



## Effect of Piracetam on Experimental Anterograde and Retrograde Amnesia

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### Abstract

Piracetam is a cyclic derivative of  $\gamma$ -aminobutyric acid (GABA) reported to improve learning and memory consolidation, facilitates inter-hemispheric transfer and increases the cerebral resistance to cognitive impairments induced by hypoxia and other noxious stimuli including ageing. Therefore, the present study was designed to investigate the effect of piracetam on scopolamine (3 mg Kg<sup>-1</sup>, i.p.), cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.), diazepam (1 mg Kg<sup>-1</sup>, i.p.), sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) and BN52021 (15 mg Kg<sup>-1</sup>, i.p.) were administered to mice before acquisition or retrieval trial using water maze test. Scopolamine, cyclohexamide and diazepam have produced only anterograde amnesia. Sodium nitrite has produced both anterograde and retrograde amnesia. BN52021 (PAF receptor antagonist) has produced only retrograde amnesia. The present results exhibit that the anterograde amnesia produced by scopolamine, cyclohexamide and sodium nitrite has been prevented by piracetam (200 mg Kg<sup>-1</sup>, i.p.). On the other hand diazepam induced anterograde amnesia has not been affected by piracetam. However, retrograde amnesia produced by sodium nitrite and BN52021 has been attenuated by piracetam (200 mg Kg<sup>-1</sup>, i.p.). It is speculated that ameliorative effect of piracetam in experimental amnesia may be due to increase in intracellular calcium, restoration of synthesis of cerebral proteins, improvement in cholinergic mechanism and consequent restoration of brain cell fluidity.

**Key words:** Scopolamine, Cyclohexamide, Diazepam, Sodium Nitrite, BN52021

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### Introduction

Anterograde amnesia is described as disruption of memory and storage of facts or events, without affecting intelligence, attention, perception or judgement [1]. Retrograde amnesia is failure of old memories before unconsciousness and it is probably related to the disruption of information stored for short term before consolidating it into long term memory [2]. Piracetam, is a cyclic derivative of  $\gamma$ -aminobutyric acid (GABA) but its mechanism of action is not related in any way to the physiological

properties of GABA [3]. Except sodium nitrite and diazepam, scopolamine and cyclohexamide administered before acquisition trial have not modulated the decrease in escape latency time (ELT) noted with ongoing acquisition trials but these four drugs have significantly attenuated the increase in time spent (TS) in target quadrant (TQ) in search of missing platform during retrieval trial. It suggests that scopolamine and cyclohexamide produced impairment only in retrieval component, whereas sodium nitrite and diazepam produced impairment in both components of trials acquisition and retrieval leading to anterograde amnesia. Scopolamine induced impairment in retrieval component is further supported by the earlier observations with scopolamine [4-5]. Similarly, scopolamine induced impairment of recall of series of nine digits [6] is in agreement with the results of present study. Scopolamine is a centrally active, muscarinic cholinergic receptor blocker [7-8]. Scopolamine induced anterograde amnesia is suggested to be due to blockade of cholinergic receptors and consequent decrease in cell membrane fluidity of brain [9]. Synthesis of cerebral proteins [10] such as cyclic AMP response element binding protein (CREB), CRB binding protein i.e. CBP [11] and Ras neuronal specific guanine nucleotide exchange factor i.e. Ras-GEF [12] have been implicated in storage of information and consolidation of memory. Rho-GTPase has been suggested to participate in development of dendrites and spines [13]. Cyclohexamide induced anterograde amnesia is suggested to be due to inhibition of cerebral proteins involved in cognition.

