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Original article

Adjuvant role of *Ocimum sanctum* hydroalcoholic extract with carbamazepine and phenytoin in experimental model of acute seizures

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ABSTRACT

Purpose: This study assessed adjuvant potential of *Ocimum sanctum* hydroalcoholic extract (OSHE) with antiepileptic drugs (AEDs) carbamazepine (CBZ) and phenytoin (PHT) in maximal electroshock seizure (MES) model in male Wistar rats.

Material and Methods: Pharmacodynamic effect of OSHE (1000 mg/kg) was assessed through seizure protection potential, neurobehavioral tests and oxidative stress estimation in MES model after 14 days administration of OSHE alone or combination with maximal (M) and sub-maximal (SM) dose of CBZ or PHT. Pharmacokinetic interaction of OSHE with AEDs was also assessed after 14 days of drug treatment.

Results: OSHE *per se* showed 50% protection against MES-induced seizures. Combination of OSHE with AEDs' SM dose enhanced its seizure protection potential. Significant reduction in duration of tonic hind limb extension was observed in CBZ-SM + OSHE as compared to control group ($p = 0.006$). Among neurobehavioral tests in Morris water maze test rats of CBZ-M + OSHE took significantly less time to reach the platform ($p = 0.022$) and spent more time in target quadrant ($p = 0.016$) as compared to other groups. Similarly, rats of PHT-SM + OSHE group spent significantly more time in the target quadrant ($p = 0.013$). In elevated plus maze test, CBZ-M + OSHE had significantly decreased transfer latency compared to other groups ($p = 0.013$). OSHE alone treated group had significantly lower oxidative stress as compared to other groups. No significant pharmacokinetic interaction was observed between OSHE and AEDs (CBZ, PHT).

Conclusion: Ocimum's potential of enhanced seizure protection and neuroprotection along with minimal drug interaction with AEDs substantiate its adjuvant role in the management of epilepsy.

1. Introduction

Despite the discovery of more than 15 newer antiepileptic drugs (AEDs) with unique mechanism of action in last 20 years, about 30% of person with epilepsy (PWE) do not respond to AEDs and experience uncontrolled seizure (Brodie et al., 2016). Amongst the older AEDs, carbamazepine (CBZ) and phenytoin (PHT) are widely used, because of their suitability for most epileptic seizure types, cost-effectiveness and long-term experience (Nolan et al., 2015). The mechanism of action of CBZ and PHT is through blockade of voltage-dependent sodium channel; thereby inhibiting the sustained repetitive firing in individual neurons (Czapiński et al., 2005). Despite better efficacy in terms of seizure control, CBZ and PHT elicit several CNS adverse effects such as dizziness, diplopia, ataxia, incoordination, and cognitive dysfunctions (Sarangi et al., 2017; Kennedy and Lhatoo, 2008). Moreover, the significant enzymes (cytochrome P450) inducing potential of these two drugs attribute to its greater propensity for possible drug interaction as compared to other AEDs (Spina et al., 2016). So, prudence is needed for concomitant use of these AEDs with other drugs and nutritional supplements. Hence, there is need of novel therapeutic agents as a stand-alone or as adjuvant to these AEDs for better seizure control as well as to counteract AEDs' adverse effects such as cognitive dysfunctions along with minimal pharmacokinetic interactions.

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The maximal electroshock seizure (MES) model of acute seizure is well-validated and one of the gold standard tests used to screen compounds with antiseizure potential in the early drug development process (Castel-Branco et al., 2009). Though, it is postulated that the MES model allows the screening of drugs acting on Na + channels such as CBZ and PHT (Meldrum, 2002), most of the standard and newer AEDs with diverse mechanism of action (e.g. phenobarbitone, topiramate) have also proven their efficacy in the MES model (Rogawski, 2006).

Traditional medicines are widely used for the management of epilepsy due to their ease of availability and broader experience (Sucher and Carles, 2015). *Ocimum sanctum* commonly known as 'Tulsi' in Hindi and 'Holy Basil' in English has been widely used in traditional systems of medicine from ancient eras with projected properties such as anticancer, antioxidant, anti-inflammatory, and immunomodulatory potential (Giridharan et al., 2011). A recent systemic review reported that leaves of Ocimum have been used for treatment of epilepsy by traditional healer in India (Emilie et al., 2019). Phytochemical analysis of *Ocimum sanctum* extract has shown presence of ursolic acid and oleanolic acid as the some of the primary constituents (Joshi et al., 2017). However, there is lack of evidence showing its possible interaction with widely used AEDs such as PHT and CBZ when used concomitantly. Previous studies have assessed the pharmacodynamics (PD) and pharmacokinetic (PK) interaction of Ocimum with valproate and levetiracetam, and found antiseizure potential of Ocimum adjuvant to these AEDs without significant drug interaction (Sarangi et al., 2020, 2017). So, in current study, we evaluated the antiseizure potential of *Ocimum sanctum* as an adjuvant to CBZ and PHT in experimental model of acute seizure i.e. maximal electroshock seizure model (MES) and its pharmacokinetic interaction with these two standard AEDs.

2. Materials and methods

2.1. Experimental protocol

Current study assessed the PD interaction of *Ocimum sanctum* hydroalcoholic leaf extract (OSHE) [botanical name: *Ocimum tenuiflorum* L.; synonym: *Ocimum sanctum* L; family: *Lamiaceae*] with CBZ and PHT in acute seizure model (i.e. MES model) in rats. Along with that pharmacokinetic (PK) interaction of the above mentioned drugs were also studied. The experimental procedure was commenced after obtaining approval from Institutional Animal Ethics Committee (IAEC) (Ethics approval no. 02/IAEC-1/2017) and conducted in accordance with 'The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)' guidelines, Department of Animal Welfare, Government of India.

2.2. Animals

Adult male Wistar rats (200–250 g), obtained from the Central Animal Facility of the All India Institute of Medical Sciences, New Delhi were housed under optimum laboratory conditions with dark and light cycle (approximately 12 h:12 h). They were provided free access to standard pellet diet and tap water. The experiment was started after 7 days of acclimatization.

2.3. Drugs and chemicals

The Ocimum extract (hydroalcoholic) was procured from one of the Pharmaceutical company (Natural Remedies Pvt. Ltd., Bangalore, India) as a gift sample. Phytochemical analysis of OSHE by high performance liquid chromatography (HPLC) has shown combined presence of ursolic acid and oleanolic acid 2.8% w/w along with rosmarinic acid (0.6% w/w) and eugenol (0.2% w/w). Glutathione was obtained from Sigma Inc., USA. CBZ and PHT active pharmaceutical ingredient were obtained from Sun Pharma Laboratories Ltd., India. All other chemicals and solvents were obtained from Merck (India) and were of analytical grade and chemicals used in HPLC were of HPLC grade.

2.3.1. Doses of drugs

***Ocimum sanctum* hydroalcoholic extract (OSHE)**: OSHE at a dose of 1000 mg/kg was used in this study. The dose selection was based on our previous study where OSHE in 1000 mg/kg was found to be the optimal dose for seizure control out of four doses of OSHE (200,400, 800 and 1000 mg/kg) (Sarangi et al., 2017).

Carbamazepine (CBZ) and Phenytoin (PHT): Two doses of CBZ (20 and 10 mg/kg) and PHT (40 and 20 mg/kg) were used in the study corresponding to the maximal and sub-maximal dose. These dose selection criteria were as per the previous study carried out in our laboratory by Pahuja et al., 2012. Dimethyl sulfoxide (DMSO) (10%) was used as vehicle to dissolve all the drugs as it was unlikely to cause any alteration in antiepileptic potentials of AEDs (Carletti et al., 2013). All of the drugs were freshly prepared in vehicle and were administered once daily orally for 14 days. OSHE was administered 30 min before administration of AEDs (CBZ or PHT) and the maximum volume of drugs administered each time was 0.5 ml/100 g of animal.

