ORIGINAL RESEARCH

Experimental studies to evaluate the protective effects of Ocimum sanctum leaf extract and its comparison with Carbamazepine in Pentylenetetrazol (PTZ)-induced convulsions in rats

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ABSTRACT

Background: Epilepsy is a prevalent neurological disorder characterised by recurrent episodes of seizures, with or without convulsions, as well as sensory or psychiatric phenomena.Ocimum sanctum L. commonly known as tulsi (synonym of Ocimumtenuiflorum L.) is widely used in Ayurveda medicine and is having multitude neuromodulatory effect.Current study investigated the protective effect of Ocimum sanctum leaf extract (OSLE) with antiepileptic drug (Carbamazepine) in Pentylenetetrazol (PTZ)- induced convulsions in rats. **Methods:** This experimental study was conducted on 54 adults healthy Wistar rats. Rats were randomly divided into 9 groups, as mentioned below. Each group contained 6 rats and underwent different treatment regime. They were assessed for 14 days. **Results:** PTZ induced changes in the oxidative parameters in rats brain was found significantly decreased ($p<0.0001$, $p<0.05$) with Ocimum sanctum (500mg/kg and 1000mg/kg) in a dose dependent manner. PTZ(30mg/kg) induced significant decrease in the antioxidant enzymes (SOD). The lipid peroxidation marker (MDA) significantly increases ($p<0.05$) after PTZ(30mg/kg) in the rats brain which was found reversed by pre-treatment with Ocimum sanctum. The markers of inflammatory cytokines (pro-inflammatory-IL-1β and anti-inflammatory- IL-4) were found significantly increase in the PTZ-treated (30mg/kg)($p<0.0001$). Molecular marker (NF-kβ) was significantly increased in all treated group ($p<0.0001$).The level was suppressed more in Ocimum pre-treated group as compared to the vehicle. Among neurobehavioral studies in EPM rats withOcimum sanctum and its combination(SM) dose significantly shows more entries ($p=0.0003$) and time spent in the open arm ($p<0.0001$) and also increased entries in centre squares in the open field test which reduced anxiety($p<0.0001$). **Conclusion:** On the basis of the result obtained in this study we can suggest that Ocimum sanctum may have protective/therapeutic and neuroprotective effects in seizures.

Keywords: Epilepsy,Ocimum sanctum, Carbamazepine, Pentylenetetrazol, Elevated plus maze, Open Field test

INTRODUCTION

Epilepsy is a prevalent neurological disorder characterised by recurrent seizures, with or without convulsions, as well as sensory or psychiatric phenomena. [1-2] Epilepsy has a focal origin in the brain, and its manifestations are dependent on the location of this origin, the regions into which the discharges extend, and the postictal depression of these regions. Epilepsy is a severe condition that can affect individuals of any age.

In India, 0.7% of the population has epilepsy. Up to 70% of patients with epilepsy in developed nations respond to treatment. In developing countries, there is a high rate of mortality, and 75% of patients do not receive treatment due to a lack of available drugs. In recent years, due to their potent pharmacological activities, low toxicity, and economic viability, the therapeuticrole of plants was examined in light of scientific advancements around the globe.[5]

Epilepsy is characterised by an imbalance of excitatory and inhibitory neurotransmitters, such as

GABA and Glutamate, over the activation of Na+ and Ca++ channels. GABA is an inhibitory neurotransmitter in the brain that maintains an inhibitory tone to counteract neuronal excitation.[5] Approximately 30% of people with epilepsy had no effect to AEDs and have uncontrolled seizures despite of more than 15 newly innovated AEDS with noval mechanisms of action. [1] Carbamazepine (CBZ) and phenytoin (PHT) are two most commonly used older AEDs because of their effectiveness, low expense, and long history of use. [8]

Medicinal plants used in conventional medicine for the treatment of epilepsy have demonstrated promising anticonvulsant activity in animal models, according to experimental evidence. With the current dependence on traditional medicinal plants for disease treatment and the possibility of drug discovery, it is important to look for plants that are capable, effective, and relatively safe to use as medicines. [9]

Several herbal remedies are used to treat epilepsy because they are readily accessible and have a long history of use. [10] Traditional medicine is predicated on man's continued existence. The anticonvulsant activity of hundreds of plant extracts has been demonstrated in phenotypic screens. Several of these extracts have anticonvulsant activity comparable to AEDs. [10]

Previous research indicates it has numerous neuromodulatory effects, including an anticonvulsant effect in models of acute seizures, and is therefore widely used in Ayurvedic medicine. There is evidence from both a systematic review and an Indian research that *Ocimum sanctum* leaves have been used in traditional medicine to treat epilepsy. Its potential translational worth could be enhanced by studying its function in a model of chronic seizures and its interaction with newer antiepileptic drugs. *Ocimum sanctum* with anticonvulsant potential has only been documented in a single study [17], which used an acute seizure model to prove it.