Diazepam is a benzodiazepine agonist [14] and it binds with postsynaptic benzodiazepine-receptor (BZD-R) and allosterically enhances the binding of GABA to GABA-BZD receptor complex [15], which produces postsynaptic hyper-polarisation due to influx of Cl<sup>-</sup> ions [16]. It is worthwhile to note that impairment of acquisition by diazepam in the present study has consequently affected the retrieval of memory. It suggests no retrieval is possible without a positive acquisition. Kumar *et al.*, [17] also reported the same results with diazepam. In the present study, sodium nitrite (NaNO<sub>2</sub>) administered before acquisition trial has significantly impaired the acquisition component to produce anterograde amnesia. Similar results have been reported in our earlier study [18]. NaNO<sub>2</sub> induced severe vasodilatation [19] and methemoglobinemia [20] be responsible to produce cerebral hypoxia [21]. Hypoxia is noted to release adenosine [22] and consequent inhibition of synaptic transmission [23]. Hippocampal formation is rich in adenosine A<sub>1</sub> receptors [24]. Transient hypoxia or ischemia induced release of adenosine [25] and consequent activation of A<sub>1</sub> receptors and opening of K<sup>+</sup> channels [26] may contribute to NaNO<sub>2</sub> induced anterograde amnesia. Administration of NaNO<sub>2</sub> and BN52021, a PAF receptor antagonist before retrieval trial have significantly attenuated increase in preferential stay in TQ quadrant in search of missing platform. It suggests that NaNO<sub>2</sub> and BN52021 produce retrograde amnesia. NaNO<sub>2</sub> induced retrograde amnesia and no such effect is noted on NaNO<sub>2</sub> induced anterograde amnesia [18, 27]. No per-se effect of dimethyl sulphoxide (DMSO) used as vehicle for BN52021 has been noted on normal acquisition and retrieval of memory. Therefore, the observed effect of BN52021 on retrieval of memory is not due to its vehicle. BN52021 has produced retrograde amnesia perhaps by blocking the presynaptic PAF receptors and subsequently decreasing the release of glutamate from presynaptic sites. It suggests that NaNO<sub>2</sub> and BN52021 induced retrograde amnesia appears to be presynaptic phenomenon. Piracetam improves learning and memory consolidation [28], facilitates inter-hemispheric transfer [29] and increases the cerebral resistance to cognitive impairments induced by hypoxia and other noxious stimuli including ageing [3]. Apart from a weak interaction with L-glutamate binding sites [30], piracetam does not bind to any known receptor system or affect specifically any enzyme or transporter system [3]. The precise mechanism of piracetam as a nootropic agent is not yet settled. Therefore, the present study has been designed to investigate the effect of piracetam in experimentally induced anterograde and retrograde amnesia with an objective to understand the mechanism of piracetam to improve learning and memory.

## Materials and Methods

Swiss albino mice (30±2 g) of either sex procured from Indian Veterinary Research Institute (IVRI) Izatnagar, Bareilly-243022 (Uttar Pradesh) India, were housed in animal house provided with 12 hours light and dark cycle, free access to water and standard laboratory diet (Kisan feed Ltd. Mumbai). All the animals were naive to water maze. The experiments were conducted between 10.00 to 17.30 hrs in a semi-sound proof laboratory. The research was conducted as per the guidelines of “committee for the Purpose of Control and Supervision of Experiments on Animals” (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, New Delhi.

### *Water maze test*

#### *Acquisition trials*

Morris water maze [31] was employed to evaluate learning and memory. It consisted of a circular water tank (diameter 150 cm and height 45 cm), filled with water maintained at 25°C. The water was made opaque with a white colored dye. The tank was 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<sup>2</sup>) of 29 cm height was located in the center of one of these four quadrants. The position of platform and clues were kept consistent throughout the training session. In the present study, the TQ was Q 4. Each animal was subjected to four consecutive trials on each day with an interval of 5 min, during which mouse was allowed to escape on the hidden platform and was allowed to remain there for 20 sec. In case the animal was unable to locate the hidden platform within 120 sec, it was gently guided by hand to the platform and allowed to remain there for 20 sec. ELT to locate the hidden platform in water maze was noted as an index of acquisition and learning. In preliminary study, trial was administered to familiarize the mice with the task and was not counted. Mouse was subjected to acquisition trial for four consecutive days. 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 trials*

On day 5, platform was removed and TS by animal in each of four quadrants was noted. The TS by animal in TQ (Q 4) searching for the hidden platform was noted as an index of retrieval. For evaluation of anterograde amnesia, amnesic treatments were administered in mice 30 min before acquisition trial conducted on four consecutive days (day 1 to day4) and ELT was noted as an index of acquisition and learning. Distilled water was administered 30 min before retrieval trial conducted on day 5 increased TS by animal in TQ (Q4) searching for hidden platform was noted as index of retrieval. Whereas for evaluation of retrograde amnesia, mice were administered vehicle used for drugs like distilled water or DMSO, 30 min before acquisition trial conducted on day 1 to day 4 and index of acquisition was noted. On day 5 mice were administered drugs before retrieval trial and index of retrieval was noted.

#### *Rota-rod test*

The ability of mouse to hold on to horizontally rotating rod (diameter 2.5 cm, 4 rpm) was used to assess motor co-ordination. Each mouse was used only once and total of 5 mice were used for each treatment. Motor co-ordination was considered to be impaired if animal fell-off from the rotating-rod within 90 sec. In control or drug treated groups, assessment of motor coordination is made before and after administration of vehicle or drugs during acquisition and retrieval trial. There is no difference between

treatment groups with regard to exclusion because animals that demonstrated impairment of muscle co-ordination (ataxia) with or without drug treatment were not included in study. 15-20 % exclusion rate was noted.

