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Original Article

Experimental Neurobiology

The Combination of Antidepressant Duloxetine with Piracetam in Mice does not Produce Enhancement of Nootropic Activity

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There is a strong association between depression and memory impairment. The present study aims to assess the nootropic activity of duloxetine and piracetam combination. Male Swiss Albino mice were divided randomly into 4 groups. Treatment of normal saline (10 ml/kg), duloxetine (10 mg/kg), piracetam (100 mg/kg), and duloxetine (5 mg/kg) plus piracetam (50 mg/kg) were given through intra-peritoneal route to group I-IV, respectively. Transfer latency in elevated plus maze (EPM) and time spent in target quadrant in Morris water maze (MWM) were recorded. Estimation of brain monoamines in hippocampus, cerebral cortex, and whole brain were done using HPLC with fluorescence detector. Piracetam treated group showed significant decrease in transfer latency in EPM and increase in time spent in target quadrant recorded in MWM. Combination treated group failed to produce statistically significant nootropic effect in both EPM and MWM. Combination treated group failed to increase brain monoamine levels when compared against duloxetine and piracetam treated groups, separately. But there was exception of significant increase in norepinephrine levels in hippocampi when compared against duloxetine treated group. Results indicate no cognitive benefits with piracetam plus duloxetine combination. These findings can be further probed with the aim of understanding the interaction between duloxetine and piracetam as a future endeavor.

Key words: nootropic activity, duloxetine, piracetam

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

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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 Nootrpil® 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 Dhingra et al. [22]. Time taken by each animal to reach the closed arm is recorded as the TL. In brief, 2 open arms

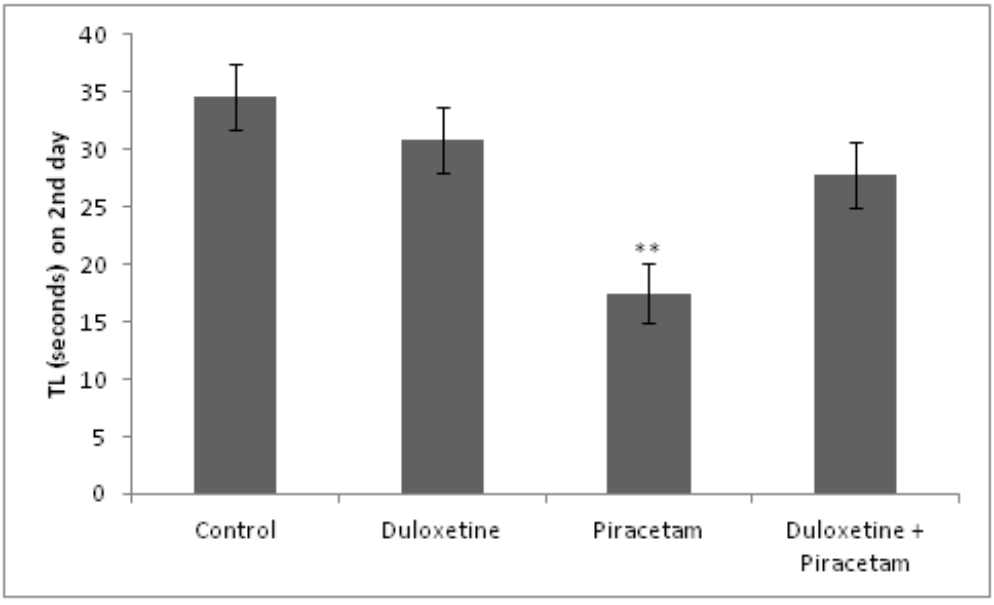

Fig. 1. Elevated plus maze - Transfer Latency (TL). TL: Transfer latency; Significant difference is denoted by **p<0.01 - as compared to the control group (n=6/group).

