RESEARCH ARTICLE

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Evaluation of memory impairment and anti-epileptic activity of carbamazepine alone and in presence of herbal nootropic

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ABSTRACT
The present study was aimed to assess the memory impairment activity of Carbamazepine (CBZ) in presence and absence of *Nardostachys jatamansi.* Memory impairments are common side effects in epileptic patients and antiepileptic drugs can worsen memory impairment. Nootropic drugs may be of potential in reducing the memory impairment when given along with antiepileptic drugs. Therefore ethanolic extract of *Nardostachys jatamansi* (ENJ) was prepared and standardized by preliminary phytochemical tests, determination and total phenolic and flavonoid content and *in-vitro* antioxidant activities like DPPH, Super oxide (SO), Hydroxyl (OH), Nitric oxide (NO) and Hydrogen peroxide (H_2O_2) scavenging activity. CBZ was administered orally for 29 days in rats to assess the memory impairment ability by employing Barne's maze on PTZ induced convulsions. ENJ exhibited potent free radical scavenging activity when evaluated by *in-vitro* methods. CBZ increased Escape Latency Time (ELT), number of errors and decreased Time spent in Target Quadrant (TSTQ) when compared to vehicle treated group in rat indicating memory impairment due to its chronic administration. When ENJ was given along with CBZ significant decrease in memory impairment induced by CBZ was observed. The anticonvulsant activity of CBZ was found to be potentiated due to the co-administration of ENJ when compared to vehicle and CBZ alone treated animals. The AChE levels in CBZ were found to increase when compared to vehicle, where as co-administration of ENJ produced a decrease in AChE levels when compared to vehicle and CBZ treated group. The combination of reduced dose of CBZ with ENJ was also shown decrease in memory impairment without altering its anticonvulsant activity. Findings of the present study supplements the above fact, however further research is required.

Key words- Carbamazepine, *Nardostachys jatamansi,* acetyl-cholinesterase, Memory impairment.

Introduction

Epilepsy is a neurological condition that in different times produces brief disturbances in the electrical functions of the brain. It refers to chronic condition characterized by recurrent seizures [(1)]. These seizures are episodes that can vary from brief and nearly undetectable to long periods of vigorous shaking [(2)]. Epilepsy affects 0.5-1% population and nearly 80% cases occur in developing countries [(3)]. The cause of epilepsy in most of the cases is unknown[(4)], although some may develop as a result of brain injury, stroke, brain tumor, drugs, misuse of alcohol, genetic mutations (rare case)[(5)]. The main symptom of epilepsy is seizures. The most common type (60%) of seizures are convulsive [(4),(6)]. Antiepileptic drugs (AED'S) also known as anticonvulsant drugs/ anti-seizure drugs are a diverse group of pharmacological agents to treat epilepsies. Memory impairment is a frequently occurring secondary consequence of epilepsy. CBZ is dibenzazepine derivative which has been primarily employed in seizures and this in turn produces memory impairment. Ethanolic extract of *Nardostachys jatamansi* (ENJ) was found to be a potent anticonvulsant nootropic based on previous findings [(4)].Various studies indicate that the increase in brain AchE levels increase the degree of memory impairment when assessed by tests of memory and information [(3)].Based on the assembled literature this study was concerned to evaluate the memory impairment potential of CBZ when administered to rat and the

protective effect of ENJ on CBZ induced memory impairment upon co-administration.

Methods:

Animals: Albino rat of either sex (150-250 g) procured from the Central Animal House Facility of JSS Medical College, Mysuru were used for the study. The animals were housed in polypropylene cages at 23–27°C with a natural light-dark cycle. The rats were fed on a standard mice pellet diet and water *ad libitum.* The animals were allowed to acclimatize to laboratory conditions for a week period before the start of the experiment. All the experiments were in accordance with the approval of Institutional Animal Ethics Committee (IAEC) of JSSCP Mysuru; the project number is 158/2014.

Drugs and plant extract: Carbamazepine was obtained as gift samples from Novartis India Ltd, Bengaluru. The roots of *Nardostachys jatamansi* (NJ) were procured from Swadeshi ayurveda bhandar, Udupi, Karnataka as gift samples.