### ***Experimental protocol***

Twenty groups and each group comprised of five mice were employed in the present study. In group I, distilled water (10 ml Kg<sup>-1</sup>, i.p.) was administered 30 min before acquisition trial conducted on four consecutive days (day1 to day 4) and 30 min before retrieval trial conducted on day 5. In groups II, III, V, VI and VII, mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.), scopolamine (3 mg Kg<sup>-1</sup>, i.p.), diazepam (1 mg Kg<sup>-1</sup>, i.p.), sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) and BN52021 (15 mg Kg<sup>-1</sup>, i.p.) respectively 30 min before acquisition trial conducted on four consecutive days (day 1 to day 4). In case of group IV, cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) was administered 60 min before acquisition trial conducted on four consecutive days (day 1 to day 4). In groups VIII, IX, X and XI, mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+scopolamine (3 mg Kg<sup>-1</sup>, i.p.), piracetam (200 mg Kg<sup>-1</sup>, i.p.)+cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.), piracetam (200 mg Kg<sup>-1</sup>, i.p.)+diazepam (1 mg Kg<sup>-1</sup>, i.p.) and piracetam (200 mg Kg<sup>-1</sup> i.p.)+sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) respectively before acquisition trial conducted on four consecutive days (day 1 to day 4). In groups VIII to XI except group IX, piracetam was administered 30 min before the administration of amnesic drug. In group IX, piracetam (200 mg Kg<sup>-1</sup>, i.p.) was concomitantly administered with cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) 60 min before acquisition trials. In all the above mentioned ten groups except group VII, distilled water (10 ml Kg<sup>-1</sup>, i.p.) was administered 30 min before retrieval trial conducted on day 5. In group VII, mice were administered vehicle for BN52021 i.e.0.5M DMSO (10 ml Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial conducted on day 5.

In groups XII, XIII, XIV, XV, XVI, XVII and XVIII, mice were administered 0.5M DMSO (10 ml Kg<sup>-1</sup>, i.p.), piracetam (200 mg Kg<sup>-1</sup>, i.p.), scopolamine (3 mg Kg<sup>-1</sup>, i.p.), cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) diazepam (1mg Kg<sup>-1</sup>, i.p.), NaNO<sub>2</sub> (75 mg Kg<sup>-1</sup>, i.p.) and BN52021 (15 mg Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial conducted on day 5. In groups XIX and XX, mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+NaNO<sub>2</sub> (75 mg Kg<sup>-1</sup>, i.p.), and piracetam (200 mg Kg<sup>-1</sup>, i.p.)+BN52021 (15 mg Kg<sup>-1</sup>, i.p.) respectively before retrieval trial conducted on day 5. In these two groups (group XIX and XX) piracetam and amnesic drug were administered 60 min. and 30 min. respectively before retrieval trial. In all the above groups (except group XVIII), mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before acquisition trial conducted on day 1 to day 4. In-group XVIII, mice were administered 0.5M DMSO (10 ml Kg<sup>-1</sup>, i.p.) 30 min before acquisition trial conducted on four consecutive days (day 1 to day 4).

### ***Statistical analysis***

All results were expressed as mean±SEM and data was analyzed using one-way ANOVA followed by post-hoc Duncan's New Multiple Range test. p<0.05 was considered to be statistically significant.