2.4. Experimental groups:

For PD assessment, the animals were randomly divided into nine groups containing six animals each. The groups along with the treatment administered were: Control (10% DMSO), OSHE (OSHE 1000 mg/kg), CBZ-M (CBZ 20 mg/kg), CBZ-SM (CBZ 10 mg/kg), CBZ-M + OSHE: (CBZ 20 mg/kg and OSHE 1000 mg/kg), CBZ-SM + OSHE: (CBZ 10 mg/kg and OSHE 1000 mg/kg), (PHT-M: PHT 40 mg/kg), PHT-SM: (PHT 20 mg/kg), PHT-M + OSHE: (PHT 40 mg/kg and OSHE 1000 mg/kg), PHT-SM + OSHE: (PHT 20 mg/kg and OSHE 1000 mg/kg).

For PK assessment, the separate group of animals were considered and divided into four groups containing six animals each as follows: CBZ-M, CBZ-M + OSHE, PHT-M, PHT-M + OSHE. The treatment administered in these groups was as per above description. The experimental protocol has been depicted in Fig. 1.

2.5. Pharamacodynamic (PD) assessment

2.5.1. Induction of seizures by maximal electroshock seizure (MES) model

The seizure was induced by MES as described by Sarangi et al, 2017, where a suprathreshold electrical stimulus (current intensity-70 mA, duration 0.2 s) was delivered via ear clip electrodes after 60 mins of AEDs and/or OSHE administration. Tonic hind limb extension [(THLE); i.e. the hind limbs of animals outstretched 180° to the plane of the body axis] was taken as end point where the animals were observed for latency, duration and occurrence of THLE. This test was performed on 14th day of administration of OSHE and AEDs.

2.5.2. Neurobehavioral tests

Neurobehavioral assessments were performed to assess cognitive functions such as learning and memory by elevated plus maze (EPM), passive avoidance (PA) and Morris water maze (MWM) as described by Sarangi et al., 2017. Tests were performed on rats with about 12 h food deprivation and after about 1 h acclimatization in a sound-proof testing room devoid of external interference like bright light. The recordings of these tests were reported as 'before MES' i.e. before MES-induced seizure on 14th day, and 'after MES' i.e. on day 15 (24 h after seizure induction).

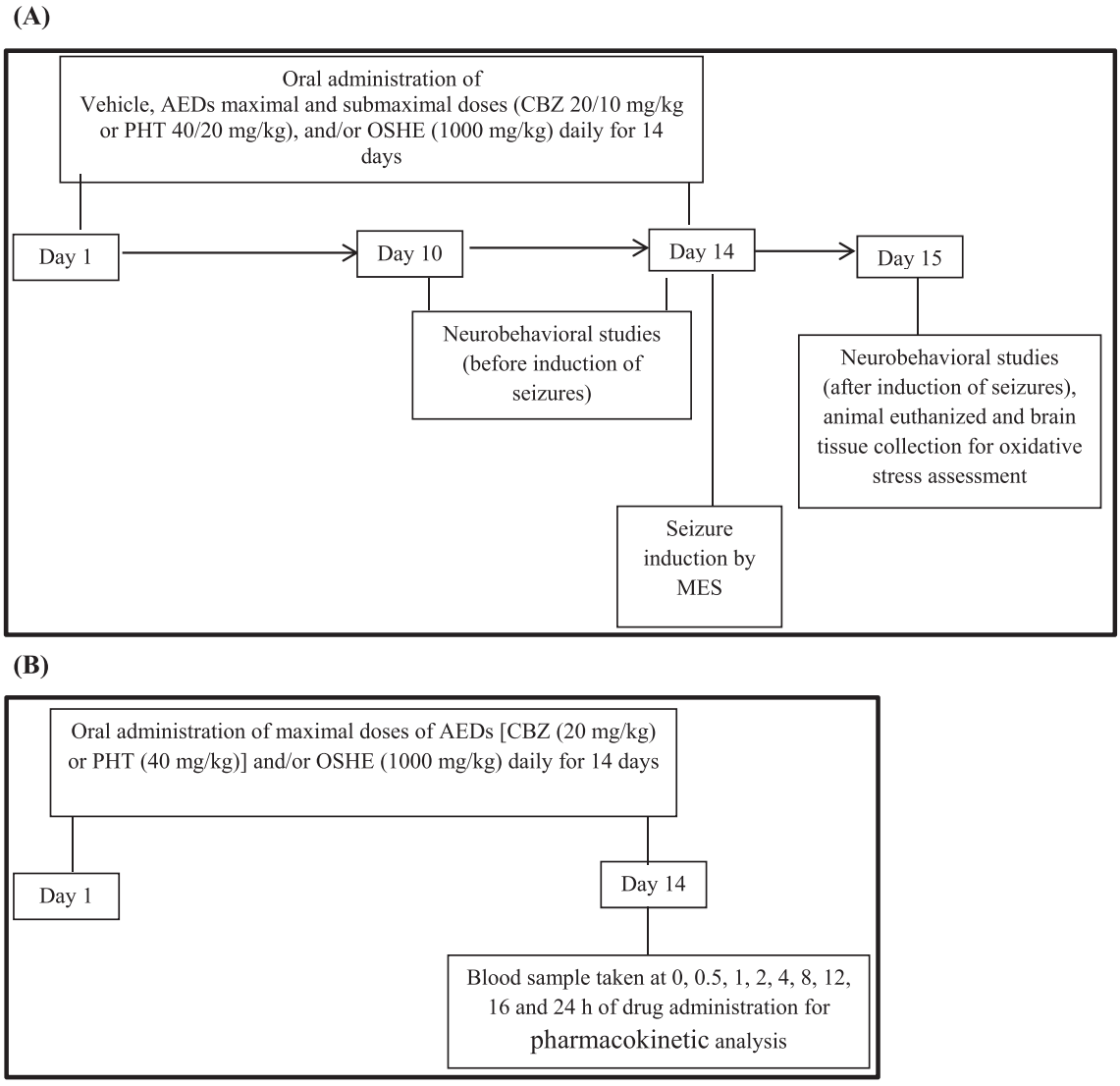

Fig. 1. Experimental protocol (A) pharmacodynamic assessment, and (B) pharmacokinetic assessment. AEDs: antiepileptic drugs, CBZ: carbamazepine, PHT: phenytoin, OSHE: *Ocimum sanctum* hydroalcoholic extract, PK: pharmacokinetic.

2.5.2.1. MWM test:. MWM test was carried out to assess the spatial memory in rats before and after induction of seizures. The MWM consisted of a large circular pool filled with water and a platform submerged 1 cm below the surface of water. The water maze was divided into four equal quadrants (1, 2, 3 and 4) and a platform was kept into 4th quadrant. A trial was initiated by placing a rat in the desired start position in the maze, facing the wall of the maze and the computer tracking program was started the moment the rat is released. Acquisition trials were carried for 4 days where trial was given from all four quadrants for which the position of the platform was fixed for each trial. On the fifth day, a spatial probe trial of 60 s duration was taken. Latency to reach the platform, time spent in target quadrant and numbers of platform crossings were noted during the probe trial. This test was performed from the 10th day to the 14th day (before induction of seizures), and a probe trial was repeated a day after seizure induction, i.e., on the 15th day.

2.5.2.2. EPM test. In EPM test, each rat was placed at one of the open arms facing outward and the latency to enter to any of the enclosed arms i.e. transfer latency (TL) was recorded on 13th day. Twenty-four hours later, i.e. on day 14, the retention TL was measured in the same manner. If a rat did not enter the enclosed arm within 60 s, the TL was assigned 60 s. TL was also measured a day after seizure induction, i.e., on the 15th day.

2.5.2.3. PA test. A step-through PA test was done to assess the memory retention potential in rats which is a hippocampus-dependent task and detect the ability of the animal to passively avoid by recalling the previously delivered foot shock. The apparatus has two parts (light and dark), training was performed on day 13 by placing the rats individually in the light compartment for a cut-off time of 60 s. The door between two compartments was raised and when the rat entered the dark compartment, the door was closed, and a single foot electric shock (50 Hz, 0.2 mA) for 3 s was delivered through the grid floor by a standard stimulator. The initial latency to enter into the dark compartment was recorded. The animal was removed from the dark chamber 10 s later. After 24 h i.e. on day 14 (before induction of seizures), retention latency was measured up to a maximum of 300 s (cut-off time), but foot shock was not delivered. The retention latency was also taken day after induction of seizures (on day 15).