Preparation and standardization of the extract: A powdered root of NJ was extracted using soxhlet extraction method using ethanol (ENJ). The cooled liquid extract was then concentrated by evaporating its liquid contents in rotary flash evaporator, with an approximate yield of 10%. It was then stored in the desiccator. The preliminary phytochemical screening was carried out on ENJ in order to find out the presence of phytochemical constituents [7-10] and total phenolic content &total flavonoid content [10] were determined.

***In-vitro* antioxidant and free radical scavenging activity of ENJ:** The ENJ was subjected to different *in-vitro* antioxidant methods, DPPH [11], super oxide [12], hydroxyl radical [13], nitric oxide [14] and hydrogen peroxide scavenging assay[15].

***In-vivo* activities:**

Memory impairment activity of CBZ by Barne's maze (BM) [16][17]:

The PTZ induced epileptic animals rats were used for the BM task. On 0th, 8th, 15th, 22nd and 29th day of treatment, the animals were evaluated for the memory impairment caused by CBZ. Then on 30th day, animals were sacrificed and the acetylcholiesterase (AChE) activity was estimated in brain homogenate. Before the start of the experiment habituation (Pre training trial) was given to rats for one week and the animals having the ability to learn this task were chosen for the study. Then acquisition and probe trial were performed to evaluate the escape latency time (ELT), number of errors and Time spent in target quadrant (TSTQ). The grouping of animals and evaluation was done as shown in Table 1.

Table 1.Memory impairment activity of CBZ by Barnes Maze model on PTZ induced convulsions. (Treatment schedule)

Group	Treatment	Evaluation
CONTROL+PTZ	Vehicle (0.5% NaCMC) was administered orally for 29 days and convulsions induced by PTZ method weekly.	Duration of convulsion induced by PTZ method was noted on 1st and 29th day and memory impairment by Barnes maze was recorded on 0th, 8th, 15th, 22th and 29th day.
PHENYTOIN+PTZ	Phenytoin (25 mg kg -1) was given as suspension in vehicle orally for 29days and convulsions induced by PTZ method weekly.	-do-
CBZ + PTZ	CBZ (20mg kg -1) was given as suspension in vehicle orally for 29 days and convulsions induced by PTZ method weekly.	do-
CBZ1/2+PTZ	CBZ 1/2 dose (10mg kg -1) was given as suspension in vehicle orally for 29 days and convulsions induced by PTZ method weekly.	do-
CBZ+ENJ+ PTZ	CBZ and ENJ (200mg kg-1) was given as suspension in vehicle orally at the interval of 2 hr and 1 hr respectively for 29 days and convulsions induced by PTZ method weekly.	do-
CBZ1/2+ENJ + PTZ	CBZ 1/2 dose and ENJ (200mg kg-1) was given as suspension in vehicle orally at the interval of 2 hr and 1 hr respectively for 29 days and convulsions induced by PTZ method weekly.	-do-
ENJ+MES	ENJ (200mg kg -1) was given as suspension in vehicle was orally for 29 days and convulsions induced by PTZ method weekly.	-do-

Evaluation of anticonvulsant activity[18]

Anticonvulsant potential of CBZ in presence and absence of ENJ was assessed on 1st and 29th day. Time before onset of clonic convulsions, duration of convulsion and the percentage of mortality were recorded (Table 1).

Estimation of brain acetyl cholinesterase activity[19]**:**

The whole brain homogenate was used to determine the AChE enzyme by Ellman method. On 30th day, the rats were decapitated and the brain homogenate was prepared and used.

RESULTS:

Preliminary phytochemical screening: Preliminary phytochemical analysis of ENJ revealed the presence of alkaloids, sterols, flavonoids, triterpenoids, tannins and reducing sugar.

The phenolic and flavonoid content of ENJ give in the Table 2.

Table 2.Total phenolic and total flavonoid content of ENJ

Extract	Total phenolic gm/mg gallic acid	Total flavonoid gm/mg quercetine
ENJ	45.25±0.0006	40.92±0.004

Values are Mean±SEM, n=3

***In-vitro* antioxidant and free radical scavenging activity of ENJ:**

DPPH radical scavenging assay: Free radical scavenging activity of ENJ by DPPH radical scavenging assay is shown in Table 3. ENJ was found to be a potent scavenger of DPPH radical with the IC_{50} of 52.81±0.17 µg/ml when compared to ascorbic acid, used as standard which showed IC_{50} value of 03.07±0.04 µg/ml.

