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Research Article

ANTIAMNESIC EFFECT OF PETROLEUM ETHER EXTRACT OF MURRAYA KOENIGII (LINN) LEAVES INVOLVING POSSIBLE ANTICHOLINESTERASE AND CHOLESTEROL LOWERING MECHANISM

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ABSTRACT

Alzheimer's disease (AD) is said to be the leading cause of dementia in elderly individuals exhibit a marked decline in cognitive functions. There are several factors involves in the pathogenesis of AD e.g. involvement of cholinesterase enzyme, oxidative free radicals and high cholesterol level promoting risk for and exacerbating AD pathology. In the light of this, the present study was undertaken to investigate the influence of petroleum ether extracts (300 and 500mg/kg p.o.) of *Murraya koenigii* leaves (MKL) on memory in aged mice. The study was also undertaken to evaluate for cholinesterase and cholesterol level. The result of the present study indicates that administration of MKL for 15 days produce significant dose dependant improvement of memory. The results also indicate to reduce the brain cholinesterase activity and total cholesterol levels respectively. Thus it concludes that the underlying mechanism of action for the observed nootropic effect may be attributed to anticholinesterase and a cholesterol lowering property of MKL. Therefore, it would be worthwhile to investigate specifically the therapeutic potential of MKL in the management of Alzheimer patients.

Key words: Cognitive performance, Murraya koenigii, Alzheimer's, Acetyl cholinesterase and cholesterol.

INTRODUCTION

Alzheimer's disease (AD) is one of the examples which said to be the leading cause of dementia in elderly individuals. Individuals suffering from AD exhibit deterioration in mental functions rendering them incapacitated to perform normal daily activities [1, 2]. Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions. Cholinergic neuronal loss in hippocampal area is the major feature of AD and enhancement of central cholinergic activity by use of anticholinesterase is presently the mainstay of the pharmacotherapy of dementia in AD [3, 4].

There is also suggested evidence that cholesterol may play a role in the pathogenesis of AD [5, 6, 7]. In addition to the putative involvement of cholesterol in promoting risk for and exacerbating AD pathology, recent findings have highlighted a direct biochemical impact of cholesterol on amyloid precursor protein (APP) processing, resulting in increased production of β-amyloid peptides [6]. The main histological features of AD include extracellular protein deposits termed β-amyloid (Aβ) plaques in blood vessels and intraneuronal neurofibrillary tangles [6]. Abnormal accumulation of cholesterol levels increases Aβ in cellular and most animal models of AD; and drugs that inhibit cholesterol synthesis lower Aβ in these models [5, 6]. A number of epidemiological studies indicated that high levels of cholesterol contribute to the pathogenesis of AD [5, 7]. Therefore, a new therapeutic strategy aimed at reducing blood cholesterol levels is gathering momentum for the management of AD.

In the light of this, the present study was undertaken to investigate the influence of *Murraya koenigii* leaves (MKL) on memory in aged mice. *Murraya koenigii* (L.) family rutaceae is an aromatic more or less deciduous shrub or a small tree up to 6m. in height found throughout India and is commonly known as Meethi neem and karry tree, is used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier [8, 9, 10]. The whole plant is considered to be a tonic and stomachic. The leaves are used extensively as a flavoring agent in curries and chutneys [9, 10]. Phytochemical screening of M. koenigii reveled the presence of some vitamins, carbazole alkaloid, terpenoids, phenolic compounds and mineral content such as calcium, iron, zinc and vanadium etc. in addition, carbazole alkaloid present in M. koenigii [11, 12, 13]. Ayurvedic literature as well as recent studies highlights the potential of MKL as an antidiabetic agent [14]. Leaf extracts of *Murraya koenigii* have also been reported to possess antifungal[15], antineoplastic[16], nitric oxide scavenging[17], antihypercholesteremic [18] and antioxidant activities [19].

The present study was designed to investigate antiamnesic effect of petroleum ether extract of Murraya koenigii leaves in scopolamine induced and sodium nitrite intoxication leading to impairment of learning and memory in aged mice. The effects of *Murraya koenigii* leaves on total serum cholesterol levels and brain acetylcholinesterase activity were also studied.

