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Evaluation of anti-epileptic activity of *Coriandrum sativum* fruit extracts

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Abstract
Epilepsy is a disease of the brain characterized by recurrent spontaneous seizures. All anti-epileptic drugs (AEDs) are of synthetic origin and known to present many side effects such as sedation, hepatotoxicity, anxiety, and depression. In our study, pentylenetetrazol (PTZ) kindling model of epilepsy was usedwith Sodium valproate as the standard. Anti-convulsive adequacy of fruit extracts of *Coriandrum sativum* (CAE and CHE) at a dose of 250mg /kg b.w and 500 mg/kg b.w. was evaluated. MDA levels in both cortical and hippocampal tissues of PTZ group were significantly higher than those of the control animals. *C. sativum* extracts had a significant effect on PTZ kindled mice.

Keywords: CAM, *Coriandrum sativum*, epilepsy, kindling, PTZ, valproate

Introduction

Epilepsy is a disease of the brain defined by at least two unprovoked seizures occurring more than 24 h apart or a diagnosis of an epilepsy syndrome [1]. This devastating multi-causal chronic disease is characterized by recurrent spontaneous seizures (a seizure is a transient occurrence signs due to abnormal excessive or synchronous neuronal activity in the brain) that affects 70 million persons worldwide, 80% of them lived in low- and middle-income countries where, in many cases, epilepsy remains untreated. [2] The disease is classified between 3 aetiologies; genetic, structural/metabolic, of unknown cause and different types can be found. Epilepsy seizures are categorized in generalized (convulsive seizures, absences) and focal (motor, partial, complex, sensitive, and secondarily generalized) seizures. Some epilepsies cannot be classified into generalized or focal, as temporal lobe epilepsy, generalized tonic-clonic seizures with generalized spike-wave, both focal impaired awareness seizures and absence seizures in a patient. Anti-epileptic treatment is based upon the use of drugs according to the type of seizures. All anti-epileptic drugs (AEDs) are synthetized, none are derived from natural products. Most prescribed AEDs are phenobarbital, carbamazepine, phenytoin, and sodium valproate. The wide variety of mechanisms of action of AEDs makes it possible to target seizures with a different molecular mechanism. Association of anti-epileptic drugs might not be recommended [3, 4]. Finally, about 30% of persons with epilepsy (PWE) are drug-resistant, representing a socioeconomic burden at the individual, familial and societal levels. Moreover, these drugs are known to present many side effects such as sedation, hepatotoxicity, anxiety, and depression, and only few of them can be administered to pregnant women (lamotrigine, levetiracetam, phenytoin and oxcarbamazepin) are the most tolerated whereas sodium valproate must be avoided). Epileptic condition is linked to an increased risk of depression, anxiety as well as impaired quality of life due to stigmatization, mental and physical impairment, work absenteeism, work-related accident, poor work efficiency and family dysfunction [5].

Pentylenetetrazole (PTZ) as convulsant: One of the most widely used methods for anticonvulsant screening is systemic injection of pentylenetetrazole (PTZ). The apparent arguments for this choice include the ease of performance, dependability, and convenience of communicating findings, as well as the shorter time required to create convulsions. Trimethadione's unique protective capacity to prevent seizures, presented pentylenetetrazole (Pentamethylenetetrazole, metrazoI, cardiazol, leptazol) for anticonvulsant screening [1]. Plants derived drugs are widely used and believed to be safe, cost effective with fewer side effects. Many medicinal plants provide relief of symptoms comparable to that obtained from allopathic medicine. Since herbal medicines that are one of the most popular forms of complementary and alternative medicines (CAMs) are effective and safe in nature, their usage in the treatment of neurological diseases and disorders is also gaining importance [6].

The rationale for the performance of these remedies extends from circumstantial to clinical testing; analogously, pathways and active compounds on all herbal remedies are being analysed, meanwhile the exact nature of activity is uncertain.

Herbal formulations exert their poly-pharmacological effects by acting on the multiple targets using its multi-component framework. 45Trend of the treatment of EP using CAMs is not new and nearly 50% of the patients are still using this strategy for the treatment [7, 8].

