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Research Article

REVERSAL EFFECT OF PHYLLANTHUS EMBLICA (EUPHORBIACEAE) RASAYANA ON MEMORY DEFICITS IN MICE

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ABSTRACT

The methanolic extract of fruit of *Phyllanthus emblica* Linn (Amla) was investigated for its reversal effect on memory deficits in mice. Two doses of the extract (75 and 150 mg/kg, i.p.) were administered for seven consecutive days. Scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) were used to induce memory deficits (amnesia). Elevated plus maze (EPM) and Morris water maze (MWM) were used to evaluate short and long term memory respectively. Scopolamine and sodium nitrite treatment produced significant impairment of elevated plus maze and Morris water maze performance indicating impairment of memory. The methanolic fruit extract significantly improved EPM and MWM performance of scopolamine and sodium nitrite treated mice. The results indicated potential of the plant in relieving memory deficits.

Keywords: Amnesia; Piracetam; Scopolamine; Amla; Sodium Nitrite, Dementia

INTRODUCTION

Memory is a process by which one encodes, stores and retrieves information. The ability to find objects, recall previous locations and navigate throughout the world is dependent upon spatial learning and memory[1]. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states such as Alzheimer's disease (A.D.). A.D. is characterized by the deposition of senile plaques mainly composed of β–amyloid (Aβ) fragment and neurofibrillary tangles [2, 3].

Senile dementia is a clinical syndrome affecting the elderly persons with loss of memory and cognition [4, 5]. The occurrence of dementia falls in the range of 10% for presenile and 38.6% for senile [6]. Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the brain because the brain is believed to be particularly vulnerable to oxidative stress due to a relatively high rate of oxygen free radical generation without commensurate levels of anti-oxidative defenses[7, 8], which may be responsible for the development of dementia or Alzheimer's disease in elder persons [9, 10]. Nootropic agents are used to improve memory, mood and behaviour [11, 12] but the side effects associated with these agents limit their use [13]. Despite intensive advancement in research, available therapeutic options are limited, thus increasing demand for new drugs. In the recent past, medicinal plants attracted attention due to their potential role in dementia [14, 15, 16].

The fruit of *Phyllanthus emblica* Linn of Euphorbiaceae family (Syn: *Emblica officinalis* Gaertn, Emblic myrobalan, Indian gooseberry, Amla) is widely used in Ayurvedic preparations (*Chyavanprash*, *Kanchnar* and *Triphala Guggulu)* for glandular swellings and weight reduction. It is used both as medicine and as a tonic to build up lost vitality and vigour. The fruit contains Ellagitannins (Chebulagic acid, Phyllanemblinin C, geraniin) and hydrolysable tannins. Tannins are antioxidants [17, 18] often characterized by reducing power [19] and free radical scavenging activities [20, 21]. The fruit possesses anti-tumor [22], immunomodulatory, expectorant, cardiotonic, anti-pyretic [23], anti-oxidative [24], anti-viral, anti-emetic, anthelmintic, anti-tussive [25] and gastroprotective properties[26]. It is used as hepatoprotective, aphrodisiac, anticytotoxic, antigenotoxic, anticlastogenic, anticarcinogenic and antimutagenic in viral hepatitis [27, 28], leucorrhoea, atherosclerosis[29], hyperacidity, low grade fevers [30] and prevent hepatotoxicity and nephrotoxicity produced by lead and aluminum [31, 32]. Therefore, the present study was designed to investigate the memory enhancing effect of *Phyllanthus emblica* Linn rasayana to prevent the scopolamine and sodium nitrite induced impairment of memory in mice using elevated plus-maze and Morris water maze as exteroceptive behavioural models.

MATERIAL AND METHODS

Animals: Swiss albino male mice (30±2 g) were procured from Indian Institute of Toxicology Research (IITR), Lucknow (Uttar Pradesh) India. They were housed in animal house provided with 12 hours light and dark cycles at 25±2ºC and had free access to water and standard laboratory diet (Ashirwad Industries, Chandigarh, India).

