Research Article

Protective Effect of Anti-Convulsant Nootropics on Memory Impairment Induced by Pregabalin

Asher John Mohan, Krishna KL*, Jisham KM, Ramesh B. Nidavani, Mahalakshmi AM
Department of Pharmacology, JSS College of Pharmacy, JSS University, Sri Shivarathreeshwara Nagar, Mysore, Karnataka, India.
***Corresponding author's E-mail:** krishpahrm@hotmail.com

Accepted on: 23-02-2014; **Finalized on:** 30-04-2014.

ABSTRACT

Memory disorders are common in patients suffering from epilepsy and it can also be aggravated due to the administration of anti-epileptic drugs (AED) which is meant to scale down the occurrence of seizures. This study was undertaken to evaluate the memory impairment potential of Pregabalin (PGBL) on mice. The study also involves in the alleviation of the above said memory disorder due to PGBL by combining it with hydro-alcoholic extract of *Ocimum sanctum* (OSHAE) and levetiracetam (LEV), both are antiepileptic nootropic agents. Memory impairment activity of PGBL was evaluated by step-down passive avoidance task using mice as the experimental animal. Animals were observed for step down latency (SDL) and brain acetylcholine esterase (AChE) level were determined. PGBL was found to impair memory by decreasing SDL when compared to the normal group animals upon chronic administration. The extent of PGBL induced memory impairment was same as that of phenytoin an AED well reported for its memory impairment activity. Reversal of PGBL induced memory impairment was found when co-administered with OSHAE and LEV, also the co-administration potentiated the anti-epileptic activity of PGBL. AChE levels were found to increase in PGBL alone treated group when compared to normal whereas the results were opposite when PGBL was co-administered with OSHAE and LEV when compared to both normal and PGBL alone treated groups. Therefore this particular study can be used as a reference to correct AED induced impairment of memory by co-administration with nootropics without compromising the anti-epileptic potential of the AED under treatment.

Keywords: Levetiracetam, Memory disorder, Pregabalin, Tulsi.

INTRODUCTION

Patients suffering from epilepsy are often complaining of impaired memory. Anti-epileptic drug (AED) medication is the mainstay of treatment and in certain cases the AED prescribed for treatment might worsen the above said adverse effect.[1] This might in turn lead to treatment abruption due to patient intolerance to the mentioned adverse effect.[2] Therefore, there is a need to reduce the impairing of memory, which can be achieved by co-administration of nootropics taking into account of not manipulating the anti-epileptic potential of the drug under treatment. A medical guide established by Pfizer and approved by United States Food and Drug Administration (USFDA) inscribed impaired memory/concentration as the most common side effect of pregabalin (PGBL). Till date, scientific literature available to report the memory impairment potential PGBL is nil.

Hydro-alcoholic extract of leaves of *Ocimum sanctum* (OSHAE) has been proven as to enhance memory and possess anti-epileptic activity.[3-5] Levetiracetam (LEV) is a broad-spectrum antiepileptic agent and has potential benefits as a nootropic in Alzheimer's disease.[5-7] Number of neurotransmitters play an important role in memory regulation out of which only a few have been reported to participate in the above said process. Studies show that reduction in brain acetylcholine esterase decrease the degree of memory impairment when assessed by tests of memory and information.[8] Thus, brain AChE levels were determined in order to study the extent of memory impairment.

Based on the compiled literature this study was conceived to evaluate the memory impairment potential of PGBL when administered to mice. The protective effects of OSHAE & LEV were also assessed on PGBL induced memory impairment by co-administration.

MATERIALS AND METHODS

Animals

Swiss albino mice of either sex (20-35 g) procured from the Central Animal House Facility of JSS Medical College, Mysore were used for the study. The animals were housed in polypropylene cages at 23–27°C with a natural light-dark cycle. The mice were fed with standard mice pellet diet and water *ad libitum*. The animals were allowed to acclimatize to laboratory conditions for a week before the starting of experiment. Experiments protocol were in accordance with the approval of Institutional Animal Ethics Committee (IAEC) of JSSCP Mysore; bearing the project number 123/2012.