MATERIALS AND METHODS

Plant: The fresh leaves of Murraya koenigii were collected in the month of November 2008 from its natural habitat at Sakoli village in Nagpur region, Maharastra, India. The plant was authenticated by Dr. N. M. Dongarwar of Botany Department; RTM Nagpur University, Nagpur India. A voucher specimen (No: 9439) was deposited at Herbarium, Department of Botany, RTM Nagpur University Nagpur.

Experimental animals: All the experiments were carried out in aged (12-15 month's) male Swiss Albino mice weighing about 40-45gm. The animals had free access to food and water, and they were housed in a natural (12 hrs each) light–dark cycle. The animals were acclimatized to the laboratory conditions for at least 5 days before behavioral experiments. Experiments were carried out between 0900 h and 1800 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 729/02/a/ CPCSEA).

Material: Scopolamine butyl bromide (Sigma-Aldrich, USA),, Cholesterol estimation kit (Himedia Laboratory, Mumbai), Acetylcholine chloride (Himedia Laboratory, Mumbai), Hydroxylamine hydrochloride, Piracetam, Doneprezil, Simvastatin.

Preparation of Extracts of *Murraya koenigii* leaves: The collected leaves of Murraya koenigii were dried under shade and undergone crushing in electric blender to form powdered and subjected to extraction by using Soxhlet's extractor. The percent yield of petroleum ether (60 Grade) extract yield 6.1% w/w. The extract was concentrated by evaporation at room temperature and was used for pharmacological studies.

Administration of Extract: Suspension petroleum ether extract were prepared in 0.5% carboxymethyl cellulose using tween 20 (0.2% v/v) as a suspending agent. The extract was administered at a dose of 300 and 500mg/kg respectively to aged mice for 15 days by orally. Control groups were given only 0.5% carboxymethyl cellulose with tween 20 (0.2% v/v).

Administration of drug: Piracetam 400mg/kg, Simvastatin 5mg/kg, Donepezil 0.5 mg/kg were prepared for administration in the same manner as that for plant extract as explained above.

EXPERIMENTAL DESIGN

Acquisition and retrieval memory in Elevated plus maze (EPM)

Mice were randomly divided into 5 groups of 6 animals each. The total treatments period were 15 days in which group1 and 2 served as a control and received vehicle; group 3 and 4 received petroleum ether extract of MKL (300 and 500mg/kg p.o); group 5 served as a standard and received Piracetam (400 mg/kg p.o) respectively.

All the groups of animals except group 1 were injected with scopolamine (0.5mg/kg i.p.) 30 min after treatment on 15th day and 60 min after injection TL was recorded respectively. The procedure, technique and end point for testing memory were followed using the parameters described as our previous work as well as similar to the earlier investigators [20, 21].

EPM was employed for measurement of transfer latency (TL). It consists of two open (16× 5 cm) and two enclosed arm (16× 5 ×12 cm) facing each other with an open roof. The maze was elevated at a height of 25 cm. from the ground. The animals were placed individually 90 min after of above treatment at the end of open arm facing away from central platform and the time it took to move from open arm to either enclosed arm (TL) was recorded on the 15th day of treatment (training session). The TL was again recorded 24 hr after 1st exposure (i.e. on 16th day). The TL measure on 1st and 2nd exposure served as parameter for acquisition and retrieval memory respectively [20].

Sodium nitrite intoxication:

Mice were randomly divided into 5 groups of 6 animals each. The total treatments period were 15 days in which group1 and 2 served as a control and received vehicle; group 3 and 4 received petroleum ether extract of MKL (300 and 500mg/kg p.o); group 5 served as a standard and received Piracetam (400 mg/kg p.o) respectively. After 90 min of treatments on 15th all the animals were exposed to special two chamber cage and day first reading was noted respectively this was 1st retention test. Immediately after 1st retention test before the animal being placed in home cage all the animals except group 1 were injected with sodium nitrite (95 mg/kg s.c) and 24 after injection 2nd retention test was noted as per our previous work as the similar with earlier investigators [22, 23].

