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Research Article

Evaluation of the Memory and Learning Improving Effects of *Mimusops elengi* in Mice

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Abstract

Management of cognitive disorders like dementia and Alzheimer's disease has been challenging since no potential drug is available with proved efficacy. Some nootropic drugs like piracetam, aniracetam and cholinesterase inhibitors such as Donepezil® have found to exhibit severe toxic effects in elderly. In the present study we assessed the nootropic potential of ethanol extract of *Mimusops elengi* Linn. in mice. *M. elengi* [100 and 200 mg/kg] was administered orally for 8 successive days to both young and aged mice. Elevated plus maze and Passive avoidance paradigm were employed to assess short term and long term memory respectively. To delineate the possible mechanism through which *M. elengi* elicits the anti-amnesic effect, we investigated its influence on central cholinergic activity by estimating the whole brain acetylcholinesterase activity. *M. elengi* [100 and 200 mg/kg, p.o.] significantly attenuated amnesic deficits induced by diazepam [1 mg/kg, i.p.], scopolamine [0.4 mg/kg, i.p.] and natural aging. *M. elengi* [100 and 200 mg/kg] decreased transfer latencies and increased step down latencies significantly in the aged mice. It also reversed amnesia induced by diazepam and scopolamine in young mice. *M. elengi* also decreased whole brain acetyl cholinesterase activity significantly. *M. elengi* can be a useful memory restorative agent in the treatment of dementia.

Key Words: Acetylcholin, ayurveda, *Mimusops elengi*, memory, scopolamine.

Introduction

Cognition is that operation of mind by means of which, we become aware of our surroundings, objects and thoughts. Cognitive disorders such as delirium, dementia and amnestic disorders are common in elderly individuals. Memory is vulnerable to a variety of pathologic processes including neurodegenerative diseases, strokes, tumors, head trauma, hypoxia, cardiac surgery, malnutrition, attention deficit disorder, depression, anxiety, the side effects of medication, and normal ageing [1]. As such, memory impairment is commonly seen by physicians in multiple disciplines including neurology, psychiatry, medicine, and surgery [2]. Memory loss is often the most disabling feature of many disorders, impairing the normal daily activities of the patients and profoundly affecting their families. The key features of these dreaded disorders are memory impairments, deterioration of language, visuospatial, motor, sensory abnormalities, gait disturbance and seizures. There are around 30 million patients suffering from Alzheimer's disease (AD) which is the major cause of dementia, all over the world [3]. In India, AD patients are estimated to be around 3 million [4]. Presently, there are no satisfactory diagnostic procedures and therapeutic regimens available for the management of these cognitive disorders. Despite the severity and high prevalence of these diseases, Allopathic system of medicine is yet to provide a satisfactory remedy. Therefore, neurobiologists all over the world are looking for new directions and alternative strategies for managing cognitive disorders.

The most common cause of dementia in the elderly is probably Alzheimer's disease [AD], a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language [5]. 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 such as diarrhea, insomnia, nausea, bronchitis, loose stools, muscular cramps and other known side effects [5-6], have made their use limited and it is worthwhile to explore the utility of traditional medicines in the treatment of various cognitive disorders.

Mimusops elengi L. [Sapotaceae] is known as bakula in ayurveda [7]. It is a small to large evergreen tree found all over India and is cultivated in gardens as an ornamental tree and is used in the ayurvedic system of medicine for the treatment of various neurological disorders [8-9]. Stem bark of *Mimusops elengi* possesses cardiotonic, stomachic, anthelmentic and astringent properties [10]. The bark powder along with 50 g alum, 5 g sodium chloride, is warmed and used for massaging on teeth in the treatment of pyorrhea by the locals [11]. The fine powder is sniffed to relieve headache, the decoction is used as a general tonic and flower in perfumery [12]. Phytochemical review of the bark of *M. elengi* reveals the presence of taraxerol, taraxerone, ursolic acid, betulinic acid, quercitol, lupeol [13], alkaloid isoretronecyl tiglate and mixture of triterpenoid saponins [14-15]. *M. elengi* is reported to possess anti-ulcer [16] and hypotensive [17]

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activities. The present study was undertaken to evaluate the effects of ethanol extract of bark of *M. elengi* on scopolamine and ageing induced amnesia in mice.

