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EFFECT OF BACOPA ON MEMORY DEFICIT PRODUCED BY CHRONIC ADMINISTRATION OF TOPIRAMATE IN RATS

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ABSTRACT

Epilepsy is a most common disease mainly occurring in children and elderly patients. Cognitive disorders are common in patients, who are under the treatment of epilepsy. Topiramate is, one of the widely used anticonvulsants, is known to adversely affect cognitive function. In this context we have studied the memory deficit function of Topiramate on chronic administration in albino rats. Topiramate was administered for successive 14 days in rats. *Bacoppa monniera extract powder (BM) 20%* was co-administered along with topiramate from 8th day of treatment. Morris water maze was employed to evaluate learning and memory using parameter like Escape Latency Time (ELT), Time Spent in Target quadrant (TSTQ) and estimation of brain Acetyl cholinesterase level. Anticonvulsant activity of Topiramate was evaluated in presence of BM on PTZ induced convulsion and MES induced convulsion in rat model. Topiramate has significantly produced memory deficit in rats as it increases ELT, decreases TSTQ and increases AchE levels. When BM was given along with Topiramate from the 8th day of treatment, significantly reversed Topiramate induced impairment as it decreases ELT, increases TSTQ and decreases AChE levels. Even when BM co-administered with Topiramate, it did not interact with Topiramate and Topiramate retain its anticonvulsant activity. The results provide evidence for potential corrective effect of BM in cognitive deficit associated with TP.

Key words: Epilepsy, Topiramate, BM and Cognitive function

INTRODUCTION

Cognitive disorders are common in patients, who are under the treatment of epilepsy[1]. Nootropic agents are used to correct observed cognitive deficits. Topiramate (TP) is one of the widely used anticonvulsants[2]. It might be beneficial to assess the use of nootropic agents along with antiepileptic therapy for possible protection against cognitive deficits. In the Indian sub-continent, many plant based formulations are available which are used for memory enhancing properties. From these formulations *Bacoppa monniera* (BM) is the most reputed and already proven nootropic agent[3]. It has been extensively investigated and used for its neuropharmacological effects and a number of confirming reports are available for its nootropic action. It is interesting that BM has recently been reported to possess anticonvulsant activity[4]. In view of all the above reports, we chose to evaluate *Bacoppa monniera* extract powder 20 % on Topiramate induced cognitive dysfunction on rats.

In the present work, Topiramate was studied alone and in combination with BM for its cognitive deficit effects on rats in the Morris water maze task, which is a method for screening memory enhancing drugs[5] and in the Pentylenetetrazole (PTZ) and maximal-electroshock (MES)-induced convulsions[5,6] in rats. In addition, the effect of the drugs on brain AchE was also ascertained to rule out the level of Acetyl cholinesterase enzyme in brain[5].

Role of AchE in relation with memory impairment: Memory starts as a biochemical response in the brain following sensory input.

Acetylcholine is a well known neurotransmitter, plays a critical synaptic role in the initial formation of memory. A transient change in neurostransmitter levels at synapses can be resulted in a **short-term memory**. It has been reported that levels of acetylcholine are deficient in the brains of patients with Alzheimer's disease, and what little acetylcholine is produced is quickly broken down by the acetylcholinesterase (AchE) enzyme contributing to the loss of memory and other cognitive functions. Previous studies have demonstrated that acetylcholinesterase (AChE) promotes the assembly of amyloid-b-peptides into neurotoxic amyloid fibrils and is toxic for chick retina neuronal cultures and neuroblastoma cells. Moreover, AChE is present in senile plaques in Alzheimer's disease brains. Treatments for memory-deficit problems, like Alzheimer's, in humans often involve enhancing the retention of acetylcholine in brain synapses. Aricept (donepezil hydrochloride) is commonly used to prevent memory problems, acts by inhibiting the action of the enzyme in the brain that breaks down acetylcholine (acetylcholinesterase). People with moderate levels of dementia can show marked improvement with this sort of treatment[7-9].

MATERIALS AND METHODS

Animals and experimental design: Albino rats (150-200gm) were procured from Animal House, JSS Medical College, Mysore-15, and Karnataka, India. They were acclimatized for laboratory condition for 7 days and randomly divided into six groups each having six animals. The animals were housed under standard laboratory conditions and maintained under a 12-h light- dark cycle and had free access to drinking water and diet for eight weeks. Institutional Animal Ethical Committee approved all the procedures (proposal no 034/2009).

Treatment:

Group I: Vehicle 0.5% Na CMC (1ml/kg body wt.) was administered orally for fourteen successive days.