Spatial two-chambered cage was used with the dimension 16 inch length, 11 inch breadth and 5 inch height. A partition placed at a distance of 6 inches from one of the end of cage; divide the cage into a smaller and larger chamber. A water feeding bottle was kept in smaller chamber. The animals were water deprivation for 24 hrs. The animal was placed in the larger chamber and allowed to explore the cage. Ones the water deprived animal locate the bottle, it was allowed to drink the water for 30 second, the time required to locate the water bottle was noted as retention time. After 24 hrs later the animal was again placed in the larger chamber of two-chamber cage. The time required to locate the water bottle was noted as a day second reading (2nd retention test). But this time the water bottle was kept empty [22].

Biochemical Estimations for brain cholinesterase and serum cholesterol

Mice were randomly divided into 4 groups of 6 animals each. Group1 served as a control and received vehicle; group 2 and 3 received petroleum ether extract of MKL (300 and 500mg/kg p.o); group 4 served as a standard and received Donepezil (0.5 mg/kg p.o) and group 5 received Simvastatin (5mg/kg p.o) for 15 days respectively.

90 min after treatments on 15th day blood was collected by retro orbital plexus for estimation of cholesterol while brain obtained after sacrificed by cervical dislocation for determination of cholinesterase activity

Estimation of brain cholinesterase: Brain were isolated and homogenized with chilled phosphate buffer and centrifuged. The supernatant was used as a source of cholinesterase enzyme (ChE); the assay was performed as per the method of Augustinsson 1957, and as mention in our previous work [24].

Estimation of serum cholesterol: Readymade kit was used for the estimation of serum total cholesterol. In this method, the blank sample, standard sample and test sample were pipetted into the respective reaction test tube using a micropipette. For the blank sample, 15 µL of distilled water and 3000 µL of working reagent were mixed. For the standard sample, 15 µL of standard cholesterol and 3000 µL of working reagent, while for the test sample, 15 µL of serum and 3000 µL of working reagent were mixed. These mixtures were placed immediately in boiling water bath for 90 sec. and cool immediately in running tap water. The absorbance was read at 560 nm against the blank sample.

RESULTS AND DISCUSSION

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions [4, 21]. In the present study cholinergic muscarinic antagonist scopolamine the drug most widely used to induce amnesia in experimental model was used [21]. Anticholinesterase which enhance the availability of acetylcholine in synaptic cleft are able to reverse the scopolamine induce deficit indicating a neurotransmitter role of acetylcholine in learning and memory [21].

In the present study EPM was used because it is widely employed and accepted for measurement of transfer latency (TL) [20, 21]. TL measured on 1st and 2nd day served as parameter for acquisition and retrieval memory respectively. In the present study animals from all the groups showed decrease in TL on 2nd day indicating the paradigm of learning and memory in aged mice. Scopolamine produced a significant ($p< 0.01$) increase in TL on day 1st compare to control indicating impairment of memory. Scopolamine induced increased in TL was however significantly ($p < 0.001$) reversed by dose dependant 15 days prior administration with petroleum ether extract of MKL respectively which were comparable to standard cholinergic agent Piracetam (400mg/kg p.o.) indicating the MKL improve the learning and memory of aged mice (Figure 1).

Hypoxic condition induced by $NaNO_2$ cause a major change in various neurotransmitter levels and also the level of various molecules like ATP, AMP, CGMP, etc [22, 23]. Memory impairment by sodium nitrite is similar to memory impairment in aged mice [22, 23] which involve the impairment of Ach synthesis in brain [25]. The evidences can be obtained from the various experiments conducted on animals that were exposed to a learning paradigm in hypoxic condition [25, 26]. The results of the present study showed that the mean time required to locate the water bottle decreased by all drug treated as well as control aged mice respectively on day 2 as compared to day 1 reading (Figure 2). Sodium nitrite treated mice took significant ($p< 0.01$) more time to locate water bottle on 2nd retention test compared to 1st retention test compare to control indicating the impairment of memory. While 15 days prior treatment with petroleum ether extract of MKL (300 and 500 mg/kg) reversed the effect of $NaNO_2$ dose dependently compared to sodium nitrite on day 2nd which were comparable with standard Piracetam (400mg/kg p.o.) respectively (Figure 2). Thus Murraya koenigii leaves extract could be said to improve the learning capabilities of the aged mice in hypoxic condition as indicated by a better performance of animals in the learning task.

