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THE MEMORY IMPAIRMENT ACTIVITY OF OXCARBAZEPINE IN PRESENCE AND ABSENCE OF *OCIMUM SANCTUM* IN RAT MODEL

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ABSTRACT

Memory impairments (MI) are common in patients with treatment of epilepsy and it is believed that co-administration of nootropic agent along with antiepileptic drugs may decrease these MI. The present study was conceived to assess the MI activity of oxcarbazepine (OXZ) in presence and absence of tulsi (*Ocimum sanctum)*. MI of OXZ was determined by chronic administration employing, Radial Arm Maze (RAM) and Morris Water Maze (MWM) task on pilocarpine induced temporal lobe epileptic (TLE) rats. In RAM task both working memory error & reference memory error and in MWM task Escape Latency Time (ELT) and Time Spent in Target Quadrant (TSTQ) were determined. The brain anti-cholinesterase level and antioxidant enzymes were determined at the end of the experiment. Results: The MI of OXZ was found to be dose dependent and was decreased when half dose of OXZ was administered. When tulsi was given along with OXZ, significant decrease in MI induced by OXZ was observed and the anticonvulsant activity of OXZ was found to be synergized when compared to control and OXZ alone treated animals. Co-administration of tulsi also produced a decrease in AChE and TBARS and increased SOD & GSH levels when compared to control and OXZ alone treated group. The combined therapy was found to have very potent anti-epileptic activity. The findings of present study reveal the MI potential of OXZ and correction by co-administration of tulsi and synergistic antiepileptic activity. However, further study is required to establish the molecular mechanism involved in MI caused by OXZ

INTRODUCTION

Epilepsy is a chronic neurodegenerative disorder of the brain which is characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological and social consequences.[1] A seizure occurs due to discharge of nerve cells in the brain which may cause a combination of symptoms, minor physical signs, a physical convulsion, or thought disturbances.[2] Epilepsy was one of the first brain disorder and affecting more than 50 million patients worldwide.[3] Among 50 million patients with epilepsy all over the world, nearly 12 million are expected to reside in India which contributes to nearly one-sixth of the global burden.[4] Due to complex conditions, sometimes it is difficult to identify the various symptoms of epilepsy.

Memory problems or memory impairments are one of the major problems associated with epileptic patients.[5] It is all due to the neurotransmitters or neuromodulators which involve in neuronal excitation. The abnormal functions of these neurotransmitters which include either excitatory or inhibitory neurotransmitters of the brain lead to stress, tiredness in the brain which in turn cause a lapse in memory. Recent literature suggests that, the co-administration of nootropic agents will reduce the MI induced by epilepsy and antiepileptic drug treatment. [6] Based on the literature the present study was undertaken with the objectives of evaluation of MI activity of Oxcarbazepine (OXZ) in normal and reduced dose. An attempt was made to investigate the effect of co-administration of *Ocimum sanctum* on MI induced by OXZ as well as antiepileptic activity.

2.0 Materials and methods

2.1 Animals –

Wister rats of either sex (200-250 gm) procured from *In-vivo* Biosciences, Bengaluru (Reg. no. 971/bc/06/CPCSEA) were used for the research study. The animals were housed in polypropylene cages and fed on a standard pellet diet and water *ad libitum*. The temperature maintained at 23–27°C with a natural light-dark cycle. The rats were acclimatized for a week before the treatment. Eight rats are used to each group of the experiments. All the experiments were in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of JSS College of Pharmacy, Mysore. (Approved No. JSSCPM 239/2017)

2.2 Hydroalcoholic extract of Tulsi-

The hydroalcoholic extract of Tulsi leaf was obtained from Natura Biotechnol, Bengaluru as gift sample. It was a pale green to greenish-brown colored powder with characteristic odor and taste.

2.3 Experimental Design

The animals were grouped and treated as shown in Tables 1 & 2.

