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Clerodendron phlomidis linn improves short term memory of chemically and naturally induced amnesia in mice

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ABSTRACT

The present work was undertaken to asses the potential of *Clerodendron phlomidis* as a antiamnesic agent in mice. Elevated plus maze was employed to evaluate short term memory in mice. Scopolamine and diazepam were used to induce amnesia in mice. 100 and 200mg/kg, p.o. of aqueous extract of *C. phlomidis* were administered for 6 successive day to both young and aged mice. *C. phlomidis* decreased transfer latencies indicating improvement in learning and memory and it also reversed amnesia induced by scopolamine, diazepam and natural ageing. Hence *C. phlomodis* can be employed as a memory restoration agent in patient suffering from amnesia.

KEYWORDS

Clerodendron phlomidis;
Amnesia;
Scopolamine.

INTRODUCTION

Memory function is vulnerable to a variety of pathologic processes including neurodegenerative diseases, strokes, tumors, head trauma, hypoxia, cardiac surgery, malnutrition, attention-deficit disorder, depression, anxiety[1], the side effects of medication, and normal ageing[2]. Normal ageing is known to deteriorate memory in human beings[3]. Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer's disease (AD) in elderly[4]. AD is a progressive neuro degenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and ultimately death[5]. It is the most common form of onset of adult dementia and attention deficit disorders. Nootropics represent a new class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory.

AD is the most common cause of a medical condition known as dementia, which affects the brain and hence memory. It is a chronic, progressive organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language[6]. AD has been identified as a protein misfolding disease due to the accumulation of abnormally folded amyloid β protein in the brains of AD patients[7]. Amyloid β, also written Aβ, is a short peptide that is an abnormal proteolytic byproduct of the transmembrane protein amyloid precursor protein(APP), whose function is unclear but thought to be involved in neuronal development[8].

AD begins as a deficiency in the production of the neurotransmitter acetylcholine. 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[9]. Amnesic mild cognitive impairment represents a transitional state between the cognitive changes of normal ageing and the earliest critical features of Alzheimer's disease[10]. Although there is no cure for dementia of AD type at present, alternative pharmacologic treatment modalities can reduce the symptoms of cognitive impairment and slow disease progression[11]. Nootropic agents like, piracetam, fosracetam, nefiracetam aniracetam and cholinesterase inhibitors like, Donepezil® are commonly used for improving memory, mood and behavior. However, the resulting adverse effects of these drugs such as diarrhea, insomnia, nausea, bronchitis, loose stools, muscular cramps and other known side effects[12], has made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders. Indian system of medicine emphasizes use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders.

Clerodendron phlomidis(*C.phlomidis* Linn) (Family-verbenaceae) is known as Agnimantha in Sanskrit. Bark of the plant is used for treating various nervous disorders[13]. A new chalcone glycoside, together with pectolinarigenin, 7-hydroxyflavone and 7-hydroxyflavanone 7-O-glucoside have been isolated from the flowers and leaves of *C.phlomidis*. The structure of the chalcone glycoside has been established as 4,2',4'-trihydroxy-6'-methoxychalcone 4, 4'-D-diglucoside by spectroscopic and degradative methods[14]. A decoction of *C.phlomidis* leaves is used along with other parts for inflammation and is effective in treating bronchitis, headache, weakness, drowsiness and digestive problems[15]. *C.phlomidis* reported for antidiarrhoeal activity[16] and antifungal activity. In the present study *C.phlomidis* was investigated for its potential as a nootropic agent in amnesic mice. Elevated plus maze were the exteroceptive behavioral models to assess short-term memory.

MATERIALS AND METHODS

The plant material and preparation of extract

The bark of *C.phlomidis*(Family-Verbenaceae) was obtained from Dharwad, Karnataka, India. The plant was authenticated and identified by qualified botanist at Department of Botany, Karnataka University, Dharwad. The specimen has been kept at dept. of pharmacognosy, SET'S College of Pharmacy, Dharwad, Karnataka, India. The bark was dried in shade, cleaned, powdered and aqueous extract was prepared by simple maceration process using 1000g of powder. The extract was concentrated using rotary flash evaporator followed by freeze drying. The yield of the dry extract from crude powder of *C.phlomidis* was 2% w/w. A suspension was prepared using tween 80 and administered orally.