The experimental protocol was approved by the Institutional Animal Ethical Committee (no. 1279/ac/09/CPCSEA) and experiments were conducted according to the CPCSEA guidelines on the use and care of experimental animals. Experiments were carried out between 09:00 and 17:00 hours. Efforts were made to minimize animal suffering and optimum number of animals used.

Drugs: Piracetam, scopolamine and sodium nitrite were procured from M/S Sigma-Aldrich, Poole U. K., M/S Cadila Health Care (Ahmedabad) and Central Drug House (New Delhi) respectively. All other chemicals used in the study were purchased from M/S S. D. Fine Chemicals Ltd. (Boisar, India). All the drug solutions were prepared freshly before use.

Plant material: The fruit of *Phyllanthus emblica* Linn was collected, from near by areas of Meerut, in the month of July 2006. The fruits were authenticated by Dr. H. B. Singh, Head and Taxonomist, Raw Materials, Herbarium and Museum at National Institute of Science Communication and Information Resources (NISCAIR, CSIR) New Delhi-110067, India. A voucher specimen no: NISCAIR/RHMD/consult/06/721/38 is deposited for the plant in the same herbarium.

Extraction: Air dried fruits (20 gm) of the plant were coarsely powered and extracted with methanol by continuous hot percolation method using Soxhlet apparatus[33]. The per cent yield of the methanolic fruit extract was found to be 32.97. The extract was suspended in normal saline solution (0.9% w/v sodium chloride solution).

Exteroceptive Behavioural Models

a) Elevated plus maze apparatus

Elevated plus-maze serves as the exteroceptive behavioural model to evaluate acquisition and retention of memory in mice [34]. Transfer latency time (TLT) taken by the mouse to move from the open arm to the covered arm with all its four legs in elevated plus maze was noted. The mouse was allowed to explore the maze for another two minutes and then returned to its home cage. After 24 hours of acquisition trials, the TLT was again noted as an index of retrieval.

b) Morris water maze

Morris water maze was employed to evaluate learning and memory [35]. It consists of a circular water tank (diameter 150 cm and height 45 cm), filled with water maintained at 25°C. The water is made opaque with a white coloured dye. The tank is divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm^2) of 29 cm height is located in the centre of one of these four quadrants. The position of platform and clues were kept consistent throughout the training session. In the present study, target quadrant was Q4.

Acquisition trials: Each animal was subjected to four consecutive trials on each day with an interval of five minutes, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 seconds. In case of the inability of the animal to locate the hidden platform within 90 seconds, it was gently guided by hand to the platform and allowed to remain there for 20 seconds. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In preliminary study, trial was conducted to familiarize the mouse with the task and was not counted. Mouse was subjected to acquisition trials for four consecutive days. The starting position on each day to conduct four acquisition trials was changed as follows:

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Retrieval trial: On the next day, platform was removed and each mouse was allowed to explore the pool for 90 seconds. Mean time spent by the mouse in each of four quadrants was noted. The mean time spent by the mouse in target quadrant (Q4) for searching the hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory, serving as prominent visual clues was not disturbed during the total duration of study.

Interoceptive behavioural models: (a) Scopolamine induced amnesia (b) Sodium nitrite induced amnesia.

Scopolamine hydrochloride (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) were administered interaperitoneally to induce experimental amnesia in male albino mice.

Experimental protocol: The animals were divided into twenty six groups (shown in Table 1 and 2). Each group comprised of six animals. In group I (control), normal untreated mice were exposed to EPM for measuring TLT on first day and again after 24 hours i.e., on the second day. In group II, III, IV and V, mice were administered 0.9 % w/v sodium chloride solution i.e vehicle (10 ml/kg, i.p.), scopolamine hydrochloride (0.4 mg/kg, i.p.) sodium nitrite (75 mg/kg) and piracetam (400 mg/kg), In group VI and VII, mice were injected low and high doses of methanolic fruit extract (75 and 150 mg/kg, i.p., respectively) for seven days. In groups II to VII except groups VI and VII, TLT was recorded after 45 minutes and then after 24 hours (the second day) using elevated plus maze. In group VI and VII, TLT was recorded after 60 minutes and then after 24 hours (the second day) using elevated plus maze. In groups VIII, IX, X, XI, XII and XIII, mice were administered piracetam (400 mg/kg) and scopolamine hydrochloride (0.4 mg/kg, i.p.), piracetam (400 mg/kg,) and **s**odium nitrite (75 mg/kg, i.p.), methanolic fruit extract (75 and 150 mg/kg) and scopolamine, methanolic fruit extract (75 and 150 mg/kg) and **s**odium nitrite (75 mg/kg, i.p.) 60 and 45 min respectively, before first day exposure on elevated plus maze. TLT was recorded on the first day and on the second day.