2.5.3. Brain tissue collection and estimation of oxidative stress

On the 15th day of experiment, animals of each groups were euthanized by decapitation after neurobehavioral assessment, and their brains were quickly removed, cleaned by rinsing with chilled normal saline and stored at −80 °C for analysis of various oxidative stress parameters such as malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) levels. Briefly, the brain tissue was homogenized with ten times (w/v) sodium phosphate buffer (pH 7.4) and the homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was collected and kept at −20 ^{0}C until used for analysis.

MDA in tissue was estimated by the method of Ohkawa et al. (1979) with slight modification (Ohkawa et al., 1979; Sarangi et al., 2020). Tetraethoxy propane was used as an external standard to obtain the standard curve and the absorbance was recorded at 532 nm using Multi-Mode Microplate Readers (SpectraMax® M Series, Molecular Devices, California, United States) and the MDA concentration was expressed as nmol/g of brain tissue. GSH concentration was measured by the method described by Ellman (1959) with minor modifications (Ellman, 1959; Sarangi et al., 2020). GSH concentration was expressed as μg/g of brain tissue and the absorbance was recorded at 412 nm within 15 min of sample preparation. The SOD activity was expressed as percentage inhibition of autoxidation of pyrogallol by observing the increase in absorbance at 420 nm for 3 min at the interval of 30 s and the method of estimation was a little modification of the method described by (Marklund and Marklund, 1974; Sarangi et al., 2020).

2.6. Pharmacokinetic (PK) assessment

Various PK parameters were assessed and compared among AEDs maximal dose alone treated rats (CBZ-M, PHT-M) and AEDs + OSHE combination groups. Nearly, 0.5 ml of blood samples were collected in heparinized tubes on 14th day at each time points i.e. 0 h (trough sample) and after 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h of drugs administration. The blood samples were drawn from the *retro*-orbital plexus of rats under mild ether anesthesia. During PK study to replenish fluid loss, dextrose-normal saline (1 ml/100 g of animal) was injected intraperitoneally after 1, 4 and 12 h of blood sampling to avoid risk of hypovolemia and changes in volume and electrolytes of the central compartment in animals. Serum was separated from the collected samples and kept at −80 °C until the estimation AEDs level by Reverse Phase-High-Performance Liquid Chromatography (RP-HPLC) (Agilent Technologies, 1200 series HPLC with Chemstation software) with reverse phase analytical C-18 column (column temperature = 25° C). The method of estimation is as described by Liu et al., 1993 with slight modification. The mobile phase consisted of potassium dihydrogen orthophosphate buffer, acetonitrile, methanol in a ratio of 58:21:21 v/v with runtime of 15 mins and with a flow rate of 1 ml/min. The serum was mixed with acetonitrile, vortexed and later centrifuged at 15000 rpm for protein precipitation. Then supernatant was filtered and injected at a volume of 10 μL to RP-HPLC system through automatic sampler and the detection was done by a UV detector with wavelength of 210 nm. The lower limit of quantification (LLOQ) and limit of detection (LOD) for CBZ are 0.4 and 0.1 ppm respectively. The LLOQ and LOD for PHT are 0.5 and 0.2 ppm respectively. Non-compartmental model was used for PK analysis using WinNonlin software [Phoenix® WinNonlin® 7.0 (Certara USA, Inc.)].

2.7. Statistical analysis

All the data were analyzed by using SPSS (version 23.0). Data were represented as mean ± SD (for normally distributed data) or median and interquartile range (for non-normally distributed data). One way Analysis of variance (ANOVA) followed by Bonferroni's Post Hoc test or Krushkal -Wallis test with multiple comparison (pairwise) were performed to compare different groups for parametric and non-parametric data respectively. Chi square test was done for analysis of categorical variable i.e. % protection of seizures. Wilcoxon rank sum test was used to compare different parameters before and after seizures induction within a group. A p value <0.05 was considered as statistically significant. PK parameters were analyzed using WinNonlin software [Phoenix® WinNonlin® 7.0 (Certara USA, Inc.)]. Comparison of PD parameters was done between following groups: a- Control, OSHE and CBZ treated groups (CBZ-M, CBZ-SM, CBZ-M + OSHE and CBZ-SM + OSHE); b- Control, OSHE and PHT treated groups (PHT-M, PHT-SM, PHT-M + OSHE and PHT-SM + OSHE). PK parameters were compared between only AED treated groups vs. combined AED + OSHE treated group.

3. Results

3.1. Effect of OSHE, CBZ and PHT on MES-induced seizures

MES-induced seizures was observed in all the rats of control group. Seizure protection was 100% in maximal AEDs dose-treated groups (CBZ-M and PHT-M). OSHE group showed 50% protection against seizures and the combination of OSHE and maximal dose of CBZ and PHT did not enhance seizure protection as already 100% protection was achieved. However, combination of OSHE with sub-maximal dose of AEDs enhanced seizure protection (66.67 to 83.33% in CBZ-SM + OSHE and 33.33 to 66.67% in PHT-SM + OSHE group) as compared to sub-maximal dose of AEDs alone treated groups. Rats of control groups showed significantly low latency time to THLE as compared to OSHE treated groups ($p < 0.001$). Rats of OSHE treated groups had significantly increased latency to THLE and decreased duration of THLE as compared to PHT-SM ($p < 0.001$ and 0.038, respectively) and PHT-SM + OSHE groups ($p < 0.001$ and 0.038, respectively) [Table 1].

3.2. Effect of OSHE, CBZ and PHT on neurobehavioural parameters (MWM, EPM and PA)

In MWM test, rats of control group took significantly more time to reach the target quadrant and spent less time in the target quadrant after MES as compared to before MES ($p = 0.046$ for both). Similarly, OSHE alone treated rats spent significantly less time in the target quadrant after MES as compared to before MES ($p = 0.028$). However, no significant difference was observed in AEDs (CBZ and PHT) treated group after MES as compared to before MES.

The comparison of control, OSHE and CBZ treated groups revealed that rats of CBZ-M + OSHE took significantly less time to reach the platform (overall p value = 0.022) and spent more time in target quadrant (overall p value = 0.016) as compared to other groups after MES. In EPM test, CBZ-M + OSHE had significantly decreased transfer latency to enter into enclosed arm as compared to control and other drug treated groups after MES ($p = 0.013$). However, significant difference was not observed between control and CBZ-M group. These results denote that combination OSHE is leading to less neurobehavioral adverse effect than CBZ alone treatment [Fig. 2].

Similarly, the comparison of OSHE with phenytoin treatment in EPM test has shown that, OSHE alone treated rats had significantly decreased transfer latency as compared to PHT-SM treated group ($p = 0.047$). However, PA test did not reveal any significant difference in retention latency to enter into dark chamber after MES among different groups. In MWM test, Rats of PHT-SM + OSHE

Table 1
Effect of OSHE, CBZ and PHT on MES-induced seizures.