Drugs and chemicals

All chemicals used for the study were procured from various suppliers and all were of analytical grade. PGBL and LEV were obtained as gift samples from Kenvista, Ahmadabad and Hetero Drugs, Hyderabad, India respectively. Hydro-alcoholic extract of leaves of *Ocimum*

sanctum was procured from Sri Nidhi Industries, Mysore, Karnataka as a gift sample.

Preliminary phytochemical tests

The preliminary phytochemical screening was carried out on OSHAE in order to find out the presence of various phytoconstituents.[9-13]

Memory impairment activity of PGBL on mice

Evaluation of memory impairment activity of PGBL was done by using step down passive avoidance task. The animals were divided into 5 groups of 6 animals each and treated as shown in Table 1. Each mouse was gently placed on the wooden platform set in the centre of a grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range (2-15 sec) during the first test were used for the second session and the retention test.

The second-session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 hr in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec.[14]

Table 1: Memory impairment activity of PGBL by Step down passive avoidance on Pentylenetetrazole induced convulsions. (Treatment schedule)

Group	Treatment	Evaluation
Normal + PTZ	0.5% sodium CMC was administered orally for 29 days.	Durations of convulsions induced by PTZ method on 1st and 29th day after 1 hour of treatment and memory impairment activity on 8th 15th, 22nd and 29th day were noted.
Phenytoin + PTZ	Phenytoin (25 mg/kg) was administered orally for 29 days	----do-------
PGBL + PTZ	PGBL (150 mg/kg) was administered orally for 29 days.	----do-------
PGBL + OSHAE + PTZ	OSHAE (200 mg/kg) and PGBL (150 mg/kg) was administered orally for 29 days at the interval of 1 hr.	----do-------
PGBL + LEV + PTZ	LEV (400 mg/kg) and PGBL (150 mg/kg) was administered orally for 29days at the interval of 1 hr.	----do-------

Note- All group animals were subjected for PTZ induced convulsion on 8th, 15th, 22nd and 29th day of study

Anticonvulsant activity of PRBL

The animals were treated as per the schedule (TABLE 1) for 29 days. Anticonvulsant potential of PGBL in absence/presence of OSHAE/LEV was assessed on 1st & 29th day. Clonic convulsions were induced by injecting by *i.p* injection Pentylenetetrazole (PTZ) 40 mg/kg. The OSHAE (200mg/kg) and LEV (400 mg/kg) as well as PGBL were co-administered orally 2 hr and 1 hr before administration of PTZ respectively. Time before onset of clonic convulsions i.e onset of action (OAA), duration of convulsion i.e duration of action (DOA) and the percentage of mortality were recorded.[15]

Estimation of brain AchE activity

On 29thday, the animals were euthanized by cervical dislocation carefully to avoid any injuries to the brain tissue. The whole brain AChE activity was measured using the Ellman method. The cloudy supernatant liquid (0.5 ml) was pipetted out into 25ml volumetric flask and dilution was made with a freshly prepared dithiobisnitrobenzoic acid (DTNB) solution (10 mg DTNB in 100 ml of phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Substrate solution (1 ml) (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both tubes and incubated for 10 min at 30°C. The solution in the tube containing phosphate buffer was used for zeroing the colorimeter. The resulting yellow colour is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per minute of the sample was read at 420nm.[15]

RESULTS AND DISCUSSION

Preliminary phytochemical screening of OSHAE

Phytochemical screening of OSHAE as shown in TABLE 2 indicated presence of alkaloids, triterpenoids, tannins, flavonoids; cardiac glycosides and trace amounts of carbohydrates (published).[5]

In-vitro antioxidant activity of OSHAE

In-vitro antioxidant and free radical scavenging activity revealed that, OSHAE was found to have good antioxidant and potent scavenger of superoxide, hydroxyl, nitric oxide and DPPH radicals. Additionally it exhibited good ferric reducing power when evaluated by ferric reducing antioxidant power assay (published).[5]

