for dopamine production [10]. These changes are accompanied by declines in dopamine concentration and dopamine and serotonin receptor availability in the frontal cortex [16, 17]. Together these age related declines in volume of PFC and concentrations of neurotransmitters are associated with declines in cognitive performance among aging adults [16].
Calotropis procera [Ait] R. Br, (family: Asclepiadaceae) is well known for its medicinal as well as toxic properties [18, 19]. The plant produces milky white latex that exhibits pleiotropic effects in various animal models [20] even the accidental exposure to the latex produces contact dermatitis, Keratitis and toxic iridocyclitis [21-23]. In the present study the potential nootropic effects of C. procera were investigated. Whole brain Acetyl cholinesterase activity was also assessed.

Methods

Plant material and Preparation of extract
The aerial parts of Calotropis procera was collected from Dharwad, Karnataka, India. The plant was authenticated and identified by Dr. Hebbar, Department of Botany, Karnataka University, Dharwad. The latex was collected from aerial parts and it was dried under shade at ambient temperature, afterwards 5 gm or 6.25 ml of latex was mixed in 50 ml of water and was centrifuged at 100 rpm/once for 10 min. Then the centrifugate was separated and filtered using whatman paper and then different concentration of extract was prepared.

Drugs and Chemicals
Scopolamine hydrobromide [Sigma Aldrich, Lt Louis MO, USA], Diazepam (Ranbaxy Ltd., India), Piracetam [Nootropil®, UCB India Pvt. Ltd. Vapi. India] and phenytoin [Zydus Neurosciences, Ahmedabad, India] were diluted in normal saline and injected intraperitoneally (i.p). volume of injection was 1 ml/100 g.

Animals
Swiss mice of either sex weighing around 18 gm (younger ones, aged 8 weeks) and 25 gm (older ones, aged 28 weeks) were used in present study. Animals were procured from disease free animal house, Bioneeds Pvt. Ltd., Tumkur, Karnataka. They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and maintained under 12:12 hr light and dark cycles. All experiments were carried out during daytime from 09:00 to 19:00 hrs. Institutional animal’s ethics committee [IAEC] approved the experimental protocol and care of the animals was taken as per guidelines of CPCSEA Dept of animal welfare, Govt. of India.

Memory Model

Elevated Plus Maze: The elevated plus maze served the extroceptive behavioral model [where in stimulus existed outside the body] to elevate learning and memory in mice. The apparatus consists of two open arms [16 cmx15 cm] and two covered arms [16 cmx5 cmx12 cm] the arms extended from a central platform [5 cmx5 cm] and maze is elevated to a height of 25 cm from the floor. On the first day each mouse is placed at the end of open arm facing away from the central flat form. Transfer latency [TL] is taken as the time taken by the mouse to move into one of the covered arm with all its 4 legs. TL was recorded on the first day. If the animal does not enter into one of the covered arm within 90 sec, it is gently pushed into one of the two covered arms and the TL is recorded as 90 sec. The mouse is allowed to explore the maze for 10 sec & then returned to its home cage. Memory retention is examined 24 hrs after the first day trial and again on the second day [15, 24].

Acute toxicity studies
Calotropis procera extract at different doses was administered intraperitoneally to young and aged mice during the first 4 hours after the drug administration. The animals were studied for gross behavioral changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedations, hypothermia and mortality were observed and the doses selected were 100 and 200 mg/kg.

Estimation of brain acetylcholinesterase
The time frame of cholinesterase activity estimation was similar to behavioral tests, i.e. 8 a.m.–11 a.m. on each day. On the ninth day animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using Ellman method [25]. The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5,5’-dithionitrobenzoic acid (DTNB), and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of 3 min was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

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R = \frac{S.O. D \times \text{Volume of Assay (3 ml)}}{E \times \text{Mg of Protein}}
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Where \( R \) = Rate of enzyme activity in ‘n’ model of acetylcholine iodide hydrolyzed/minute/mg protein.
S. O. D. = Change in absorbance / minute. \( E \) = Extinction Coefficient= 13600/M/Cm.
Statistical Analysis

All the results were expressed as Mean ± SEM. The data were analyzed by ANOVA followed by Tukey-Kramer test. P < 0.05 was considered as statistically significant.