Superoxide anion radical scavenging (SO) assay: Free radical scavenging activity of ENJ by Superoxide anion radical scavenging assay is shown in Table 3. ENJ was found to be a potent scavenger of superoxide anion radical with the IC_{50} of 390.37±0.09 µg/ml value when compared to ascorbic acid, used as standard which showed IC_{50} value of 60.51±0.13 µg/ml.

Hydroxyl radical scavenging assay: Free radical scavenging activity of ENJ by hydroxyl radical scavenging assay is shown in Table 3. ENJ was found to be a potent scavenger of hydroxyl radical with the IC_{50} of 709.7±0.23 µg/ml. Ascorbic acid, used as standard showed IC_{50} value of 65.57±0.21 µg/ml.

Nitric oxide scavenging assay: ENJ was found to be a potent scavenger of hydroxyl radical with the IC_{50} of 628.57±0.14 µg/ml. Ascorbic acid, used as standard showed IC_{50} value of 66.06±0.19 µg /ml. (Table 3).

Hydrogen peroxide scavenging assay: Free radical scavenging activity of ENJ by hydrogen peroxide scavenging assay is shown in Table 3. ENJ was found to be a potent scavenger of hydroxyl radical with the IC_{50} of 296.05±0.11µg/ml when compared to ascorbic acid, used as standard which showed IC_{50} value of 09.35±0.7µg/ml.

Table 3. Antioxidant activity of ENJ and ascorbic acid (IC_{50} values in µg/ml)

Methods	IC_{50} values (µg/ml concentration)	
	ENJ	Ascorbic acid
DPPH method	52.81±0.17	03.07±0.04
Super oxide anion method	390.37±0.09	60.51±0.13
Hydroxyl radical method	709.7±0.23	65.57±0.21
Nitric oxide method	628.57±0.14	66.06±0.19
Hydrogen peroxide method	296.05±0.11	09.35±0.7

Values are Mean±SEM, n=3

***In-vivo* activities:**

Evaluation of memory impairment activity of Carbamazepine by Barnes maze:

CBZ induced memory impairment and the protective effect of ENJ is represented in Table 4, 5 and 6. Escape latency time (ELT), number of errors, time spent in target quadrant (TSTQ) and whole brain acetyl cholinesterase activity were the parameters used to assess the memory impairment potential of CBZ.

Table 4. CBZ induced Memory deficit and protective effect of ENJ in rat (Escape Latency Time in seconds)

Group	Day 0	Day 8	Day 15	Day 22	Day 29
Control	10.32±0.57	16.58±0.18	21.32±.20	24.11±0.60	32.48±0.09
PHT	11.32±0.53[a]	20.72±0.27	31.35±0.10[a]	43.86±0.29[a]	51.22±0.5[a]
CBZ	11.056±0.65[a]	18.60±0.79	27.29±0.09[a,b]	39.28±0.08[a]	48.32±0.22[a,b]
CBZ½	10.54±0.9[a,b]	17.21±0.51[a,b]	19.51±0.13[a,b]	22.70±0.24[a,b]	30.61±0.62[a,b]
ENJ	12.01±0.82	11.34±0.39[a,b]	09.56±0.02[a,b]	07.62±0.45[a,b]	04.08±0.088[a,b]
ENJ+CBZ	12.52±0.69	11.78±0.7[a,b]	10.45±0.09[a,b]	08.91±0.06[a,b]	05.89±0.50[a,b]
ENJ+CBZ½	12.32±0.65[a]	10.34±0.39[a,b]	08.49±0.015[a,b]	06.13±0.17[a,b]	04.34±0.043[a,b]

Values are Mean ±SEM, n=6, Statistical analysis – Two way ANOVA

a - Significant when compared with control animals (P<0.05)

b - Significant when compared with phenytoin treated animals (P<0.05)