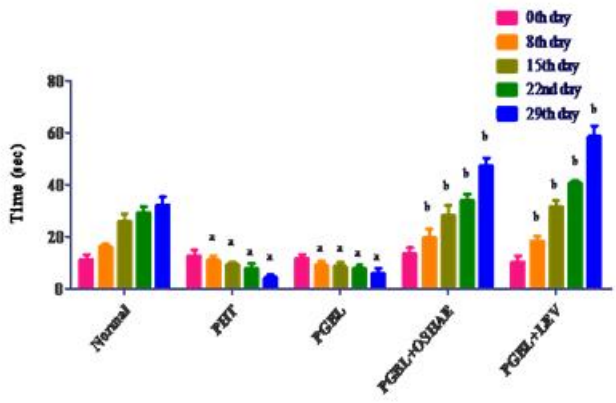
In-vivo activities

Memory impairment activity of PGBL

The memory impairment induced by chronic administration of PGBL both treated alone and in combination with OSHAE and LEV are depicted in Fig. 1&3. The activity was done by Step-down passive avoidance task using mice as experimental model. Here SDL and whole brain AChE levels were the parameters evaluated. In this model it was observed that when PGBL administered orally for 29 days on mice induced with PTZ convulsions, produced a significant decrease in SDL value (5.96±1.67 sec) when compared to the normal group (32.20±3.00 sec). Memory impairment of PRBL was found to be almost similar to Phenytoin (an antiepileptic drug that has been proven to produce memory impairment in animal models). However co-administration of OSHAE along with PGBL in PTZ induced convulsions resulted in a significant increase in the SDL value (47.30±3.02 sec) when compared to normal, phenytoin and PGBL alone treated group. Co-administration of LEV along with PGBL on PTZ induced convulsions resulted in a significant increase SDL value (58.50±4.18 sec) when compared to normal, phenytoin and PGBL alone treated group. The results obtained clearly proves that OSHAE and LEV improve learning and memory in mice and reverse the PGBL induced memory impairment with LEV having an upper hand in nootropic activity.

Table 2: Preliminary phytochemical screening of OSHAE

Chemical tests	Sub tests	Inference
Test for alkaloids	Wagner's test Mayer's test Dragendorff's test Hager's test	Positive Negative Positive Positive
Tests for Sterols	Salkowski tests Liebermann – burchard test	Negative Negative
Tests for triterpenoids	Salkowski tests Liebermann burchard test	Positive Positive
Tests for tannins	Ferric chloride test Gelatin test Vanillin - HCl test Match stick test	Positive Positive Positive Positive
Tests for flavonoids	Shinoda tests Ferric chloride test Lead acetate test Zinc – HCl test NaOH test	Positive Positive Positive Positive Positive
Test for Reducing sugar		Positive
Test for anthraquinone glycosides		Negative
Test for cardiac glycosides		Positive
Tests for Carbohydrates	Fehling's test Molisch's test	Positive Negative



Values are Mean ±SEM, n=6; a - Significant when compared with normal animals (P<0.05); b - Significant when compared with PGBL treated animals (P<0.05)

Figure 1: Memory impairment activity of PGBL and protective effect of OSHAE and LEV (Step-down Latency in seconds-SDL)

PGBL treated group showed a significant decrease in SDL by 51.24%, 65.34% 73.08%, and 78.15%, which is almost similar to the decrease in SDL value produced by phenytoin (whose memory impairment activity has been already proven)[17] which shows decrease in SDL by 33.46%, 62.71%, 71.70%, and 86.08% on 8th, 15th, 22nd and 29th day of treatment respectively when compared with the normal treated group.