There is extensive evidence linking the central cholinergic system to memory [27, 28]. Cognitive dysfunction has been shown to be associated with reduced cholinergic transmission and the facilitation of central cholinergic transmission with improved memory [27, 28]. Selective loss of cholinergic neurons and a decrease in cholinesterase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type [3, 4]. Several research findings have also displayed a link between memory improving effect and cholinesterase inhibition [29].

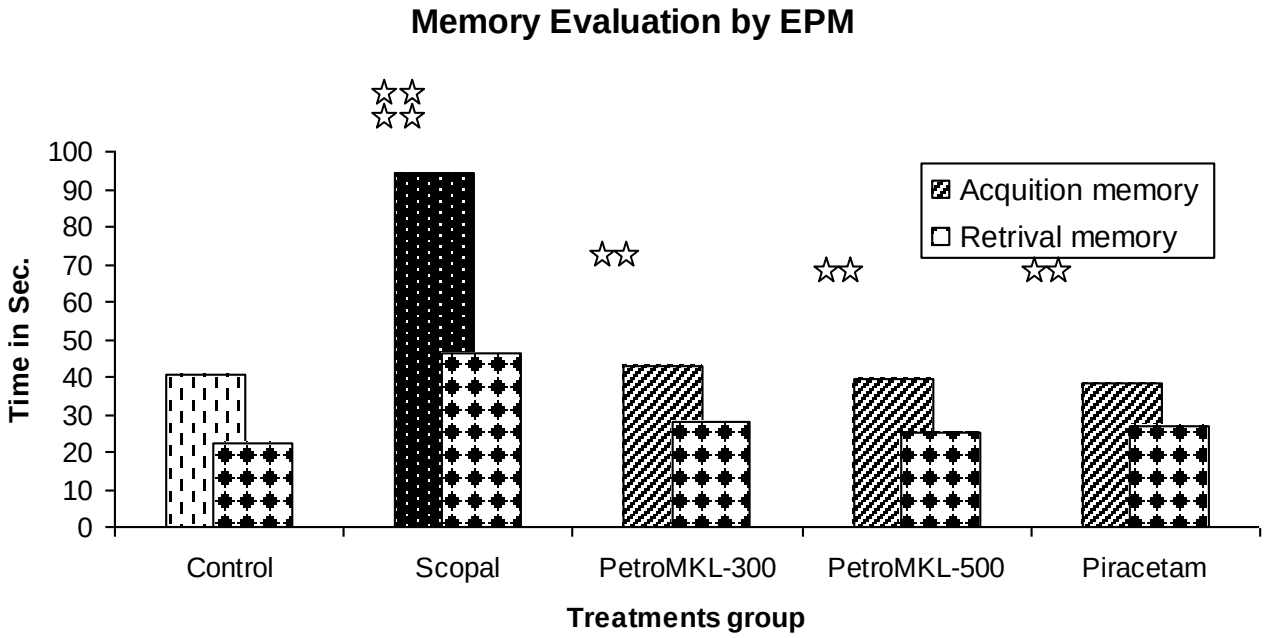

Figure -1: Effect of petroleum ether extracts of Murraya koenigii leaves (PetroMKL) on Transfer latency (TL) in aged mice using Elevated Plus maze (EPM). Piracetam (400 mg/kg, p.o.) was used as standard. Values are mean ± SD (n = 6). ☆ denotes significantly ($p < 0.001$) protect from amnesic effect of scopolamine. ☆☆ denotes Scopolamine produced significant ($p< 0.01$) amnesia in aged mice which represent by increased in TL on day 1st compare to control. (Student's unpaired t-test)

