INTRODUCTION

Memory impairment is common in depression [1-3]. The prevalence of memory impairment is 22% in depressed patients [4]. Modulation of neurotransmitters in hippocampus and cerebral cortex plays a vital role in cognitive functioning [1, 5]. Antidepressant treatment also helps in relieving depression associated short-term memory deficit [6, 7]. Duloxetine is a potent and reuptake inhibitor of serotonin (5-HT) and norepinephrine (NE). The dual action makes it an interesting option in the treatment of depression associated cognitive impairments. Eight weeks of duloxetine treatment resulted in significant improvement of cognition in depressed patients [8]. Comparison of fluoxetine, paroxetine, and venlafaxine against duloxetine showed better safety, tolerability with fewer side effects, and efficacy with the latter drug [9-12]. Duloxetine showed reversal of cognitive deficit.
at dose range of 10–30 mg/kg in rodents [13]. In forced swim test, the immobility period was significantly reduced at 10 mg/kg dose [14, 15]. In addition to 5-HT and NE reuptake inhibitors, nootropic agents like piracetam [16] also play an important role in cognition. The dose range of piracetam described in package inserts of Nootrpf® is 2.4–12 gm per day [17]. The resulting dose range would be 34 to 171 mg/kg for an adult of 70 kg. Navarro et al. [17] showed therapeutic activity of piracetam at 100 mg/kg dose. Therefore, piracetam monotherapy was considered at 100 mg/kg dose in present study. The precise mechanism of action of piracetam is not clear [18]. Reports suggest better mitochondrial functioning and metabolism of glucose with piracetam treatment [19]. It also has ability to restore the cell membrane alteration in the aging brain [20, 21]. The combination of piracetam and duloxetine may result in augmentation of nootropic activity due to their different mechanism of action. Therefore, the present study was planned to investigate the nootropic effect of piracetam combination with duloxetine in mice.

MATERIALS AND METHODS

Animals
Male Swiss Albino mice weighing 25–30 gm (3 months old) were procured from Bharat Serum Ltd, Thane. They were stored kept in a temperature (22–24°C) and humidity (50–60%) controlled central animal house facility under light (12 h) and dark (12 h) illumination cycle. Animals were given free access to standard food and water. Experiments were performed between 12.00–16.00 h. Each animal model i.e. Elevated plus maze (EPM), Morris water maze (MWM) and brain monoamine estimation was conducted on separate set of animals. In each set, animals were randomly distributed into 4 groups (n=6/group; 1 set=24 animals; 3 experiments=72 animals). The arena of EPM was cleaned using 70% ethyl alcohol solution before placing each mouse. Experimental protocols used in present were approved by the Institutional Animal Ethics Committee (Project approval number CPCSEA/IAEC/SPTM/P-08-2013), Government of India, New Delhi.

Drug solutions and treatment
Drugs were administered through intra-peritoneal route. Normal saline (0.9% w/v NaCl) was used to prepare drug solutions. Each animal received treatment 1 h before test session in EPM (on 2nd day) and Morris water maze (MWM- on 6th day). Euthanasia was performed 1 h before treatment in the estimation of brain monoamine. Each animal model had 4 groups. Control group (Group I) received normal saline (10 ml/kg). Treatment of duloxetine (10 mg/kg; Dr. Reddy’s Laboratories Ltd.), piracetam (100 mg/kg; UCB India Pvt. Ltd.), and combination of duloxetine (5 mg/kg)+piracetam (50 mg/kg) were given to Group II, III, and IV, respectively.

Spatial memory tests
EPM
The protocol used to evaluate transfer latency (TL) in EPM was described by Dhandra et al. [22]. Time taken by each animal to reach the closed arm is recorded as the TL. In brief, 2 open arms