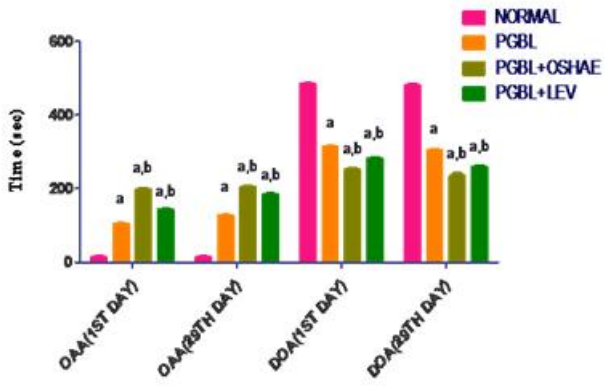
OSHAE increased SDL significantly by 145.18%, 210.37%, 251.85% and 350.37% while LEV treated group increased SDL by 178.64%, 307.76%, 393.20% and 567.96% on 8th, 15th, 22nd and 29th day of treatment when compared with the normal treated group (Figure.1). Co-administration of OSHAE and LEV orally for 29 days significantly reversed memory deficit induced by PGBL. The present study indicates that OSHAE and LEV possesses nootropic activity in view of its facilitator effect on retention of acquired learning.

These results supplements the recent findings that, PGBL was found to induce memory impairment when administered on MES induced epileptic mice, which can be reversed by co-administration of OSHAE and LEV.[5]

Anti convulsant activity

Time OAA and DOA were the parameters used to evaluate anticonvulsant activity of PGBL on PTZ induced convulsion. When PGBL was administered orally at a dose of 150mg/kg for 29 days to mice, it produced protection against PTZ induced convulsion as it increased the onset of convulsion and decreased duration of convulsions significantly when compared to normal animals. When OSHAE (200mg/kg) and LEV (400mg/kg) was co-administered with PGBL slight protection was observed which may be attributed to the synergistic activity of these anti-convulsant nootropics to PGBL. These findings prove that OSHAE and LEV when co-administered with PGBL produce no significant interaction with respect to the anticonvulsant activity (Figure 2). PRBL retains its

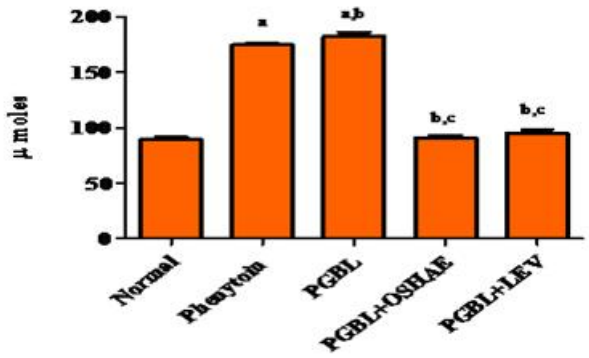
antiepileptic activity when co-administered with OSHAE & LEV, which is very much needed.



Values are Mean ±SEM, n=6; a-Significant when compared with Normal animals (P<0.05); b- Significant when compared with Pregabalin animals (P<0.05).

Figure 2: Anticonvulsant activity of PRBL in presence and absence of nootropics on mice by PTZ induced convulsions

It was found that with chronic treatment of PGBL for 29 days on PTZ induced convulsions in mice significantly reduced the duration of convulsion by 157.4% and delayed the onset of convulsion by 10.35% on 29th day when compared with normal treated group animal (Figure 2). When OSHAE and LEV were co-administered with PGBL, we observed the significant reduction in duration of action by 130.04% and 117.30% and delayed the onset of action of clonic convulsion by 61.51% and 67.98% respectively when compared to PGBL alone treated animals.



Values are Mean ±SEM, n=6; a - Significant when compared with Normal animals (P<0.05); b - Significant when compared with Phenytoin animals (P<0.05); c - Significant when compared with PGBL animals (P<0.05).

Figure 3: Memory impairment activity of PGBL and protective effect of OSHAE and LEV (Brain AChE levels)

Estimation of brain AChE levels

It was observed that administration of PGBL on PTZ induced convulsions resulted in a significant increase in AChE value 152.40±3.60 µmoles when compared to normal group. The co-administration of OSHAE and LEV with PGBL on PTZ induced convulsions, significantly decreased AChE value to 91.24±2.11 and 95.14±3.52 µmoles respectively. All the AChE values are comparable with phenytoin (an antiepileptic drug which has been proven to produce memory impairment in animal models) treated group in which a significant increase of AChE value of 174.70±1.60 µmoles was observed. This indicates that, low levels of acetylcholine in PGBL treated group, which was restored by co-administration of OSHAE and LEV.