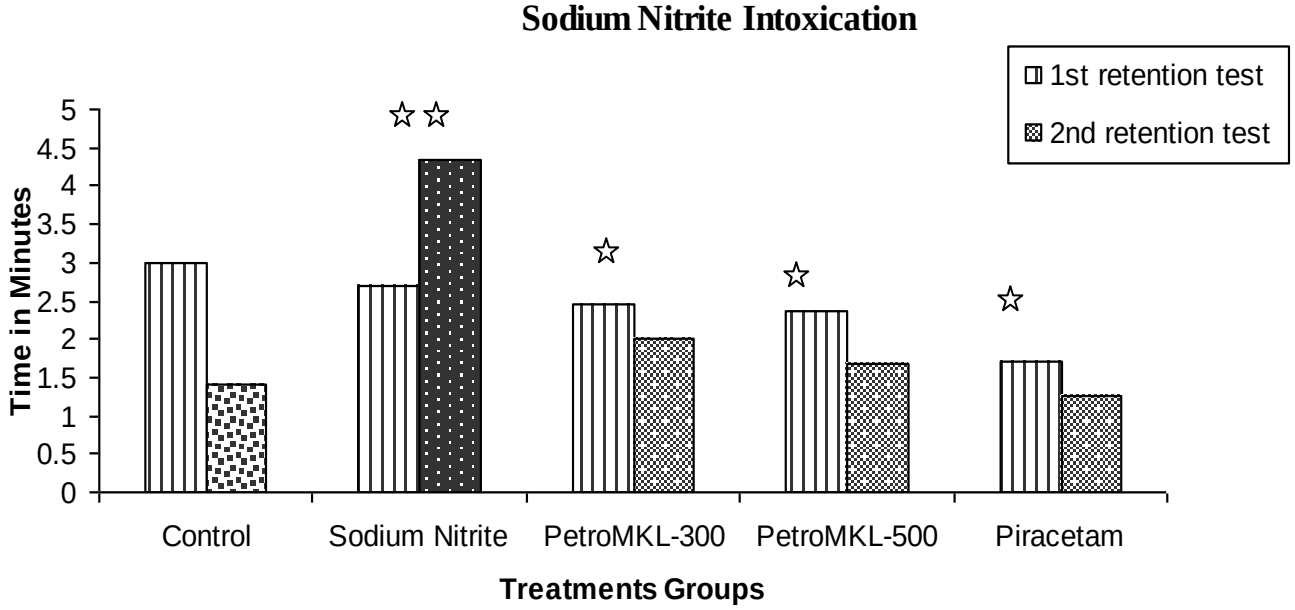

Figure -2: Effect of petroleum ether extracts of Murraya koenigii leaves (PetroMKL) on retention memory/test in aged mice using sodium nitrite induce hypoxic model in aged mice. Piracetam (400 mg/kg, p.o.) was used as standard. Values are mean ± SD (n = 6). ☆ denotes significantly ($p < 0.001$) protect from hypoxic effect of sodium nitrite which represent by decreased in time required to locate water bottle on day 2nd. ☆☆ denotes sodium nitrite produced significant ($p< 0.01$) hypoxia in aged mice which represent by increased in time required to locate water bottle on day 2nd compare to 1st retention test in control. (Student's unpaired t-test)