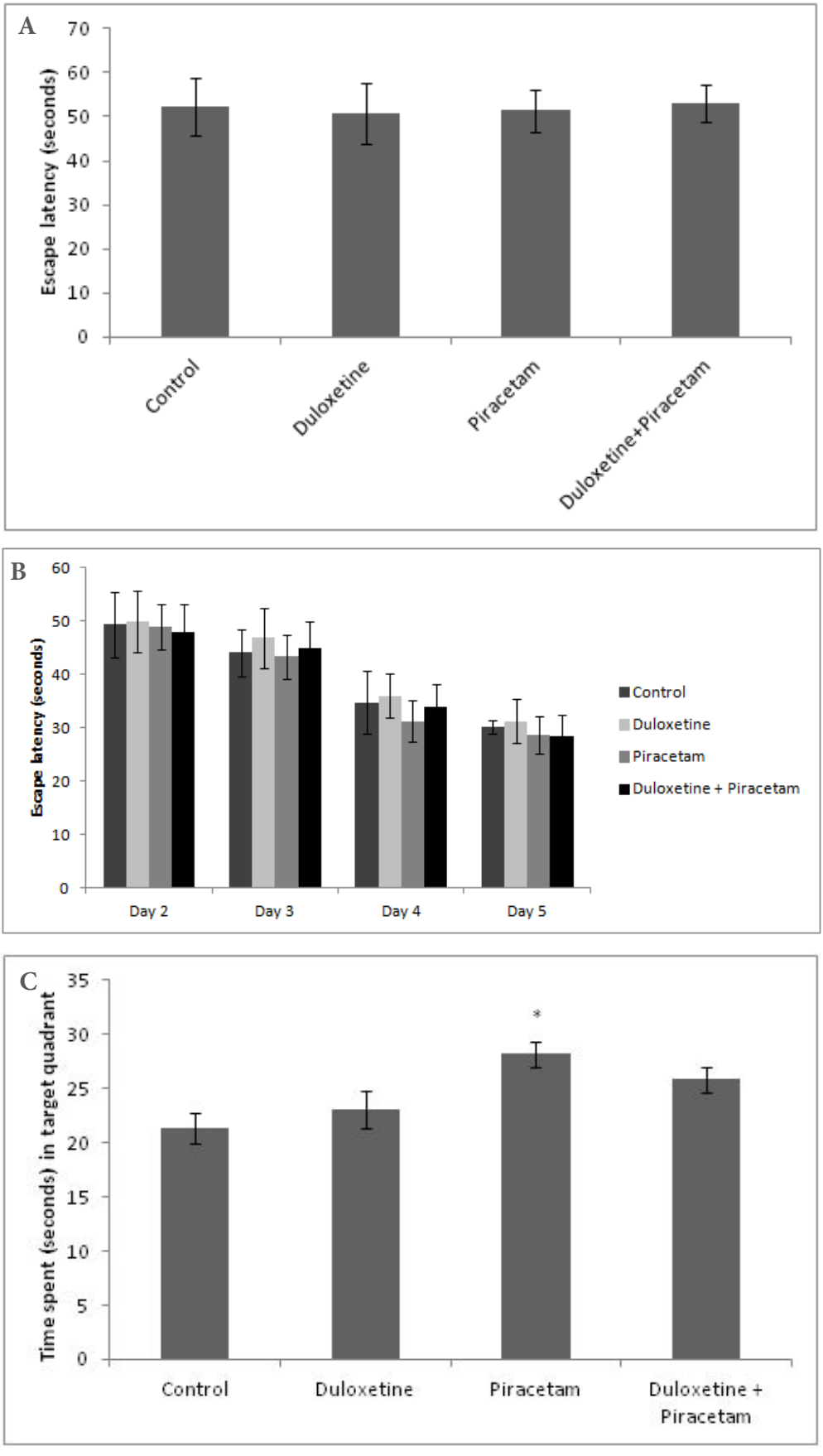

Fig. 2. Morris water maze. (A) Escape latency (visible platform) measured on 1st day. (B) Escape latency (invisible platform) measured between 2nd and 5th days. (C) Time spent in target quadrant on 6th day. Data is presented as mean±SEM (n=6/group). Significant difference is denoted by *p<0.05 - as compared against the control group.

