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Pharmacokinetic and pharmacodynamic interaction of hydroalcoholic extract of *Ocimum sanctum* with valproate

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ABSTRACT

For effective control of seizures, antiepileptic drugs (AEDs) are administered at higher dose which is associated with several adverse effects. This study envisaged antiepileptic and neuroprotective potential of Tulsi, a commonly used herb for its immunomodulatory property. The optimal dose of *Ocimum sanctum* hydroalcoholic extract (OSHE) was determined using maximal electroshock seizure (MES)- and pentylenetetrazol (PTZ)-induced seizure models in Wistar rats (200–250 g) after administering OSHE (200–1000 mg/kg) orally for 14 days. For interaction study, OSHE optimal dose in combination with maximum and submaximal therapeutic doses of valproate was administered for 14 days. Serum levels of valproate were estimated using HPLC for pharmacokinetic study. For pharmacodynamic interaction, antiepileptic effect on above seizure models, neurobehavioral effect using Morris water maze, passive avoidance and elevated plus maze tests, and antioxidant capacity were assessed. *Ocimum sanctum* hydroalcoholic extract 1000 mg/kg was found to be optimal providing 50% protection against both MES- and PTZ-induced seizures. Combination of OSHE with valproate did not alter antiepileptic efficacy of valproate significantly. However, the combination showed better memory retention potential in neurobehavioral tests and protection against oxidative stress compared with valproate-alone-treated groups. Pharmacokinetic parameters did not reveal any significant change in combination group compared with valproate alone. *Ocimum*, although having per se antiepileptic action, did not affect antiepileptic action of valproate in combination. However, combination treatment has an edge over valproate alone—better neurobehavioral function and reduced oxidative stress—predicting adjuvant potential of *Ocimum* in epilepsy treatment.

1. Introduction

Epilepsy is one of the most prevalent noncommunicable neurologic conditions worldwide (lifetime prevalence was 7.60 per 1000 persons) [1] and also in India (10 million people, 1% of population) [2]. Despite availability of several newer AEDs, 30% of patients with epilepsy still suffer from uncontrolled seizures and many experience sudden deaths [3]. For effective control of seizure, antiepileptic drugs (AEDs) are administered alone or in combination for years together, which is associated with several adverse drug effects. Cognitive dysfunction is commonly seen with all major AEDs at therapeutic doses [4,5]. Valproate (VPA), a first-line and broad spectrum AED, is associated with adverse effects like hepatotoxicity, thrombocytopenia, gastrointestinal irritation, weight gain, transient alopecia, and neurological adverse effects including cognitive problems, ataxia, sedation, tremor, reversible parkinsonism, and dementia [6,7]. As epilepsy needs chronic treatment and VPA is a potent inhibitor of cytochrome P450 (CYP) enzyme system, there is a possibility of clinically significant drug interaction of VPA with other drugs and nutritional supplements. These facts definitely put forth the need for drugs having per se or adjuvant role in suppressing epileptogenesis with the capacity for reducing cognitive impairment.

In Ayurveda, *Ocimum sanctum* (Tulsi) (also called as *Ocimum tenuiflorum*) is known as 'The Incomparable One' and 'The Queen of Herbs'. Daily consumption of Tulsi is said to prevent disease and stresses of daily life and promote general health, wellbeing, and longevity [8]. The anticipatory potentials of Tulsi have been enumerated as chemoprotective, antistress [9], anticonvulsant [10], anxiolytic [11], antiulcer, antidiabetic [12], analgesic, antioxidant [13], anticancer, immunomodulatory, and antiinflammatory agent [14].

There are few previous in vivo studies [10,15–17] which have thrown light upon the anticonvulsant potential of *Ocimum* extracts using different species (*Ocimum basilicum*, *Ocimum gratissimum*, and *Ocimum sanctum*) in the maximal electroshock seizure (MES) and pentylenetetrazol (PTZ) model. However, there are only few evidences comparing *Ocimum* with standard antiepileptic drugs; moreover, the combined effect of administering these drugs together has not been characterized in terms of seizure control, neurocognition parameters, and pharmacokinetic interaction. Thus, there is a need for thorough investigation of this widely exploited plant's potential as an antiepileptic and neurocognitive beneficial agent. This study was performed to find

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out the per se antiepileptic effect of *Ocimum* and its pharmacokinetic and pharmacodynamic interaction with valproate.

2. Materials and methods

2.1. Experimental design

This experimental study for exploration of antiepileptic potential of *Ocimum sanctum* hydroalcoholic extract (OSHE) was conducted in rats using standard acute seizure models, i.e., maximal electroshock seizure (MES)- and pentylenetetrazol (PTZ)-induced seizure models. In the first phase, optimal dose of OSHE was screened using four different doses, i.e., 200, 400, 800, and 1000 mg/kg administered orally for 14 days. In the second phase, the optimal dose of OSHE from the first phase was used to determine pharmacokinetic and pharmacodynamic interaction of OSHE with valproate.

2.2. Animals

Adult Wistar rats (200–250 g), obtained from the Central Animal Facility of the All India Institute of Medical Sciences, New Delhi were housed in polyacrylic cages (38 × 23 × 10 cm) under standard laboratory conditions with dark and light cycle (approximately 12 h:12 h). They had free access to standard pellet diet and tap water ad libitum. The experiment was started after getting approval from the Institutional Animal Ethics Committee (IAEC), AIIMS, New Delhi (Ethics approval no. 917/IAEC/16) and conducted in accordance with CPCSEA guidelines, Department of Animal Welfare, Government of India.