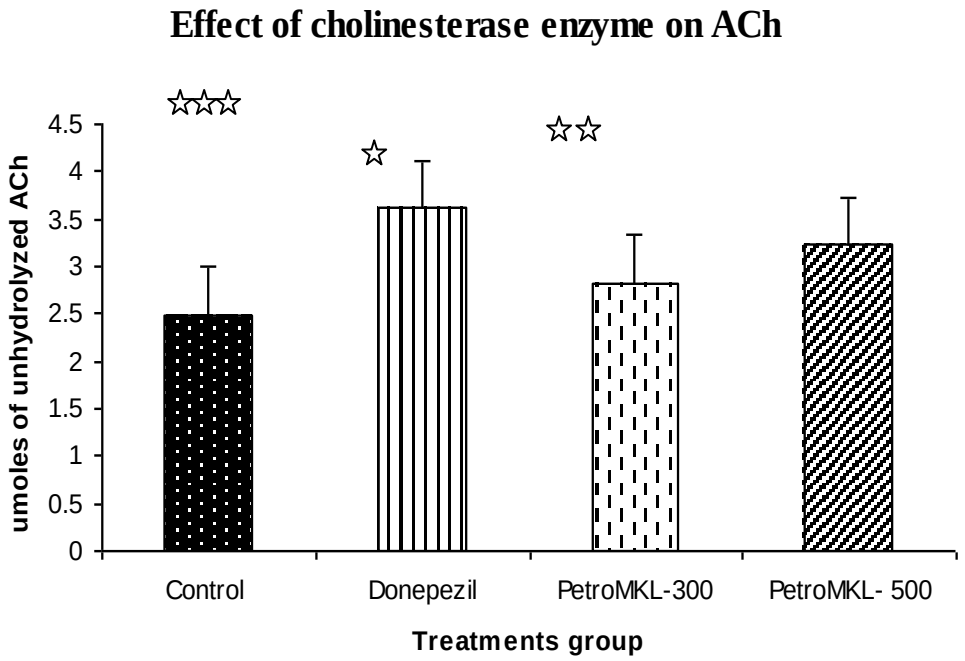

Figure -3: Effect of petroleum ether extracts of Murraya koenigii leaves (PetroMKL) on activity of cholinesterase enzyme in aged mice. Doneprezil (0.5 mg/kg, p.o.) was used as standard. Values are mean ± SD (n = 6). ☆ denotes significant ($p< 0.05$) reduction in cholinesterase activity which represent by greater unhydrolyzed Ach compare to control in aged mice. ☆☆ denotes significant ($p< 0.01$) reduction in cholinesterase activity which represent by greater unhydrolyzed Ach compare to control in aged mice. ☆☆☆ denotes significant ($p< 0.001$) reduction in cholinesterase activity which represent by highest unhydrolyzed Ach compare to control in aged mice. (Student's unpaired t-test)