Material and Methods

The stem bark of *Mimusops elengi* [ME] was collected from mature trees growing in Gullarghati, Dehradun, Uttaranchal and identified at Department of Pharmacognosy, SBS Institute of Biomedical Sciences and Research, Balawala, Dehradun. A voucher specimen [HKJ/ME-41] has been deposited in the Department. The bark was dried, cleansed and powdered. One kilogram of moderately powdered bark of ME was extracted by refluxing with 90% ethanol in soxhlet extractor for 8-10 h. The extract was evaporated to dryness under reduced pressure and temperature using rotary vacuum evaporator. The yield of dry extract from the crude powder of ME was 12 % w/w. The ethanol extract of ME was suspended in a mixture of Tween 80: Distilled Water in a ratio of 2 : 8. The suspension was orally administered to animals. The volume of administration was 1 ml/100 g, body weight of mice.

Drugs and chemicals

Scopolamine hydrobromide [Sigma Aldrich, USA] and piracetam [Nootropil®, UCB India Pvt. Ltd., Vapi, Gujarat] were diluted in normal saline and injected intraperitoneally. Phenytoin [Dilantin® suspension, Parke Davis] was administered orally. Volume of administration was 1ml/ 100 g. All the drugs were administered in the morning session i.e. 8 AM- 9 AM on each day.

Animals

Swiss mice of either sex weighing around 18 g [younger ones, aged 8 weeks] and 25 g [older ones, aged 28 weeks] were used in present study. Animals were procured from disease free animal house of CCS Haryana Agriculture University, Hisar [Haryana, India]. 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 h light and dark cycles. Institutional Animals Ethics Committee [IAEC] approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

Administration of ME: The ethanol extract of *M. elengi* [ME] at different doses [50-2000 mg/kg] was administered orally to mice with the help of a specially designed oral needle connected to a polythene tube. ME was administered at the same time on each day [i.e. 8 AM- 9 AM]. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, mortality were observed and doses selected for further studies were 100 mg/kg and 200 mg/kg.

Locomotor function:

Locomotor activity of control and drug-treated animals was measured with the help of a photoactometer (INCO, Ambala, India) [18].

Elevated plus-maze: Elevated plus-maze served as the exteroceptive behavioral model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory was followed as reported earlier [19-21]. The elevated plus maze for mice consisted of two open arms [16 cm × 5 cm] and two covered arms [16 cm × 5 cm × 12 cm] extended from a central platform [5 cm × 5 cm], and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency [TL] was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial.

Passive shock avoidance paradigm

Passive avoidance behavior based on negative reinforcement was recorded to examine the long-term memory. The apparatus consisted of a box [27 X 27 X 27 cm] having three walls of wood and one wall of Plexiglas, featuring a grid floor [3 mm stainless steel rods set 8 mm apart], with a wooden platform [10 X 7 X 1.7 cm] in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock [20V AC] was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step-down latency [SDL] was recorded. SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range [2-15 sec] during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec [22-24].

Collection of brain samples

The animals were sacrificed by cervical decapitation under light anesthesia on the 8th day, 90 mins after

administration of the last dose of ME. Immediately after decapitation whole brain was carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% w/v sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of brain acetyl cholinesterase activity.

Estimation of brain acetyl cholinesterase [AChE] activity

The time frame of cholinesterase activity estimation was similar to behavioral tests i.e. 8 AM- 11 AM on each day. On the 9th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using the Ellman method [25]. The end point was the formation of yellow color due to 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 colour compound formed during the reaction at 412 nm every minute for a period of three minutes was measured. Protein estimation was done using Folin's method. AChE activity was calculated using the following formula:

$$R = \frac{\delta \text{ O.D. X Volume of Assay [3 ml]}}{\text{E X mg of protein}}$$

Where R= rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed / min / mg protein; δ O.D. = Change in absorbance / min; E = Extinction coefficient = 13600 / M / cm.

Statistical Analysis: All the results were expressed as mean ± Standard error. The data was analyzed using ANOVA followed by Tukey-kramer test.

Results

Acute toxicity study

No mortality was observed following oral administration of ME even with the highest dose [2000 mg/kg]. ME had no toxic effect on the normal behavior of the mice. However doses more than 1500 mg/kg profuse watery stools.

Effect on locomotor activity

In the present study, ME (100 and 200 mg/kg) did not show any significant change in the locomotor function of animals (score 219±1.8 and 212±13) as compared to control group (score 215.4±11) when tested using a photoactometer.