This study will evaluate the potential of OLE as adjuvant in conjunction with CBZ to enhance their antiepileptic effect. However, current evidence demonstrating the effect of *Ocimum sanctum* with CBZ in the PTZ model is lacking. Carbamazepine (CBZ) is a commonly used antiepileptic medication because it reduces repetitive neuronal firing by prolonging the inactivation of Na+ channels. [18] However, no such evidence demonstrating its potential interaction with CBZ. Due to the fact that CBZ is a substrate for CYP 450 and has a narrow therapeutic window, CBZ has a more significant potential to interact with herbal drugs, results in toxicity or therapeutic failure. [19] Therefore, it is essential to investigate the interaction between CBZ and antiepileptic herbs such as *Ocimum sanctum*. These facts highlight the need for the current study, which will investigate the anticonvulsant potential of *Ocimum sanctum* alone and in combination with CBZ, as well as the interaction in a PTZ model of convulsions. In addition, the effect of administering *Ocimum sanctum* in comparison to CBZ will be evaluated in order to examine the anxiolytic effect on neurobehavioral status in rats.

The present study was aimed to determine the protective role of *Ocimum sanctum* leaf extract (OLE) and its interaction with the standard drug carbamazepine (CBZ) in PTZ-induced convulsions in rats.

MATERIAL AND METHODS

The institutional animal ethics council at King George's Medical University in Lucknow gave their blessing to this experiment [Project no. 156/IAEC/Pharma-2021], which was carried out in the Department of Pharmacology and Therapeutics. CPSCEA-accredited animal facilities [IITR, Lucknow] provided 54 adult Wistar rats in good condition. Standard animal home procedures were followed for their care. The rats were arbitrarily split up into the following nine categories. Six rodents per group were treated differently for 14 days and then evaluated.

In this study, *Ocimum sanctum* was administered at a dosage of 500 & 1000 mg/kg orally for14days. Our earlier research indicated that of four different doses of OLE (200, 400, 800, and 1000 mg/kg), the 1000 mg/kg dose was the most effective in preventing seizures. The intrperitoneal(i.p.) dose of pentylenetetrazol was 20 & 30mg/kg. For rats, this concentration of PTZ causes convulsions at a 100% lethal dosage.CBZ(20mg/kg) orally as a standard drug was used. MDA and SOD activity, as well as inflammatory marker (IL-4, IL-1β, and Nf-kβ) and neurobehavioral (elevated plus maze, Open field test, etc.) were measured in cerebral cortex homogenate.

STATISTICAL ANALYSIS

SPSS version 26 was used for statistical analysis after data was imported from Microsoft Excel (SPSS Inc., Chicago, IL, USA). When necessary, the continuous variables were analysed using either the mean (standard deviation) or range value. The continuous variables were presented as mean and standard deviations after being analysed with ANOVA, and Dunnett's multiple comparison test (post-hoc test) was used to compare numerous groups. It was considered statistically significant when the p-value was less than < 0.05 or 0.001.

RESULTS

After 14 days, the weight of rats was comparable in all the groups and non-significant [p=0.9973] [Table-1].

EFFECT OF OCIMUM SANCTUM ON INFLAMMATORY CYTOKINES

As compared to control group (150.8 ± 18.37ρg/ml), the mean level of Interleukin 1-β (IL-1β) was found to be significantly higher in GROUP-PTZ (30mg/kg)

[326.0 ± 22.05ρg/ml] [p<0.0001*]. The PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg) [160.6 ± 22.18 ρg/ml] and PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) [165.8 ± 21.23ρg/ml] had most efficacious and closed to controls.As compared to control group (160.3 ± 15.16 ρg/ml), the mean level of Interleukin-4 (IL-4) was found to be higher in PTZ (30mg/kg)[316.8 ± 28.21ρg/ml]. The PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg) [168.4 ± 25.10 ρg/ml] and PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) [173.6 ± 26.98 ρg/ml] had most efficacious and closed to controls. Also the mean level of Nuclear Factor Kappa B (NF-κβ) was significantly higher in GROUP-PTZ (30mg/kg) [10.6 ± 2.17ng/ml] as compared to controls [1.26 ± 0.81ng/ml]. The PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg) [4.33 ± 1.23 ng/ml] and PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) [2.33 ± 0.99 ng/ml] had most efficacious and closed to controls. Statistically, a significant difference was also observed among groups [P<0.0001*][Table-2].