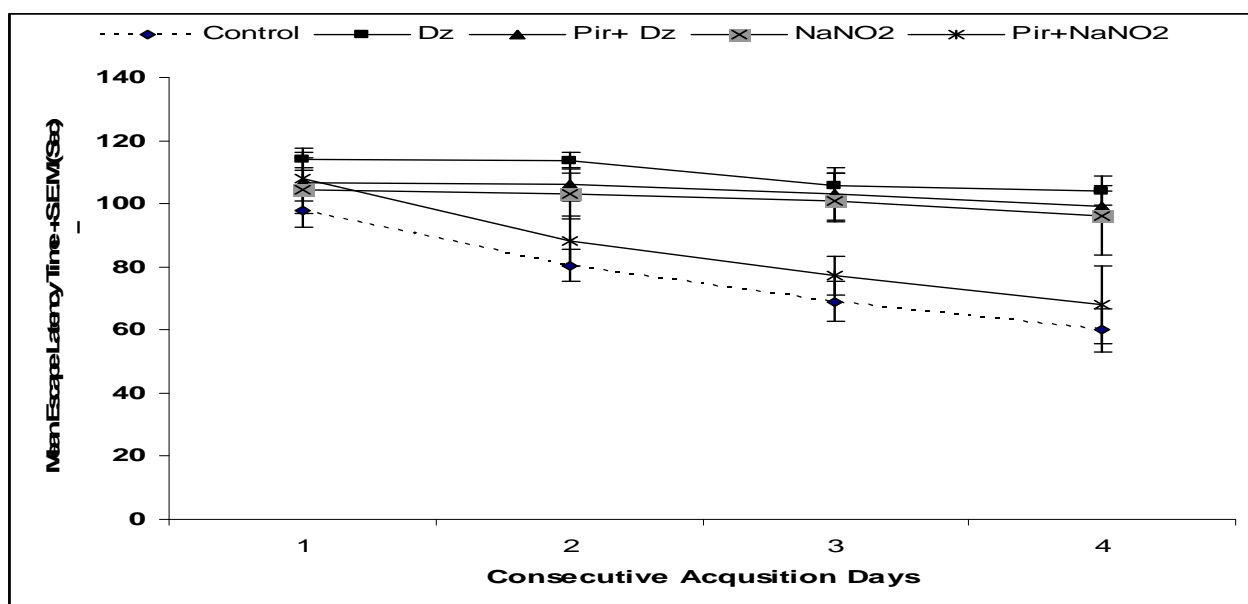
### ***Drugs and chemicals***

Piracetam (UCB India Ltd., VAPI, India), scopolamine (German Remedies Ltd., Mumbai, India), cyclohexamide (HI-MEDIA Laboratory, Pvt Ltd. Mumbai, India), diazepam (Ranbaxy Laboratory Ltd., New-Delhi, India), sodium nitrite (SD Fine Chem. Ltd., Boisar, India) 0.5M dimethylsulphoxide (Poicha, Taluka Salvi, Baroda, India) and BN52021 (Dr. P. Branquet, Institute Henri Beaufour, France) were used in present study. All the drug solutions were freshly prepared before use.

## Results

### *Effect of piracetam on ELT during acquisition trial and TS in TQ (Q 4) during retrieval trial*

In control group, mice administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before acquisition trial demonstrated significant decrease in ELT as compared to its value noted on day 1 with successive acquisition trial conducted on day 2, 3 and 4 (Fig. 1). Moreover, mice administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial conducted on day 5 spent significantly more time in TQ (Q 4) in search of missing platform as compared to TS in other quadrants (Q1, Q 2 and Q 3) during retrieval trial (Fig. 2). Piracetam (200 mg Kg<sup>-1</sup>, i.p.) administered 30 min before acquisition trial produced no marked effect on decrease in ELT and increase in TS in TQ (Q 4) noted in control group during acquisition and retrieval trial conducted on day 1 to day 4 and day 5 respectively (Fig. 1 and 2).



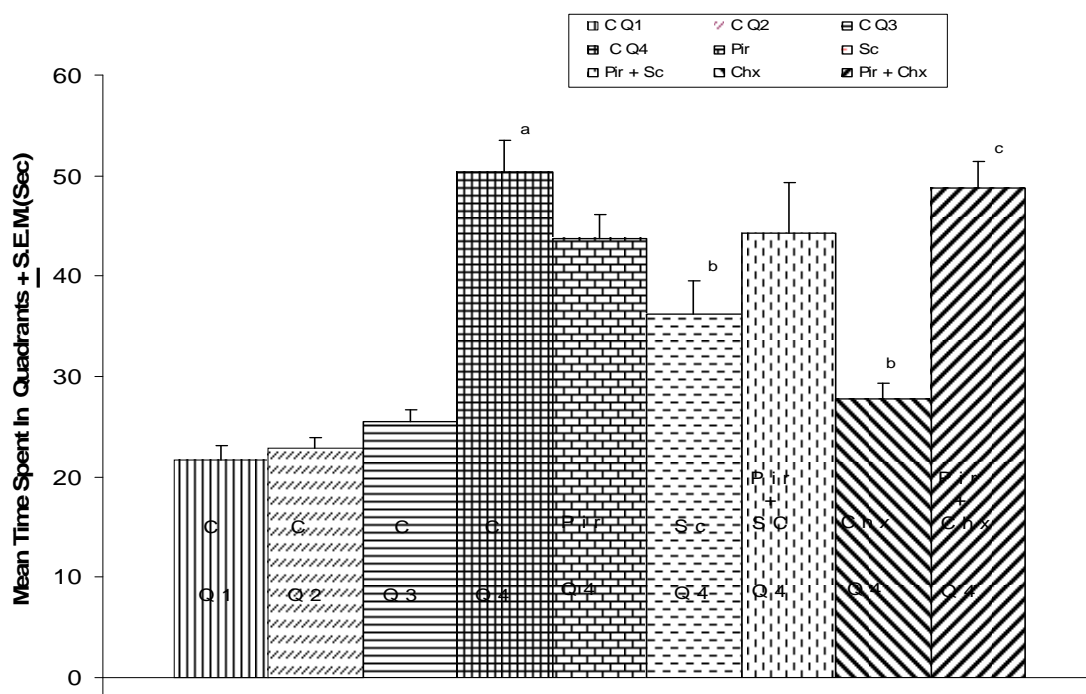
**Fig. 1 Effect of piracetam on diazepam and sodium nitrite induced anterograde amnesia (acquisition component)**