Temporal lobe epilepsy (TLE) was induced by administration of Pilocarpine (30 mg/kg, i.p.) on every 7th day of treatment. Lithium chloride (127 mg/kg, s.c.) is given 24hrs before pilocarpine administration to minimize the mortality rate. First 30 days, the MI activity was assessed on 8th, 15th, 22nd and 29th day by RAM. Next 30 days, the MI activity was measured every 8th, 15th, 22nd and 29th day by MWM. The anti-epileptic activity was assessed every 7th day for entire treatment period.

2.3.1 Racine Scales were used to measure temporal lobe epilepsy in the experiment.[10]The control group was given diazepam (10mg/kg, i.p.) to stop the TLE at stage 4 to save the life of the animal.[11]

2.3.2 Behavioural parameters (memory impairment)-

The behavioral parameters were done by 8 arm Radial arm maze and Morris water maze.

2.3.3 Biochemical Parameter -

Estimation of acetyl cholinesterase activity (hippocampus)-

The *acetyl cholinesterase* activity was measured on the 67th day of the treatment by Ellman's method in the hippocampus homogenate.[12]

Antioxidant enzymes in brain homogenate

Antioxidant enzymes SOD, reduced glutathione and TBARS were determined in the brain homogenate isolated at the end of the study (67th day of the treatment).[13,14,15]

3.0 RESULTS

The effect of tulsi on OXZ induced WMI is represented in Table (3). In this study, it was observed that when Pilocarpine was administered to Control group there was a significant increase in the working memory error score when compared to Normal. OXZ (ND) was administered there was a significant increase in the working memory error score when compared to Control group, PHT group, OXZ (RD) group. When OXZ (ND) + tulsi were administered there was a significant increase in the working memory error score when compared to OXZ (RD) + tulsi. Tulsi alone was administered that showed significant decrease in the working memory error score when compared to control. The effect of tulsi on OXZ induced RME is represented in Figure (1) the Pilocarpine was administered to Control group there was a significant increase in the working memory error when compared to Normal. OXZ (ND) was administered there was a significant increase in the working memory error score when compared to Control group, PHT group

and OXZ (RD) group. When OXZ (ND) + Tulsi was administered there was a significant increase in the working memory error score when compared to OXZ (RD) + Tulsi. When Tulsi was administered there was a significant decrease in the working memory error score when compared to control.

The effect of tulsi on OXZ induced MI (ELT) is represented in Table (4) Pilocarpine was administered to Control group there was a significant increase in the ELT when compared to Normal. When OXZ (ND) was administered there was a significant increase in the ELT when compared to Control group, PHT group, OXZ (RD) group. When OXZ (ND) + tulsi was administered there was a significant increase in the ELT when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant decrease in the ELT when compared to control.