EFFECT OF OCIMUM SANCTUM ON OXIDATIVE STRESS

After administration of PTZ, the mean of MDA was increased in GROUP-PTZ (30mg/kg) [8.5 ± 3.07] as compared to controls [3.2 ± 1.02nmoles /mg]. The PTZ (20mg/kg) + OLE best dose(1000mg/kg) [4.1 ± 0.97] and PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) [4.3 ± 2.11nmoles /mg] were showed most efficacious and closed to controls. Furthermore, the mean level of SOD was observed to besignificantly lower in GROUP-PTZ (30mg/kg) [10.6 ± 3.02μ/mg] as compared to controls [21.8± 5.23 μ/mg].The PTZ (20mg/kg) + OLE best dose(1000mg/kg) [20.97 ± 4.34 μ/mg] and PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) [19.27 ± 4.34 μ/mg] had significantly increased in SOD level and were showed most efficacious and closed to controls. [Table-3, Figure-1(a) and 1(b)]

EFFECT OF OCIMUM SANCTUM ONNEUROBEHAVIOURAL PARAMETERS

The racine score, seizure latency, and seizure duration were significantly increased while administration of PTZ into mice. On treatment with other modalities, maximum efficiency was found in PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) followed by PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg). Mortality were also decreased while administration of OLE, CBZ or both. [Table-4]

While comparing the PTZ induced behavioural changes in Open field test in rats, as compared to control group, Corner square entry and defecation were significantly increased in PTZ (30mg/kg) group. However, the Centre square entry, rearing and grooming were significantly decreased in PTZ (30mg/kg) group as to control group. [Table-5]

As compared to control group, number of closed arm entry, and time spent in the open arm were significantly increased in GROUP-PTZ (30mg/kg). However, the number of open arm entry and time spent in closed arm were significantly decreased in GROUP-PTZ (30mg/kg). On treatment with other modalities, maximum efficiency was found in PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) followed by PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg). [Table-6]

Table-1: Mean baseline weight of rats of different study Groups.

Groups	Weight (kg)
Control	161.3 ± 6.37
PTZ (30mg/kg)	163.2 ± 6.75
[PTZ (20mg/kg)]	162.8 ± 7.21
[PTZ (20mg/kg) + OLE (500mg/kg)]	160.9 ± 5.53
[PTZ (30mg/kg) + OLE (1000mg/kg)]	162.5 ± 7.25
[PTZ(30mg/kg) + CBZ (20mg/kg)]	163.6 ± 6.44
[PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg)]	161.7 ± 7.63
[PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)]	162.2 ± 5.99
[PTZ (20mg/kg) + OLE best dose (1000mg/kg)]	163.8 ± 7.37
P-VALUE	F=0.1343 p=0.9973

Table-2: Effects of Ocimum sanctum and Carbamazepine on Interleukins and NF-ƙβ level in brain homogenate(cortex) in rats.

Groups	Interleukin 1-β (ρg/ml)	P Value	Interleukin -4 (ρg/ml)	P Value	NF-ƙβ levels (ng/ml)	P-Value
Control	150.8 ± 18.37		160.3 ± 15.16		1.26 ± 0.81	
PTZ (30mg/kg)	326.0 ± 22.05	**p<0.0001***	316.8 ± 28.21	**p<0.0001***	10.6 ± 2.17	**<0.0001***
PTZ (20mg/kg)	297.0 ± 19.15	**p<0.0001***	261.4 ± 21.45	**p=0.0001***	9.66 ± 2.02	**<0.0001***
PTZ (20mg/kg) + OLE (500mg/kg)	306.0 ± 30.06	**p<0.0001***	216.8 ± 24.22	**p=0.0006***	8.33 ± 1.91	**<0.0001***

PTZ (30mg/kg) + OLE (1000mg/kg)	292.0 ± 31.01	**p<0.0001***	199.7 ± 19.42	**p=0.0260***	7.86 ± 1.73	**<0.0001***
PTZ (30mg/kg) + CBZ (20mg/kg)	198.8 ± 13.29	**p=0.0032***	179.0 ± 20.30	p=0.5950	6.06 ± 1.07	**<0.0001***
PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg)	160.6 ± 22.18	p=0.9668	168.4 ± 25.10	p= 0.9909	4.33 ± 1.23	**0.0079***
PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)	165.8 ± 21.23	p=0.7748	173.6 ± 26.98	p=0.8722	2.33 ± 0.99	**0.7575**
PTZ (20mg/kg) + OLE best dose(1000mg/kg)	202.1 ± 13.13	**p=0.0015***	189.0 ± 19.10	p=0.1704	5.00 ± 1.21	**0.0009***
P-VALUE	F=62.41 **p<0.00001***		F=31.01 **p<0.0001***		F=26.42 **p<0.0001***	

Table-3: Effects of Ocimum sanctum and Carbamazepine on MDA and SOD level in PTZ induced convulsions in rats.