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 (i.e. ageing-induced amnesia). Piracetam (200 mg/kg, ip) pretreatment for 8 days decreased transfer latency on 8th day and after 24 hr, i.e. on 9th day as compared to distilled water treated group, indicating improvement in both learning and memory (Fig 1). Scopolamine (0.4 mg/kg) and diazepam (1 mg/kg) increased TL significantly (P< 0.01) in young mice on first and second day as compared to control, indicating impairment of memory (Fig 2).

ME (100 mg/kg, po) decreased the TL on 8th day and 9th day in young and aged mice (P < 0.05) when compared to control groups. Higher dose of ME (200 mg/kg, po) more significantly enhanced the learning and memory of aged animals rather than the young mice as reflected by marked decrease in TL on 8th day and 9th day when subjected to elevated plus maze tests (Fig 1). The higher dose of ME pretreatment for 8 days successively protected young mice (P < 0.001) against scopolamine, diazepam and ageing induced amnesia (Fig 2).

Effect on SDL using Passive avoidance paradigm

ME [100 and 200 mg/kg, p.o.] profoundly increased step down latency [SDL] significantly as compared to control group on the second day indicating improvement in memory of young mice (Fig 3). Scopolamine hydrobromide [0.4 mg/kg, i.p.] decreased SDL on second day after training, indicated impairment of memory. ME [200 mg/kg, p.o.] administered orally for 8 days significantly [P<0.001] reversed amnesia induced by both scopolamine and natural aging (Fig. 4).

Effect on whole brain acetylcholinesterase activity

The whole brain AChE activity with phenytoin [12 mg/kg, p.o.] demonstrated significant rise in AChE activity as compared to control and piracetam [200 mg/kg, p.o.]. ME [100 and 200 mg/kg, p.o.] significantly [P<0.001] lowered AChE activity [Fig. 5].

Discussion

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities and severe behavioral abnormalities such as irritability, aphasia, apraxia, agnosia and restlessness [26]. Alzheimer patients frequently have non-cognitive symptoms, such as depression, apathy and psychosis, which impair their day-to-day activities [27-28]. Enhancement in the life-span of human beings in developed and developing countries has resulted in proportionate increase in the number of patients suffering from senile dementia. Alzheimer's disease (AD) is said to be the leading cause of dementia in elderly individuals. AD individuals exhibit deterioration in mental functions rendering them

incapacitated to perform normal daily activities. However, evidence shows that AD can also afflict young individuals as early as 40 years of age [29]. Neuritic plaques (consisting of a core of β- amyloid aggregates covered by dead neurons, microglia and apolipoprotein E) and neurofibrillary tangles are the major pathological hallmarks of an Alzheimer brain [30]. Cholinergic drugs such as Donepezil® improve learning, memory and attention. The non-cognitive aspects of dementia however are linked to serotonin and dopamine rather than acetylcholine because these neurotransmitter systems most probably influence mood, emotional balance and psychosis [31].

The symptoms of dementia are oxidative damage, impaired neurotransmission and degeneration of neuronal circuits in the affected brain areas [32]. Oxidative damage accompanies Alzheimer's disease [AD], and cholinesterase inhibitors are recommended for use in mild-to moderate Alzheimer's disease [33]. In exteroceptive behavioral models, the stimulus lies outside the body whereas; it lies within the body in case of interoceptive behavioral models. Passive avoidance behavior is a classic paradigm to assess memory with strong aversive component, based on negative reinforcement and is used in the present study to examine long-term memory [34]. Interoceptive behavioral models such as diazepam, scopolamine and natural aging induced amnesia are widely cited as models simulating human dementia in general and Alzheimer's disease in particular [35].

Nootropics are a class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system, particularly on intellectual performance, learning capability and memory [36-37]. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals [38].

Acetylcholine is considered the most important neurotransmitter involved in the regulation of cognitive functions. Cognitive dysfunction has been shown to be associated with reduced cholinergic transmission and the facilitation of central cholinergic transmission with improved memory [38-39]. Selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type [40-41]. There are extensive evidences linking the central cholinergic system to memory [42-46].

Anticholinesterases such as Metrifonate [47], Physostigmine, Tacarine, Donepezil [29], Huperzine-A [48],Rivastigmine[49], Galantamine [50] and Eptastigmine [51] have all been shown to reverse amnesia produced by disruption of cholinergic system. Enzyme choline acetyltransferase is involved in the synthesis of acetylcholine and acetylcholinesterase is involved in the degradation of acetylcholine. In the present study, M. elengi, significantly lowered the whole brain AChE activity thereby elevating acetylcholine levels in the brain.