Fig 1: Coriandrum Sativum Fruit

Across many scenarios, botanical therapies under contemporary or traditional practice are indeed ingested as consumables or culinary seasoning, oftentimes on a massive scale. Coriander (*Coriandrum sativum*), popularly described as *Cilantro* and used throughout the old World, itself is an exemplar (Figure 1). Coriander possesses diaphoretic, diuretic, carminative, and stimulating properties and is typically used to alleviate gastrointestinal, pulmonary, and urinary related problems. Coriander is said to have a host of medicinal properties, notably antioxidative, anti-diabetic, lipid lowering effect, and antifungal activity. The volatile oils composition of ripe and dried coriander fruits spans around 0.03 and 2.3 percent, whereas the fatty chemical composition varies between 9.9 and 27.7 percent. Monoterpene hydrocarbons, such as a pinene, limpnene, terpinene, p-cymene, borneol, citronellol, camphor, geraniolacetate, heterocyclic components such as pyrazine, pyridine, thiazole, furan and tetera-hydrofuran derivatives, iso-coumarins, flavonoids, and sterols, constitute the principal components of Cilantro [9-11].

Many treatment strategies were used across time to treat epilepsy. The bulk of the remedies used in traditional system of medicine are found in nature, and many have proven to be efficacious. It was also indicated that Coriandrum sativum possesses anticonvulsant activity, albeit suitable studies have still not been undertaken in this regard. This research aimed to validate its anticonvulsant efficacy in a mouse models of epilepsy triggered by PTZ. [1, 3].

Materials and Methods

Drug Solutions and Reagents: Pentylenetetrazole (PTZ) was procured from Sigma Aldrich, Mumbai, India. Sodium bicarbonate, Sodium citrate, Thiobarbituric acid, Trichloro acetic acid, Sodium carbonate, Sodium hydroxide, Pot. dihydrogenortho phosphate, Carbon tetrachloride, Chloroform, Ethanol, Formalin, Epinephrine bitartrate, EDTA, HCl, DTNB (5,5′-dithiobis-(2-nitrobenzoic acid) were obtained from HI Media Mumbai, India. Other reagents were analytical grade and double-distilled, deionized water was used for all studies. Gallic acid and quercetin were purchased from LOBA Chemie and SD Fine Chemicals, Mumbai, India, respectively.

Collection and authentication of plant material: The study was conducted using aqueous extract of *C. sativum* fruits. Fruits/seeds were purchased from local market, of Dehradun, Uttarakhand, India, in the month of January and authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai, Maharashtra, India. The voucher specimen has been deposited under the accession number SKW. 6872.

Preparation of hydro-alcoholic extract (CHE): Coriander fruits were washed thoroughly and air dry (35-40˚C). Dried fruits were pulverized, and powder was successively extracted with 70% ethanol using a Soxhlet apparatus. Extract was filtered and concentrated *in-vacuo*. Dried CHE was stored capped glass vials at −20 °C until use.

Preparation of aqueous extract (CAE): Powdered drug was extracted using infusion technique. In brief, about 200gm of dried coriander fruits were soaked in about one and a half litres of double distilled water for 24 hours. The mixture was brought to boil and concentrated, until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to screen its. Desiccated CAE was kept at 20 °C in properly labelled airtight glass jars till it was required.

Procurement of animals and ethics approval: Male inbred Swiss Albino mice (weighing 20–25 g; age: 7–8 weeks) were used for the study. Well before experiment, the animals were acclimatized to lab conditions for one week. The animals were house in standard propylene cages and maintain under control RT (25±2˚C) and relative humidity (55±5%) with 12:12 hour light – dark cycle and provided standard pellet feed and water *ad-libitum* both being retracted only prior to experimentation. Use of animal studies during this project was sanctioned by Institutional Animal Ethics Committee (CPCSEA/IAEC/273/CPCSEA/SBS/005/2016-17). These studies complied with the prescribed guidelines of CPCSEA, MoEFCC, Government of India.

Induction of epilepsy and experimental design: Kindling procedure, for kindling, mice received a single dose of 35 mg\kg PTZ dissolved in 1 ml of normal saline intraperitoneally (i. p.), every 48 hr. A total of 12-15 doses of PTZ will be giving to each mouse. The convulsive behaviour was observed for 20 min after each PTZ injection. The seizures were classified according to the score as follows:

Stage 0. No response.
Stage 1.ear and facial twitching.
Stage 2. Myoclonic jerks without upright position.
Stage 3. Myoclonic jerks, upright position with bilateral forelimb clonus.
Stage 4. Tonic-clonic seizures.
Stage 5. Generalized tonic-clonic seizures, loss of postural control.