Groups	MDA (nmoles /mg of protein)	P-value	SOD (µ/mg of protein)	P-value
Control	3.2 ± 1.02	-	21.8 ± 5.23	-
PTZ (30mg/kg)	8.5 ± 3.07	**0.0009***	10.6 ± 3.02	**0.0001***
PTZ (20mg/kg)	7.8 ± 3.15	**0.0046***	9.5 ± 3.03	**<0.0001***
PTZ (20mg/kg) + OLE (500mg/kg)	6.7 ± 2.10	**0.0473***	16.3 ± 4.01	0.1252
PTZ (30mg/kg) + OLE (1000mg/kg)	6.2 ± 2.02	0.1169	14.13 ± 4.02	**0.0132***
PTZ (30mg/kg) + CBZ (20mg/kg)	5.1 ± 2.02	0.5404	18.34 ± 4.04	0.5621
PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg)	4.7 ± 1.04	0.7675	19.20 ± 5.03	0.8196
PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)	4.3 ± 2.11	0.9368	19.27 ± 4.34	0.8377
PTZ (20mg/kg) + OLE best dose(1000mg/kg)	4.1 ± 0.97	0.4270	20.97 ± 4.34	=0.7710
P-VALUE	F=3.645 **p=0.0024***		F=6.288 **p<0.0001***	

Table-4: Effect on changes in Racine score, Seizure latency, Seizureduration, and Mortality (%) in PTZ induced convulsions in rats

Groups	Racine score	Seizure latency	Seizure duration	Mortality (%)
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
PTZ (30mg/kg)	4.59 ± 1.22	270.8 ± 33.27* **[p<0.0001]**	261.6 ± 27.92	83.33%
PTZ (20mg/kg)	4.33 ± 1.21	261.62 ± 23.78* **[p<0.0001]**	255.8 ± 28.20* **[p<0.0001]**	66.66%
PTZ (20mg/kg) + OLE (500mg/kg)	3.43 ± 1.16	196.6 ± 21.33* **[p<0.0001]**	165.0 ± 15.62* **[p<0.0001]**	33.33%
PTZ (30mg/kg) + OLE (1000mg/kg)	2.81 ± 0.70* **[p=0.0141]**	190.8 ± 25.23* **[p<0.0001]**	133.3 ± 14.21* **[p<0.0001]**	16.66%
PTZ (30mg/kg) + CBZ (20mg/kg)	1.33 ± 0.81* **[p<0.0001]**	121.23 ± 18.82* **[p<0.0001]**	58.3 ± 4.10* **[p<0.0001]**	0.00
PTZ (20mg/kg) + OLE	1.65 ± 0.71*	41.65 ± 8.66*	31.6 ± 6.00*	16.66%

(500mg/kg) + CBZ (20mg/kg)	**[p<0.0001]**	**[p<0.0001]**	**[p<0.0001]**	
PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)	1.41 ± 0.62* **[p<0.0001]**	43.39 ± 7.31* **[p<0.0001]**	27.5 ± 5.5* **[p<0.0001]**	0.00
PTZ (20mg/kg) + OLE best dose(1000mg/kg)	2.16 ± 0.96* **[p<0.0001]**	83.36 ± 13.57* **[p<0.0001]**	93.3 ± 13.33* **[p<0.0001]**	33.33%
P-VALUE	F=16.98 **p<0.0001***	F=156.6 **p<0.0001***	F=223.9 **p<0.0001***	--

Table-5: Effect of Ocimum sanctum and Carbamazepine in PTZ induced behavioural changes in Open field test in rats.

Groups	Corner square entry	Centre square entry	Rearing	Grooming	Defecation
Control	12.66 ± 1.66	10.36 ± 0.54	6.33 ± 1.20	7.66 ± 1.37	1.16 ± 0.16
PTZ (30mg/kg)	15.33 ± 1.33* **[p=0.0054]**	6.5 ± 1.48* **[p=0.0006]**	2.66 ± 0.73* **[p<0.0001]**	4.56 ± 0.89* **[p=0.0007]**	3.33 ± 0.83* **[p<0.0001]**
PTZ (20mg/kg)	14.0 ± 1.03	5.5 ± 1.65* **[p<0.0001]**	2.54 ± 0.33* **[p<0.0001]**	3.66 ± 0.88* **[p<0.0001]**	2.33 ± 0.33* **[p<0.0001]**
PTZ (20mg/kg) + OLE (500mg/kg)	12.75 ± 1.55	7.5 ± 1.57* **[p=0.0149]**	3.53 ± 0.41* **[p<0.0001]**	6.75 ± 1.28	1.55 ±0.28
PTZ (30mg/kg) + OLE (1000mg/kg)	11.6 ± 1.83	9.2 ± 1.54	5.26 ± 0.40* **[p<0.0228]**	5.41 ± 1.57* **[p<0.0198]**	1.40 ±0.30
PTZ (30mg/kg) + CBZ (20mg/kg)	11.83 ± 0.89	9.66 ± 1.40	5.16 ± 0.37* **[p<0.0106]**	6.09 ± 1.28	1.35 ± 0.23
PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg)	12.08 ± 0.94	9.8 ± 1.89	5.82 ± 0.48	6.62 ± 1.27	1.29 ±0.23
PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)	11.83 ± 0.95	10.16 ± 1.60	5.95 ± 0.31	6.59 ± 1.42	1.25 ±0.21
PTZ (20mg/kg) + OLE best dose(1000mg/kg)	12.5 ± 0.96	9.75 ± 1.72	3.75 ± 0.63* **[p<0.0001]**	5.08 ± 1.07* **[p<0.0056]**	1.50 ±0.00
P-VALUE	F=5.346 **p<0.0001***	F=7.965 **p<0.0001***	F=34.90 **p<0.0001***	F=6.056 **p<0.0001***	F=23.58 **p<0.0001***