Both piracetam and *M. elengi* ME meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit [52]. 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 [53]. Cholinergic deficits occur in the brain of patients with AD and vascular dementia [54-55]. Phenytoin is known to reduce hippocampal ACh concentration and causes cognitive impairment [56-57]. In the present study, the aqueous extract of *M. elengi* 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, the specific memory improving effects of *M. elengi* reported here is of enormous interest and deserves further investigations using more experimental paradigms for further confirmation of memory improving potential of *M. elengi* in the treatment of various cognitive disorders.

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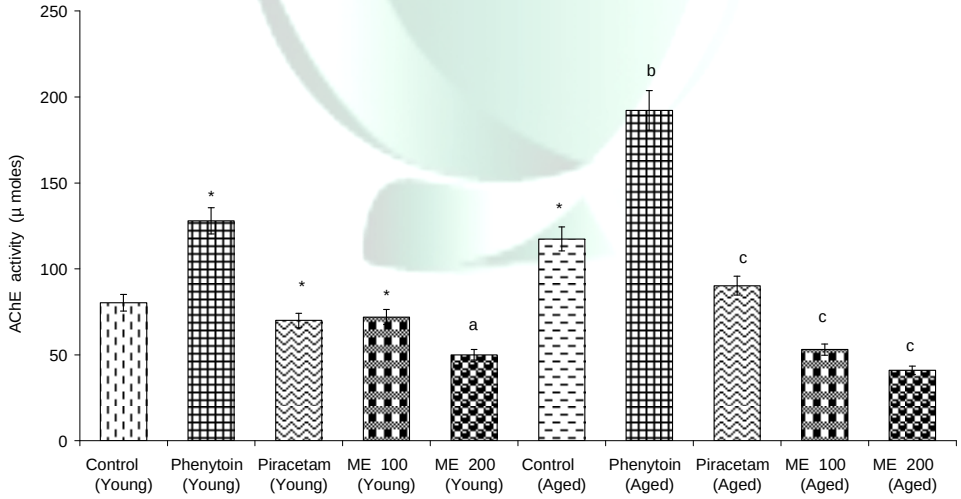

Fig. 5 Effect of *M. elengi* (ME, 100 and 200 mg/kg, p.o.) on brain cholinesterase (AChE) activity of young and aged mice using Ellman's colorimetric method. Phenytoin ((12 mg/kg, p.o.) was used as negative control.

Values are mean ±S.E.M. (n=6). (One way ANOVA followed by Tukey-kramer multiple comparison tests)
***** indicates P< 0.01 as compared to control group of young mice. **a** indicates P< 0.001 as compared to control group of young mice.
b indicates P< 0.01 as compared to control group of aged mice. **c** indicates P< 0.001 as compared to control group of aged mice.

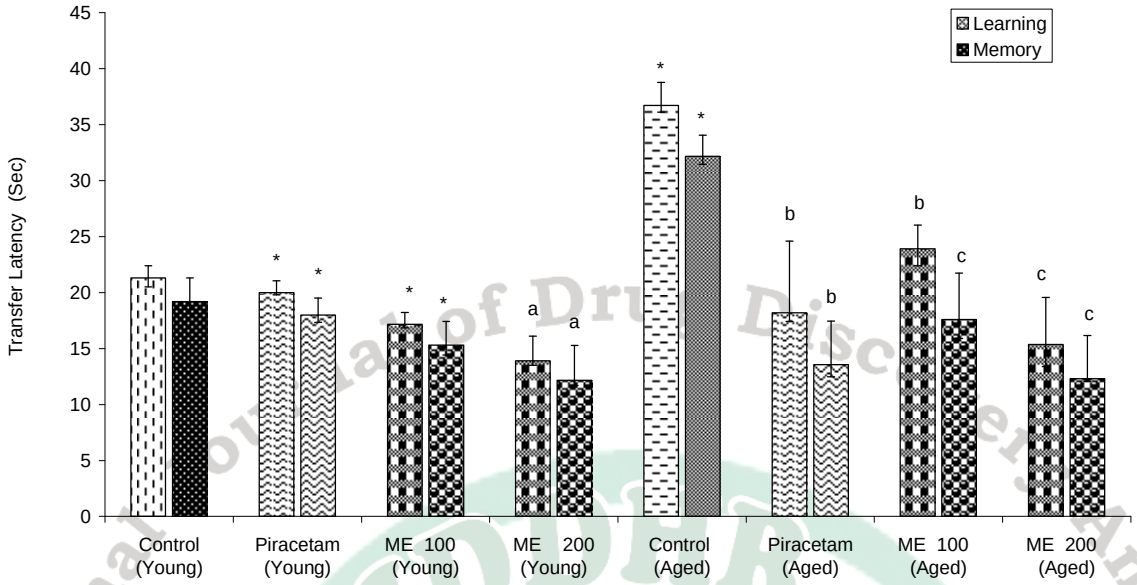

Fig. 1 Effect of *M. elengi* (ME, 100 and 200 mg/kg, p.o.) on transfer latency of young and aged mice using elevated plus maze.