Control, Dz and NaNO<sub>2</sub> represents that mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.), diazepam (1 mg Kg<sup>-1</sup>, i.p.) and sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) respectively, 30 min before acquisition trial (day 1 to day 4). Pir + Dz and Pir + NaNO<sub>2</sub> represents that mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+diazepam (1 mg Kg<sup>-1</sup>, i.p.) and piracetam (200 mg Kg<sup>-1</sup>, i.p.)+sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.), 60 and 30 min respectively before acquisition trial (day 1 to day 4). In all above mentioned groups, mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial (day 5). Each value represents mean±S.E.M (n=5). a=p<0.05 Vs ELT on day 1. b=p<0.05 Vs ELT of control group for same day.

### *Effect of piracetam on scopolamine, cyclohexamide, diazepam and sodium nitrite induced anterograde amnesia*

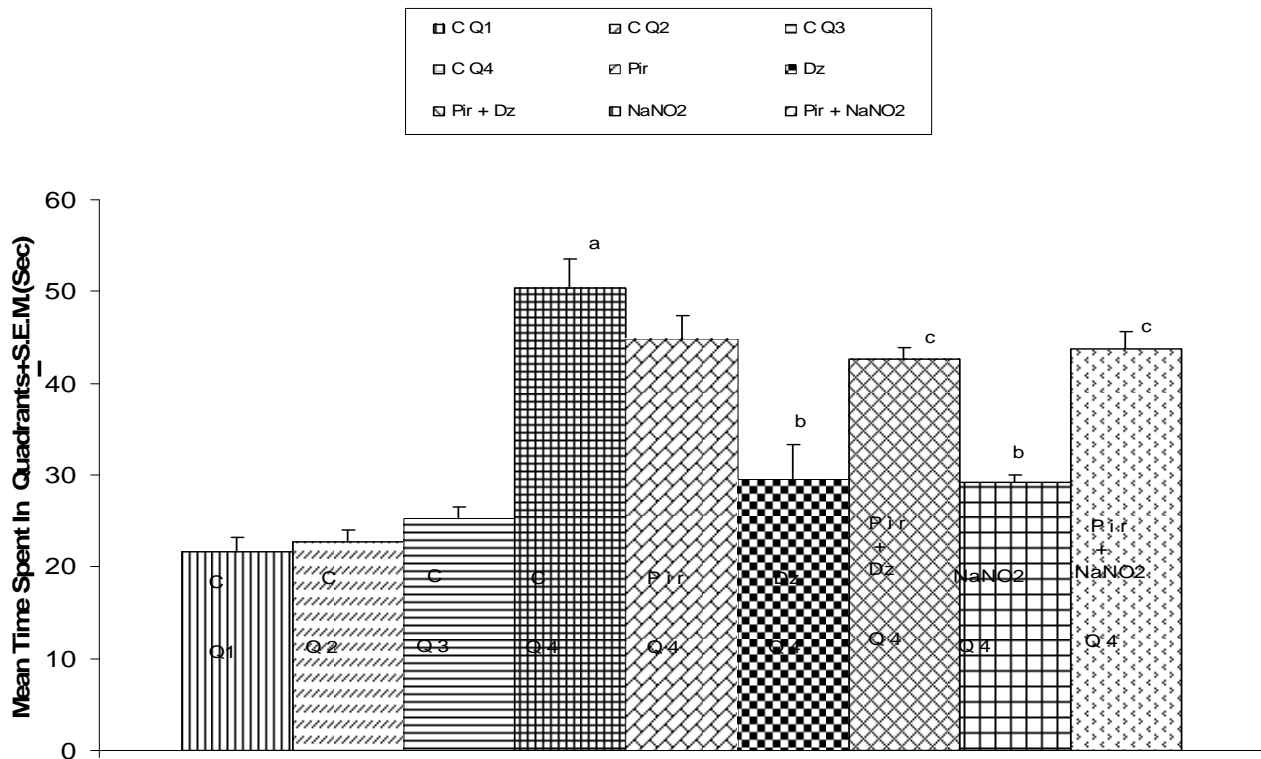
Scopolamine (3 mg Kg<sup>-1</sup>, i.p.) and cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.), did not significantly modulate the decrease in ELT noted as result of successive acquisition trials conducted on day 1 to day 4 but markedly attenuated the increase in TS in TQ (Q 4) in search of missing platform during retrieval trial conducted on day 5 (Fig. 2). It suggests that scopolamine and cyclohexamide induced poor retrieval may not be due to impairment of acquisition. Piracetam (200 mg kg<sup>-1</sup>, i.p.) prevented scopolamine and cyclohexamide induced attenuation of increase in TS in TQ (Q 4) in search of missing platform during retrieval trial conducted on day 5 (Fig. 2). The effect of piracetam was significant only in case of cyclohexamide. Diazepam (1 mg Kg<sup>-1</sup>, i.p.) and sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) significantly