The effect of tulsi on OXZ induced MI (Time spent in the target quadrant in sec.) is represented in Figure (2). In this study, when Pilocarpine was administered to control group there was a significant decrease in the TSTQ when compared to normal. When OXZ (ND) was administered there was a significant decrease in the TSTQ when compared to Control group, PHT group and OXZ (RD) group. When OXZ (ND) + tulsi was administered there was a significant decrease in the TSTQ when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant increase in the when compared to control. The evaluation of AChE is represented in Figure (3) the level of AChE in the temporal lobe brain homogenate was determined. It was observed that when Pilocarpine was administered to Control group there was a significant increase in the AChE level when compared to Normal. When OXZ (ND) was administered there was a significant increase in the AChE level when compared to Control group, PHT group, OXZ (RD) group. When OXZ (ND) + Tulsi was administered there was a significant increase in the AChE level when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant decrease in the AChE level when compared to control. The evaluation of antioxidant enzymes (TBARS) is represented in Table (5). In this study the level of TBARS in the temporal lobe brain homogenate was determined. When Pilocarpine was administered to Control group there was a significant increase in the TBARS level when compared to Normal. When OXZ (ND) was administered there was a significant increase in the TBARS level when compared to control group, PHT group, OXZ (RD) group. When OXZ (ND) + Tulsi was administered there was a significant increase in the TBARS level when compared to OXZ (RD) + Tulsi. When Tulsi was administered there was a significant decrease in the TBARS when compared to control. The evaluation of antioxidant enzymes (SOD) is represented in Figure (4). Pilocarpine was administered to Control group there was a significant decrease in the SOD level when compared to Normal. When OXZ (ND) was administered there was a significant decrease in the SOD level when compared to Control group, PHT group, OXZ (RD) group. When OXZ (ND) + tulsi were administered there was a significant decrease in the SOD level when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant increase in the SOD level when compared to control. The evaluation of antioxidant enzymes (reduced Glutathione) is represented in Table (6) Pilocarpine was administered to Control group there was a significant decrease in the GSH level when compared to Normal. When OXZ (ND) was administered there was a significant decrease in the GSH level when compared to Control group, PHT group and OXZ (RD) group. When OXZ (ND) + tulsi was administered there was a significant decrease in the GSH level when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant increase in the GSH level when compared to control. Figure 5. Shows that the anti-epileptic activity of OXZ was assessed on every 7th day. It was observed that when Pilocarpine was administered to Control group there was a significant increase in the AES when compared to OXZ (ND). When OXZ (ND) was administered there was a significant decrease in the AES when compared to PHT group, OXZ (RD) group. When OXZ (ND) + tulsi were administered there was a significant decreased in the AES when compared to OXZ (RD) + tulsi.

Table 1. Grouping, treatment and evaluation

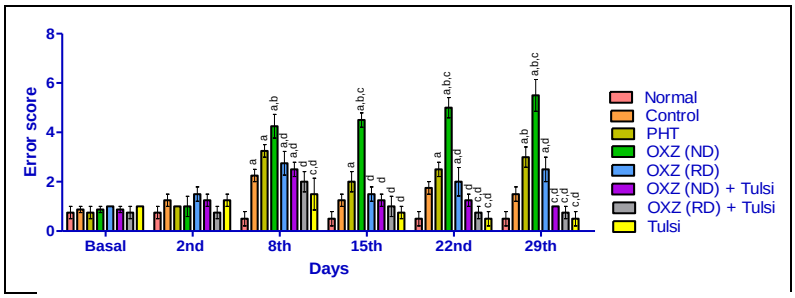
OXZ- Oxcarbazepine, PHT- Phenytoin, ND- Normal dose, RD- Reduced dose, RAM- Radial arm maze, MWM- Morris water maze

Group	No. of animals	Treatment	Evaluation
Normal	8	Vehicle (0.5% Na CMC) orally for 60 days.	Antiepileptic activity on every 7th day followed by MI on every 8th day for the first 30 days by RAM and next 30 days with MWM task. Estimation of acetylcholinesterase and antioxidant enzymes in brain homogenate at the end of the study.
Control	8	Vehicle orally for 60 days + Pilocarpine (30 mg/kg, i.p.) on every 7th day after 24hr of Lithium Chloride (127mg/kg, s.c.) administration.[7]	
Phenytoin	8	PHT (25 mg/kg) orally daily for 60 days + Pilocarpine on every 7th day.[8]	
OXZ (ND)	8	OXZ (30 mg/kg) orally daily for 60 days + Lithium chloride and Pilocarpine on every 7th day.[9]	
OXZ (RD)	8	OXZ (15 mg/kg) orally daily for 60 days. + Lithium chloride and Pilocarpine on every 7th day.[9]	
OXZ (ND) + Tulsi	8	OXZ (30 mg/kg) orally and Tulsi (200 mg/kg) orally for 60 days + Lithium chloride and Pilocarpine on every 7th day.[9]	
OXZ (RD) + Tulsi	8	OXZ (15 mg/kg) orally and Tulsi (200 mg/kg) orally for 60 days + Lithium chloride and Pilocarpine on every 7th day.[9]	
Tulsi	8	(200 mg/kg) orally for 60 days + Lithium chloride and Pilocarpine on every 7th day.[6]	
Total number of animals= 64			
Radial arm maze model			
Animals were trained for 5 days without treatment			

