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RESEARCH ARTICLE

Evaluation of the effect of volatile oil extract of *Nigella sativa* seeds on maximal electroshock-induced seizures in albino rats

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ABSTRACT

Background: Epilepsy is one of the most common serious neurological disorders, responsible for substantial morbidity and mortality due to the seizures and the available medications. Natural products from folk remedies have contributed significantly in the discovery of modern drugs with novel structures and better safety and efficacy profiles. In this regard, one such plant is *Nigella sativa.* **Aims and Objectives:** (i) To evaluate the anticonvulsant activity of volatile oil extract of *N. sativa* seeds by maximal electroshock (MES)-induced seizure model of epilepsy in albino rats; (ii) To evaluate the influence of volatile oil extract of *N. sativa* seeds on the anticonvulsant activity of sodium valproate in albino rats. **Materials and Methods:** Male Albino rats (150-200 g) were randomly selected, from Central Animal Facility, Mysore Medical College and Research Institute, Mysore. The anticonvulsant activity was screened using MES-induced seizure model. Albino rats were divided into six groups of six rats each. Six groups were treated with gum acacia 0.5 ml (control group), sodium valproate 300 mg/kg (standard group), Groups 3, 4, and 5 were administered the test drug, volatile oil extract of *N. sativa* seeds at doses of 200, 400, and 600 mg/kg, respectively, and Group 6 was treated with the combination of test drug, volatile oil extract of *N. sativa* seeds 200 mg/kg and sodium valproate 150 mg/kg. All the drugs were dissolved in gum acacia and administered intraperitoneally 30 min prior to induction of seizures. **Results:** The volatile oil extract of *N. sativa* seeds showed the anticonvulsant activity in electroshock-induced seizure model at the dose of 400 and 600 mg/kg body weight and the potentiation of anticonvulsant activity of sodium valproate. The anticonvulsant activity of volatile oil of *N. sativa* seeds was less when compared to sodium valproate. **Conclusions:** The *N. sativa* seeds showed the anticonvulsant activity in MES-induced seizure model of epilepsy. This study showed that volatile oil of *N. sativa* seeds potentiated the effect of sodium valproate.

KEY WORDS: Epilepsy; *Nigella Sativa* Seeds; Volatile Oil; Maximal Electroshock; Sodium Valproate; Tonic Hind Limb Extension

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INTRODUCTION

An epileptic seizure has been defined as a paroxysmal discharge of cerebral neurons accompanied by clinical phenomena apparent to the patient or an observer. The phenomena can be motor, sensory, or autonomic, and there may also be impairment or complete loss of consciousness.

Motor disturbances may include convulsions - Which are involuntary, violent, and spasmodic or prolonged contraction of skeletal muscles.[1]

Epilepsy is one of the most common serious neurological disorders, responsible for substantial morbidity and mortality due to the seizures and the available medications. The prevalence of epilepsy is around 0.5-1%, and its overall annual incidence ranges from 50 to 70 cases/100,000 in industrialized countries and up to 190/100,000 in developing countries. Around 80% of people with epilepsy reside in developing countries. The high incidence in developing countries is attributed to poor obstetric services and the greater risk of intracranial infections and head injuries. Furthermore, in these countries, 80-90% of epileptic patients have difficulties in accessing treatment. This treatment gap has been mainly ascribed to inefficient and unevenly distributed health-care systems, cost of treatment, cultural beliefs, and unavailability of antiepileptic drugs.[2]

In India, the prevalence rate is about 5-6/1000, which means approximately more than 45 lakhs Indians suffers from this disease.[3]

Phenytoin was the first antiepileptic drug discovered using an animal seizure model. Phenytoin was synthesized, in 1908, and was recognized as a first nonsedating antiepileptic drug after the pioneering studies of Merritt and Putnam using an electroshock-induced seizure model in cats. Trimethadione, the first treatment specifically for absence seizures was licensed in the1940s, following laboratory evaluation with the pentylenetetrazole (PTZ) animal seizure model by Richards and Everett, in 1944, and clinical evaluation by Lennox in 1945.[4]

Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizure.[5]

Pharmacoresistant Epilepsy[2,4]

Pharmacoresistance may be defined as poor seizure control despite accurate diagnosis and carefully monitored pharmacologic treatment. Clinically available anticonvulsant drugs fail to control seizures in around 30% of epileptic patients. About 75% of patients diagnosed with mesial temporal lobe epilepsy have pharmacoresistant seizures and more than 50% of patients with Lennox-Gastaut syndrome are classified as pharmacoresistant. The condition is more complicated in certain brain abnormalities, for example, when hippocampal sclerosis is combined with focal dysplasia. Despite the introduction of new drugs, the problem of pharmacoresistance has not been solved, although most of the new drugs have better safety profiles than those of older drugs. Surgical treatment of epilepsy may be an alternative, but at present, surgery is possible in only a small proportion of pharmacoresistant patients, and after the surgery, most of the patients are still prescribed antiepileptic drugs for full seizure control.[4]