attenuated the decrease in ELT noted as a result of successive acquisition trial conducted on day 1 to day 4 (Fig. 1) and also markedly reduced the increase in TS in TQ (Q 4) in search of missing platform during retrieval trial conducted on day 5 (Fig. 3). It suggests that diazepam and NaNO<sub>2</sub> induced poor retrieval may be due to impairment of acquisition. Piracetam (200 mg Kg<sup>-1</sup>, i.p.) significantly prevented NaNO<sub>2</sub> (75 mg Kg<sup>-1</sup>, i.p.) induced attenuation of decrease in ELT (Fig. 1) and increase in TS in TQ (Q 4) noted during acquisition and retrieval trial conducted on day 1 to day 4 and 5 respectively (Fig. 3). However, piracetam (200 mg Kg<sup>-1</sup>, i.p.) did not modulate diazepam (1 mg Kg<sup>-1</sup>, i.p.) induced attenuation of decrease in ELT noted during acquisition trial. On the other hand, piracetam (200 mg Kg<sup>-1</sup>, i.p.) significantly prevented diazepam (1mg Kg<sup>-1</sup>, i.p.) induced increase in TS in TQ (Q4) noted during retrieval trial conducted on day 5 (Fig. 3). It suggests that piracetam prevented diazepam induced poor retrieval occurred as a result of impaired acquisition.



**Fig. 2 Effect of piracetam on scopolamine and cyclohexamide induced anterograde amnesia (retrieval component)**

On day 5, TS by animal in each of four quadrants was noted. Q represents the quadrant. C, Pir, Sc and chx, represents control, piracetam, and scopolamine treated groups, in which mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.), piracetam (200 mg Kg<sup>-1</sup>, i.p.), scopolamine (3 mg Kg<sup>-1</sup>, i.p.) and cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) respectively, 30 min before acquisition trial (day 1 to day 4). Pir+Sc represents that mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+scopolamine (3 mg Kg<sup>-1</sup>, i.p.) 60 and 30 min respectively before acquisition trial (day 1 to day 4). Chx represents that mice were administered cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) 60 min before acquisition trial (day 1 to day 4). Pir+Chx represents that mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+cyclohexamide (28 mg Kg<sup>-1</sup>, i.p.) 60 min before acquisition trial (day 1 to day 4). In all above-mentioned groups, mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial (day 5). Each value represents mean±S.E.M. (n=5). a=p<0.05 Vs TS in other quadrants (Q 1, Q2 and Q3). b=p<0.05 Vs TS in TQ (Q 4). c=p<0.05 Vs TS by cyclohexamide treated mice in TQ. Calculated F value 6.34 for D.F. (5, 24) is not less than tabular value 2.62. There are differences between groups.