Table 2. The animal study protocol followed

DAY 1-30	Animals were treated with regular treatment as shown in Table 1. TLE was induced by pilocarpine. MI activity was measured on every 8th day and antiepileptic activity on every 7th day.
Morris water maze	
DAY 31- 35	Animal was trained for 5 days without treatment
DAY 36- 66	Animals were treated with regular treatment as shown in Table 1. TLE was induced by Pilocarpine. MI activity was measured on every 8th day and antiepileptic activity on every 7th day.
DAY 67	Animals were sacrificed at the end of the study for the estimation of biochemical parameters.

Figure 1 Evaluation of MI activity of OXZ by RAM task (Reference memory error)



The data were analyzed by two way ANOVA; Bonferroni's test. p≤0.05,
Values are expressed at MEAN±SEM, n=8, significant when compared to Normal, significant when compared to control, significant when compared to PHT, significant compared to OXZ

Table 3. Evaluation of MI activity of OXZ by RAM task

(Working memory error)

The data were analyzed by two way ANOVA; Bonferroni's test.
Values are expressed at MEAN±SEM, n=8, p≤0.05,

Days	Normal	Control	PHT	OXZ (ND)	OXZ (RD)	OXZ (ND) + Tulsi	OXZ (RD) + Tulsi	Tulsi
Basal	0.50±0.40	0.52±0.50	0.49±0.25	0.47±0.40	0.51±0.25	0.52±0.25	0.48±0.40	0.49±0.00
2nd	0.45±0.04	0.65±0.25	0.62±0.08	0.63±0.25	0.55±0.29	0.63±0.50	0.60±0.25	0.45±0.25
8th	0.40±0.29	1.25±0.25 a	2.00±0.41a	4.25±0.48a,b	2.00±0.29d	1.75±0.29a,d	1.50±0.4d	0.42±0.65c,d
15th	0.30±0.29	1.50±0.29 a	2.50±0.29a	4.50±0.29a,b,c	2.10±0.58a,d	1.40±0.29d	1.00±0.4d	0.37±0.25d
22nd	0.25±0.25	1.75±0.25 a	3.00±0.41a,b	5.00±0.41a,b,c	2.50±0.50a,d	1.25±0.25d	0.75±0.50c,d	0.25±0.50c,d
29th	0.20±0.25	2.25±0.25 a	3.25±0.25a	5.50±0.65a,b,c	2.75±0.48a,d	0.85±0.25c,d	0.60±0.00c,d	0.18±0.29c,d

a- significant when compared to Normal,
b- significant when compared to control,
c- significant when compared to PHT,
d- Significant compared to OXZ

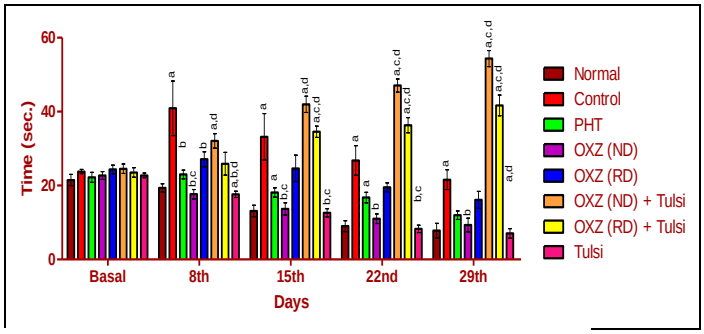
Table 4. Evaluation of MI activity of OXZ by MWM task