Table-6: Effect of Ocimum sanctum and Carbamazepine in PTZ induced behavioural changes in Elevated plus maze in rats.

Groups	No of closed arm entry	No of open arm entry	Time spent in closed arm	Time spent in open arm
Control	5.66 ± 1.33	7.83 ± 1.30	228.3 ± 23.27	71.66 ±13.27
PTZ (30mg/kg)	8.33 ± 2.33* **[p=0.0197]**	4.33 ± 1.34* **[p=0.0016]**	193.3 ± 16.66* **[p=0.0122]**	186.6 ± 6.66* **[p<0.0001]**
PTZ (20mg/kg)	7.33 ± 2.66	4.08 ± 1.57* **[p=0.0007]**	185.0 ± 12.86* **[p=0.0012]**	175.0 ± 12.81* **[p<0.0001]**
PTZ (20mg/kg) + OLE (500mg/kg)	7.08 ± 1.40	4.25 ± 1.25* **[p=0.0012]**	172.5 ± 18.53* **[p<0.0001]**	147.5 ± 18.53* **[p<0.0001]**
PTZ (30mg/kg) + OLE (1000mg/kg)	6.92 ± 1.24	4.61 ±1.24* **[p=0.0042]**	174.0 ± 20.78	156.0 ± 16.78
PTZ (30mg/kg) + CBZ (20mg/kg)	6.46 ± 1.30	5.33 ± 1.33* **[p=0.0392]**	116.6 ± 15.42	153.3 ± 15.44
PTZ (20mg/kg) + OLE (500mg/kg) + CBZ (20mg/kg)	6.32 ± 0.21	6.66 ± 1.41	199.0 ± 15.07	82.07 ± 9.06
PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg)	6.51 ± 0.23	6.83 ± 2.37	207.8 ± 23.91	75.3 ± 9.80
PTZ (20mg/kg) + OLE	5.98 ± 0.28	5.75 ± 1.48	234.5 ± 14.9	105.0 ± 6.19*

best dose(1000mg/kg)				**[p<0.0003]**
P-VALUE	F=1.745 p=0.1141	F=4.706 **p=0.0003***	F=21.99 **p<0.0001***	F=73.93 **p<0.0001***

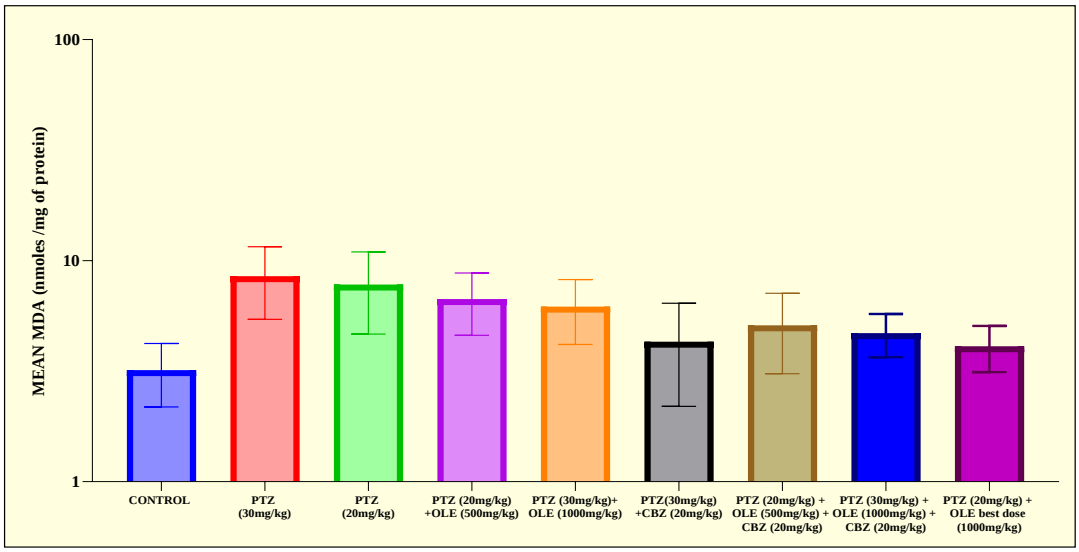

Figure-1(a): Effects of *Ocimum sanctum* and Carbamazepine on biochemical parameters (MDA) level in PTZ induced convulsions in rats.