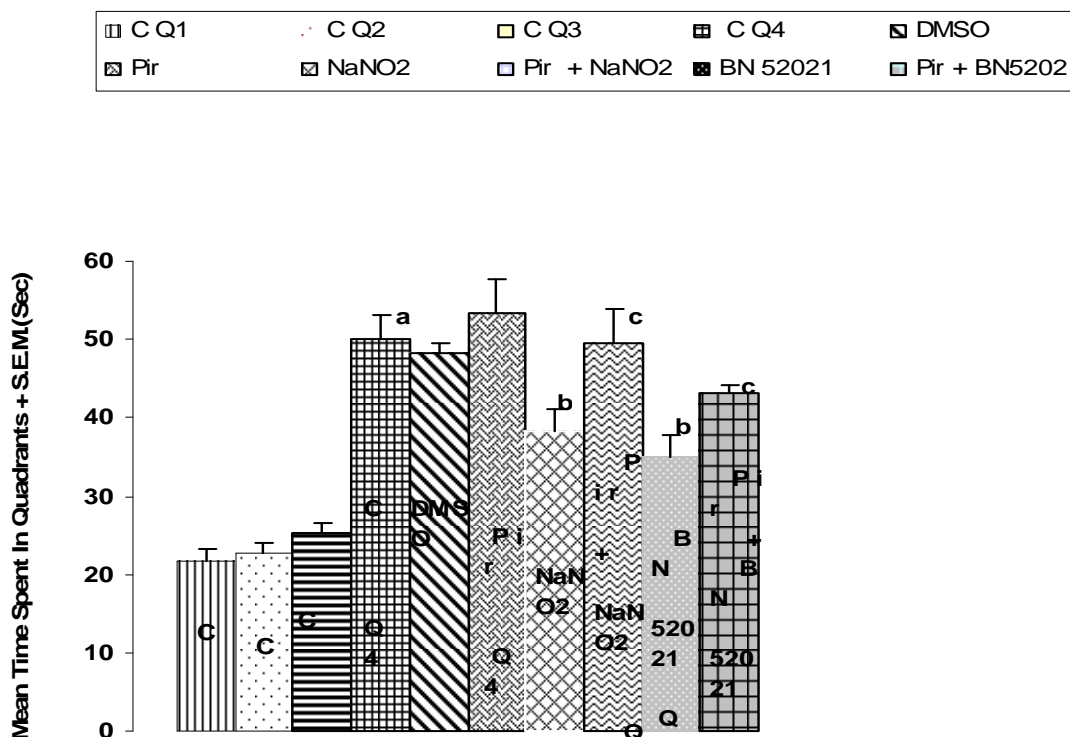


**Fig. 3 Effect of piracetam on diazepam and sodium nitrite induced anterograde amnesia (retrieval component)**

On day 5, TS by animal in each of four quadrants was noted. Q represents the quadrant. C, Pir, Dz and NaNO<sub>2</sub> represents control, piracetam, diazepam and sodium nitrite treated groups, in which mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.), piracetam (200 mg Kg<sup>-1</sup>, i.p.), diazepam (1 mg Kg<sup>-1</sup>, i.p.) and sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.), 30 min before acquisition trial (day 1 to day 4). Pir+Dz and Pir+NaNO<sub>2</sub> represents that mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+diazepam (1 mg Kg<sup>-1</sup>, i.p.) and piracetam (200 mg Kg<sup>-1</sup>, i.p.) and sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) 60 and 30 min respectively before acquisition trial (day 1 to day 4). In all above mentioned groups, mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before retrieval trial (day 5). Each value represents mean±S.E.M (n=5). a=p<0.05 Vs TS in other quadrants (Q1, Q2 and Q3). b=p<0.05 Vs TS in TQ (Q 4). c=<0.05 Vs TS by diazepam or sodium nitrite treated mice in TQ (Q 4). Calculated F value 9.91 D.F. (5, 24) is not less than tabular value 2.62. Hence there are differences between groups.

#### **Effect of piracetam on sodium nitrite and BN52021 induced retrograde amnesia**

NaNO<sub>2</sub> (75 mg Kg<sup>-1</sup>, i.p.) and BN 52021 (15 mg Kg<sup>-1</sup>, i.p.) administered 30 min before retrieval trial significantly attenuated increase in TS in TQ (Q 4) in search of missing platform during retrieval trial conducted on day 5 (Fig. 4). These observations suggest that NaNO<sub>2</sub> and BN52021 produced retrograde amnesia. Moreover, scopolamine, cyclohexamide and diazepam did not produce retrograde amnesia. Piracetam (200 mg Kg<sup>-1</sup>, i.p.) significantly prevented BN52021 (15 mg Kg<sup>-1</sup>, i.p.) and NaNO<sub>2</sub> (75 mg Kg<sup>-1</sup>, i.p.) induced attenuation of increase in TS in TQ (Q 4) in search of missing platform during retrieval trial conducted on day 5 (Fig. 4).