Days	Normal	Control	PHT	OXZ (ND)	OXZ (RD)	OXZ (ND) + Tulsi	OXZ (RD) + Tulsi	Tulsi
Basal	20.39±2.69	22.42±1.87	21.70±2.93	22.94±3.21	25.27±2.30	23.48±2.87	22.94±2.03	20.30±2.32
8th	16.25±1.55	25.74±2.01	31.28±2.39^{a}	44.03±2.73^{a,b}	29.96±2.39^{a}	20.70±2.27^{a,}	19.73±1.75^{c,d}	16.23±1.79^{c,d}
15th	15.58±1.49	31.63±2.0^{a}	46.32±5.12^{a}	69.69±2.09^{a,b,}	38.86±2.15^{a,b,}	18.22±2.10	14.51±3.06^{b,c,}	13.89±2.28^{c,d}
22nd	13.12±1.80	54.93±2.4^{a}	65.84±1.57^{a,}	76.87±2.20^{a,b,}	58.47±3.60^{a,d}	14.51±2.27^{b,}	12.46±1.42^{b}	11.60±2.10^{b,c,d}
29th	8.28±1.69	60.95±2.96	73.98±4.00^{a}	81.81±3.07^{a,b}	62.19±2.49^{a,c,}	13.49±2.30^{b,}	10.52±1.53	9.07±1.26^{b,c,d}

(Escape latency time in sec.)

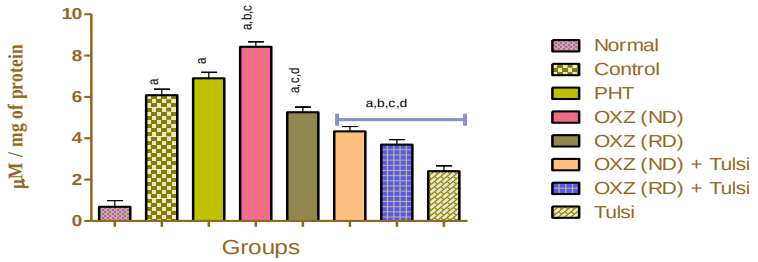
The data were analyzed by two way ANOVA; Bonferroni's test. Values are expressed at MEAN±SEM, n=8, p≤0.05, Significant when compared to Normal, b- significant when compared to control, c- significant when compared to PHT , d- Significant compared to OXZ

Figure 2. Evaluation of MI activity of OXZ by MWM task (Time spent in the target quadrant in sec.)



The data were analyzed by two way ANOVA; Bonferroni's test. Values are expressed at MEAN±SEM, n=8, p≤0.05, a-significant when compared to Normal, significant when compared to control, c- significant when compared to PHT, d-significant compared to OXZ

Figure 3. Evaluation of AChE activity

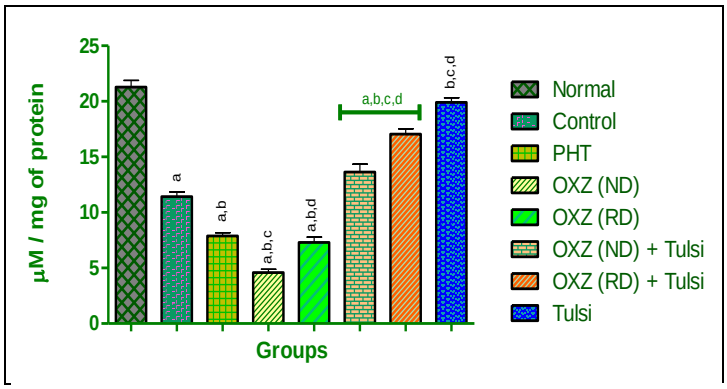


The data were analyzed by two way ANOVA; Bonferroni's test.
Values are expressed at MEAN±SEM, n=8, p≤0.05, a-significant when compared to Normal, b- Significant when compared to control, c-significant when compared to PHT, d-significant compared to OXZ