Group XIV (control) of normal untreated mice was subjected to MWM for measuring ELT (from day 1 to day 4) and time spent in target quadrant (TSTQ) on the fifth day. In group XV, XVI, XVII and XVIII, mice were administered normal saline solution (10 ml/kg, i.p.), scopolamine hydrochloride (0.4 mg/kg, i.p.) sodium nitrite (75 mg/kg) and piracetam (400 mg/kg, i.p.) respectively 45 minutes before acquisition trial conducted on four consecutive days (from day 1 to day 4). In case of group XIX and XX, low and high doses of methanolic fruit extract (75 and 150 mg/kg, i.p., respectively) were administered 60 minutes for seven days before acquisition trials conducted on four consecutive days (from day 1 to day 4). In groups XXI, XXII, XXIII, XXIV, XXV and XXVI mice were administered piracetam (400 mg /kg, i.p.) and scopolamine hydrochloride (0.4 mg/kg, i.p.), piracetam (400 mg /kg, i.p.) and **s**odium nitrite (75 mg/kg, i.p.), methanolic fruit extract (75 and 150 mg/kg, i.p.) and scopolamine, methanolic fruit extract (75 and 150 mg/kg, i.p.) and **s**odium nitrite (75 mg/kg, i.p.) respectively, before acquisition trials conducted on four consecutive days (from day 1 to day 4). In groups XXI to XXII, piracetam and in groups XXIII to XVI, methanolic fruit extract (M. Pe) were administered 60 minutes before the administration of amnestic drug. In all the above mentioned groups, 0.9 % w/v sodium chloride solution (10 ml/kg, i.p.) was administered 45 minutes before retrieval trial conducted on the fifth day.

Rota-rod test: Each mouse was used only once and total of six mice were used for each treatment. Motor co-ordination was considered to be impaired if the animal fell-off from the rotating-rod within nine secounds. In control or drug treated groups, the assessment of motor coordination was made before and after administration of vehicle or drugs during acquisition and retrieval trials.

Statistical analysis: Results are expressed as means $\pm$ standard error of the mean (SEM). The data was analyzed with Graph Pad Prism statistical analysis using two-way analysis of variance followed by Bonferroni post-hoc test except in retrieval trial of Morris water maze for which the data was analysed by one way analysis of variance followed by Tukey's test. The p value of less than 0.05 was considered to be statistically significant.

RESULTS

Effect of scopolamine and sodium nitrite on TLT of mice using elevated plus-maze

Transfer latency time (TLT) of first day reflected learning behavior of animals whereas, TLT of next day reflected retention of learning behaviour. TLT of control group animals decreased significantly on the second day i.e. after 24 hours of training on elevated plus-maze. Scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) administered in respective groups III and IV before elevated plus maze exposure on the first day, significantly increased ($p<0.05$) the first and the second day TLT, when compared to the respective first and second day TLT in control group (Table 1).

Effect of Piracetam and methanolic fruit extract (M. Pe) on scopolamine and sodium nitrite induced amnesia using elevated plus-maze

Pretreatment for seven days with piracetam (group V) *per se* at the dose of 400 mg/kg, i.p. did not produce any significant effect on the second day transfer latency time of mice as compared to transfer latency time of control group on the first day. Pretreatment for seven days with M. Pe. (group VI and VII) *per se* at the doses of 75 and 150 mg/kg, i.p. significantly ($p<0.05$) decreased the transfer latency time in mice as compared to transfer latency time of control group on the second day (Table 1). The extract *per se* at the dose of 150 mg/kg, i.p. exhibited improvement in normal short-term memory by 58% (Fig. 1). Pretreatment for seven days with piracetam (400 mg/kg, i.p.), standard drug and M. Pe. (150 mg/kg, i.p.) has significant increase in per cent retention by 61% and 54%; 83% and 74% respectively in scopolamine and sodium nitrite treated mice with respective control indicating the reversal of scopolamine and sodium nitrite induced amnesia.