Groups	% (number of animals) with protection against THLE	No. of animals showing THLE	Latency to THLE (sec) Mean ± S.D.	Duration of THLE (sec) Mean ± S.D.
Control	0 (0/6)	6/6	4.16 ± 1.16$	6.16 ± 0.75
OSHE	50 (3/6)	3/6	8.66 ± 1.15	4.66 ± 0.57
CBZ-M	100 (6/6)	0/6	–	–
CBZ-SM	66.67 (4/6)	2/6	4.25 ± 0.95	6.25 ± 0.50
CBZ-M + OSHE	100 (6/6)	0/6	–	–
CBZ-SM + OSHE	83.33 (5/6)	1/6	6	4
Overall p value	*NS		0.001	<0.01
PHT-M	100 (6/6)	0/6	–	–
PHT-SM	33.33 (2/6)	4/6	3.25 ± 0.5$	7 ± 1.41$
PHT-M + OSHE	100 (6/6)	0/6	–	–
PHT-SM + OSHE	66.67 (4/6)	2/6	4 ± 0.81$	7 ± 0.81$
Overall p value	*NS		<0.01	<0.05

"-" means none of the animals in the group showed Tonic hind limb extension (THLE). OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), CBZ-M: carbamazepine maximal dose (20 mg/kg), CBZ-SM: carbamazepine sub-maximal dose (10 mg/kg), PHT-M: phenytoin maximal dose (40 mg/kg), PHT-SM: phenytoin sub-maximal dose (20 mg/kg). Overall p-value determined by comparison among Control, OSHE, and AED treated (CBZ and PHT separately) groups: % protection against THLE [NS (not significant)] as per chi-square test, and latency and duration of THLE as per One-way ANOVA. $p value vs. OSHE as per Bonnferonni post-hoc test: in Latency to THLE $p < 0.001$ and in Duration to THLE $p < 0.05$.

spent significantly more time in the target quadrant (overall p value = 0.013) and PHT-SM group had significantly more number of platform crossing (overall p value = 0.015) after MES as compared to other groups. However, no significant difference was found in EPM and PA test among different groups **[**Fig. 3**]**.

3.3. Effect of OSHE, CBZ and PHT on oxidative stress (MDA, GSH and SOD)

Analysis of different oxidative stress parameters (MDA, GSH and SOD activity) by one-way ANOVA showed significant difference among different groups (overall p-value < 0.001). Post-hoc analysis revealed significantly increased MDA level in rats of control group as compared to other drug treated groups ($p < 0.001$). Rats of OSHE alone treated group had significantly lower MDA as compared to other AEDs treated groups. GSH level was significantly lower in control group as compared to other drug treated groups ($p < 0.001$). OSHE alone treated rats had significantly higher GSH level as compared to CBZ-M group ($p = 0.008$). In SOD activity test, CBZ-M + OSHE had significantly higher protection as compared to CBZ-SM + OSHE group ($p = 0.004$) **[**Figs. 4 and 5**]**.

3.4. Effect of OSHE on various PK parameters of CBZ and PHT

The PK analysis by HPLC did not reveal any statistically significant difference in various PK parameters such as C_{max}, T_{max}, area under curve (AUC), $T_{1/2}$, Vd and CL among CBZ-M and combination (CBZ-M + OSHE) group. Similarly, no statistically significant difference was found in above parameters when comparison was done between PHT-M and PHT + OSHE group **(**Table 2**)**.

4. Discussion

The aim of the study was to assess the pharmacodynamic interaction of OSHE with AEDs such as CBZ and PHT in acute seizures model. As per a recent review article, the pentylenetetrazole seizure (PTZ) model and the maximal electroshock seizure (MES) model are widely used for screening of anticonvulsant potential of a test compound. Both PTZ and MES models display efficacy and predictability into humans across different AEDs with different mechanisms of actions. However, as per review articles comparing MES and PTZ models (Yuen and Trocóniz, 2015; Löscher, 2011), AEDs such as CBZ and PHT show false negative results and are not effective in PTZ model, whereas they have better efficacy in MES model. Further it has been stated that MES model has less variability and better precision of human dose prediction as compared to PTZ model. Taking these opinions into consideration this study chose MES model for pharmacodynamic assessment.

In the current study, antiseizure potential of Ocimum *per se* has been demonstrated using OSHE treatment in MES model of seizure which resulted in 50% protection against THLE. Maximum dose of AED (both CBZ and PHT) resulted in 100% protection. Moreover, combination of OSHE with maximum dose of AEDs also resulted in similar protection (100%) without any change. This is in line with our previous study, where OSHE effectively reduced pentylene-tetrazole (PTZ) and MES-induced seizures in rats; however, it did not exhibit any additional benefit in combination with valproate (Sarangi et al., 2017). In another study, chronic treatment with OSHE either alone or in combination with AED levetiracetam for 54 days significantly decreased the seizure score in rats in kindling model of seizure (Sarangi et al., 2020). Moreover, current study demonstrated that OSHE potentiated the antiseizure action of CBZ and PHT when administered concomitantly with submaximal dose. As per previous study, higher AED dose or its level are associated with several neurobehavioral adverse effects in PWE and these adverse effects may be reduced or reversed by lowering the dose of AED (Witt and Helmstaedter, 2017). The current study result thus put forth the adjuvant potential of Ocimum, which may aid in lowering of AED dose in treatment of epilepsy.

CBZ and PHT exert their antiseizure action by prolonging inactivation of Na+ channel and thereby reduce repetitive neuronal firing (Czapiński et al., 2005). OSHE has exerted its beneficial role in MES model of acute seizures in the current study and previous studies also (Sarangi et al., 2017; Jaggi et al., 2003). This study also opines the adjuvant potential of OSHE with AEDs to potentiate their antiepileptic effect. From these finding it may be hypothesized that OSHE may have a role on voltage gated Na+ channel thereby potentiating the antiseizure action of AED. However, previous studies have also shown that, Ocimum demonstrated antiepileptic potential in PTZ induced acute seizure and kindling model (Sarangi et al., 2017, Sarangi et al., 2020), which primarily involves modulation of GABA. Also, it has potentiated the antiepileptic effect of AEDs like valproate which has broad spectrum of action, levetiracetam which acts through synaptic vesicle protein. These study findings suggest that Ocimum's antiepileptic potential may involve multiple mechanisms including modulation of Na^+ channel and GABA. However, further mechanistic studies are required to establish these findings.

Evidence suggests that epilepsy itself as well as treatment with AEDs lead to several neurobehavioural dysfunctions such as