In this study the day 0 of respective group (basal reading) was considered as the normal group and it was observed that when CBZ (20 mg/kg) administered orally for 29 days on rat with PTZ induced convulsions, there was a significant increase in ELT (48.02±0.22 seconds) when compared to the control group (10.32±0.57 seconds) as shown in Table 4. Memory impairment of CBZ was found to be almost similar to PHT (an antiepileptic drug that has been proven to produce memory impairment in animal models).It was observed that administration of CBZ half dose 10mg/kg along with ENJ with PTZ induced convulsions resulted in a significant decrease in ELT of 4.348±0.043 seconds on 29th day when compared to the CBZ, PHT and control group. Administration of ENJ at the dose 200mg/kg along with CBZ with PTZ induced convulsions resulted in a significant decrease in ELT value (5.89±0.50 seconds)when compared to the CBZ, PHT as well as control group on 29th day. The memory impairment induced by CBZ was found to be dose and duration dependant. Finally it has been proven that ENJ has shown improvement in learning and memory in rat and reversed the CBZ induced memory impairment.

Table 5. Effect of herbal nootropic- ENJ on CBZ induced memory deficit in rat
(Total number of errors)

Group	Day 0	Day 8	Day 15	Day 22	Day 29
Control	06.66±0.49	08.66±0.41	09.66±0.31	10.67±0.45	11.67±0.41
PHT	07.5±0.61	11.50±0.4[a]	15.5±0.3[a]	19.50±0.4[a]	23.50±0.39[a]
CBZ	08.50±0.12[a]	09.50±0.1[b]	12.50±0.42[a,b]	15.50±0.32[a,b]	18.50±0.52[a,b]
CBZ½	08.83±0.5[a]	09.83±0.13[b]	11.83±0.23[a,b]	13.8±0.41[a,b]	15.84±0.09[a,b]
ENJ	08.50±0.06[a]	05.50±0.24[a,b]	04.50±0.52[a,b]	03.50±0.55[a,b]	01.83±0.61[a,b]
ENJ+CBZ	08.06±0.08[a]	07.16±0.23[a,b]	06.16±0.11[a,b]	05.16±0.71[a,b]	04.16±0.04[a,b]
ENJ+CBZ½	10.17±0.32[a]	07.00±0.61[a,b]	06.02±0.13[a,b]	05.00±0.07[a,b]	04.00±0.07[a,b]

Values are Mean ±SEM, n=6, Statistical analysis- Two way ANOVA
a - Significant when compared with control animals (P<0.05)
b - Significant when compared with phenytoin treated animals (P<0.05)

It was observed that when CBZ (20 mg/kg) administered orally for 29 days on rat with PTZ induced convulsions, there was a significant increase in number of errors (18.50±0.52 seconds) when compared to the control group (6.66±0.49 seconds) as shown in Table 5 on 29th day. It was observed that administration of ENJ at the dose 200mg/kg along with CBZ with PTZ induced convulsions resulted in a significant decrease in error value (4.16±0.04seconds)when compared to the CBZ, PHT as well as control group. It was observed that administration of CBZ half dose 10mg/kg along with ENJ with PTZ induced convulsions resulted in a significant decrease in ELT of 4.00±0.07 seconds when compared to the CBZ, PHT and control group. The memory impairment induced by CBZ was found to be dose and duration dependant. Finally it has been proven that ENJ has shown improvement in learning and memory in rat brain and reversed the CBZ induced memory impairment.

Table 6. Effect of herbal nootropic- ENJ on CBZ induced memory deficit in rat
(Time Spent in Target Quadrant)

Group	Day 0	Day 8	Day 15	Day 22	Day 29
Control	83.61±0.11	79.18±0.23	74.45±0.81	69.02±0.21	63.09±0.04
PHT	76.61±0.08[a]	63.44±0.21[a]	50.27±0.14[a]	37.10±0.27[a]	23.93±0.09[a]
CBZ	71.91±0.13[a]	66.04±0.61[a]	54.17±0.32[a]	42.30±0.31[a,b]	30.43±0.05[a,b]
CBZ ½	79.30±0.12[a]	68.44±0.71[a,b]	57.68±0.02[a,b]	46.92±0.62[a,b]	36.16±0.21[a,b]
ENJ	77.24±0.33[a]	80.00±0.12[b]	83.76±0.41[a,b]	87.89±0.16[a,b]	93.65±0.02[a,b]
ENJ+CBZ	76.79±0.26[a]	77.86±0.11[b]	79.93±0.05[a,b]	82.40±0.17[a,b]	85.47±1.002[a,b]
ENJ+CB ½	77.83±0.08[a]	79.26±0.32[b]	81.69±0.26[a,b]	84.12±0.05[a,b]	86.550±0.35[a,b]