Group	TBARS (µg/mg of protein)
Normal	0.71±0.28
Control	14.21±0.48a
PHT	16.86±0.43a,b
OXZ (ND)	19.69±0.58a,b,c
OXZ (RD)	15.26±0.48a,b,d
OXZ (ND) + Tulsi	10.83±0.30a,b,c,d
OXZ (RD) + Tulsi	4.57±0.42a,b,c,d
Tulsi	0.84±0.26a,b,c,d

The data were analyzed by two way ANOVA; Bonferroni's test. Values are expressed at MEAN±SEM, n=8, p≤0.05, a-significant when compared to Normal, b-significant when compared to control, c-significant when compared to PHT, d-significant compared to OXZ

Figure 4. Evaluation of antioxidant enzymes (SOD)



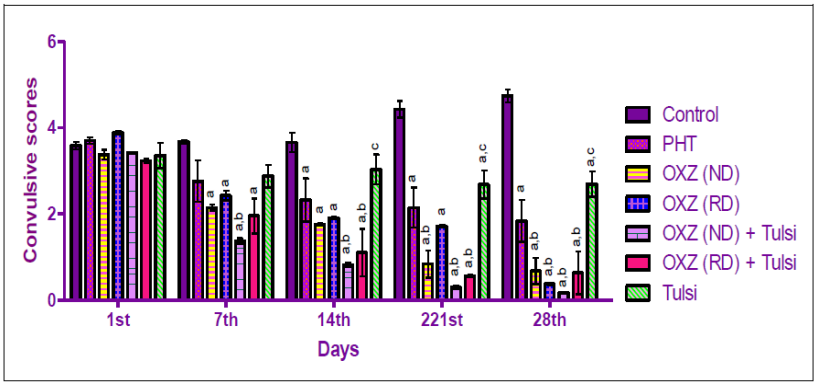
The data were analyzed by two way ANOVA; Bonferroni's test.
Values are expressed at MEAN±SEM, n=8, p≤0.05,
a-significant when compared to Normal,
b- significant when compared to control,
c- significant when compared to PHT,
d-significant compared to OXZ

Table 6. Evaluation of antioxidant enzymes (reduced glutathione)

Group	GSH (µg/mg of protein)
Normal	19.40±0.49
Control	11.05±0.57
PHT	8.44±0.39a,b
OXZ (ND)	2.54±0.29a,b,c
OXZ (RD)	5.02±0.30a,b,c,d
OXZ (ND) + Tulsi	11.96±0.48a,c,d
OXZ (RD) + Tulsi	14.92±0.39a,b,c,d
Tulsi	20.90±0.48

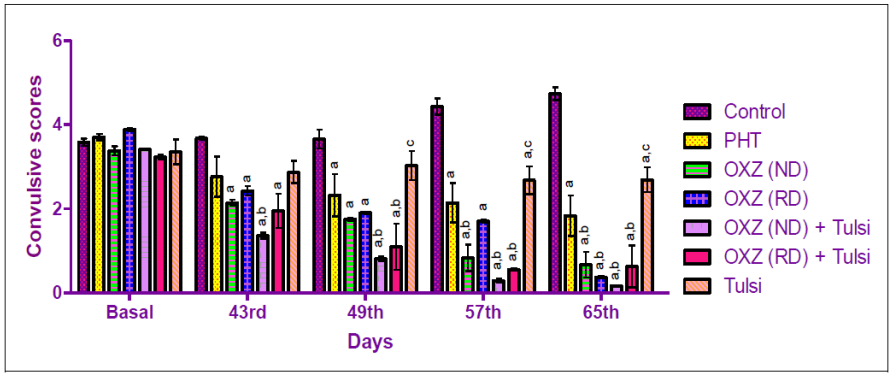
The data were analyzed by two way ANOVA; Bonferroni's test. Values are expressed at MEAN±SEM, n=8, p≤0.05, a-significant when compared to Normal, b-significant when compared to control, c-significant when compared to PHT, d-significant compared to OXZ