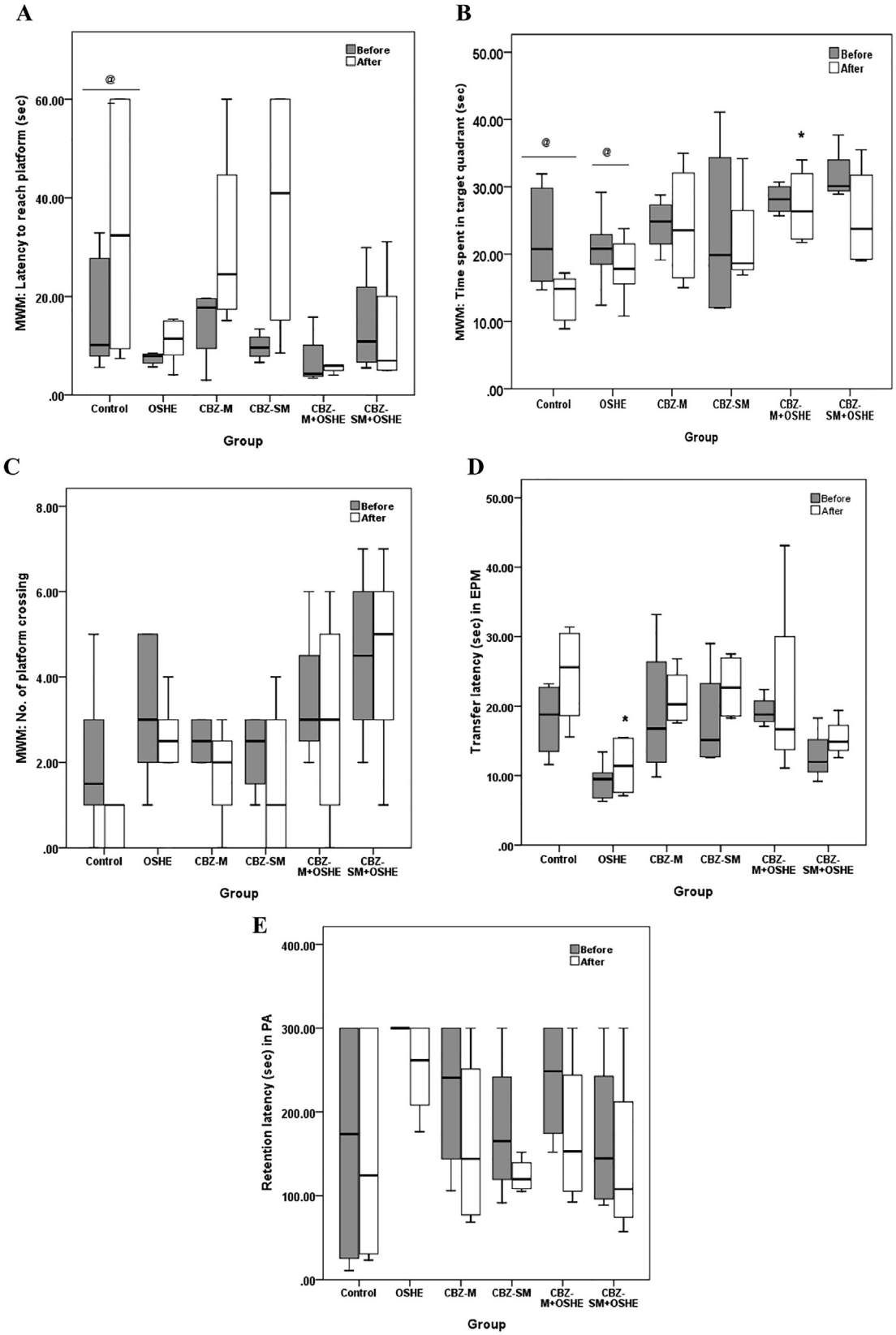

Fig. 2. Effect of OSHE and CBZ on neurobehavioral parameters (MWM, EPM and PA). Morris water maze (MWM) [A- Latency to reach platform, B- Time spent in target quadrant, C- No. of platform crossings]; Elevated plus maze (EPM) [D- Transfer latency]; Passive avoidance (PA) [E- Retention latency]. 'Before' means before seizure induction (14th day); 'After' means after seizure induction (15th day). OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), CBZ-M: carbamazepine maximal dose (20 mg/kg), CBZ-SM: carbamazepine sub-maximal dose (10 mg/kg). Overall p-value as per Kruskal-Wallis test significant ($p < 0.05$) in case of MWM- latency to reach platform and time spent in target quadrant 'After data', EPM transfer latency both 'Before and After data'. *p value vs. Control as per Kruskal-Wallis multiple comparison test. @p value between 'Before and After data' within a group as per Wilcoxon Sign Ranks test.

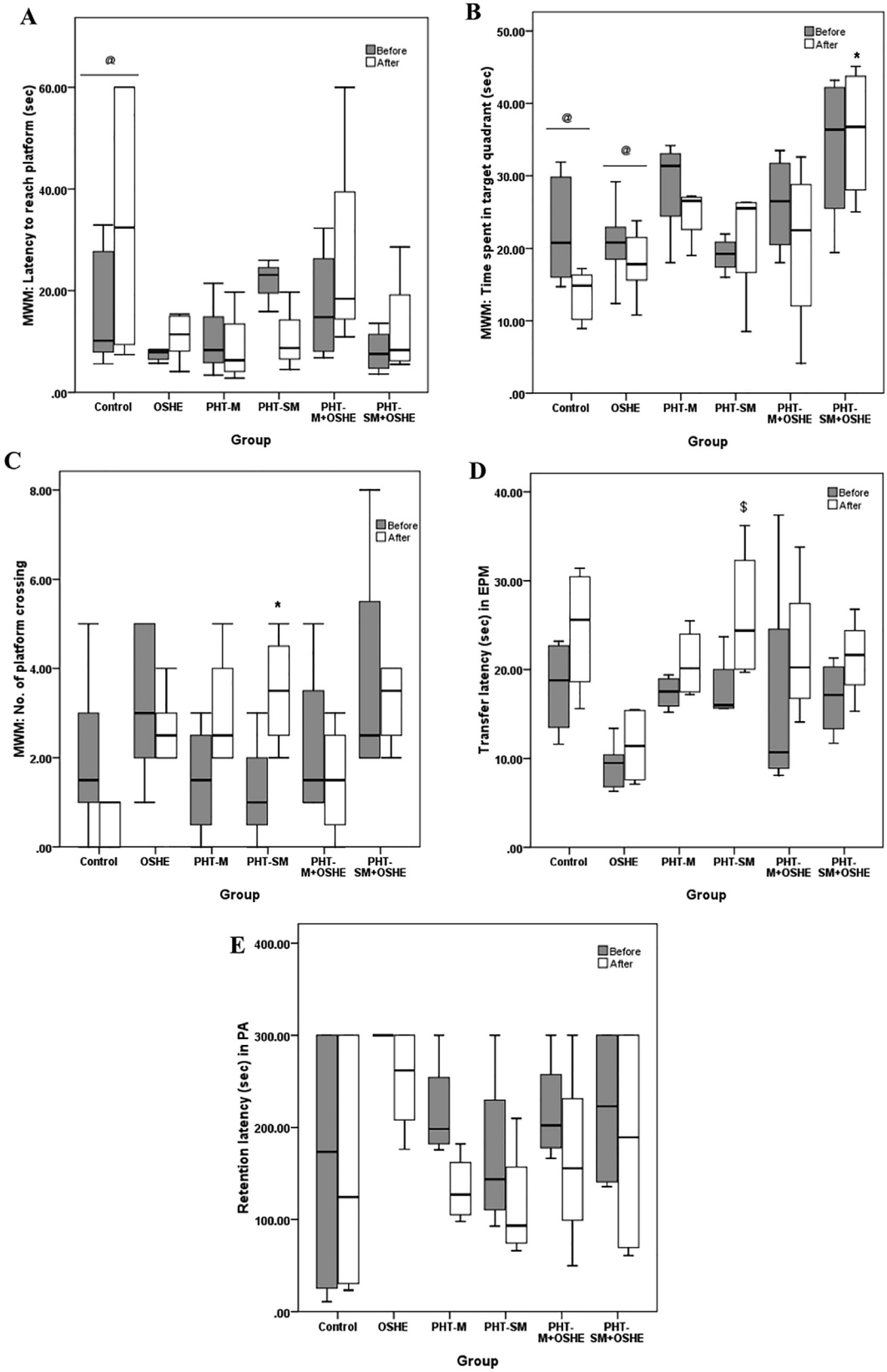

Fig. 3. Effect of OSHE and PHT on neurobehavioral parameters (MWM, EPM and PA). Morris water maze (MWM) [A- Latency to reach platform, B- Time spent in target quadrant, C- No. of platform crossings]; Elevated plus maze (EPM) [D- Transfer latency]; Passive avoidance (PA) [E- Retention latency]. 'Before' means before seizure induction (14th day); 'After' means after seizure induction (15th day). OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), PHT-M: phenytoin maximal dose (40 mg/kg), PHT-SM: phenytoin sub-maximal dose (20 mg/kg). Overall p-value as per Kruskal-Wallis test significant ($p < 0.05$) in case of MWM- time spent in target quadrant and no. of platform crossing 'After data', EPM transfer latency both 'Before and After data'. *p value vs. Control and $p value vs. OSHE as per Kruskal-Wallis multiple comparison test. @p value between 'Before and After data' within a group as per Wilcoxon Sign Ranks test.