To check the maintenance of kindling state, the animals were challenged with a sub-convulsive PTZ dose (35 mg\kg) 10 days after the last kindling injection. Only the mice showing generalized tonic- clonic seizures were used as kindled such as myoclonic and tonic seizures.

Treatment protocol Experimental epilepsy was pentylenetetrazol (PTZ) kindling model of epilepsy was used and the experimental animals were differentiated into different groups (either control or PTZ-kindled groups). In the experiment the animals were divide into seven groups

(n=6). Valproic acid was used as reference standard. The dose of Valproic was selected based on previous reports. [1, 12-14]

Group 1: Normal control group received CMC

Group 2: Positive control received PTZ in normal saline (35 mg\kg)

Group 3: kindled animals treated with standard (Valproic acid, 200mg\kg)

Group 4: kindled animals treated with CAE in CMC (dose 250mg\kg)

Group 5: kindled animals treated with CAE in CMC (dose 500 mg\kg)

Group 6: kindled animals treated with CHE in CMC (dose 250 mg\kg)

Group 7: kindled animals treated with CHE in CMC (dose 500 mg\kg)

At the day 0 and 15 seizure latency time noted: At the day 0 and 15 of treatment challenge dose of PTZ was administered at dose 35 mg\kg and behavioural parameter was noted. From this data increase in latency time and decrease in the severity was noted.

Methods for estimation of different biochemical parameters in PTZ induced convulsive mice.

Blood was collected by retro-orbital puncture and on the last day of experiment animals were sacrificed by decapitation under chloroform anaesthesia. Immediately following decapitation, brain was removed, freed from adhering tissues, washed with ice-cold normal saline solution until bleached of all blood and blotted dry. Weight of the organ was measured after drying the tissue. After mincing into small homogenate was centrifuged at 10,000 rpm for 20 min at 4˚C and the supernatant was used for biochemical estimation of the following parameters:

Estimation of Gamma Aminobutyric Acid Determination and Glu of brain tissue: After last PTZ injection, the rodents (n=3) were euthanized 50 minutes. Brain was sliced and weighted after it had been frozen. Concentrations of GABA and Glu assessed utilizing reference standards by HPLC [15].

Estimation of Serum MDA Level (Non-enzymatic Oxidative stress parameter): Methodology was standardised based on the guidelines of Satohk's method. Protocol comprises of sample collection, inoculation with TCA, Centrifugation, Washing with H_2SO_4, incubation with TBA, and removal of contaminants. Its concentration was determined by reading the absorbance through the spectrophotometer at 245 nm (ε245 nm = 13,700 L·mol−1·cm−1) [16, 17].

Cerebral Histopathology: At the conclusion of the experiment, mice from each group were euthanized and the brain was harvested, segregated, and preserved in a 10% formalin solution. Dewatering and tissue cleansing followed naturally Haematoxylin and Eosin (H&E) and Gomeri aldehyde-fuchsin (GAF), a beta cell specific staining, were being used to dye tissue samples. Stained segments were evaluated quantitatively (Morphometrically) and subjectively (morphologically) [3, 17].

Statistical Analysis: The statistical analysis was carried out using Graph Pad Prism 5.0 software. All values were presented as Mean± SEM. Multiple comparisons between different groups was performed using Analysis of Variance (ANOVA) followed by Turkeys Multiple Comparison Test. Difference level at P< 0.01 and P<0.001 was considered statistically significant condition.

Results and Discussion

Extraction, antiepileptic, and other pharmacological investigations were done on the fruits (seeds) of the herb Coriandrum sativum, and it is a chloride channel blocker associated to the GABA receptor in PTZ-treated rodents. The anticonvulsant activity of *Coriandrum sativum* was evaluated using aqueous (CAE) and hydroalcoholic (CHE) solutions. To evaluate the effect of such interventions in kindled mice, brain tissue homogenates were used to estimate several biochemical parameters [1, 4, 17].

Table 1: Effect of *Coriandrum sativum* of different (aq. & hydro)) doses on latency of seizures at day 0 and day 15 in PTZ kindled mice.