![Fig. 1. Elevated plus maze - Transfer Latency (TL). TL: Transfer latency. Significant difference is denoted by **p<0.01 - as compared to the control group (n=6/group).](http://dx.doi.org/10.5607/en.2014.23.3.224)
Fig. 2. Morris water maze. (A) Escape latency (visible platform) measured on 1st day. (B) Escape latency (invisible platform) measured between 2nd and 5th days. (C) Time spent in target quadrant on 6th day. Data is presented as mean±SEM (n=6/group). Significant difference is denoted by *p<0.05 - as compared against the control group.
(30×5 cm) and 2 closed arms (30×5×12 cm) of EPM were arranged so that the 2 closed arms kept opposite to each other with an open roof. Each animal was placed at the end of open arm facing away from central platform (5×5 cm). On the first day (the acquisition session), each animal was exposed to EPM for 90 seconds. Time taken by animal to reach the closed arm was recorded as the transfer latency (TL). Animals failed to enter in closed arm in 90 seconds were excluded from study. On second day (the retention session), each animal was put into the open arm and the TL was recorded for maximum 90 seconds. The SMART v2.5.21 video-tracking system (Panlab Harvard Apparatus, spain) was used to evaluate TL.

**Morris water maze (MWM)**

MWM test is used to evaluate the hippocampal-dependent learning, including acquisition of spatial memory and long-term spatial memory. The protocol of MWM described by Bromley-Brits et al. [23] was used to determine the percent time spent in target quadrant. Drug treatments were given to mice 60 min before test on 6th trial day. In brief, the pool having 150 cm diameter and, 50 cm depth was constructed of seamless black polyethylene. The clear plastic escape platform (10 cm diameter, 31 cm high) could be positioned in the any 1 of 4 quadrant position in the pool. The water temperature was maintained at room temperature (22–24°C). Each animal went through training trials (5 trials every day) from day 1 to day 5. On 1st day, platform was visible (1 cm above water level) and placed in south-west, north-west, north-east, centre, and south-west positions in 5 trials, respectively. Starting directions of animal in 5 trials were south (S), north (N), S, east (E), and west (W), respectively. On 2–5th days, platform was made hidden (at water level) and kept in S-W position. The starting locations of each animal in 5 trials were W-S-N-E-S (2nd day), N-E-W-W-S (3rd day), N-E-W-S-N (4th day), and E-S-W-E-N (5th day). On the 6th day, only 1 trial was performed having N as starting location of animal and without platform. The time spent in target quadrant (SW) was noted as index of retrieval or memory. Video camera was fixed on the ceiling to record the behavior of the mice in the pool. It was interfaced with the SMART v2.5.21 video-tracking system (Panlab Harvard Apparatus, spain).

**Brain monoamine estimation by HPLC with fluorescence detector (HPLC-FD) method**

Heads were dropped in ice cold perchloric acid (0.1 M) immediately after euthanasia. After weighing brain, separation of cerebral cortex, hippocampus, and remaining brain parts were separated, weighed, and homogenized in 2 ml of ice cold 0.1 M perchloric acid. Analysis of monoamine levels in cerebral cortex, hippocampus, and whole brain (whole brain=cerebral cortex+hippocampus+remaining brain tissue) was performed using method described by Choudhary et al. [24] and Madepalli et al. [25] (HPLC-Shimadzu, LC-2010C HT, autosampler with FDR-20A-prominence, Shimadzu). The method was optimized in-house [26]. Homogenized mixture was centrifuged at 20817×g (Eppendorf 5810 R, Rotor F-45-30-11) for 30 min (4°C) and the obtained supernatant was filtered through 0.45 μm membrane. Filtered supernatant was stored at -80°C until the time of analysis. After sample injection, the chromatographic separation was achieved on reversed-phase analytical column (KROMASIL 100, C18, 5 μm, 25 mm × 0.46 mm) at room temperature. LC Solution® software was used to process acquired data. The composition of mobile phase (flow rate - 1.3 ml/min) includes sodium acetate (0.02 M), ethylenediaminetetraacetic acid (0.2 mM), methanol (16%), di-n-butylamine (0.01%) and heptane sulfonic acid (0.055%), adjusted at pH 3.92 with phosphoric acid. The prepared mobile phase was filtered through a 0.45-mm membrane (PALL® Pall corporation, India). Monoamines were detected at an excitation wavelength of 280 nm and an emission wavelength of 315 nm. Retention of time of standard and sample were used to identify peaks. Monoamine concentration was estimated according their area under curve using their straight line equation. The linearity of monoamines was in the range 0.99–0.996. Obtained data was expressed as ng/g of wet weight of tissue.