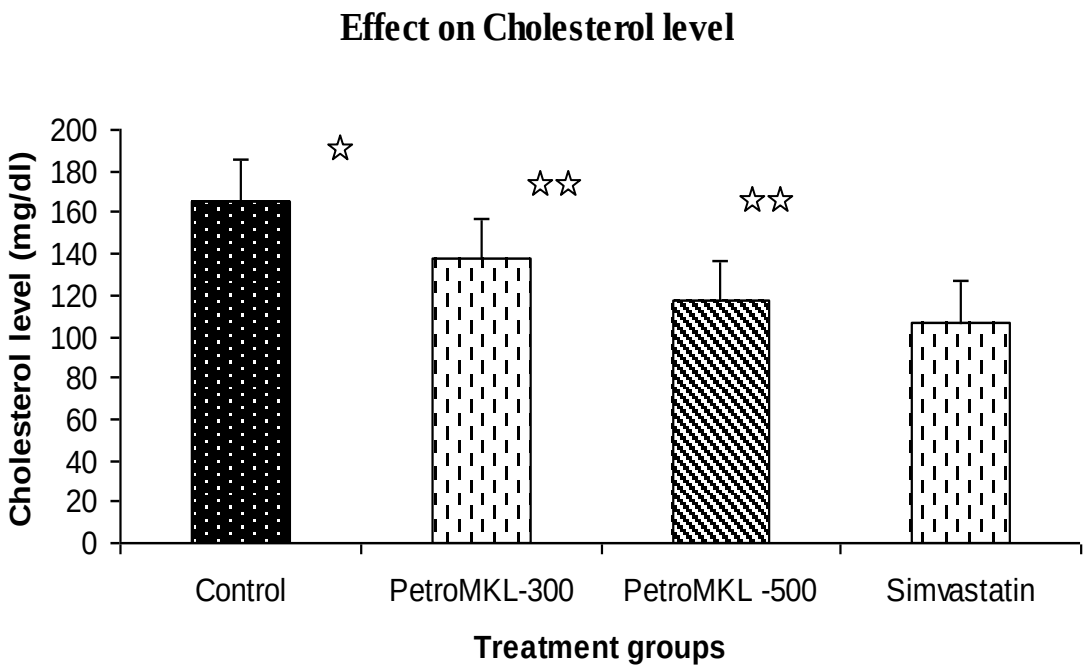

Figure -4: Effect of petroleum ether extracts of Murraya koenigii leaves (PetroMKL) on Cholesterol level in aged mice. Simvastatin (5 mg/kg, p.o.) was used as standard. Values are mean ± SD (n = 6). ☆ denotes significant ($p< 0.05$) reduction in cholesterol level compares to control in aged mice. ☆☆ denotes significant ($p< 0.01$) reduction in cholesterol level compares to control in aged mice. (Student's unpaired t-test)

Recently newer antichonesterase such as doneprezil which used as a standard in the present investigation, have been introduced in the European market for the symptomatic treatment of AD with fewer side effect [30]. In the present investigation the results of cholinesterase assay showed that 15 days treatments with petroleum ether (300 and 500mg/kg p.o.) extract of Murraya koenigii leaves remarkable reduce the brain cholinesterase activity which shown by of more unhydrolyzed Ach in extract of MKL compare with those of their control groups in aged mice. At 300 mg/kg the result was found to be significant at ($p < 0.05$) and at 500 mg/kg it was at ($p < 0.01$) respectively. While standard Doneprezil (0.5mg/kg p.o.) reduce more cholinesterase activity which represent by significant greater ($p < 0.001$) unhydrolyzed Ach compare to control groups in aged mice as shown in figure 3. The study indicated that high levels of cholesterol contribute to the pathogenesis of AD. Individuals with elevated levels of plasma cholesterol have an increased susceptibility to AD [5, 6, 7]. Therefore, a new therapeutic strategy aimed at reducing blood cholesterol levels is gathering momentum for the management of AD. In the light of this, the present study was also undertaken to investigate hypocholesteremic effect of MKL and the result of the study indicates that MKL decreases the cholesterol level dose dependently in aged mice. At 300 mg/kg the result was found to be significant at ($p < 0.05$) and at 500 mg/kg it was at ($p < 0.01$) respectively which were comparable to standard cholesterol lowering agent Simvastatin (5mg/kg p.o.) figure 4. The results of these studies are also supported with earlier findings of Murraya koenigii leaves for hypocholesteremic activity in various animal models [18, 31, 32].

Oxygen free-radicals are also implicated in the process of age-related decline in cognitive performance and may be responsible for the development of Alzheimer's disease in elderly persons [33, 34]. Oxygen-free radicals and other by-products of oxidative metabolism have been shown to be neurotoxic [35] and antioxidant rich diets improved cerebellar physiology and motor learning in aged rats [36]. Chemical examination of MKL indicates to content carbazole alkaloids major phytochemical constituents [11-13, 15, 16, 37] responsible for various biological activities like antioxidant, antimicrobial, antidiabetic, lipid lowering etc [14-19]. Thus there may be possibility of the same phytochemical constituents carbazole alkaloids which are reported previously[11-13,15,16,37] responsible for present antichonesterase and hypocholesteremic mechanism in antiamnesic effect of petroleum ether extract of Murraya koenigii leaves. The results of the present study also supported with recent finding of Vasudevan [32] shown to increase the memory of mice after 30 days consumption of various concentration MKL powder in diet.

In the present study, it was observed that MKL lowered serum cholesterol in mice, inhibited brain acetylcholinesterase enzyme and thereby elevated the acetylcholine concentration in brain homogenate and ultimately improved memory in aged mice. Thus, a combination of anticholinesterase and cholesterol lowering effect exhibited by MKL may be the factors responsible for this memory improving effect observed in the present study.

CONCLUSION

Thus from the results of present investigation, it conclude that petroleum ether extracts of MKL lowered serum cholesterol in aged mice and also inhibited brain acetylcholinesterase enzyme and thereby elevated the acetylcholine concentration in brain homogenate and ultimately improved memory in aged mice. Thus, a combination of anticholinesterase and cholesterol lowering effect exhibited by Murraya koenigii leaves may be the responsible mechanism for this memory improving effect observed in the present investigation.

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