Values are mean ±S.E.M. (n=6). (One way ANOVA followed by Tukey-kramer multiple comparison tests)
***** indicates P< 0.01 as compared to control group of young mice. **a** indicates P< 0.001 as compared to control group of young mice.
b indicates P< 0.01 as compared to control group of aged mice. **c** indicates P< 0.001 as compared to control group of aged mice.

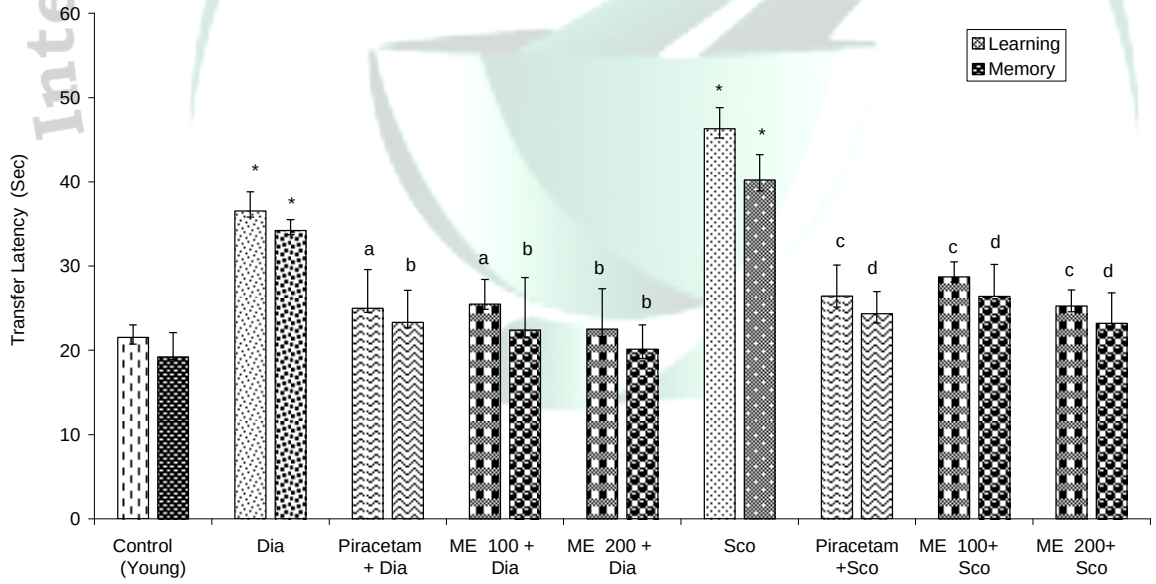

Fig. 2 Effect of *M. elengi* (ME, 100 and 200 mg/kg, p.o.) on diazepam (Dia, 1 mg/kg, i.p.) and scopolamine (Sco, 0.4 mg/kg, i.p.) induced amnesia in young mice using elevated plus maze.

Values are mean ±S.E.M. (n=6). (One way ANOVA followed by Tukey-kramer multiple comparison tests)
***** indicates P< 0.01 as compared to control group of young mice. **a** indicates P< 0.01 as compared to diazepam (Dia) group alone.
b indicates P< 0.001 as compared to diazepam (Dia) group alone. **c** indicates P< 0.01 as compared to scopolamine (Sco) group alone.
d indicates P< 0.001 as compared to scopolamine (Sco) group alone