Drugs and chemicals

Scopolamine hydrobromide (Sigma Aldrich, USA), diazepam(Valium,® Ranbaxy laboratories Ltd., Mumbai, India) and piracetam(Nootropil® UCB India pvt. Ltd., Vapi, India) were diluted in normal saline and injected intraperitoneally (i.p.). Volume of injection was 1ml/100 g body weight of the mouse.

Animals

Swiss mice of either sex weighing around 18g (younger, 8 weeks old) and 25g (older, 28 weeks old) were used in the present study. Animals were procured from disease free animal house, BLDEA Medical College, Bijapur. They were acclimatized to the laboratory conditions for 5days before behavioral studies. The animals had free access to food and water and maintained under 12:12h light and dark cycles. All experiments were carried out during day time from 0900 to1900 hours. The Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of experimental animals, dept. of animal welfare, ministry of environment and forests, Govt. of India.

Acute toxicity studies

C.phlomidis aqueous extract at different doses(50-1000 mg/kg) was administered orally to the mice with the help of a specially designed oral needle connected to a polythene tube. Mice, which received extracts in doses above 1000mg/kg, exhibited ptosis(dropping of upper eyelids) and were found lethargic. The param-

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eters such as hyperactivity, grooming, convulsions, sedation, hypothermia and mortality were observed. The doses selected were 100mg/kg and 200mg/kg/day.

Memory models

Exteroceptive behavioral model

Elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model(where in stimulus existed outside the body) to evaluate learning and memory in mice[17]. The apparatus consists of two open arms(16cm×5cm) and two covered arms(16cm×5cm×12cm). The arms extended from a central platform(5cm×5cm), and maze is elevated to a height of 25cm from the floor. On the first day(i.e. 6th day of drug treatment), each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency(TL) was defined as the time (in seconds) taken by mouse to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec, it is gently pushed into one of the two covered arms and the TL was assigned as 90 seconds. The mouse was allowed to explore the maze for 2min and then returned to its home cage. Memory retention was examined on the second day(i.e. 7th day), 24 hours after the first day's trial[18].

Interoceptive behavioral models

Scopolamine induced amnesia- amnesia was induced by administration of Scopolomine hyadrobromide on 6th day and TL recorded. Retention was recorded after 24hr. *C.phlomidis*(100 and 200mg/kg, p.o.) and piracetam(200mg/kg, i.p.) were administered for 6days successively. On 7th day, after 45min of administration of doses, Scopolomine(0.4mg/kg, i.p.) was administered and TL was noted after 45min.

Diazepam induced amnesia- Diazepam was administered to young mice and TL was noted after 45min of injection on 6th day and after 24hr. C.*phlomidis*(100 and 200mg/kg, p.o.) and piracetam(200mg/kg, ip) were administered for 6th day, diazepam was administered. TL were noted after 45min of administration of diazepam and after 24hr[19].

Statistical analysis

All the results were expressed as mean ± Standard error. The data was analyzed using ANOVA followed by Tukey-kramer test. P<0.01 was considered as statistically significant.

RESULTS

Effect of transfer latency(TL) using elevated plus maze

Aged mice showed higher transfer latency(TL) values on first day and second day(after 24hr) as compared to young mice, indicating impairment in learning and memory. Piracetam(200mg/k, i.p.) pretreatment for 6 days decreased TL on 6th day and after 24hrs i.e. on 7th day as compared to control, indicating improve-

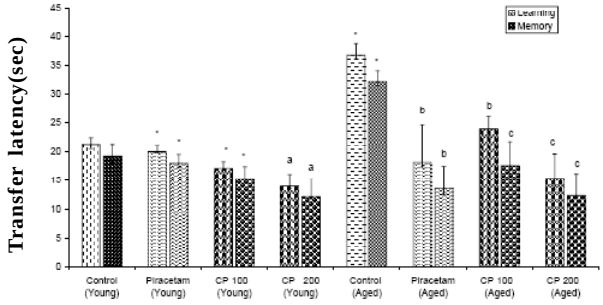

Figure 1 : Effect of *C.phlomidis* (CP) on transfer latencies of young and aged mice.