Group II: Topiramate (40 mg kg $^{-1}$) was administered orally for 14 successive days. MES was induced after 1 hour administration of Topiramate and durations of convulsion were noted

Group III: BM (40 mg kg $^{-1}$) and Topiramate (40 mg kg $^{-1}$) was administered orally before 2 hr and 1 hr respectively, before induction of MES-seizures for 14 days and duration of convulsion were noted. The same animals were used to assess the amnesic activity of Topiramate on Morris water maze model on 15th day.

Group IV: Vehicle 0.5% sodium CMC was administered orally for 14 days. PTZ (45 mg/kg body weight) was given after 1 hour administration of vehicle on 14th day

Group V: Topiramate (40 mg kg $^{-1}$) was administered orally for 14 days. PTZ (45 mg/kg body weight) was given after 1 hour administration of Topiramate on 14th day.

Group VI: Bacoppa monnari extract powder 20% (40 mg kg -1) and Topiramate (40 mg kg -1) was administered orally before 2 hr and 1 hr respectively, before administration of PTZ on14th day.

Memory deficit activity[10]: Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days, during which they were allowed to escape on to the hidden platform and to remain there for 20 seconds. If the rat failed to find the platform within 120 sec, it was guided gently on to the platform and allowed to remain there for 20 sec. Escape latency time (ELT) was defined as the time taken by the animal to locate the hidden platform. ELT was noted as an index of learning.

Rat was placed in water maze and allowed to explore the maze for 120 sec. Mean time spent in all the three quadrants i.e. Q1, Q2 and Q3 was recorded and the time spent in the target quadrants (TSTQ) in search of the missing platform provided as an index of retrieval. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory. Both ELT and TSTQ were recorded on 15th day of the experimentation.

Estimation of Brain AChE Activity[11]: On the 15th day 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[12]. The end point was the formation of the yellow colour because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5-dithionitrobenzoic acid (DTNB), and the optical density (OD) of the yellow colour compound formed during the reaction at 420 nm every minute for a period of 3 min was measured.

Protein estimation was done using semi Auto analyzer (Micro lab 300).

Maximal-electroshock seizures (MES)[13]: Tonic and clonic convulsions were induced by giving MES (150 mA for 0.2 s) using an electroconvulsometer via crocodial ear electrodes. Different stages of convulsions (a) tonic flexion, (b) tonic extensor, (c) clonic convulsions, (d) stupor, (e) recovery or death are noted and time spent by the animal in each phase of the convulsions is also noted.

Pentylenetetrazole induced seizures[13]: Clonic convulsions were induced by administering PTZ (45mg/kg) i.p. on 14th day. BM as well Topiramate were given orally 2hr and 1 hr before administration of PTZ respectively. Onset of convulsion and duration of convulsion were evaluated.

Drugs and Chemicals: Topiramate (Unichem Laboratories Ltd. UP). Bacoppa Monniera (Indfrag Ltd. Bangalore). PTZ (Sigma Chemical Company, USA). DTNB (Sigma Aldrich, USA). Acetylcholine iodide (Himedia, Mumbai).

Data Analysis: Data in the manuscript are expressed as Mean ± SEM. Comparison between groups were made using one-way ANOVA. Statistical significance was assumed at $P< 0.001$.

RESULTS

The effects of BM on Topiramate induced memory impairment are presented in Table 1. The memory improvement effect of BM was assessed by using *in vivo* model of Topiramate induced memory deficit in rats by Morris water maze. Escape Latency Time (ELT), Time Spent Target Quadrant (TSTQ) and total brain Acetyl cholinesterase activity were the parameters used to assess the nootropic activity. It was observed that when Topiramate (40 mg/kg) administered orally, it has significantly increased ELT as compared to the normal group (Fig 1). It was observed that administration of BM along with Topiramate resulted in a significant decreased ELT as compared to the Topiramate treated group.

The Time Spent in Target Quadrant was the one more parameter considered for evaluating memory improving activity of BM. It was observed that chronic administration of Topiramate resulted in a significant decreased TSTQ as compared to the normal group (Fig 2).

When BM co-administered with Topiramate resulted in a significant increase in TSTQ as compared to the Topiramate treated group. (Table 1) In this study we have determined the level of AChE in the whole brain homogenate of all group animals, which was used to assess the memory improving activity (Table 1). It was observed that administration of Topiramate resulted in a significantly increased AChE as compared to the normal group (Fig 3).

When the BM was co-administered with Topiramate, it has significantly decreased AChE as compared to the Topiramate treated group. This study clearly shows that, BM is having potent memory improving activity on Topiramate induced cognitive impairment in rats.

The anticonvulsant effect of Topiramate is presented in Table 2 and 3. The anticonvulsant activity was assessed by using *in vivo* model of Maximal Electroshock induced seizures and Pentylenetetrazole induced seizures in rats. Flexion, extension, clonus, stupor, death or recovery parameters were evaluated in MES induced convulsion.