Values are Mean ±SEM, n=6, Statistical analysis- Two way ANOVA
a - Significant when compared with control animals (P<0.05) b - Significant when compared with phenytoin treated animals (P<0.05)

The time spent in target quadrant (TSTQ) is another parameter recorded for evaluating memory deficit property of CBZ and its improving activity due to co-administration of ENJ. It was observed that chronic administration of CBZ in rat for 29 days with PTZ induced convulsions resulted in significant decrease in TSTQ to 30.43±0.05 seconds when compared to the control group of 83.61±0.11 seconds. Memory impairment of CBZ was found to be almost similar to PHT in this parameter (an antiepileptic drug that has

been proven to produce memory impairment in animal mode). Co-administration of ENJ extract at the dose of 200 mg/kg with CBZ on MES induced convulsions resulted in a significant increase in TSTQ of 85.47±1.002 seconds when compared to the CBZ treated group (Table 6). ENJ alone group showed significant increase in TSTQ 93.65±0.02 seconds when compared to normal and CLZ. This shows that, the ENJ has potent memory improving activity on CBZ induced memory deficit. The memory impairment induced by CBZ was found to be dose and duration dependant. The results hence prove the ability of ENJ in reducing the memory deficit produced by anticonvulsant drug CBZ.

Anticonvulsant activity by PTZ method:

Table 7. Anticonvulsant activity of CBZ in presence and absence of ENJ on rat by PTZ induced convulsions (Onset of action (OOA) and Duration of action (DOA) in seconds)

Day	1st day		29th day	
Group	**OAA**	**DOA**	**OAA**	**DOA**
Control	014.88±0.31	302.2±1.23	011.21±0.51	310.09±0.31
PHT	128.10±1.06[a]	180.1±1.02[a]	147.10±0.98[a]	153.08±1.03[a]
CBZ	145.8±0.23[a,b]	157.2±0.52[a,b]	168.02±1.19[b]	121.5±0.62[a,b]
CBZ½	136.5±0.43[a,b]	176.5±1.42[a,b]	155.9±1.04[a,b]	140.1±0.01[a,b]
ENJ	140.0±1.06[a,b]	164.12±0.1[a,b]	159.30±0.9[a,b]	132.7±1.03[a,b]
ENJ+CBZ	176.8±0.24[a,b]	106.90±0.4[a,b]	216.4±0.11[a,b]	33.56±0.05[a,b]
ENJ+CBZ½	160.0±0.31[a,b]	135.5±0.33[a,b]	195.01±1.7[a,b]	72.91±0.51[a,b]

Values are Mean ±SEM, n=6, Statistical analysis- Two way ANOVA

a - Significant when compared with control animals (P<0.05) b - Significant when compared with phenytoin treated animals (P<0.05)

The anticonvulsant effect of CBZ is presented in Table 7. When CBZ was administered at a dose of 20mg/kg for 29 days orally to rat, it produced protection against PTZ induced convulsion as it increased the onset of convulsion and decreased the duration of convulsions when compared to control animals by 168.00±1.005seconds and 121.5±0.03seconds respectively. Additionally when ENJ was co-administered with CBZ increased the onset of convulsion (216.40±0.11) and decreased the duration of convulsions (33.56±0.07) when compared to PHT and CBZ treated animals which can be attributed to the synergistic activity of ENJ to CBZ. So it was proven that the nootropic herb ENJ when co-administered with CBZ produced no significant interaction with respect to the anticonvulsant activity.