Treatment (p.o.\i.p.)	Latency of seizures on day 0 (min)	Latency of seizures on day 15 (min)
Group 1	3.23±0.307	3.21±0.251
Group 2	3.21±0.342	3.21±0.250
Group 3	5.76±0.520^{a},^{b}**	7.21±0.421^{a},^{b}**
Group 4	5.15±0.553^{a},^{b}**	5.51±0.461^{a},^{b}*
Group 5	5.71±0.551^{a},^{b}*	6.19±0.469^{a},^{b}*
Group 6	4.21±0.551^{a},^{b}**	4.59±0.321^{a},^{b}**
Group 7	5.21±0.206^{a},^{b}*	5.05±0.125^{a},^{b}**

Results are expressed as Mean±SEM. a: as compared to normal, b: as compared to PTZ control group. Significant level was considered at P<0.01 and P<0.001.

*:P<0.01

**:P<0.001

Different dosages of plant extract impacted the observable traits of epileptic mouse.

The behavioural parameters level 0–stage 5 was exploited in the current research to evaluate the epileptogenic existence of mouse model on day zero and after a fortnight. Cognitive function including such seizure postponement initially and day fifteen suggests treatment group is antiepileptic. There was indeed a significant increase in seizures delayed in the treated group with standard drug Valproic acid, CAE at dose levels of 250mgkg and 500mgkg, and CHE at dose level 500mgkg respectively contrasted to the PTZ experimental group on day zero and day fifteen. Furthermore, on fifteenth day, the latency period with CHE extract at 500mg /kg dose levels was risen exponentially. [1, 18]

Various fractions of *Coriandrum sativum* were evaluated in a study to treat epilepsy caused by PTZ in rats. CAE at 250mgkg was ineffective against minimal clonic seizures (MCS) and generalized tonic-clonic seizures (GTCS). The CAE at 500 mg/kg increased MCS latency, but the outcome really was not significant statistically (See Table1 along

with figures Fig. 2 and Fig. 3 effect on latency of arriving to phase 5 of seizures on day "0" and day "15" because of the treatment respectively) [3, 17].

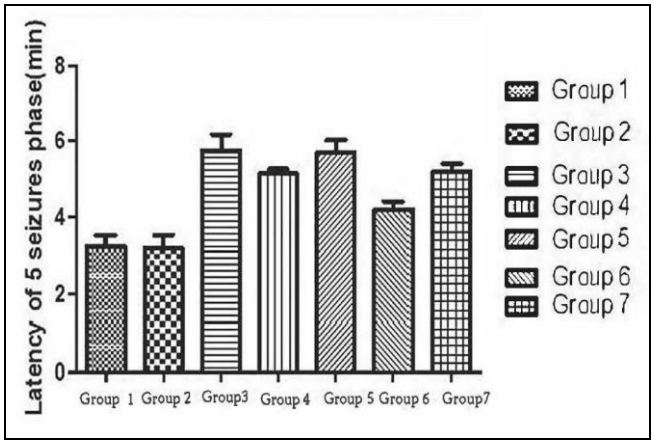

Fig 2: On 0 day: Effect of Standard (Valproic acid) and *Coriandrum sativum* (CAE & CHE) doses of plant extract on latency of arriving to phase 5 of seizures. (n= 6).

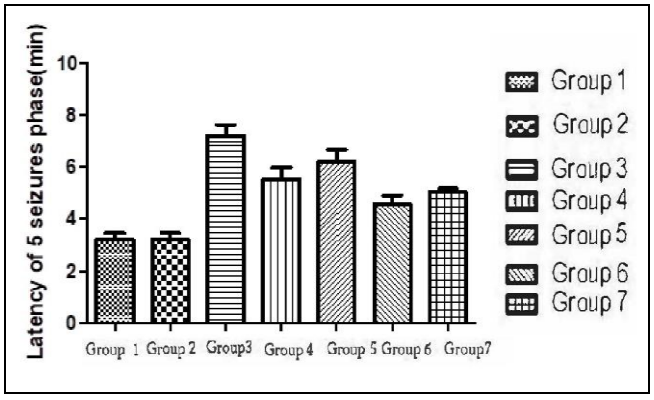

Fig 3: On 15 day: Effect of Standard (Valproic acid) and *Coriandrum sativum* (CAE & CHE) doses of plant extract on latency of arriving to phase 5 of seizures. (n= 6).