**Fig. 4 Effect of piracetam on sodium nitrite and BN 52021 induced retrograde amnesia**

On day 5, TS by animal in each of four quadrants was noted. Q represents the quadrant. C, DMSO, Pir NaNO<sub>2</sub> and BN52021 represents control, dimethylsulphoxide, piracetam sodium nitrite and BN52021 treated groups, in which mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.), 0.5M dimethylsulphoxide (10 ml Kg<sup>-1</sup>, i.p.) used as vehicle for BN52021, piracetam (200 mg Kg<sup>-1</sup>, i.p.), sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) and BN52021 (15 mg Kg<sup>-1</sup>, i.p.), 30 min before acquisition trial (day 1 to day 4) and retrieval trial (day 5) respectively. Pir+NaNO<sub>2</sub> and Pir+BN52021 represents that mice were administered piracetam (200 mg Kg<sup>-1</sup>, i.p.)+sodium nitrite (75 mg Kg<sup>-1</sup>, i.p.) and piracetam (200 mg Kg<sup>-1</sup>, i.p.)+BN 52021 (15 mg Kg<sup>-1</sup>, i.p.), 60 and 30 min before retrieval trial (day 5) In sodium nitrite or piracetam+sodium nitrite treated group, mice were administered distilled water (10 ml Kg<sup>-1</sup>, i.p.) 30 min before acquisition trial (day1 to day 4). In BN52021 or piracetam+BN52021 treated groups, mice were administered 0.5M DMSO (10 ml Kg<sup>-1</sup>, i.p.) 30 min before acquisition trial (day1 to day 4). Each value represents mean±S.E.M (n=5). a=p<0.05 Vs TS in other quadrant (Q1, Q2, and Q3). b=p<0.05 Vs TS in TQ (Q 4). c=p<0.05 Vs TS sodium nitrite or BN52021 treated mice in TQ (Q 4). Calculated F value 4.14 for D.F. (6 28) is not less than tabular value (2.45). There are differences between groups.

## Discussion

The marked decrease in escape latency time with ongoing acquisition trial and an increase in time spent in target quadrant in search of missing platform during retrieval trial indicate normal acquisition and retrieval of memory noted in present study with control mice. Piracetam (200 mg kg<sup>-1</sup>, i.p.) has prevented scopolamine-induced attenuation of increase in time spent in target quadrant in search of missing platform during retrieval trial. Piracetam is not observed to bind with muscarinic cholinergic receptors [30, 32] but it is documented to increase the number of muscarinic cholinergic receptors [33] and to enhance the neuronal uptake of choline in hippocampus which is a precursor of acetyl choline [34-35]. Piracetam enhances hydrocarbon core fluidity of hippocampal membrane obtained from Alzheimer patients [36]. It may be speculated that prevention of scopolamine induced anterograde amnesia with piracetam may be due to restoration of brain cell fluidity through increase in cholinergic receptors and biosynthesis of acetylcholine.

Piracetam is a cyclic GABA derivative but it has no significant effect on GABA receptors [30], the synaptosomal uptake of GABA [37] and GABA concentration in either brain or plasma [38]. Perhaps that's why piracetam has not prevented diazepam induced anterograde amnesia in the study at hand. It is interesting to note that piracetam has selectively prevented diazepam induced poor retrieval occurred as a result of impaired acquisition. However, piracetam has not affected diazepam-induced impairment of acquisition component of anterograde amnesia. It suggests that diazepam induced poor retrieval may not be mediated through GABA-BZD complex. Piracetam stimulates adenylyl cyclase [39], decreases potassium efflux [40], increases calcium influx [41] and IP<sub>3</sub> mediated intracellular Ca<sup>+2</sup>, which may consequently prevent the noted anterograde amnesia with NaNO<sub>2</sub>. Piracetam is reported to increase IP<sub>3</sub> mediated release of Ca<sup>+2</sup> from endoplasmic reticulum [40] and may be responsible to prevent BN52021 induced retrograde amnesia.

## Conclusion

Scopolamine, cyclohexamide and diazepam produced impairment only in the retrieval component, whereas sodium nitrite produced impairment in both acquisition as well as retrieval components. Scopolamine, cyclohexamide and diazepam have produced only anterograde amnesia. Sodium nitrite has produced both anterograde and retrograde amnesia. BN52021, a PAF receptor antagonist has produced only retrograde amnesia. Piracetam is able to prevent anterograde amnesia experimentally induced by scopolamine, cyclohexamide and sodium nitrite. Moreover, retrograde amnesia produced by sodium nitrite and BN52021 was also attenuated by piracetam. On the other hand diazepam induced anterograde amnesia has not been affected by piracetam atleast in mice species.

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