Estimation of brain acetyl cholinesterase (AChE) activity:

Table 8.Estimation of brain AChE levels in PTZ model

Group	AChE levels in µmoles/mg protein
Normal	10.54±0.24
Control	15.04±0.19[a]
PHT	20.73±0.15[a,b]
CBZ	17.84±0.117[a,b,c]
CBZ1/2	15.86±0.21[a,b,c]
ENJ	07.64±0.13[a,b,c]
ENJ+CBZ	10.12±0.43[a,b,c]
ENJ+ CBZ1/2	09.08±0.217[a,b,c]

Values are Mean ±SEM, n=6, Statistical analysis- One way ANOVA

a - Significant when compared with Normal treated animals (P<0.05)

b - Significant when compared with Phenytoin treated animals (P<0.05)

c- Significant when compared with Carbamazepine treated animals (P<0.05)

The brain acetylcholine level is responsible for memory and level of acetylcholine and depends on the activity of metabolizing enzyme acetylcholine esterase (AChE). In this study we have determined the level of AChE in whole brain homogenate of all animal groups (Table 8). It was observed that administration of CBZ on PTZ induced convulsions resulted in a significant increase of AChE value 17.84±0.11 µmoles/mg protein. The co-administration of ENJ with CBZ on PTZ induced convulsions at the dose of 200mg/kg, significantly decreased AChE value to 10.12±0.43 µmoles/mg protein. Similarly ENJ on PTZ induced convulsions at the dose of 200mg/kg significantly decreased AChE value to 7.64±0.20 µmoles/mg protein. All the AChE values are comparable with PHT (an antiepileptic drug which has been proven to produce memory impairment on animal models) in which a significant increase of AChE value of 20.73±0.15µmoles/mg protein was

observed. Thus it was confirmed that ENJ can reverse or minimize the adverse effect on memory impairment by CBZ.

Discussion:

Epilepsy refers to a disorder that is best viewed as a symptom of disturbed electrical activity in the brain caused by a variety of etiologist. Epilepsy can cause impaired cognition. Many factors contribute to this impairment, including the adverse effects of AEDs [20]. The main side effect of CBZ is memory impairment which accounts for about 90%[21]. The exact mechanism of action of CBZ in causing cognitive impairment is unknown. The probable mechanism may be it inhibits release of endogenous glutamate evoked by 4-aminopyridine in hippocampal synaptosomes. It may also decrease the level of acetylcholine in brain[22]. Herbs having both inotropic as well as anticonvulsant activity can be used for improvement of memory impairment caused by anticonvulsant drugs as well potentiate the anticonvulsant activity of the CBZ.

By preliminary phytochemical screening it was found that the ENJ contain alkaloids, sterols, triterpenoids, tannins and flavonoids which may have been responsible for the observed antioxidant activity. These compounds are known to be biologically active and therefore could be responsible for their therapeutic effect.

DPPH is usually used as a substrate to evaluate anti-oxidative activity of antioxidant. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non-radical form DPPH by the reaction. The extract was able to reduce the stable radical DPPH to yellow colored diphenyl picryl hydrazine [12]. ENJ has shown potent free radical scavenging activity by DPPH method and it was found to be dose dependent. Superoxide anion is a weak oxidant it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Numerous biological reactions generate superoxide anions which are highly toxic species [13]. Scavenging activity of ENJ has shown potent activity when compared to ascorbic acid. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to the cell, the model used is ascorbic acid- iron- EDTA model of OH generating system. This is totally aqueous system in which ascorbic acid, iron and EDTA conspire with each other to generate hydroxyl radicals [13]. In this study, ENJ shows better dose dependent prevention towards generation of hydroxyl radicals with IC_{50} of 709.57±0.23µg/ml. NO is a very unstable species under the aerobic condition. It reacts with oxygen to produce stable products. In the presence of test compound the amount of nitrous acid will decrease [15]. In this study, ENJ shows better dose dependent prevention towards generation of nitrite ion radicals. The extract was examined for its ability to act as OH radical scavenging agent. The hydroxyl free radical generated from hydrogen peroxide is scavenged by antioxidant compound which leads to decrease in absorbance which is measured at 230 nm. Best scavenging potential was shown by ENJ and the activity was found to be dose dependent.