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(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 FD-RF-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 for 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

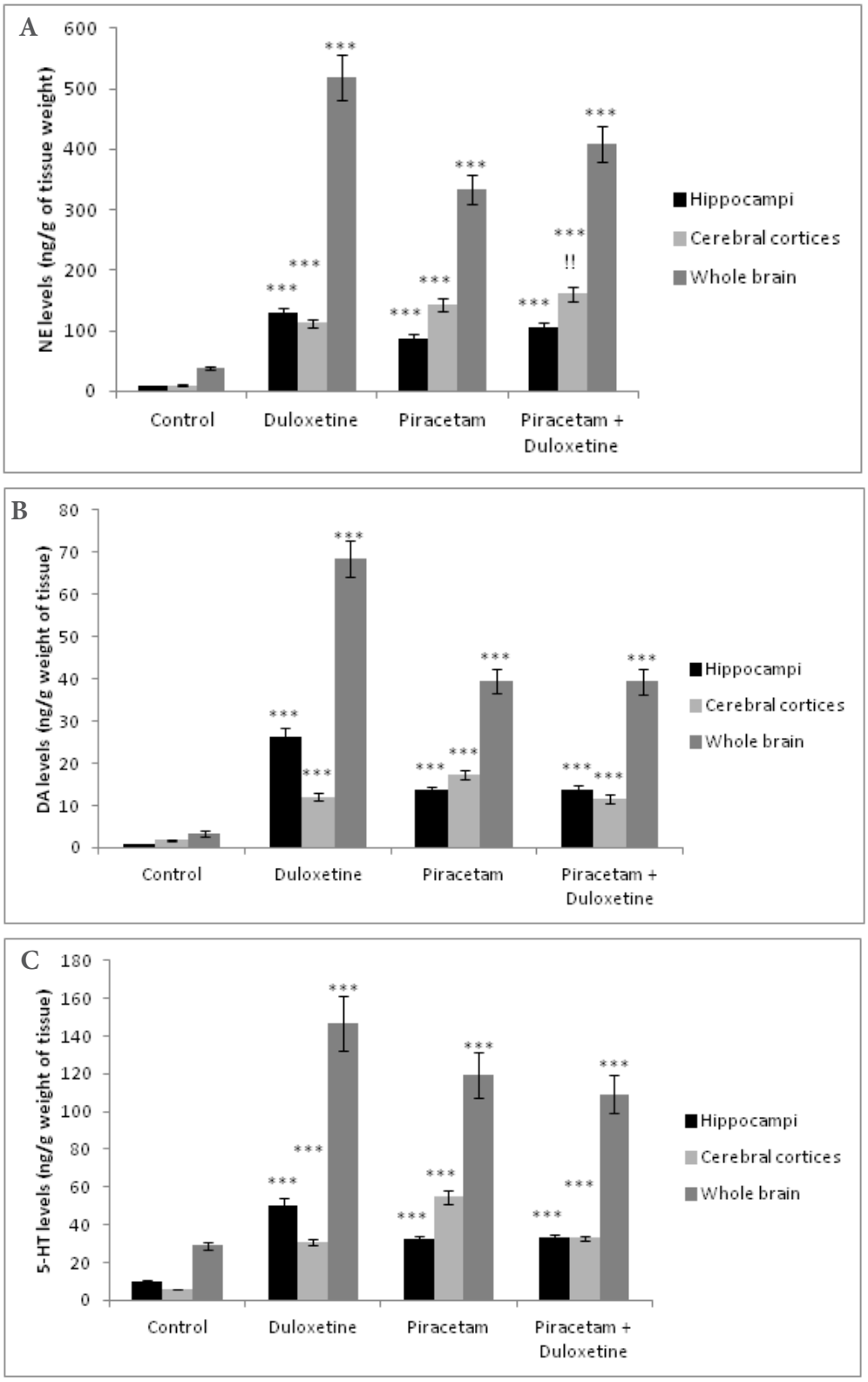

Fig. 3. Brain monoamine levels (ng/g of tissue weight). (A) NE: Norepinephrine; (B) DA: Dopamine; (C) 5-HT: Serotonin; Significant difference is denoted by ***p< 0.001 - as compared to the control group; !!p< 0.01 - as compared to duloxetine treated group (n=6/group).