2.3. Drugs and chemicals

Pentylenetetrazol and glutathione were obtained from Sigma Inc., USA. Valproate was obtained from HiMedia Laboratories Pvt. Ltd., Mumbai. Chemicals used in HPLC were of HPLC grade. All other chemicals and solvents were obtained from Merck (India) and were of analytical grade.

2.3.1. Doses of drugs

The standardized *Ocimum sanctum* hydroalcoholic extract (OSHE) was obtained from Natural Remedies Pvt. Ltd., Bangalore, India as a gift sample. *Ocimum sanctum* hydroalcoholic extract phytochemical analysis by HPLC has shown combine presence of ursolic acid and oleanolic acid 2.8% w/w. Four doses of OSHE, viz. 200, 400, 800, and 1000 mg/kg were used for the study. A freshly prepared suspension was made in distilled water before administering OSHE orally daily for 14 days.

Two doses of valproate were used. One dose was equivalent to the maximum recommended human therapeutic dose (MHRD) designated as VPA-M and the other was half of it, i.e., submaximal therapeutic dose (VPA-SM). Maximum recommended human therapeutic dose of valproate is 60 mg/kg [18]. Equivalent dose for rat was calculated as per the following formula: rat dose in mg/kg = human dose in mg/kg × 6.2, where 6.2 is the conversion factor considering body surface area [19,20]. Accordingly, the doses used in rats were 370 mg/kg (VPA-M) and 185 mg/kg (VPA-SM). Valproate solution was freshly prepared in distilled water before oral administration daily for 14 days. A time gap of 30 min was maintained between administration of OSHE and valproate, and the maximum volume administered each time was 0.4 ml/100 g animal.

2.4. Pharmacodynamic studies

2.4.1. Assessment of anticonvulsant action

2.4.1.1. Maximal electroshock seizure (MES)- and pentylenetetrazol (PTZ)-induced seizure. Seizure was induced on the 14th day, 60 min after the administration of last treatment, i.e., OSHE or valproate. Maximal electroshock seizure test was carried out as described [21] by delivering suprathreshold electrical stimulus (current intensity: 70 mA, duration: 0.2 s) via ear clip electrodes using electroconvulsiometer (Ugo Basile, Germany). Animals were observed for occurrence, latency, and duration of tonic hind limb extension (THLE), i.e., the hind limbs of animals outstretched 180° to the plane of the body axis.

Pentylenetetrazol test was carried out as described [21]. Pentylenetetrazol was prepared freshly in normal saline and administered at a dose of 60 mg/kg, i.p. This dose of PTZ is considered as 100% convulsant dose with minimal mortality in rats [22]. The latency to myoclonic jerks and occurrence and the latency and duration of generalized tonic–clonic seizures (GTCS) with loss of righting reflex were noted. Animals were observed for 60 min after seizure induction.

2.4.2. Neurobehavioral tests

During neurobehavioral study, only one animal was tested at a time. These studies were performed in a calm and quiet room devoid of any external interference like high volume noise and bright light. The rats were deprived of food 12 h before the behavioral testing, as this is known to enhance their motivation to perform the test [23].

2.4.2.1. Morris water maze test. Morris water maze (MWM) test was performed as described earlier [24]. Acquisition trials were carried for 4 days. On the fifth day, a spatial probe trial of 60-s duration was done to detect spatial memory of the animal. Latency to reach the target quadrant and latency to reach the platform during the probe trial was noted. This test was performed from the 10th day to the 14th day, and a probe trial was repeated a day after seizure induction, i.e., on the 15th day.

2.4.2.2. Passive avoidance test. Memory retention deficit was evaluated by a step through passive avoidance (PA) apparatus [23]. On the acquisition trial, initial latency (IL) to enter the dark chamber was recorded. Rats exhibiting an initial latency time of more than 60 s were excluded from further experiments. After 24 h, transfer latency (TL) was measured in the same way as in the acquisition trial, but foot shock was not delivered. This test was performed on the 13th and 14th days, and TL was also measured a day after seizure induction, i.e., on the 15th day.

2.4.2.3. Elevated plus maze test. Acquisition and retention of memory processes were assessed using EPM [23]. On the 1st day, transfer latency (TL) was recorded. Twenty-four hours later, 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. This test was performed on the 13th and 14th days, and TL was also measured a day after seizure induction, i.e., on the 15th day.

2.4.3. Oxidative stress

The oxidative stress markers malondialdehyde (MDA), reduced glutathione levels (GSH), and superoxide dismutase (SOD) were estimated in the brain cortical tissue homogenate of a rat made in phosphate buffer (pH 7.4). The rats were euthanized by decapitation under ether anesthesia on the 15th day after neurobehavioral assessment, and their brains were quickly removed, cleaned by rinsing with chilled normal saline and stored at −80 °C for analyses within a week.

2.4.3.1. Measurement of lipid peroxidation. Malondialdehyde (MDA) level was estimated as described [25] with little modification. To 1 ml of brain cortical tissue homogenate, the reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%), and 0.2 ml sodium dodecyl sulfate (8.1%) were added. The mixture was then heated at 100 °C for 60 min and cooled and 5 ml of n-butanol: pyridine (15:1% v/v) was added. The mixture was vortexed vigorously and centrifuged at 4000 rpm for 10 min. After centrifugation, the organic layer was withdrawn,