It is not well established, why and how epilepsy becomes drug resistant in some patients while others with seemingly identical seizure types and epilepsy syndromes can achieve seizure control with medication. Thus, there is a clear need to understand the pathological process involving epilepsy and for an ideal antiepileptic agent with properties such as broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability, and low cost.[4,5] Three major mechanisms have been proposed to explain pharmacoresistance in around 30% of patients:

1. Disease-related: Two key hypotheses have been proposed as disease-related mechanisms. The target hypothesis proposes the alterations of pharmacological targets of antiepileptic drugs in the brains of pharmacoresistant patients that lead to the failure of antiepileptic drugs to block excitatory sodium or calcium currents or to enhance the gamma-aminobutyric acid-mediated inhibition, whereas the transporter hypothesis proposes that excessive expression of multidrug transporters could remove antiepileptic drugs from epileptogenic brain regions.
2. Genetics: Genetic alterations due to, for example, polymorphisms in drug efflux transporters may also lead to poor seizure control in these patients.
3. Drug-related mechanism: Finally, tolerance as a drug-related mechanism may be responsible for lower efficacy of antiepileptic drugs in these patients.[2]

Intensive research is being carried out based on these hypotheses. However, the detailed mechanisms leading to pharmacoresistance is still unknown.[2] The aim of treating with an antiepileptic drug is not only to abolish the occurrence of seizures but also to lead a self-sustained life. Hence, search should continue to develop newer more effective and safer neuroprotective agents for the treatment of epilepsy.[6]

The use of indigenous plant medicines in developing countries became the World Health Organization policy since 1970. Of the 520 new drugs approved in the period 1983-1994 by either the US Food and Drug Administration or comparable entities in other countries, 30 drugs came directly from natural product sources, 173 were either semi-synthetics or synthetics originally modeled on a natural parent product.[7]

Nigella sativa is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea, Pakistan, and India. For thousands of years, this plant has been used in many Asian, Middle Eastern, and Far Eastern Countries as a spice and food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders.[7]

The seed of this plant is commonly known as black seed and is referred by the Prophet Muhammad as having healing powers. The Prophet Muhammad (Peace be upon him) once stated that the black seed can heal every disease except death. It is also included in the list of natural drugs of "Tibb-e-Nabavi" or "Medicine of the Prophet Muhammad (Peace be upon him)" according to the tradition "hold onto the use of black seeds for healing all diseases."[8]

Other names for the seed include black caraway seed: Habbat Al Sawda and Habat Al Baraka, and the blessed seed:[7] Ajemuz, neguilla,[9] kalaunji, upakunchika, ajaji, kalvanjika, kalika, and kunchika.[10]

N. sativa seeds contain two types of oils, i.e., fixed oil (30-36% w/w) and volatile oil (0.43-0.72% w/w).[11]

The fixed, or fatty, oil is rich in unsaturated fatty acids, mainly linoleic acid (44.7-56%), oleic acid (20.7-24.6%), and eicosadienoic acid (3%). Saturated fatty acids (palmitic, stearic acid) amount to about 30% or less.[12]

Volatile oil of *N. sativa* seeds is composed mainly of thymoquinone (2-isopropyl-5- methyl- 1,4- benzoquinone) and monoterpenes. Thymoquinone contents range from 18.4% to 24% w/w of the volatile oil. The monoterpenes in the volatile oil amount to 46% w/w. The major components of these monoterpenes are p-cymene (isopropyl toluene), which comprises 31.7% of the volatile oil, and *alpha*-pinene, which comprises 9.3% of the volatile oil. Other components include phenols (1.7%), esters (16%), thymol, dithymoquinone, and thymohydroquinone.[11]

Black seed's constituents, in particular, its major constituent thymoquinone, have recently shown antiepileptic effects in mice. Literature has indicated that the whole oil from black seeds is effective against PTZ-induced kindling in mice. Some other studies have pointed out that the treatment of mice with thymoquinone reduced the duration of myoclonic seizures and effectively protected the mice from mortality.[11]

Here, we have evaluated the antiepileptic activity of volatile oil extract of *N. sativa* seeds with the standard sodium valproate in electrically induced seizures. We have also evaluated the influence of *N. sativa* volatile oil on sodium valproate.

Aims and Objectives

i. To evaluate the anticonvulsant activity of volatile oil extract of *N. sativa* seeds in albino rats by electrically induced seizure model
ii. To evaluate the influence of volatile oil extract of *N. sativa* seeds on the anticonvulsant activity of sodium valproate in albino rats by electrically induced seizure model.

MATERIALS AND METHODS

The study was conducted after the Institutional Animal Ethical Committee clearance.

Materials

Equipment

Electroconvulsiometer with accessories, 1 ml tuberculin syringe, electronic weighing balance, animal weighing balance.

Chemicals

- Sodium valproate was obtained from Sun Pharmaceuticals. Dose used was 300 mg/kg[13] and was given intraperitoneally. Vehicle used was 5% gum acacia
- Volatile oil of *N. sativa* (200, 400, and 600 mg/kg body weight)[11]
- Gum acacia: 5%
- Distilled water.

Collection of seeds

The seeds of *N. sativa* were obtained from a local market in Mysore, Karnataka state and authenticated by the Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore. The seeds were crushed and grounded into a fine powder. The powdered sample was stored in clean and dry container until used.