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),

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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.

REFERENCES

1. Eriksson TM, Delagrange P, Spedding M, Popoli M, Mathé AA, Ögren SO, Svenningsson P (2012) Emotional memory impairments in a genetic rat model of depression: involvement of 5-HT/MEK/Arc signaling in restoration. Mol Psychiatry 17:173-184.
2. Burt DB, Zembar MJ, Niederehe G (1995) Depression and memory impairment: a meta-analysis of the association, its pattern, and specificity. Psychol Bull 117:285-305.
3. Biringer E, Mykletun A, Dahl AA, Smith AD, Engedal K, Nygaard HA, Lund A (2005) The association between depression, anxiety, and cognitive function in the elderly general population--the Hordaland Health Study. Int J Geriatr Psychiatry 20:989-997.
4. Kim JM, Stewart R, Shin IS, Choi SK, Yoon JS (2003) Subjective memory impairment, cognitive function and depression--a community study in older Koreans. Dement Geriatr Cogn Disord 15:218-225.
5. Logue SF, Gould TJ (2014) The neural and genetic basis of executive function: attention, cognitive flexibility, and response inhibition. Pharmacol Biochem Behav 123C:45-54.
6. Sternberg DE, Jarvik ME (1976) Memory functions in depression. Arch Gen Psychiatry 33:219-224.
7. Glass RM, Uhlenhuth EH, Hartel FW, Matuzas W, Fischman MW (1981) Cognitive dysfunction and imipramine in outpatient depressive. Arch Gen Psychiatry 38:1048-1051.
8. Raskin J, Wiltse CG, Siegal A, Sheikh J, Xu J, Dinkel JJ, Rotz BT, Mohs RC (2007) Efficacy of duloxetine on cognition, depression, and pain in elderly patients with major depressive disorder: an 8-week, double-blind, placebo-controlled trial. Am J Psychiatry 164:900-909.
9. Hunziker ME, Suehs BT, Bettinger TL, Crismon ML (2005) Duloxetine hydrochloride: a new dual-acting medication for the treatment of major depressive disorder. Clin Ther 27:1126-1143.
10. Stahl SM, Grady MM, Moret C, Briley M (2005) SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. CNS Spectr 10:732-747.
11. Thase ME, Pritchett YL, Ossanna MJ, Swindle RW, Xu J, Detke MJ (2007) Efficacy of duloxetine and selective serotonin reuptake inhibitors: comparisons as assessed by remission rates in patients with major depressive disorder. J Clin Psychopharmacol 27:672-676.
12. Zomkowski AD, Engel D, Cunha MP, Gabilan NH, Rodrigues AL (2012) The role of the NMDA receptors and l-arginine-nitric oxide-cyclic guanosine monophosphate pathway in