Figure 5. Anticonvulsant activity of OXZ in presence and absence of tulsi for the first 30 days of RAM task (Convulsive scores)



The data were analyzed by two way ANOVA; Bonferroni's test. Values are expressed at MEAN±SEM, n=8, p≤0.05, a-significant when compared to Control, b-significant when compared to PHT, c- significant when compared to OXZ (ND), d-significant compared to OXZ (RD)

Figure 6. Anticonvulsant activity of OXZ in presence and absence of tulsi for the last 30 days of MWM task (Convulsive scores)



The data were analyzed by two way ANOVA; Bonferroni's test.
Values are expressed at MEAN±SEM, n=8, p≤0.05,
A-significant when compared to Control, b-significant when compared to PHT,
c- significant when compared to OXZ (ND), d-significant compared to OXZ (RD)

When tulsi was administered there was a significant decrease in the AES when compared to control. Figure 6: Shows that the anti-epileptic activity of OXZ was assessed on every 7th day. when Pilocarpine was administered to Control group there was a significant increase in the AES when compared to OXZ (ND). When OXZ (ND) was administered there was a significant decrease in the AES to PHT group and OXZ (RD) group. When OXZ (ND) + tulsi was administered there was a significant decreased in the AES when compared to OXZ (RD) + tulsi. When tulsi was administered there was a significant decrease in the AES when compared to control.

4.0 Discussion

Epilepsy is a chronic disorder mostly occurring in children and elderly patients and AEDs are used in the treatment of epilepsy. Epilepsy can cause impaired cognition and many factors contribute to this impairment, including the adverse effects of AEDs.[2] OXZ is a structural derivative of carbamazepine, adding an extra oxygen atom to the benzylcarboxamide group with an anticonvulsive property. As a prodrug, OXZ is converted to its active metabolite, 10-hydroxycarbazepine.[16] Even though, the MOA has not been completely explained, electrophysiological studies express this agent blocks voltage-gated sodium channels, thus decreasing the propagation of synaptic impulses, stabilizing hyper-excited neural membranes, and inhibiting repetitive neuronal firing.[17] One of the side effects of OXZ is MI and decreases the level of acetylcholine in brain.[18] Since OXZ belongs to dibenzazepine family, therefore, the mechanism involved in causing MI might be same as that of Carbamazepine. Tulsi, commonly called as Holy basil, or *Ocimum sanctum*, is an aromatic perennial plant in the family Lamiaceae can be used for improvement of MI caused by antiepileptic drugs as well as potentiates the antiepileptic activity of the OXZ.[19,20]

CONCLUSION

In the present study, we found that chronic administration of OXZ for 60 days significantly induced MI in rats. OXZ adversely affected cognitive function which was observed by RAM and MWM task. In RAM the errors were significantly increased when compared to control animals. In MWM, the ELT was remarkably increased and TSTQ was remarkably decreased when compared to control group. When OXZ was given in reduced dose, the extent of MI was also reduced, however it was more than disease alone (control) group. When tulsi was given along with OXZ, significant reversal of OXZ induced memory deficit was observed. Both the acquisition and retention of memory had shown improvement. Tulsi substituted the reduce dose of OXZ without altering the antiepileptic effect. Thus a conclusion can be made based on the observation and results obtained that tulsi can be used for alleviating the MI caused by antiepileptic drug, OXZ. However, further research is required to investigate the usefulness of these nootropics in various animal models and clinical studies are required to explore the full potential of tulsi in correcting OXZ induced cognitive deficits and finding a place in the current AED therapy. Our study gives a platform for further extensive research on nootropics for the correction of AED induced MI. Future directives are a reduction in dose of an AED when combined with anticonvulsant nootropics thereby reducing the extent or degree of adverse effect produced by the AED used for treatment.

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