When Topiramate was administered orally, significantly produced 100% protection in extension phase and decreased the duration of stupor as compared to normal group on MES convulsion. (Table 2) It was observed that when BM administered along with Topiramate, resulted significant protection in extension phase and decreased duration of stupor when compared to normal.

Pentylenetetrazole induced seizures in rats is one more parameter used to assess the anticonvulsant activity of Topiramate in presence BM. Onset of convulsion and duration of convulsion parameters are evaluated in PTZ model to assess the anticonvulsant activity.

It was shown that when Topiramate was administered, significantly produces 100% protected against PTZ induced convulsion when compared to normal animals (Table 3). It was shown that when BM was co-administered with Topiramate resulted similar effect to that of Topiramate treated group.

DISCUSSION

Epilepsy is the most common disease generally occurring in children and aged people. Cognitive impairment is frequently observed in children with epilepsy syndromes. Topiramate causing memory deficit in patients by mechanism that they act by potentiating GABA and antagonizing glutamate receptor at the N-methyl-D aspartate (NMDA)

receptors. Their potentiating action at the level of neurotransmission system of GABA produces inhibitory action in the frontal lobe.

Nootropic herbs can be used for improvement of memory deficit caused by anticonvulsant drugs and being used by physicians. Many well known brain tonics such as *Albizia lebbeck*[14], Ocimum sanctum[15], Vitis Vinifera[16], *Bacopa monnieri*[17], *Centella asiatica*[18], *Withania somnifera*[19], *Acorus calamus*[20] and *Celastrus paniculatus*[21] are beneficial in cognitive disorders.

In order to investigate the beneficial effects of BM on TP -induced memory deficit we used Morris water maze apparatus. The results of the present study showed that Topiramate has significantly increased the ELT by 733% and decrease TSTQ by 56.28% compare to normal group that adversely affected cognitive impairment in the MWM task (Table 1). These findings reconfirm the well documented memory-impairing effects of TP.

When BM was given along with Topiramate significantly reversed ELT by 611.11% and TSTQ by 42% when compared to Topiramate. (Table 1) BM is known to have nootropic effects on acquisition and retention of memory in varied experimental models[22]. The results obtained by this study on MWM task are in agreement with these reports.

It was observed that administration of Topiramate resulted in a significant increased AChE level as compared to the normal group. When the BM was co-administered with Topiramate, has significantly decreased AChE as compared to the Topiramate group. These results also support the beneficiary affect of nootropic herbs on Topiramate induced memory loss.

In the present study we have evaluated anticonvulsant activity of TP in presence of nootropic herbs in both type of epilepsy- grandmal epilepsy (MES induced) and petitmal epilepsy (PTZ induced).

Some recent studies report anticonvulsant effects of BM against MES induced seizures. We found the potentiating effects in BM against MES as it has reduced the extension phase and stupor duration by 10% compared to TP alone (Table 2).

It was observed that administration of BM along with Topiramate did not show any inhibition or potentiating of anticonvulsant activity on PTZ induced convulsions.

These results suggest that BM can be systematically used as an add-on therapy for improving the cognitive functions of patients being treated with TP. The precise mechanism by which BM elicits its nootropic effects is probably that the sulphydryl and polyphenol components have shown to impact the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoxygenase activity and reducing divalent metals.

It lowers norepinephrine (NE) and increases 5-hydroxytryptamine (5HT) levels in the hippocampus, hypothalamus and cerebral cortex. BM may thus modify ACh concentrations. Further, BM has been shown to enhance protein kinase activity, which could also contribute to its nootropic action[23].

CONCLUSION

In the present study we found that Topiramate showed significant memory deficit activity on Morris Water Maze as experimental animal model. Three parameters were used for evaluation, ELT, TSTQ and brain AChE activity. In all parameters Topiramate shows memory deficit activity. But when BM was co-administered along with Topiramate, has significantly reversed memory deficit caused by Topiramate. Further study also demonstrates that BM does not interact dynamically with Topiramate as it does not influence/inhibit the anticonvulsant activity of Topiramate so this drug can be used as add on therapy with other anticonvulsant drug to improve antiepileptic drug- induced cognitive impairment.

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Table 1: Effect of BM on Topiramate induced memory deficit in rats.
(ELT, TSTQ, AChE)

Sl .no	Group	ELT (in seconds)	TSTQ (in seconds)	AChE levels (µ moles)
1	Normal	9±0.40	66±0.05	124.93±0.66
2	TP	75±1.08[a]	28.85±0.66[a]	211.76±1.00[a]
3	TP + BM 20%	20±0.81[b]	57.1±0.05[b]	159.03±0.33[b]

Values are Mean ±SEM, n=6,
[a]p< 0.001 significantly increased ELT when compared to normal group.
[b]p<0.001 significantly improved ELT, compared to TP treated group.
[a]p<0.001 significantly decreased the TSTQ when compared to normal group.
[b]P<0.001 significantly improved TSTQ when compared to Topiramate treated group.
[a]p<0.001 significantly increased the AChE level when compared to normal group.
[b]p<0.001 significantly decreases AChE level compared to Topiramate treated group.