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)

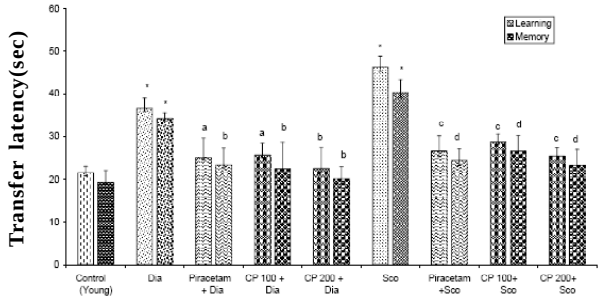

Figure 2 : Effect of *C.phlomidis* (CP) on transfer latencies of diazepam and scopolamine induced mice

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

ment in both learning and memory (figure 1). Scopo lamine(0.4mg/kg, i.p.) and Diazepam(1mg/kg, i.p.) increased TL significantly($P<0.05$) in young mice on first day and second day as compared to control, indicating impairment of memory(figure 2).

C.phlomidis (100 and 200mg/kg, p.o.) decreased the TL on 6th day and 7th day in young and aged mice ($P<0.05$) when compared to control groups. Higher doses of. *C.phlomidis*(200mg/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 6th and 7th day when subjected to elevated plus maze tests. The higher doses of *C.phlomidis* pretreatment for 6 days successively to young mice protected them against scopolamine, diazepam and ageing induced amnesia.

DISCUSSION

Alzheimer's disease, an age related neurodegener ative disorder is characterized by a progressive loss of memory and cognitive function, resulting in severe dementia. Neuropathlogically, AD is defined by the accumulation of two types of insoluble fibrous material, i.e., extracellular amyloid-β peptide deposited in senile plagues and intracellular neurofibrillary tangles composed principally of abnormal and hyperperphos phorylated tau protein. Amyloid-β is a proteolytic product of the single membrane spanning protein, amyloid precursor protein, amyloid precursor protein(APP)[20]. The APP gene is located on chromosome [21]. Tau is normal brain phosphoprotein that promotes the assembly and stability of neuronal axons by binding to microtubules[22]. In AD, numerous phosphorylation sites on the tau protein have been identified. In its hyperpho sphorylated state, tau protein looses its ability to stabilize microtubules, causing axonal instability, which contributes to the dysfunction in their transport ability[23].

AD is further characterized pathologically by regionalized neuronal death and loss of synaptic connection within selective brain regions. The other cause of AD is based on the genetic observation from familial Alzheimer disease[24]. This research showed that mutation of the gene of amyloid precursor protein[25], presenillin-1 and presenillin-2[26] that cause inherited AD lead to increased accumulation of fibrillary β amyloid in the brain. Several hypotheses have been proposed to explain AD pathogenesis including: amyloid cascade, excitoxicity, oxidative stress, and inflammation[27]. There is accumulating evidence that suggests a key role of oxidative stress in the pathophysiology of AD. Free radicals produced during oxidative stress are speculated to be pathologically important in AD and other neurodegenerative diseases[28]. Oxidative stress leads to oxidative injury of dorsal root ganglion neurons, mitochondria being a specific target[29]. The central nervous system is specially vulnerable to oxidative stress as a result of the brains high oxygen consumption, abundant lipid content, and relative paucity of antioxidant compounds compared with other tissues[30]. Presently, the allopathic system of medicine principally relies on nootropic agents, such as piracetam, aniracetam, fosracetam, nefiracetam, etc., and anticholinesterases, such as Donepezil® and tacrine are commonly used for improving mood and behavior. However the Donepezil® have adverse side effect on cholinergic symptoms particularly gastrointestinal symptoms like nausea, vomiting and diarrhea. Tacrine have adverse effect on liver toxicity, as shown by elevated serum amino transferases. Since allopathic system of medicine is yet provide a radical cure of AD, it is worthwhile to look for new direction, which would minimize the memory loss of patients with neuropsychiatric disorder. The utility of traditional medicines may be explored for treating patient with dementia. In present study, *C.phlomidis* extract administered orally for 6 days improved the memory of mice as reflected by diminished TL as compared to control animal and pretreatment with *C.phlomidis* for 6 days protect the animal from memory deficits produced by Scopolamine, diazepam and ageing induced amnesia. These findings suggest the possible neuroprotective role of *C.phlomidis.*

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