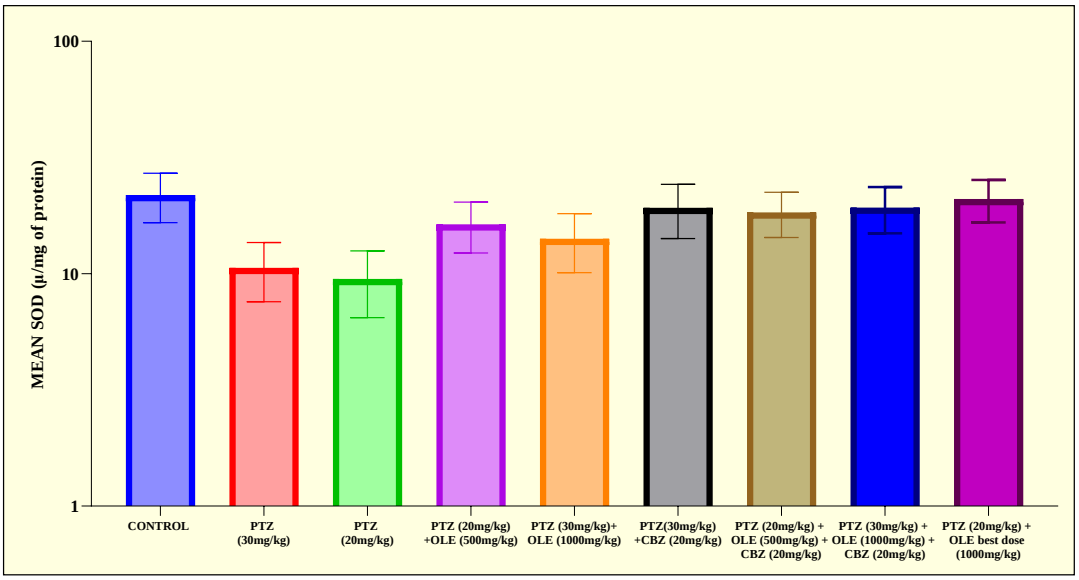

Figure-1(b): Effects of *Ocimum sanctum* and Carbamazepine on biochemical parameters (SOD) level in PTZ induced convulsions in rats.

DISCUSSION

Epilepsy is most serious neurological disorder of recurrent, unprovoked seizures. It increases the risk of morbidity and mortality in the world. Seizures mainly occur due to an imbalance between excitation and inhibition in the brain. The imbalance results from the alteration of brain function, from genes and subcellular signaling cascades to neuronal circuits. Recently, the benefits of herbal preparation for epilepsy treatment have been prominent.*Ocimum sanctum* has the properties of anti-inflammatory, antianxiety, antioxidant and neuroprotective activity. The purpose of this study was to evaluate the protective effect of *Ocimum sanctum* and its interaction with Carbamazepine in rats in PTZ-induced convulsions.

Recent research has found that the PTZ paradigm is widely used to evaluate a drug's anticonvulsant potential. There is conclusive evidence that PTZ harms neural membranes. PTZ regulates potassium and calcium channels and triggers the release of calcium ion repertoires from within cells. Chloride permeability caused by neurotransmitters is also reduced by PTZ [20, 21]. Because drugs that work

against PTZ-induced seizures are also very helpful in treating mild to no epilepsy [22]. So, *O. sanctum* leaf extract has shown promise in preventing PTZ-induced epilepsies and could be helpful in the management of absence seizures [23].

In this study, in order to investigate the behavioral changes in experimental rats, we determine the parameters such as- Elevated plus maze, Open field test and Racine's score for the staging of convulsions along with seizure latency, seizure duration, mortality were considered to evaluate the effect of treatment drugs on PTZ-induced convulsions.

Treatment of epilepsy ratsmodel withOLE and CBZ of various doses showed no significant change in the weight of rats as compared to control group. In experimental models of epilepsy, the Racine scale is a common approach for assessing seizure intensity. This study indicated that *Ocimum sanctum* has neuroprotective benefits against PTZ-induced seizures. 30mg/kg and 20mg/kg of PTZ showed the increased Racine score, Seizure latency, and Seizure duration. Treatment of rats with PTZ (20mg/kg) + OLE (500mg/kg)] and PTZ(30mg/kg) + OLE(1000mg/kg)] showed reduced racine score, seizure latency, and seizure duration and also decreases anxiety as compared to PTZ alone. CBZ treatment showed much better result in reduction of racine score, seizure latency, and seizure duration as compared to OLE treatment.