Table 2: Anticonvulsant activity of Topiramate in presence of BM
(Maximal Electro Shock induced convulsions)

Sl no.	Group	Duration (in second)			
		Flexion	Extension	Clonus	Stupor
1	Normal	1.22±0.02	15.0±0.40	18.3±0.04	240.94±0.00
2	TP treated	1.4±0.02	0.00±0.00[a]	16.8±0.02	100.19±0.03[a]
3	TP + BM	1.6±0.08	0.00±0.00[b]	17.0±0.20	90.12±0.02[b]

Values are Mean ±SEM, n=6,
[a]p<0.001 significantly abolishes the extension phase and reduced duration of stupor compared to normal.
[b]p<0.001 significantly abolishes the extension phase and reduced duration of stupor compared to TP treated.

Table 3: Anticonvulsant activity of Topiramate in presence of BM
(PTZ induced convulsions)

Sl no	Drug	Onset of convulsion (sec)	Duration of convulsion (sec)	% mortality
1	PTZ Control	90.666±4.889	650.666±24.493	33.33%
2	TP	0.00±0.00[a]	0.00±0.00[a]	0.00%
3	TP + BM 20%	0.00±0.00[a]	0.00±0.00[a]	0.00%

Values are mean ±SEM, n=6,

[a]p<0.001. Significantly given 100% protection when compared to normal group

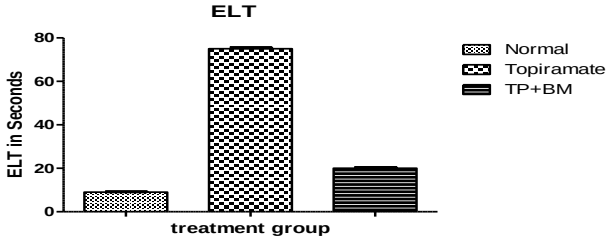

Figure 1: Effect of BM on Topiramate induced memory deficit in rats (Escape Latency Time)

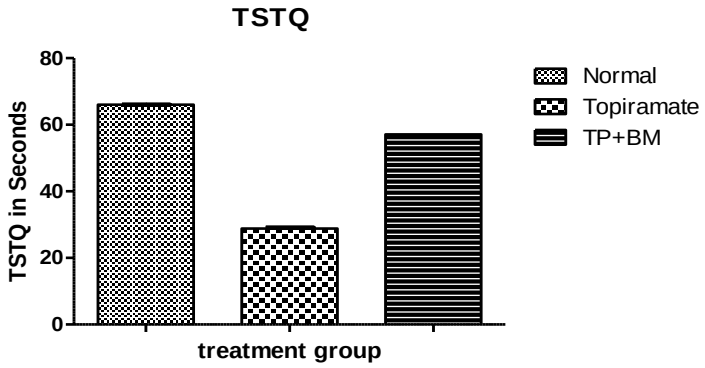

Figure 2: Effect of BM on Topiramate induced memory deficit in rats
(Time Spent Target Quadrant)

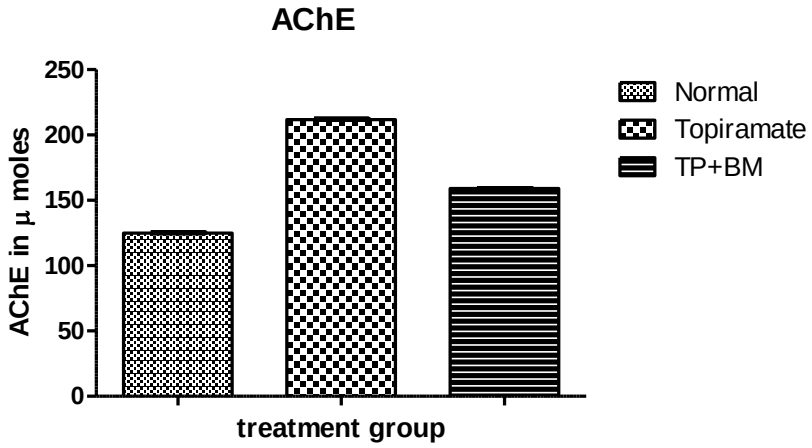

Figure 3: Effect of BM on Topiramate induced memory deficit in rats
(Brain AChE levels µ moles)

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