Results

Effect on transfer latency (TL) using elevated plus maze: Aged mice showed higher transfer latency (TL) values on first day and on second day (after 24 hr) as compared to young mice, indicating impairment in learning and memory. Piracetam (200 mg/kg, i.p.) pretreatment for 3 days decreased TL on 3rd day and after 24 hrs i.e. on 4th day as compared to control, indicating improvement in both learning and memory (fig.1).

![Graph showing transfer latency (TL) comparison between control and treated groups.](image)

All values are mean ± SEM : ANOVA followed by Tukey- Kramer test
* denotes P<0.01 as compared to control (Young)
a denotes P<0.001 as compared to control (Young)
b denotes P< 0.01 as compared to control (Aged)
c denotes P<0.001 as compared to control (Aged)

Scopolamine (0.4 mg/kg) and Diazepam (1 mg /kg) increased TL significantly (P<0.05) in young mice on first day and second day as compared to control, indicating impairment of memory (fig.2).

C. procera(100 and 200 mg/kg, p.o.) decreased the TL on 3rd day and 4th day in young and aged mice (P<0.05) when compared to control groups. Higher doses of CP (200 mg/kg, p.o.) more significantly enhanced the learning and memory of aged animals rather than the young mice as reflected by marked decrease in TL on 3rd and 4th day when subjected to elevated plus maze tests. The higher dose of CP pretreatment for 3 days successively to young mice protected them against scopolamine, diazepam and ageing induced amnesia.

Effect on whole brain acetylcholinesterase (AChE) activity: The whole brain AChE activity with phenytoin (12 mg/kg, p.o.) exhibited significant elevation to AChE activity as compared to control and piracetam (200 mg/kg, p.o.). C. procera (200 mg/kg, p.o.) significantly reduced AChE activity (fig.3).
**Discussion**

The symptoms of all types of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the brain areas affected [26] cognitive deterioration occurring in patients with probable Alzheimer’s disease (AD) is associated with a progressive loss of cholinergic neurons and a consequent decline in the levels of acetylcholine (ACh) in the brain particularly in the temporal and parietal neocortex and hippocampus [27]. Acetylcholine is believed to affect the memory, sleep, and concentration abilities, and also to be involved in some severe diseases such as Alzheimer, Parkinson and epilepsy [28, 29].
Despite the severity and high prevalence of this disease, Allopathic System of Medicine is at to provide a radical cure for AD. Therefore, we were motivated to explore the potential of medicinal plant to manage AD. In the present study C. procera extract administered i.p for 3 days improved the memory of mice as reflected by decrease in TL values as compared to control mice.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. There is extensive evidence linking the Central Cholinergic System to Memory [30]. The symptoms of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the brain areas of affected [26]. Cognitive deterioration occurring in patients with probably AD is associated with progressive loss of cholinergic neurons and consequent decline in levels of acetylcholine (ACH) in the brain [27]. Selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be characteristic feature of senile dementia of the Alzheimer’s type [31]. C. procera (50, 100, 200 mg/Kg i.p) significantly decreased whole brain AchE activity indicating its potentials in the attenuation of severity of Alzheimer’s disease.

The present study indicates that C. procera is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of acquired learning. Cognitive deterioration occurring in patients with probably AD is associated with progressive loss of cholinergic neurons and consequent decline in levels of acetylcholine (Ach) in brain. Cholinergic deficits occur in the brain of patients with AD and vascular dementia [32-33]. Altered hippocampal neurogenesis may also play a pathophysiological role in neurodegenerative disorders such as AD [34]. Phenytoin is known to reduce hippocampal Ach concentration and causes cognitive impairment [35]. The aqueous extract of C. procera significantly inhibited the ACHE activity in the whole brain homogenate of mice, indicating its potential in the attenuation of learning and memory deficits especially in aged mice. Considering the lack and need of drugs with proven effectiveness in improving learning and memory[36] the specific memory improving effects of C. procera reported in the present study can be of enormous interest and deserves further investigations using more experimental paradigms for further confirmation of memory improving potential of C. procera in the treatment of various cognitive disorders. Considering the lack and the need of the drugs with proven effectiveness in improving learning and memory, the specific memory improving, anticholinesterase effects of C. procera can be of enormous use in the management of preliminary symptoms of dementia and Alzheimer’s disease.

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References