Table 2: Effect on different group of animals in the presence of extract of *Coriandrum sativum* (aq. & hydro) by estimating Glutamate neurotransmitter and GABA.

Treatment (p.o.\i.p.)	Glutamate#	GABA# ()
Group 1	9.11±0.351	1.99±.121
Group 2	11.12±0.165	2.01±.201
Group 3	12.01±0.791[a,b**]	3.92±.911[a,b**]
Group 4	10.56±0.221[a,b*]	3.51±.416[a,b*]
Group 5	10.01±0.215[a,b**]	3.12±.316[a,b**]
Group 6	10.98±0.191[a,b*]	2.92±.27[a,b**]
Group 7	10.00±1.25[a,b**]	2.47±.199[a,b**]

- brain homogenate

Results are expressed as Mean±SEM. a: as compared to normal, b: as compared to PTZ control group. Significant level was considered at P<0.01 and P<0.001.

*: P<0.01

**:P<0.001

Results of present study are summarized in Table 2 and indicate significant decreases in brain level of GABA an inhibitory neurotransmitter involved in kindling process. Significant decrease in GABA level was observed in group received PTZ alone when compared with normal control group. Group treated with standard drug showed significant increase in GABA level. Groups treated with CAE 250mg\kg and CHE 500mg\kg showed significant increase in GABA level as compared to group treated with PTZ alone. Group treated with 500mg/kg of CAE and 250 mg/kg of CHE however, does not show any significant increase in GABA level.

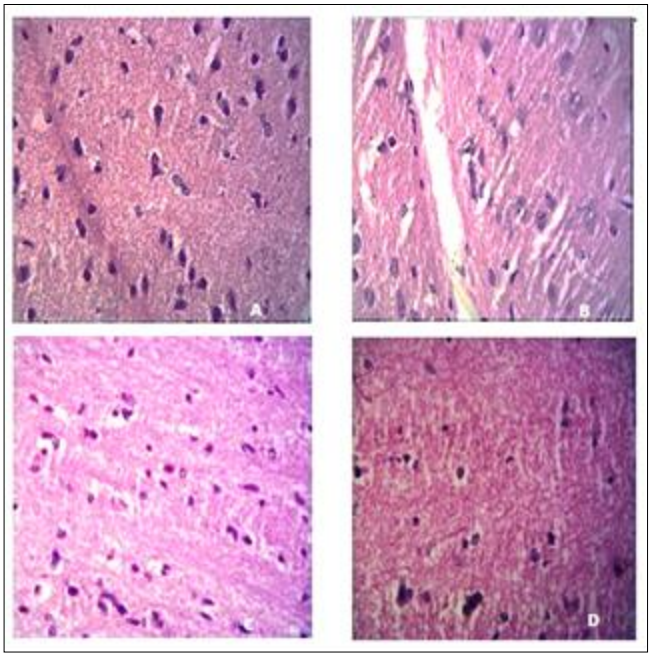

Fig 4: Histopathology of mice brain after treatment. Figure 4.A Histologically normal Brain tissue. Figure 4.B Degenerated neuronsdue to PTZ induced epilepsy. Figure 4.C Effect of Valproic acid on mice tretated with PTZ. Figure 4.D Effect of coriander extracts on mice treated with PTZ and improved histopathological profile of the brain tissue.

Effects of hydroalcoholic extract of *C. sativum* on plants on brain tissues oxidative damages following seizures induced by PTZ was investigated in rats. Malondialdehyde (MDA) levels in both cortical and hippocampal tissues of PTZ group were significantly higher than those of the control animals. This study mainly indicated its anticonvulsant and antioxidant effect against PTZ inducing kindling. [17,20] Figure 4 shows the histology of brain. Figure 4. A shows normal cells of Group 1, while Figure 4.B indicates the effect of PTZ in Group 2 caused significant increase in chronic seizures in brain presence of large number of neuronal damage cells was reported. However, treatment with Valproic acid and *Coriander* decreases the number of neuronal damage cells and epilepsy in brain as indicated in figure 4. C and 4. D, respectively.

Conclusion

C. sativum extracts *(*aq. & hydro) at dose 250mg\kg and 500mgkg are useful in treatment of kindled mice. Thus, it can be concluded that antiepileptic potential of CHE maybe due to presence of similar compounds present in the CAE also.

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