Effect of Piracetam and M. Pe. on ELT and TSTQ during retrieval trial of memory using Morris water maze In control group, the normal untreated mice demonstrated significant decrease in the ELT as compared to the first day during the acquisition trials (Table 2). Moreover, these mice spent significantly more time in target quadrant (Q4) in search of the missing platform as compared to the time spent in other quadrants (Q1, Q2 and Q3) during the retrieval trial conducted on the fifth day (Fig. 1). Control group shows normal retrieval of memory. Piracetam (400 mg/kg, i.p.), M. Pe. (150 mg/kg, i.p.) administered 60 minutes and saline solution (10 ml/kg, i.p.), vehicle used for plant drug (M. Pe) administered 45 min before the

acquisition trials did not produce any significant *per se* effect on decrease in ELT during the acquisition trials conducted from day 1 to day 4 (Table 2) and an increase in the time spent in Q4 target quadrant during the retrieval trial conducted on the fifth day noted in control group (Fig. 1). The above mentioned treatments did not alter the normal retrieval of memory.

Effect of scopolamine and sodium nitrite on acquisition and retrieval of memory using Morris water maze

Scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) significantly attenuated the decrease in the ELT during the successive acquisition trials conducted from day 1 to day 4 (Table 2) and also reduced the increase in the time spent in target quadrant (Q4) markedly in search of missing platform during the retrieval trial conducted on the fifth day (Fig. 1). Scopolamine and sodium nitrite both produced amnesia.

Effect of Piracetam and M. Pe. on scopolamine and sodium nitrite induced amnesia using Morris water maze

Pretreatment of mice for seven days with piracetam (400 mg/kg) and M. Pe. (150 mg/kg, i.p.) 60 min before training significantly decreased the ELT $\left[F_{Scopolamine}\ (3,40) = 169.1;\ F_{Sodium\ nitrite}\ (3,40) = 39.60\right]$ during the acquisition trials conducted on four consecutive days (day 1 to day 4) in scopolamine and sodium nitrite treated mice (Table 2). Like piracetam, the extract significantly increased the time spent in target quadrant (Q4) $\left[F_{Scopolamine}(3,20) = 3.83;\ F_{sodium\ nitrite}(3,20) = 10.87\right]$ by the mice treated with scopolamine and sodium nitrite during the retrieval trial on the fifth day (Fig. 1).

DISCUSSION

A significant decrease in the transfer latency time (TLT) of mice noted on the second day as compared to their transfer latency time on the first day indicated normal memory in elevated plus maze test. Similarly, a marked decrease in escape latency time (ELT), during subsequent trials as compared to first exposure on MWM, denotes normal learning ability whereas an enhancement in the time spent by the animal in the target quadrant (in search of the missing platform) reflects successful retention of learned task (or memory).

The Morris water maze-test[36] and elevated plus-maze test [37] have been used extensively to investigate learning and memory in rodents. Since, both of these different memory models produced uniform results on memory scores, the built-in limitation if any, present in an individual experiment model was automatically taken care of. Pilot studies indicated that a single dose administration of M. Pe. had no acute behavioural effects, hence it was administered interaperitoneally at two dose levels (75 and 150 mg/kg, i.p.).