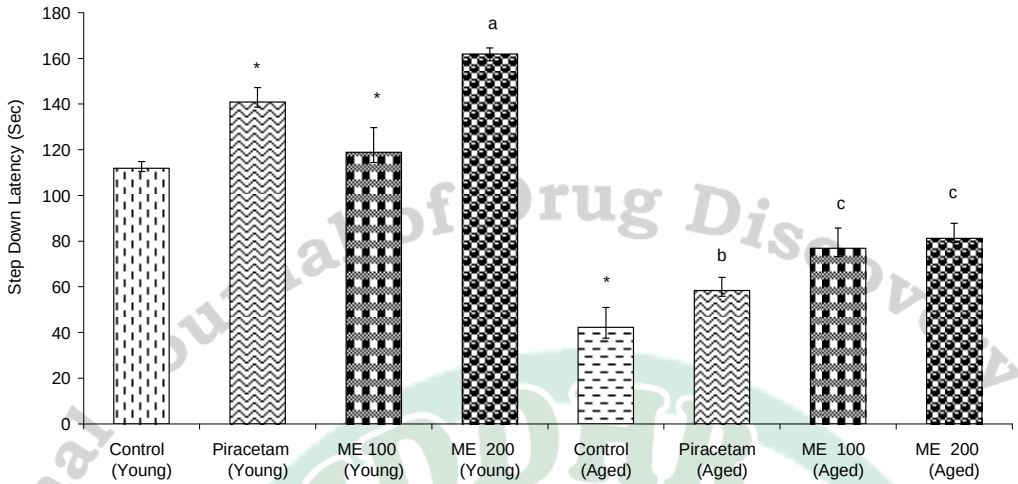

Fig. 3 Effect of *M. elengi* (ME, 100 and 200 mg/kg, p.o.) on step down latency of young and aged mice using passive avoidance apparatus.

Values are mean ±S.E.M. (n=6). (One way ANOVA followed by Tukey-kramer multiple comparison tests)

***** indicates P< 0.01 as compared to control group of young mice. **a** indicates P< 0.001 as compared to control group of young mice.

b indicates P< 0.01 as compared to control group of aged mice. **c** indicates P< 0.001 as compared to control group of aged mice.

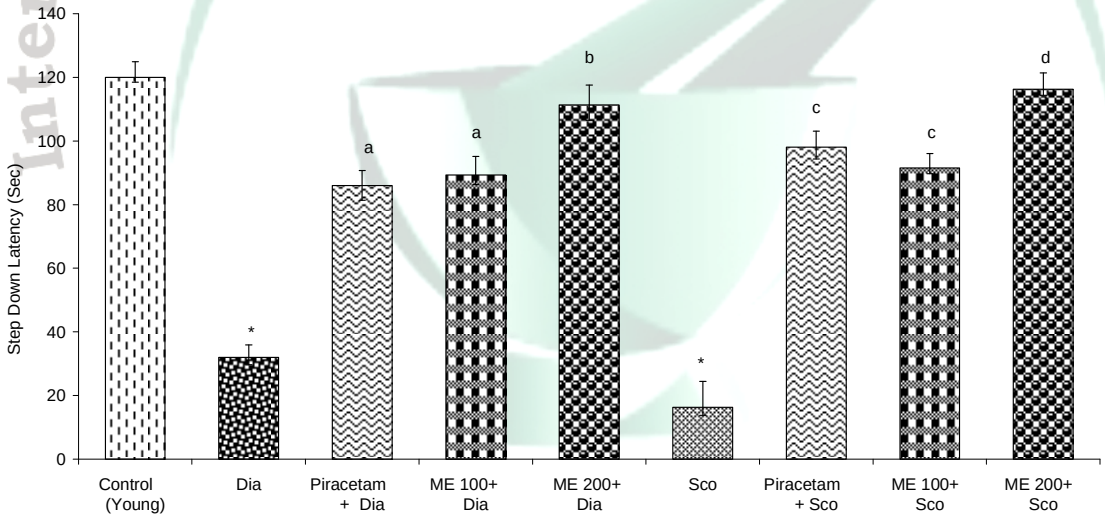

Fig. 4 Effect of *M. elengi* (ME, 100 and 200 mg/kg, p.o.) on diazepam (Dia, 1 mg/kg, i.p.) and scopolamine (Sco, 0.4 mg/kg, i.p.) induced amnesia in young mice using passive avoidance apparatus.

Values are mean ±S.E.M. (n=6). (One way ANOVA followed by Tukey-kramer multiple comparison tests)

***** indicates P< 0.01 as compared to control group of young mice. **a** indicates P< 0.01 as compared to diazepam (Dia) group alone.

b indicates P< 0.001 as compared to diazepam (Dia) group alone. **c** indicates P< 0.01 as compared to scopolamine (Sco) group alone.

d indicates P< 0.001 as compared to scopolamine (Sco) group alone