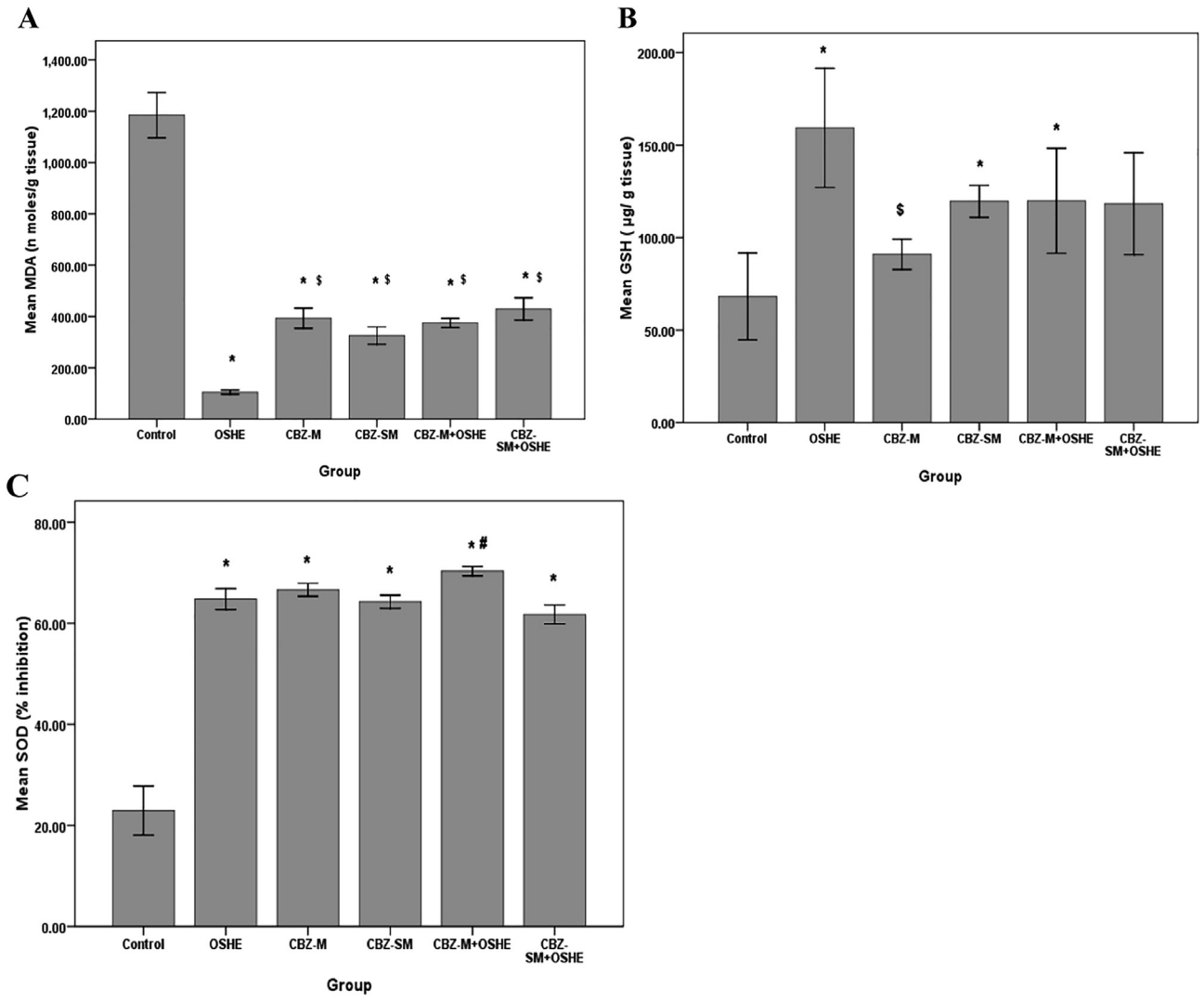

Fig. 4. Effect of OSHE and CBZ on oxidative stress parameters (MDA, GSH and SOD). [A]: MDA: Malondialdehyde, [B]: GSH: glutathione reduced, [C]: SOD: superoxide dismutase. OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), CBZ-M: carbamazepine maximal dose (20 mg/kg), CBZ-SM: carbamazepine sub-maximal dose (10 mg/kg). Overall p-value for MDA, GSH and SOD as per one-way ANOVA are < 0.001 for each. As per Bonferroni post-hoc analysis: *p value < 0.001 vs. Control, $p value < 0.01 vs. OSHE, and #p value < 0.001 vs. CBZ-SM + OSHE.

impairment in learning and memory in PWE (20 to 50%) (Subramaniam et al., 2020; Reeta et al., 2010). There are large bodies of literatures reporting significant association of cognitive decline and AEDs therapy such as CBZ and PHT in PWE (Subramaniam et al., 2020; Eddy et al., 2011). Further, the risk is increased with higher doses of AEDs. Subramaniam et al (2020) reported CBZ at a dose of 1000 mg per day significantly deteriorated immediate memory performance in PWE. In the current study, though there was no significant difference, there was relatively higher memory impairment in CBZ-M group than CBZ-SM group. Current study demonstrated that OSHE treatment alone or along with CBZ has improved memory as compared to control group in the MWM and EPM neurobehavioral tests. Similarly, OSHE treatment alone or along with PHT has improved memory in MWM test only. This is in support of our previous study finding where combined treatment of OSHE and VPA had better memory retention potential as compared to VPA alone treated rats (Sarangi et al., 2017). In another study, *Ocimum sanctum* showed nootropic effect and improved cognitive functions in terms of decreased transfer latency to enter into enclosed arm in EPM and increased retention latency to enter into dark chamber in PA in mice (Joshi and Parle, 2006). These facts suggest that Ocimum may have the potential to attenuate the cognitive dysfunctions associated with AEDs therapy when used in combination.

Oxidative stress is common phenomenon which occurs due to an imbalance between antioxidant and production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) leading to cellular and tissue damage (Ullrich and Kissner, 2006). Increased oxidative stress has been found in surgically resected brain tissue of epileptic patients which prompts neuronal hyperexcitability and degeneration (Pearson-Smith and Patel, 2017). Animal models of seizures have also demonstrated seizure-induced oxidative damage to mitochondrial and hippocampal DNA and proteins leading to neurodegeneration and neuronal death (Jarrett et al., 2008; Liang et al., 2000). Increased oxidative stress may modulate the synthesis and release of several neurotransmitters which plays a key role in cognition. Current study found that treatment with OSHE at a dose of 1000 mg/kg significantly ameliorated the oxidative stress by MES-induced seizures in terms of decreased MDA, higher GSH level in rats as compared to control and AED treated groups. This is at par with the previous studies where Ocimum *per se* has shown antioxidant effects in various disease models