CONCLUSION

The study thereby concludes that chronic administration of PGBL can induce memory impairment in mice when assessed by Step-down passive avoidance task. The extent or degree of memory impairment produced by PGBL was found to be similar to that of Phenytoin a standard antiepileptic drug which has been very well established for memory impairment as its adverse effect. Based on the findings in the study, OSHAE & LEV can be employed as anti-epileptic nootropics to correct the memory deficit induced by PGBL without compromising on its anti-epileptic activity. Co-administration of OSHAE has synergized the anti-epileptic activity of PGBL on PTZ induced epileptic mice. However further research on this combination study is a necessity to obtain an anti-epileptic therapy devoid of impaired memory as the adverse effect.

Acknowledgement: The authors sincerely thanks Dr. H.G. Shivakumar, Principal, JSS College of Pharmacy, Mysore, for his support and encouragement. Our gratitude goes to JSS University, Mysore, for providing all the necessary facilities.

REFERENCES

1. Adam Z, Narinder K, Marilyn J, Epilepsy and Memory, Oxford University Press, 397-405.
2. Joyce AC, Scott M, Adverse effects of antiepileptic drugs: a brief overview of important issues, Expert Review of Neurotherapeutics, 10(6), 2010, 885-891.
3. Joshi H, Parle M, Cholinergic basis of memory improving effect of *Ocimum tenuiflorum* Linn, Indian Journal of Pharmaceutical Sciences, 68(3), 2006, 364-365.
4. Jaggi RK, Madaan R, Singh B, Anticonvulsant potential of holy basil, *Ocimum sanctum* Linn. and its cultures, Indian Journal of Experimental Biology, 41(11), 2003, 1329-1333.
5. Asher John Mohan, Krishna KL, Jisham KM, Seema Mehdi, Ramesh B Nidavani, Protective effect of tulsi and levetiracetam on memory impairment induced by pregabalin on mice, IOSR Journal of Pharmacy and Biological Sciences, 9 (1), 2014, 46-52.
6. Ulloa CM, Towfigh A, Safdieh J, Review of levetiracetam, with a focus on the extended release formulation, as adjuvant therapy in controlling partial-onset seizures, Neuropsychiatric Disease and Treatment, 5, 2009, 467-476.
7. Internet search- Accessed on December 15th, 2012- http://www.pnas.org/content/suppl/2012/08/06/1121081109.DCS supplemental

8. Elaine KP, Michael C, David JD, Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease, Journal of Neurology, Neurosurgery and Psychiatry, 48, 1985, 413-421.

9. Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy. NiraliPrakashan: Pune, 1996.

10. Finar IL, Organic chemistry, Longman Scientific and Technical, London, 1975.

11. Trease GE, Evans WC, Pharmacognosy, Elsevier Health Sciences, London, 1989.

12. Geinssman TA, Flavanoids, In Modern Methods of Plant Analysis, Berlin: Springer Verlag, 1955.

13. Cromwell BT, Alkaloids. In Modern Methods of Plant Analysis, Berlin: Springer Verlag, 1955.

14. Joshi H, Parle M, *Zingiberofficinale*: Evaluation of its nootropic effect in mice, African Journal of Traditional Complementary and Alternative Medicines, 3(1), 2006, 64-74.

15. Kulkarni SK, Hand book of Experimental Pharmacology. 3rd edition. M K Jain, VallabhPrakashan, Delhi, 2005, 142-144.

16. Ellman GL, Courtney KD, Valentino A, Featherstone RM, A new and rapid colorimetric determination of Acetylcholin esterase activity, Biochemical Pharmacology, 7, 1961, 88-95.

17. Vohora D, Pal SN, Pillai KK, Protection from phenytoin-induced cognitive deficit by *Bacopa monniera*, a reputed Indian nootropic plant, Journal of Ethnopharmacology, 71(3), 2000, 383-390.

Source of Support: Nil, **Conflict of Interest:** None.