Scopolamine and sodium nitrite treatments produced a significant learning and memory deficits as indicated by a decrease in EPM and MWM performance. These findings are in line with earlier reports [38, 39, 40]. Central cholinergic system plays a crucial role in the process of learning and memory. Cholinomimetic drugs have been shown to enhance memory, whereas centrally acting, muscarinic cholinergic receptor blockers like scopolamine [41, 42] are reported to impair memory and therefore have been widely used as animal models to study the anti-amnestic potential of new drugs on animals [43, 44]. The fruit of *Phyllanthus emblica* Linn is not only useful as anti-oxidant[45, 46] and anti-inflammatory [47], but has adaptogenic activity[48]. It is used clinically for degeneration caused by Alzheimer's disease[49, 50]. Sodium nitrite has been reported to induce severe vasodilatation[51] and methemoglobinemia[52] which may be responsible to produce cerebral hypoxia[53] that initiates the generation of free radicals and may damage hippocampus. Hypoxia is noted to release adenosine[54] which consequent leads to inhibition of synaptic transmission[55, 56]. Hippocampus formation is rich in adenosine A_1 receptors [57]. Transient hypoxia or ischemia induced release of adenosine [58, 59] and consequent activation of A_1 receptors and opening of K^+ channels[60] may contribute to sodium nitrite induced amnesia. The enriched low molecular weight hydrolysable tannins, namely emblicanin A and emblicanin B along with punigluconin and pedunculagin (both medium molecular weight gallo-ellagi tannins), and rutin (a flavanol glycoside), present in berries, constitute 'CAPROS' anti-oxidative mixture having oxygen radical captodative properties which are at least three times higher than vitamin C[61].

The antioxidant capabilities of tannins depend on the extent of their colloidal state, the ease of interflavonoid bond cleavage or its stereochemical structure, the ease of pyran ring (C-ring) opening, and the relative numbers of - OH groups on A and B rings[62]. Compounds with a trihydroxyl structure in the B-ring display the greatest antioxidant activity[63, 64]. The strong antioxidant activity of rasayana was found to be 1000 times more potent than ascorbic acid, α-tocopherol, and probucol[65]. The antioxidant effect of *Phyllanthus emblica* due to ascorbic acid may be responsible to prevent the impairment of memory[66]. The memory enhancing activity of the ripe fruit extract may be attributed to its antioxidant action and presence of polyphenolic compounds and ascorbic acid leading to rejuvenation of nervous system. The administration of vehicle did not produce any modification in the EPM and MWM performance of control animals.

The treatment of piracetam and M. Pe. significantly and dose-dependently attenuated memory deficits induced by scopolamine and sodium nitrite, as reflected by improvement in the EPM and MWM performance.

Table 1: Effect of *Phyllanthus emblica* (M. Pe) on scopolamine and sodium nitrite induced changes in transfer latency time (TLT) of mice using elevated plus-maze

Groups No.	Treatments	Dose (kg^{-1})	1st Day [TLT(s)on last day of treatment]	2nd Day [TLT (s) after 24 hr]
I	Control	10 ml	30.4 ± 1.4	15.5 ± 2.4 [a]
II	Vehicle control (Saline)	10 ml	31.3 ± 1.3	17.5 ± 1.6
III	Scopolamine	0.4 mg	44.2 ± 4.2 [a]	26.4 ± 1.4 [b]
IV	Sodium nitrite	75 mg	40.4 ± 5.2 [a]	28.6 ± 2.4 [b]
V	Piracetam	400 mg	9.2 ± 0.5 [c]	7.4 ± 1.0 [b]
VI	M. Pe . (L) - Per Se	75 mg	25.4 ± 2.4	7.3 ± 1.1 [b]
VII	M. Pe . (H) - Per Se	150 mg	15.4 ±1.1 [c]	6.5 ± 1.1 [b]
VIII	Piracetam+ Scopolamine	400 mg + 0.4 mg	10.6 ± 1.7 [d]	10.4 ± 0.5 [e]
IX	Piracetam + Sodium nitrite	400 mg + 75 mg	13.8 ± 1.6 [f]	13.2 ± 1.2 [g]
X	M. Pe . (L) +Scopolamine	75 mg + 0.4 mg	37.4 ± 4.2	7.7 ± 2.4 [e]
XI	M. Pe . (H) +Scopolamine	150 mg + 0.4 mg	9.4 ± 1.6 [d]	4.5 ± 1.1 [e]
XII	M. Pe . (L) +Sodium nitrite	75 mg +75 mg	27.6 ± 1.4 [f]	9.7 ± 2.3 [g]
XIII	M. Pe . (H) +Sodium nitrite	150 mg +75 mg	16.7 ± 2.4 [f]	7.3 ± 1.6 [g]