Treatment of epilepsy rats model with OLE and CBZ of various doses showed significant decreased in racine score, seizure latency, and seizure duration as compared to PTZ (30mg/kg) treatment. This is consistent with the recent work by Sarangi, S. C. et al., 2017 [17], which demonstrated that OSHE effectively decreased pentylenetetrazol (PTZ) and MES-induced seizures in rats; however, it did not demonstrate any extra advantage when combined with valproate. A further study by Sarangi et al., 2020 [24] found that 54 days of treatment with OSHE alone or in conjunction with the antiepileptic drug levetiracetam significantly decreased the seizure score in a rat model of seizures induced by kindling. The antiepileptic properties of *O. basilicum* leaves were also confirmed by a 2014 study [25] by Modaresi, M., et al., which showed that an intraperitoneal injection of *O. basilicum* hydroalcoholic extract improved certain metrics of epileptic behaviour in mice. The results indicated that the effect of *O. basilicum* extract is dose-dependent, with varying amounts inhibiting or delaying different epileptic behaviours.

Percentage of mortality was higher in 30mg/kg of PTZ as compared to 20mg/kg of PTZ. Treatment of rats with PTZ(20mg/kg) + OLE(500mg/kg) and PTZ(30mg/kg) + OLE(1000mg/kg) showed reduction in rats mortality as compared to 30mg/kg of PTZ while PTZ + CBZ(20mg/kg) and PTZ(30mg/kg) + OLE(1000mg/kg) + CBZ (20mg/kg) treatment showed no mortality. PTZ(20mg/kg) + OLE(500mg/kg) + CBZ (20mg/kg) and PTZ (20mg/kg) + OLE best dose (1000mg/kg) also showed decreased in mortality as compared to 30mg/kg of PTZ.

Both the total number of entries in closed and open arms and the time spent in the open arms were assessed for the evaluation of locomotor activity in the elevated plus maze test, which was used to assess the effects of the test subjects on animals' anxiety levels.The number of entries of the rats and the time spent in the closed arm was significantly high in PTZ (30mg/kg) and PTZ (20mg/kg) as compared to control. PTZ + CBZ(20mg/kg) treatment showed an increase in number of entries as compared to OLE and PTZ treated group but the time spent in open arm was decreased as compared to OLE and PTZ. Treatment of PTZ(30mg/kg) + OLE(1000mg/kg) + CBZ (20mg/kg), and PTZ (20mg/kg) + OLE best dose (1000mg/kg) showed decrease in number of entries and increased in the time spent in open arm as compared to control group. These results demonstrated the anxiolytic activity of OLE. Thus, OLE has antioxidant property in brain.

Joshi and Parle, 2006 [15] showed that Ocimum sanctum had a nootropic impact and improved cognitive functions in mice by decreasing transfer latency to enter the enclosed arm in the EPM and increasing retention latency to enter the dark chamber in the PA. OSHE therapy alone or with CBZ improved EPM neurobehavioral task recall compared to the control group, according to Sarangi et al., 2020 [24]. Ocimum sanctum and AEDs may reduce brain dysfunctions.

When the rats were released into the open field, the control animals immediately began traveling along the walls and exploring primarily the corner squares; the center squares were rarely investigated. Therefore, corner square entry in control animals was high and central square entry was low. Rat treated with PTZ (30mg/kg) showed significant increase in the corner square entry and significant decreased in the centre squares entry as compared to control group. Treatment with *ocimum* and its combination group increased entries in the centre squares.Treatment with OLE and co-treatment with OLE and CBZ showed decreased in defection as compared to PTZ treated group.

Current study found that treatment with PTZ (30mg/kg) and PTZ (20mg/kg) significantly increased oxidative stress by increasing the MDA level and when treated with PTZ(20mg/kg) + OLE(500mg/kg), PTZ(30mg/kg) + OLE(1000mg/kg), PTZ + CBZ(20mg/kg)], PTZ(20mg/kg) + OLE(500mg/kg) + CBZ (20mg/kg), PTZ(30mg/kg) + OLE(1000mg/kg) + CBZ (20mg/kg), PTZ (20mg/kg) + OLE best dose (1000mg/kg) MDA level found to be decreased as compare to PTZ treated group while increased when compared to control.