In Barne's maze result showed that CBZ 20 mg/kg p.o. when administered for 29 days along with PTZ induced convulsions, it significantly increased the ELT by 68.23%, 146.83%, 255.28% and 337.05% while decreasing TSTQ by 8.16%, 24.67%, 41.18%, 57.68% and also increasing the number of errors by 11.76%, 47.06%, 82.35% and 113.85% on 8th, 15th, 22nd and 29th day of treatment respectively and when compared to control treated group the ELT was increased significantly by 10.23%, 28.00%, 62.92% and 48.77% while the TSTQ was decreased significantly by 16.60%, 27.24%, 38.71%, 51.77% and the number of errors were increased by 9.70%, 29.40%, 45.27% and 58.53%. The result clearly demonstrates that, the CBZ adversely affected cognitive impairment in the Barne's maze task in rat. When ENJ was given alone it significantly decreased ELT by 5.58%, 20.40%, 36.55%, 66.03% and number of errors by 36.49%, 53.42%, 67.20% and 84.29% while increasing TSTQ by 3.57%, 8.44%, 13.79% and 21.25%on 8th, 15th, 22nd and 29th day of treatment respectively and when compared to the CBZ alone treated group, ENJ showed decrease in ELT by 34.11%,55.16%, 68.39%, 87.44% and number of errors by 36.49%, 53.42%, 67.20%, 84.29% and increase in TSTQ by 1.04%, 12.51%, 27.34% and 48.44%. The Combination of ENJ with CBZ½ dose showed decrease in ELT by 44.41%, 54.35%, 67.04%, 76.62% and number of errors by 19.65%, 17.6%, 29.18%, 41.18% and increase in TSTQ by 20.02%, 23.70%, 27.38% and 31.06% respectively when compared to CBZ alone treated group. CBZ½+ENJ was found to decrease ELT, number of errors and increase TSTQ more than that of CBZ+ENJ proving the hypothesis of reduction in

memory impairment by reducing the dose of AED under treatment without compromising the anti-epileptic activity of the same.

AChE inhibitor's clinical efficacy is thought to result from prolonging the half-life of acetylcholine through inhibition of AChE. The whole brain AChE activity was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithio-bisnitrobenzoate ions. The rate of formation of thiocholine from acetylthiocholine in the presence of tissue cholinesterase was measured using a spectrophotometer. It was observed that administration of CBZ along with PTZ induced convulsions resulted in a significant increase in AChE value of 69.25% when compared to the normal group. When the ENJ was co-administered with CBZ in PTZ induced convulsions, it produced a significant decrease in AChE value of 43.27% compared to CBZ treated alone group. When ENJ was administered alone, it produced a decrease in AChE value by 77.98% when compared to CBZ treated alone. Similarly when CBZ ½ dose was given along with ENJ it produced a significant decrease of AChE value by 49.10% when compared to CBZ alone treated group. Here it was observed that ENJ alone produced good nootropic effect when compared to CBZ+ENJ and CBZ alone. But CBZ ½+ENJ was found to decrease AChE value more than that of CBZ+ENJ proving the hypothesis of reduction in memory impairment by reducing the dose of AED under treatment without compromising the anti-epileptic activity of the same. PTZ induced seizures represents petit mal type of epilepsy in human and generally occurs in children. Here PTZ exerts its action by acting as an antagonist at the picrotoxin sensitive site of the GABA-A receptor complex. It was found that chronic treatment of CBZ for 29 days on PTZ induced convulsions in rat significantly reduced the duration of convulsion by 47.98%, 60.81% on 1st, 29th day and delayed the onset of convulsion by 879.8%, 1398.8% on 1st and 29th day when compared with control treated group.

When ENJ was co-administered with CBZ, we observed the significant potentiation of anticonvulsant activity of ENJ which reduced duration of action by 31.99%, 72.37% and delayed the onset of action of clonic convulsion 21.26%, 28.79% when compared to CBZ treated alone on 1st and 29th day. When ENJ was given alone, we observed the significant decrease in anticonvulsant activity, which reduced duration of action by4.40%, 9.21% and decreased delay of onset of action of clonic convulsion by 3.97%, 5.18% when compared to CBZ treated alone on 1st and 29th day. When half dose of CBZ was given along with ENJ, we observed the significant increase in anticonvulsant activity of ENJ which reduced duration of action by 13.80%, 39.99% and delayed onset of action by 9.73%, 16.06% when compared to CBZ treated alone group on 1st and 29th day. This significant increase of anticonvulsant activity was not up to that of co-administration of full dose of CBZ+ENJ. This clearly demonstrates the protective effect as well as synergistic effect of ENJ on CBZ for petit mal type of epilepsy.

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