a = $p<0.05$ Vs day 1 TLT in control; b = $p<0.05$ Vs 2nd day TLT in control; c = $p<0.05$ Vs day1TLT in control; d = $p<0.05$ Vs day 1 TLT in scopolamine; e = $p<0.05$ Vs day 2 TLT in scopolamine; f = $p<0.05$ Vs day 1 TLT in sodium nitrite; g = $p<0.05$ Vs day 2 TLT in sodium nitrite.

Table. 1 The effect of administration of methanolic fruit extract of *phyllanthus emblica* (M. Pe.) on transfer latency time (TLT) of mice is exhibited. The TLT for control, vehicle (saline solution), standard drug [Piracetam], amnestic agents (scopolamine and sodium nitrite) and plant drug (M. Pe.) are shown. Note that while the amnestic agents have a significantly increasing effect on TLT, the standard and plant drugs have a reverse effect. Values are expressed as mean± S.E.M (n=6).

Table 2: Effect of Piracetam and MPe on scopolamine and sodium nitrite induced changes in escape latency time (ELT) during acquisition trials using Morris water maze

Groups No.	Treatment	Dose (kg^{-1})	ELT (s) on Acquisition days	
			Day 1	Day 4
XIV	Control	10 ml	85.75 ± 2.5	51.5 ± 7.2 [a]
XV	Vehicle control (Saline)	10 ml	87.54 ± 0.89	51.03 ± 4.3
XVI	Scopolamine	0.4 mg	89.13± 0.47	85.15 ± 1.77 [b]
XVII	Sodium nitrite	75 mg	88.60 ± 0.60	77.3 ± 2.2 [b]
XVIII	Piracetam - *per se*	400 mg	82.75 ± 3.4	50.3 ± 6.3
XIX	M. Pe. (L) - *per se*	75 mg	83.88 ± 3.5	56.2 ± 8.3 [b]
XX	M. Pe . (H) - *per se*	150 mg	81.25 ± 1.6	50.9 ± 2.4
XXI	Piracetam + Scopolamine	400 mg + 0.4 mg	89.42 ± 0.17	69.71 ± 3.5 [c]
XXII	Piracetam + Sodium nitrite	400 mg + 75 mg	89.00 ± 0.8	51.0 ± 9.1 [d]
XXIIII	M. Pe . (L) +Scopolamine	75 mg + 0.4 mg	86.1 ± 1.5	68.4 ± 4.3 [c]
XXIV	M. Pe . (H) +Scopolamine	150 mg + 0.4 mg	82.7 ± 3.0	56.18 ± 9.6 [c]
XXV	M. Pe . (L) +Sodium nitrite	75 mg +75 mg	87.08 ± 0.86	64.48 ± 5.2 [d]
XXVI	M. Pe . (H) +Sodium nitrite	150 mg + 75 mg	80.55 ± 6.6	55.7 ± 9.9 [d]

a = p<0.05 Vs day 1 ELT in control; b = p<0.05 Vs day 4 ELT in control; c = p<0.05 Vs day 4 ELT in scopolamine; d= p<0.05 Vs day 4 ELT in sodium nitrite.

Table 2 The effect of administration of methanolic fruit extract of *Phyllanthus emblica* (M. Pe.) on escape latency time (ELT) of mice during acquisition days is exhibited. The ELT of day 1 and day 4 for control, vehicle (saline solution), standard drug (Piracetam), amnestic agents (scopolamine and sodium nitrite) and plant drug (M. Pe.) are shown. Note that while the amnestic agents have a significantly increasing effect on ELT, the standard and plant drug have a reversing effect.

Control: I, II, XIV and XV; Negative control: III, IV, XVI and XVII; Protective: V and XVIII; Curative: VI, VII, XIX and XX groups.