The level of SOD was significantly lower in PTZ (30mg/kg) and PTZ (20mg/kg) treated group as compared to control. PTZ(30mg/kg) + CBZ(20mg/kg)

alone showed protective effect but less than treatment of rats with OLE and combination group significantly protected these activities. The mode of action of PTZ induced neuronal injury diminishes the level of antioxidant enzymes like SOD. Thus, our findings can possibly explain the neuroprotective effect of *Ocimumsanctum* that significantly ameliorate the PTZ induced neuronal changes. These result was similar to the previous result where *Ocimumsanctum* has shown antioxidant effects in different disease models, including epilepsy as revealed by Sarangi et al., 2020, [24].

To a similar extent, Aycicek and Iscan, 2007 [26] hypothesised that CBZ therapy may have exacerbated oxidative stress in both an animal model of seizures and epileptic patients. The antioxidant effect of Ocimum was shown by Sarangi, S. C. et al. 2017 [17], who used a MES- and PTZ-induced seizure model, respectively, and found that compared to the control and valproate-alone-treated groups, MDA levels were significantly reduced and GSH levels were significantly increased. Antioxidant function of Ocimum was shown by decreasing MDA level in hypoperfusion-induced oxidative stress, decreasing lipid peroxidation, and increasing reduced glutathione content in blood in diabetic rabbits (Yanpallewar SU, et al., 2004 [14], Gupta S, et al., 2006 [27]). In contrast to this research, Suanarunsawat T. et al. (2011) [28] found that Ocimum sanctum did not affect serum superoxide dismutase levels.

According to Erdogan, M. A et al., 2022, IL-1β [29] level was increased in PTZ-induced convulsion in rats. Our data also revealed that the level of IL-1β was significantly increased in PTZ (30mg/kg), PTZ (20mg/kg), PTZ(20mg/kg) + OLE(500mg/kg), PTZ(30mg/kg) + OLE(1000mg/kg) PTZ + CBZ (20mg/kg) and PTZ (20mg/kg) + OLE best dose (1000mg/kg) and non-significantly increased in PTZ(20mg/kg) + OLE(500mg/kg) + CBZ (20mg/kg), and PTZ(30mg/kg) + OLE(1000mg/kg) + CBZ (20mg/kg)] as compared to the control group.

The level of IL-4 was also found to be significantly increased in PTZ (30mg/kg), PTZ (20mg/kg), PTZ (20mg/kg) + OLE (500mg/kg), PTZ (30mg/kg) + OLE (1000mg/kg) treated group while non-significantly increased in PTZ (30mh/kg) + CBZ (20mg/kg) as CBZ produces an anti-inflammatory effect. Level of IL-1β and IL-4 in *Ocimum sanctum* treated group was lower when compared with PTZ treated group, which revealed that *Ocimum sanctum* has the capability to reduce inflammation.

According toKarunaweera, N et al., 2015 [30], NF-κβ plays a critical part in the release of inflammatory cytokines and chemokines during epileptic seizures, which exacerbates the damage. The purpose of this research was to determine whether or not Ocimum, has anti-inflammatory activity in PTZ-induced epilepsy. It's worth noting that in vivo PTZ-induced epilepsy is a valid and dependable model system for studying this disorder [31].

Our study showed that NF-κβ was significantly increased in all the treated group except PTZ (30mg/kg) + OLE (1000mg/kg) + CBZ (20mg/kg) treated group (non-significant increased) as compared to control. *Ocimum sanctum* showed to be decreased the level of NF-κβ as compared to PTZ treated group. Similarly, Choudhury, S. S., et al., 2014 [32] showed that *Ocimum sanctum* leaf extracts showed significant inhibition of nuclear translocation of NF-kβ in LPS stimulated THP-1 cells.

The protective effect of *Ocimum* was found more in *Ocimum*(1000mg/kg) as compared to *Ocimum*(500mg/kg). Maximum improvement was seen in the rat which were treated with *Ocimum*(1000mg/kg) in combination with CBZ (20mg/kg).

This study demonstrates that *Ocimum* significantly suppress the development and progression of PTZ induced convulsions when combined with standard drug, which is evidenced by its effect of reduction in Racine's score, improvement in antioxidant parameter and reduction in proinflammatory cytokine leve

CONCLUSION

On the basis of the result obtained in this study we can suggest that *Ocimum sanctum* may have protective/therapeutic effect and neuroprotective effect in suppression of Racine's seizure score, seizure latency, seizure duration, mortality and concurrent episodes of seizure. These effects may be mediated through oxidative stress in the brain. *Ocimum sanctum* with CBZ may be used therapeutically to prevent seizures. Further studies may be designed to evaluate these effects in humans.

ABBREVIATIONS

PTZ- Pentylenetetrazol
CBZ- Carbamazepine
OSLE- Ocimum sanctum leaf extract
SOD-Superoxide dismutase
NF-kβ-Nuclear factor kappa beta
MDA-Malondialdehyde

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