the antidepressant-like effect of duloxetine in the forced swimming test. Pharmacol Biochem Behav 103:408-417.
13. Grégoire S, Michaud V, Chapuy E, Eschalier A, Ardid D (2012) Study of emotional and cognitive impairments in mononeuropathic rats: effect of duloxetine and gabapentin. Pain 153:1657-1663.
14. Ciulla L, Menezes HS, Bueno BB, Schuh A, Alves RJ, Abegg MP (2007) Antidepressant behavioral effects of duloxetine and fluoxetine in the rat forced swimming test. Acta Cir Bras 22:351-354.
15. Rénéric JP, Lucki I (1998) Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology (Berl) 136:190-197.
16. Gouliaev AH, Senning A (1994) Piracetam and other structurally related nootropics. Brain Res Brain Res Rev 19:180-222.
17. Navarro SA, Serafim KG, Mizokami SS, Hohmann MS, Casagrande R, Verri WA Jr (2013) Analgesic activity of piracetam: effect on cytokine production and oxidative stress. Pharmacol Biochem Behav 105:183-192.
18. Winblad B (2005) Piracetam: a review of pharmacological properties and clinical uses. CNS Drug Rev 11:169-182.
19. Grau M, Montero JL, Balasch J (1987) Effect of Piracetam on electrocorticogram and local cerebral glucose utilization in the rat. Gen Pharmacol 18:205-211.
20. Keil U, Scherping I, Hauptmann S, Schuessel K, Eckert A, Müller WE (2006) Piracetam improves mitochondrial dysfunction following oxidative stress. Br J Pharmacol 147:199-208.
21. Heiss WD, Hebold I, Klinkhammer P, Ziffling P, Szelies B, Pawlik G, Herholz K (1988) Effect of piracetam on cerebral glucose metabolism in Alzheimer's disease as measured by positron emission tomography. J Cereb Blood Flow Metab 8:613-617.
22. Dhingra D, Parle M, Kulkarni SK (2004) Memory enhancing activity of Glycyrrhiza glabra in mice. J Ethnopharmacol 91:361-365.
23. Bromley-Brits K, Deng Y, Song W (2011) Morris water maze test for learning and memory deficits in Alzheimer's disease model mice. J Vis Exp (53):pii: 2920
24. Choudhary KM, Mishra A, Poroikov VV, Goel RK (2013) Ameliorative effect of Curcumin on seizure severity, depression like behavior, learning and memory deficit in post-pentylenetetrazole-kindled mice. Eur J Pharmacol 704:33-40.
25. Lakshmana MK, Raju TR (1997) An isocratic assay for norepinephrine, dopamine, and 5-hydroxytryptamine using their native fluorescence by high-performance liquid chromatography with fluorescence detection in discrete brain areas of rat. Anal Biochem 246:166-170.
26. Kale PP, Addepalli V (2014) Augmentation of antidepressant effects of duloxetine and bupropion by caffeine in mice. Pharmacol Biochem Behav 124:238-244.
27. Pereira P, Gianesini J, da Silva Barbosa C, Cassol GF, Von Borowski RG, Kahl VF, Cappelari SE, Picada JN (2009) Neurobehavioral and genotoxic parameters of duloxetine in mice using the inhibitory avoidance task and comet assay as experimental models. Pharmacol Res 59:57-61.
28. Greer TL, Sunderajan P, Grannemann BD, Kurian BT, Trivedi MH (2014) Does duloxetine improve cognitive function independently of its antidepressant effect in patients with major depressive disorder and subjective reports of cognitive dysfunction? Depress Res Treat 2014:627863.
29. Kihara T, Ikeda M (1995) Effects of duloxetine, a new serotonin and norepinephrine uptake inhibitor, on extracellular monoamine levels in rat frontal cortex. J Pharmacol Exp Ther 272:177-183.
30. Muneoka K, Shirayama Y, Takigawa M, Shioda S (2009) Brain region-specific effects of short-term treatment with duloxetine, venlafaxine, milnacipran and sertraline on monoamine metabolism in rats. Neurochem Res 34:542-555.
31. Patil RA, Jagdale SC, Kasture SB (2006) Antihyperglycemic, antistress and nootropic activity of roots of Rubia cordifolia Linn. Indian J Exp Biol 44:987-992.
32. Bhattacharya SK, Upadhyay SN, Jaiswal AK, Bhattacharya S (1989) Effect of piracetam, a nootropic agent, on rat brain monoamines and prostaglandins. Indian J Exp Biol 27:261-264.
33. Petkov VD, Grahovska T, Petkov VV, Konstantinova E, Stancheva S (1984) Changes in the brain biogenic monoamines of rats, induced by piracetam and aniracetam. Acta Physiol Pharmacol Bulg 10:6-15.
34. Everss E, Arenas MC, Vinader-Caerols C, Monleón S, Parra A (2005) Piracetam counteracts the effects of amitriptyline on inhibitory avoidance in CD1 mice. Behav Brain Res 159:235-242.

 http://dx.doi.org/10.5607/en.2014.23.3.224