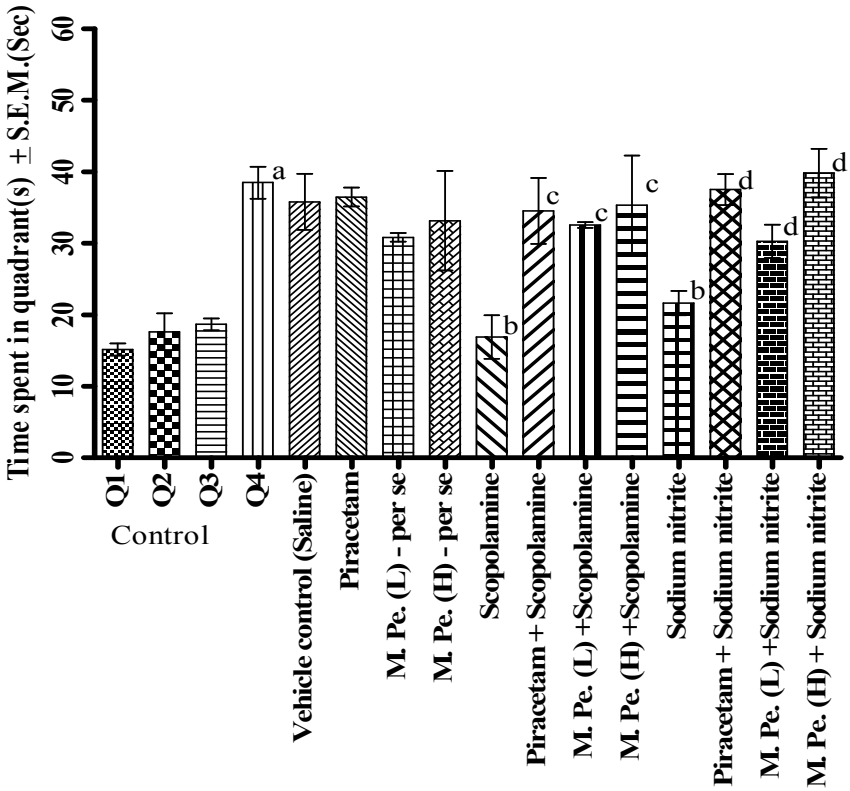

Fig. 1: Effect of Piracetam and MPe on time spent in quadrant (s) during retrieval trial using Morris water maze

a = p<0.05 Vs time spent in other quadrants in control; b = p<0.05 Vs time spent in target quadrant (TSTQ) in control; c = p<0.05 Vs time spent in target quadrant (TSTQ) in scopolamine; d p<0.05 Vs time spent in target quadrant (TSTQ) in sodium nitrite.

Fig. 1 The effect of the administration of methanolic fruit extract of *Phyllanthus emblica* (M. Pe.) on time spent by mice in quadrant(s) during retrieval trial is exhibited. Histograms of the time spent in quadrant (s) for control, vehicle (saline solution), standard drug (Piracetam), amnestic agents (scopolamine and sodium nitrite) and plant drug (M. Pe.) are shown. Note that while the amnestic agents have a significantly decreasing effect on time spent in target quadrant (Q4), the standard and plant drug have a reversing effect. Each value represents mean time spent in quadrant (s) ± S.E.M.

CONCLUSION

The methanolic fruits extract of *Phyllanthus emblica* Linn. (Euphorbiaceae) was investigated for its reversal effect on memory deficits in mice. Two doses of the extract (75 and 150 mg/kg, i.p.) were administered for seven consecutive days. Scopolamine (0.4 mg/kg, i.p.) and sodium nitrite (75 mg/kg, i.p.) were used to induce memory deficits (amnesia). Elevated plus maze (EPM) and Morris water maze (MWM) were used to evaluate short and long term memory respectively. The administration of methanolic fruit extract of the plant significantly improved learning and memory, prevented scopolamine and sodium nitrite induced experimental amnesia and may be a great potential in memory deficits. Moreover, it can be used in the treatment of dementia associated with numerous psychiatric and neurodegenerative states. However, further studies are required to elucidate the mechanisms of action.s

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