**Statistical analysis**

The Graphpad InStat for 32 bit Windows version 3.06 was used to perform statistical analysis. Comparison between different groups was performed using ANOVA followed by Tukey’s honest significant difference (HSD) post-hoc test. Data was represented as mean±SEM values (per group n=6/group).

Table 1. One way ANOVA F values of models/parameters

| Models/parameters       | One way ANOVA F values |
|-------------------------|------------------------|
| Elevated plus maze      | F (3, 20)=7.041, p=0.9909 |
| Morris water maze       | F (3, 20)=4.716, p=0.7659 |
| NE levels in hippocampi | F (3, 20)=61.531, p=0.0002 |
| NE levels in cerebral cortices | F (3, 20)=63.558, p=0.0002 |
| NE levels in whole brain | F (3, 20)=62.353, p=0.0005 |
| DA levels in hippocampi  | F (3, 20)=64.69, p=0.0001 |
| DA levels in cerebral cortices | F (3, 20)=50.483, p=0.005 |
| DA levels in whole brain | F (3, 20)=82.066, p=0.03 |
| 5-HT levels in hippocampi| F (3, 20)=48.993, p=0.0075 |
| 5-HT levels in cerebral cortices | F (3, 20)=78.09, p=0.0001 |
| 5-HT levels in whole brain | F (3, 20)=22.325, p=0.007 |

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RESULTS AND DISCUSSION

In EPM, duloxetine treatment showed no significant change in TL (Fig. 1) than control group. The escape latency on 1st day with visible platform was similar in all animals in MWM (Fig. 2A). In the training and acquisition with invisible platform (2–5 days),

Fig. 3. Brain monoamine levels (ng/g of tissue weight). (A) NE: Norepinephrine; (B) DA: Dopamine; (C) 5-HT: Serotonin; Significant difference is denoted by "***p<0.001 - as compared to the control group; "p<0.01 - as compared to duloxetine treated group (n=6/group).
there was reduction in escape latency observed from 2nd to 5th day (Fig. 2B). Duloxetine treatment showed no significant change in time spent in target quadrant (Fig. 2C) than control group. The results of duloxetine treated group in EPM (Fig. 1) and MWM (Fig. 2C) are in-line with the published reports [27]. Published report suggest no benefits with acute or sub-acute treatment duloxetine in cognition treatment [27], however clinical studies have reported cognition related benefits with 8 weeks [8] and 12 weeks [28] of duloxetine treatment in depressed patients. The significant increase in brain monoamine profile of duloxetine is in line with the published reports [29, 30]. These reports suggest that duloxetine increases DA levels not only in cerebral cortex [29, 30], but also in hippocampus [26] and nucleus accumbens region [30].

In present study, piracetam treated group showed significant decrease in TL (Fig. 1), as compared to control group. Patil et al. [31] have reported similar decrease in TL after piracetam treatment in EPM. The decrease in TL was not significant in remaining groups (Fig. 1). In MWM, the time spent in target quadrant was significantly increased in piracetam treated group, as compared to control group (Fig. 2C). One way ANOVA F values of EPM, MWM, and brain monoamine are given in Table 1. The significant increase in brain monoamine profile of lower dose of piracetam treatment in present study is in line with the published reports [32, 33]. The decrease in TL observed in EPM and the increase in time spent in target quadrant observed in MWM were not statistically significant in combination treated group, as compared to control, duloxetine, and piracetam treated groups, separately. Cortex and hippocampus regions play important role in cognition and emotions [1]. Combination treated group showed significant increase in brain monoamine levels in hippocampus, cerebral cortex, and whole brain when compared against respective control groups (Fig. 3). However, same treatment failed to increase in monoamine profile in hippocampus, cerebral cortex, and whole brain when compared against duloxetine and piracetam treated groups (Fig. 3). There was exception of NE levels in cerebral cortex when compared against duloxetine treated group (Fig. 3A).

The possible reason behind failure to produce augmentation of nootropic activity may be the interactions between piracetam and duloxetine. Everss et al. [34] reported decrease in memory and learning tasks due to interaction between piracetam and amitriptyline. However, the report hasn’t described the reason [34]. Therefore, the study focusing on the effect of acute and chronic dosing of duloxetine and piracetam combination on electrophysiological analysis, neurogenesis, biogenic amine pathway activation/deactivation, drug metabolism, and related drug interaction studies may help in understanding the present study outcomes.

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