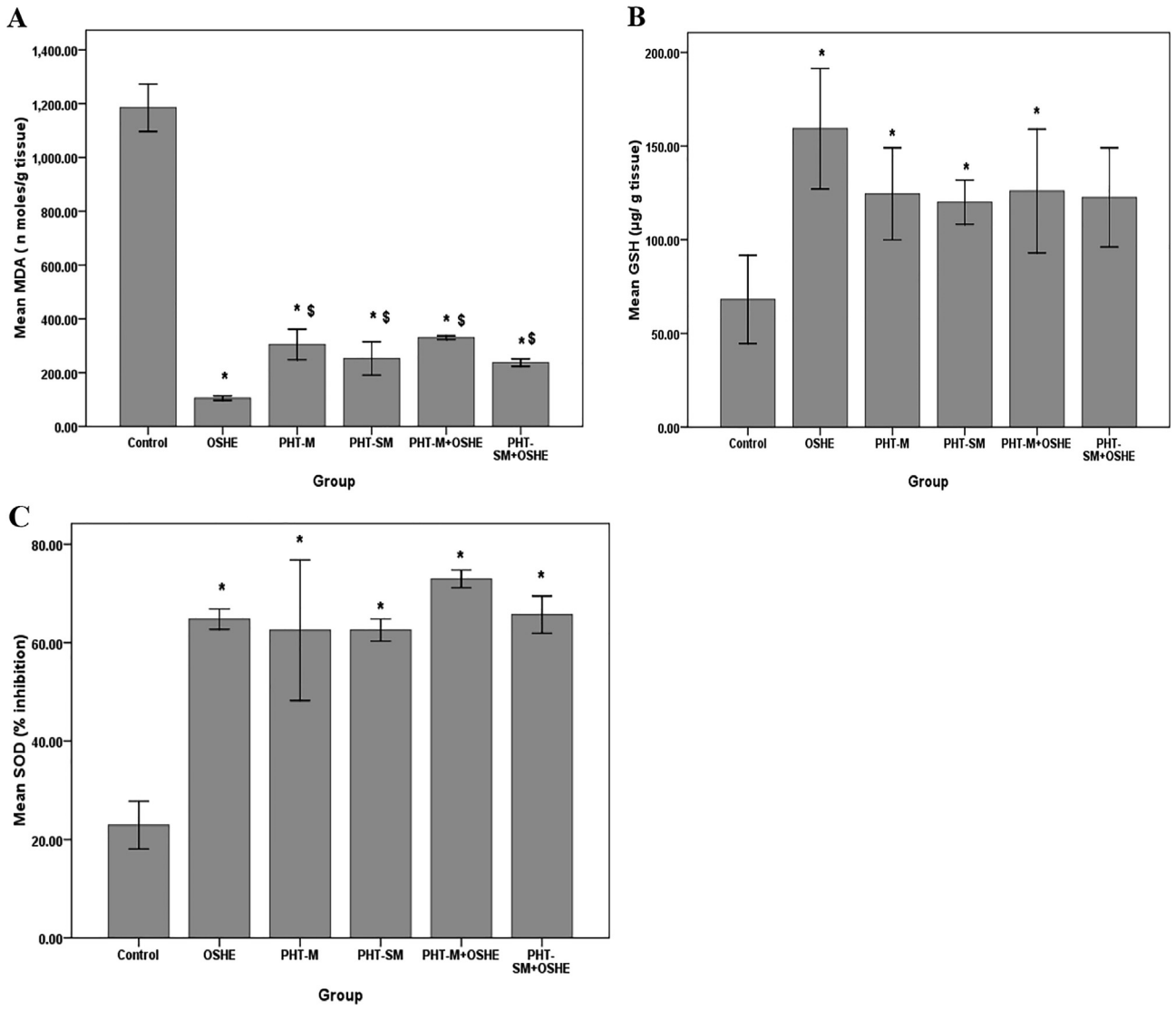

Fig. 5. Effect of OSHE and PHT on oxidative stress parameters (MDA, GSH and SOD). [A]: MDA: Malondialdehyde, [B]: GSH: glutathione reduced, [C]: SOD: superoxide dismutase. OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), PHT-M: phenytoin maximal dose (40 mg/kg), PHT-SM: phenytoin sub-maximal dose (20 mg/kg). Overall p-value for MDA, GSH and SOD as per one-way ANOVA are < 0.001 0.001 and < 0.001 respectively. As per Bonferroni post-hoc analysis: *p value vs. Control [in A and C $p < 0.001$; in B $p < 0.001$ for OSHE and $p < 0.05$ for PHT-M, PHT-SM and PHT-M + OSHE], $p value vs. OSHE [$p < 0.001$ for PHT-M and PHT-M + OSHE, $p < 0.05$ for PHT-SM and PHT-SM + OSHE].

Table 2
Effect of OSHE on pharmacokinetic parameters of CBZ and PHT.

Pharmacokinetic Parameters	CBZ-M	CBZ-M + OSHE	p-value	PHT-M	PHT-M + OSHE	p-value
C_{max}(µg/ml)	5.90 ± 1.47	8.52. ± 2.82	NS	13.60 ± 2.52	13.90 ± 1.95	NS
T_{max} (h)	6.67 ± 2.30	7.00 ± 2.00	NS	2.00 ± 0.00	2.67 ± 1.15	NS
$AUC_{0\rightarrow\infty(h}$*µg/ml)	75.29 ± 11.51	80.53 ± 6.09	NS	88.53 ± 4.12	84.81 ± 13.68	NS
$T_{1/2}$(h)	7.27 ± 2.75	5.70 ± 2.22	NS	6.66 ± 2.69	7.62 ± 2.18	NS
Vd (L)	5.88 ± 3.15	4.15 ± 1.86	NS	4.40 ± 1.95	5.18 ± 1.33	NS
CL (µg/ml/h)	0.54 ± 0.08	0.50 ± 0.04	NS	0. 45 ± 0.02	0.47 ± 0.07	NS

Values are expressed as Mean ± SD. CBZ-M: carbamazepine maximal dose (20 mg/kg), PHT-M: phenytoin maximal dose (40 mg/kg), OSHE: *Ocimum sanctum* hydroalcoholic extract (1000 mg/kg), C_{max}: maximum concentration, T_{max}: time at which maximum concentration is reached, AUC: area under curve, $T_{1/2}$: half-life, Vd: volume of distribution, CL: clearance, NS: not significant.

including epilepsy (Sarangi et al., 2020, 2017). Previous studies postulated that treatment with CBZ potentially contributed to oxidative stress in animal model of seizures as well as in epileptic patients (Aycicek and Iscan, 2007) which is in line with current study where significantly lower level of GSH was found in CBZ maximal dose-treated group as compared to OSHE alone treated group. However, this study did not find any change in oxidative stress markers in between the AED alone treated group vs. AED with OSHE combined treatment group.

Being CYP 450 substrate and narrow therapeutic window, CBZ and PHT have higher potential to cause PK interaction with other AEDs or herbal drugs which may lead to either toxicity or thera-

peutic failure (Spina et al., 2016). So, it is essential to study the PK interaction of CBZ and PHT with herbal drugs such as Ocimum which has shown antiepileptic potential (Sarangi et al., 2020, 2017; Jaggi et al., 2003). Further, these AEDs are known to show a phenomenon called as 'metabolic tolerance' where there is enhanced drug elimination with time which is usually achieved within 1 to 4 weeks of daily drug administration. This may lead to less adverse effect but may hamper their antiepileptic efficacy (Löscher, 2007). These facts highlight that pharmacokinetic interaction study with these AEDs should be performed after a period of drug administration so that metabolic tolerance could have been achieved. Hence, current study planned to evaluate the pharmacokinetic interaction of CBZ and PHT with OSHE after 14 days of drug administration.

In the current study, co-administration of Ocimum with maximal dose of CBZ (20 mg/kg) and PHT (40 mg/kg) did not show any significant alteration in various pharmacokinetic parameters of CBZ and PHT. This is at par with our previous study in which concomitant administration of Ocimum at a dose of 1000 mg/kg with levetiracetam (LEV) 300 mg/kg in rats for 14 days did not alter PK of LEV significantly except increased T_{max} in combination group (LEV + OSHE) group (Sarangi et al., 2020). Similarly, in another study, co-administration of Ocimum with valproate did not show any significant PK interaction in rats (Sarangi et al., 2017). These facts highlight that Ocimum can be administered as an adjuvant to AEDs such as CBZ or PHT with minimal drug interactions.

5. Conclusion

Current study is the first-in-kind to investigate the pharmacodynamic and pharmacokinetic interaction of *Ocimum sanctum* with two of the most widely used and high drug interacting potential AEDs such as CBZ and PHT in MES model of seizure. In this study, Ocimum *per se* exerted protection against MES-induced seizure in rats. Moreover, it showed additive antiseizure potential in combination with sub-maximal dose of CBZ and PHT. Ocimum in combination with AEDs significantly improved memory and learning. Ocimum *per se* ameliorated the oxidative stress induced by seizures in rats, but did not show any significant reduction in combination with AEDs. Further, no significant pharmacokinetic interaction was found between OSHE and CBZ or PHT. Though overall effect of OSHE has been demonstrated in this study, it would have been better to extrapolate the effect of individual ingredients of the extract to have more specific result. Further mechanistic studies are required to elucidate the mechanism behind the antiseizure potential of Ocimum through its role on neurotransmitters and ion channels to establish its adjuvant role to AEDs. Further mechanistic studies based upon neurotransmitters and ion channels and trials in human beings are required to elucidate the adjuvant potential of Ocimum along with AEDs in treatment of epilepsy.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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References

Aycicek, A., Iscan, A., 2007. The effects of carbamazepine, valproic acid and phenobarbital on the oxidative and antioxidative balance in epileptic children. Eur. Neurol. 57 (2), 65–69.

Brodie, M.J., Besag, F., Ettinger, A.B., Mula, M., Gobbi, G., Comai, S., Aldenkamp, A.P., Steinhoff, B.J., 2016 Jul. Epilepsy, Antiepileptic Drugs, and Aggression: An Evidence-Based Review. Pharmacol. Rev. 68 (3), 563–602. https://doi.org/10.1124/pr.115.012021.

Carletti, F., Ferraro, G., Rizzo, V., Cannizzaro, C., Sardo, P., 2013. Antiepileptic effect of dimethyl sulfoxide in a rat model of temporal lobe epilepsy. Neurosci. Lett. 546, 31–35. https://doi.org/10.1016/j.neulet.2013.04.031.

Castel-Branco, M.M., Alves, G.L., Figueiredo, I.V., Falcão, A.C., Caramona, M.M., 2009. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Meth. Find Exp Clin Pharmacol. 31 (2), 101–106. https://doi.org/10.1358/mf.2009.31.2.1338414.

Czapiński, P., Blaszczyk, B., Czuczwar, S.J., 2005. Mechanisms of action of antiepileptic drugs. Curr. Top Med. Chem. 5 (1), 3–14. https://doi.org/10.2174/1568026053386962.

Eddy, C.M., Rickards, H.E., Cavanna, A.E., 2011. The cognitive impact of antiepileptic drugs. Ther. Adv. Neurol. Disord. 4 (6), 385–407. https://doi.org/10.1177/1756285611417920.

Emilie A, François C, Geneviève B, Mayoura B, Jérémy J, Jaime L, Voa R et al. 2019. Herbal medicine for epilepsy seizures in Asia, Africa, and Latin America: A systematic review., J Ethnopharmacol., 2019 pii: S0378-8741, 32745-4. doi: 10.1016/j.jep.2018.12.049

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82 (1), 70–77.

Giridharan, V.V., Thandavarayan, R.A., Mani, V., Ashok Dundapa, T., Watanabe, K., Konishi, T., 2011. Ocimum sanctum Linn. leaf extracts inhibit acetylcholinesterase and improve cognition in rats with experimentally induced dementia. J. Med. Food 14 (9), 912–919. https://doi.org/10.1089/jmf.2010.1516.

Jaggi, R.K., Madaan, R., Singh, B., 2003. Antiseizure potential of holy basil, Ocimum sanctum Linn., and its cultures. Indian J. Exp. Biol. 41 (11), 1329–1333.

Jarrett, S.G., Liang, L.P., Hellier, J.L., Staley, K.J., Patel, M., 2008. Mitochondrial DNA damage and impaired base excision repair during epileptogenesis. Neurobiol. Dis. 30 (1), 130–138. https://doi.org/10.1016/j.nbd.2007.12.009.

Joshi, H., Parle, M., 2006. Evaluation of nootropic potential of Ocimum sanctum Linn. in mice. Indian J. Exp. Biol. 44 (2), 133–136.

Joshi, R.K., Setzer, W.N., Da Silva, J.K., 2017. Phytoconstituents, traditional medicinal uses and bioactivities of Tulsi (Ocimum sanctum Linn.): a review. Am J Essent Oils Nat Prod. 5 (1), 18–21.

Kennedy, G.M., Lhatoo, S.D., 2008. CNS adverse events associated with antiepileptic drugs. CNS Drugs. 22 (9), 739–760. https://doi.org/10.2165/00023210-200822090-00003.

Liang, L.P., Ho, Y.S., Patel, M., 2000. Mitochondrial superoxide production in kainate-induced hippocampal damage. Neuroscience 101, 563–570. https://doi.org/10.1016/S0306-4522(00)00397-3.

Liu, H., Delgado, M., Forman, L.J., Eggers, C.M., Montoya, J.L., 1993. Simultaneous determination of carbamazepine, phenytoin, phenobarbital, primidone and their principal metabolites by high-performance liquid chromatography with photodiode-array detection. J Chromatogr. 616 (1), 105–115. https://doi.org/10.1016/0378-4347(93)80477-l.

Löscher, W., 2011. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 20 (5), 359–368. https://doi.org/10.1016/j.seizure.2011.01.003.

Löscher, W., 2007. The pharmacokinetics of antiepileptic drugs in rats: consequences for maintaining effective drug levels during prolonged drug administration in rat models of epilepsy. Epilepsia. 48 (7), 1245–1258. https://doi.org/10.1111/j.1528-1167.2007.01093.x.

Nolan SJ, Marson AG, Weston J, Tudur Smith C. Carbamazepine versus phenytoin monotherapy for epilepsy: an individual participant data review. Cochrane Database Syst Rev. 2015 Aug 14;(8):CD001911. doi: 10.1002/14651858.CD001911.pub3

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47 (3), 469–474.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Pahuja, M., Kleekal, T., Reeta, K.H., Tripathi, M., Gupta, Y.K., 2012. Interaction profile of Zizyphus jujuba with phenytoin, phenobarbitone, and carbamazepine in maximal electroshock-induced seizures in rats. Epilepsy Behav. 25 (3), 368–373. https://doi.org/10.1016/j.yebeh.2012.08.014.

Pearson-Smith, J.N., Patel, M., 2017. Metabolic Dysfunction and Oxidative Stress in Epilepsy. Int. J. Mol. Sci. 18 (11), 2365. https://doi.org/10.3390/ijms18112365.

Reeta, K.H., Mehla, J., Gupta, Y.K., 2010. Curcumin ameliorates cognitive dysfunction and oxidative damage in phenobarbitone and carbamazepine administered rats. Eur J Pharmacol. 644 (1–3), 106–112. https://doi.org/10.1016/j.ejphar.2010.07.022.

Rogawski, M.A., 2006. Molecular targets versus models for new antiepileptic drug discovery. Epilepsy Res. 68 (1), 22–28.

Sarangi, S.C., Joshi, D., Kumar, R., Kaleekal, T., Gupta, Y.K., 2017. Pharmacokinetic and pharmacodynamic interaction of hydroalcoholic extract of Ocimum sanctum with valproate. Epilepsy Behav. 75, 203–209. https://doi.org/10.1016/j.yebeh.2017.08.018.

Sarangi, S.C., Pattnaik, S.S., Katyal, J., Kaleekal, T., Dinda, A.K., 2020. An interaction study of Ocimum sanctum L. and levetiracetam in pentylenetetrazole kindling model of epilepsy. J. Ethnopharmacol. 249, 112389. https://doi.org/10.1016/j.jep.2019.112389.

Spina, E., Pisani, F., de Leon, J., 2016. Clinically significant pharmacokinetic drug interactions of antiepileptic drugs with new antidepressants and new antipsychotics. Pharmacol. Res. 106, 72–86. https://doi.org/10.1016/j.phrs.2016.02.014.

Subramaniam, S.R., Khoo, C.S., Raymond, A.A., Che Din, N., Syed Zakaria, S.Z., Tan, H. J., 2020. Prevalence and factors of verbal learning and memory dysfunction in patients with epilepsy - A single centre study. J. Clin. Neurosci. 73, 31–36. https://doi.org/10.1016/j.jocn.2020.02.003.

Sucher, N.J., Carles, M.C., 2015. A pharmacological basis of herbal medicines for epilepsy. Epilepsy Behav. 52 (Pt B), 308–318. https://doi.org/10.1016/j.yebeh.2015.05.012.

Ullrich, V., Kissner, R., 2006. Redox signaling: bioinorganic chemistry at its best. J. Inorg. Biochem. 100, 2079–2086. https://doi.org/10.1016/j.jinorgbio.2006.09.019.

Witt, J.A., Helmstaedter, C., 2017. How can we overcome neuropsychological adverse effects of antiepileptic drugs?. Expert Opin. Pharmacother. 18 (6), 551–554. https://doi.org/10.1080/14656566.2017.1309025.

Yuen, E.S., Trocóniz, I.F., 2015. Can pentylenetetrazole and maximal electroshock rodent seizure models quantitatively predict antiepileptic efficacy in humans?. Seizure 24, 21–27. https://doi.org/10.1016/j.seizure.2014.11.006.