Extraction of the volatile oil[14]

The steam distillation method was employed in the extraction process. 250 ml of distilled water was added to 50 g of finely powdered sample placed in a distillation flask connected to a steam distiller, condenser, and a receiver and was hydro distilled for 4 h. The distillate was collected in coated glass bottles. Diethyl ether was added to separate the water phase. Anhydrous sodium sulfate was added to the supernatant fraction with simple agitation to dry volatile oil and then filtered. The filtrate was concentrated by distilling off the solvent in a rotary evaporator. The final residue of volatile oil was stored at 4°C in coated glass bottles.

Animals: Healthy male swiss albino rats of 150-200 g

Animals

A total of 36 adult healthy male albino rats of Wistar strain of similar characteristics weighing 150-200 g were selected from the central animal facility, Mysore Medical College and Research Institute, Mysore, and divided into six groups of six each.

All the test animals were allowed food and water *ad libitum* both being withdrawn just before experimentation. The test animals were subjected to further study after a gap of 24 h to avoid any possible "kindling" effect. The drug preparations were administered intraperitoneally as shown in Table 1. To

evaluate the influence of volatile oil extract of *N. sativa* seeds on sodium valproate, a combination of subanticonvulsive dose of volatile oil of *N. sativa* seeds and sodium valporate were studied, and results obtained were compared with anticonvulsive dose of sodium valproate alone. After an interval of 30 min, animals were subjected to electroshock of 150 mA intensity for 0.2 s, through auricular electrodes (covered in cotton wool and saline moistened). The duration of different parameters namely tonic flexion of fore and hind limbs with tail erection, tonic extension of both fore and hind limbs, clonus, stupor followed by postictal depression and recovery were noted.

Maximal electroshock (MES) seizure model

The most commonly used simple model for evaluation of drugs useful in generalized seizures is the electroshock model, which has been validated both clinically and electroencephalographically. The credit for standardizing this model goes to Woodbury and Davenport.[15]

Electroshock seizures are either threshold or maximal. It is the most specifically predictive test among the available anticonvulsant screening models.

Description

MES model evaluates the ability of drugs to prevent electrically induced tonic hind limb extension (THLE) in mice or rats. Efficacy of drugs in this model has been shown to correlate with their ability to prevent partial and generalized tonic-clonic seizures in man and is said to evaluate the capacity of a drug to prevent the spread of seizures. Drugs that are active in the MES test often have a phenytoin like effect on voltage-dependent Na+ channels, viz., phenytoin, carbamazepine, phenobarbital, and valproate.

Method

All animals are maintained on an adequate diet and allowed free access to food and water, except during testing as pre-test starvation modifies the MES-induced seizure pattern (shortens tonic flexion and prolongs tonic extension). Stimulation can be carried out through directly applied corneal electrodes. However, as this method causes pain and bleeding, hence transauricular electrodes (applied to the pinna with small crocodile clips covered with cotton wool and saline-moistened) are used. Pre-test saline moistening is mandatory to ensure better contact and to reduce fatalities resulting from MES-induced seizures. Maximal seizures are evoked by supramaximal electroshock stimulation of 150 mA, 50 HZ, for 0.2 s using conventional electroconvulsiometer.

A stopwatch can be used for timing the various events of each phase.

The parameters studied were:

1. Tonic hind limb flexion
2. THLE
3. Clonus
4. Stupor ([Unconsciousness] from the end of clonus to regain consciousness)
5. Postictal depression (from the regain of consciousness till the animals starts walking) duration of each parameter was recorded in seconds.

The abolition of the hind limb tonic extension is taken as an index of anticonvulsant activity.

Table 1: Drug preparations administered

Group	Drugs administered
I (control group)	5% gum acacia 0.25 ml/100 g
II (standard group)	Sodium valproate 300 mg/kg
III (TG1)	Volatile oil extract of *N. sativa* seeds 200 mg/kg
IV (TG2)	Volatile oil extract of *N. sativa* seeds 400 mg/kg
V (TG3)	Volatile oil extract of *N. sativa* seeds 600 mg/kg
VI (TG4)	Sodium valproate 150 mg/kg+volatile oil extract of *N. sativa* seeds 200 mg/kg

N. sativa: *Nigella sativa*, TG: Test group

Statistical Analysis

Analysis of normal distribution and equivalence of variances between different phases of convulsions of MES model was confirmed using Shapiro–Wilks normality test and Barlett's Chi-square with Levene tests, respectively (Tables 2 and 4). As the phases of convulsions are naturally dependent on each other, Karl Pearson correlation analysis was used, supported by Barlett's Chi-square and Scatter plot matrix. (Table 5 and Graph 1). Multivariate analysis was used for testing the equality of sequences of vector means of different phases of convulsion across treatment groups simultaneously, which indicated that vector means are significant (Table 7). As multivariate test provide the significance in the vector sequences, it was customary to find out which of the convulsion phases contribute for the overall all significance. As a result, one-way ANOVA (Table 8a) was used for multiple comparisons since the normality assumptions were met by the variables, followed by *post-hoc* test for comparison between groups.

RESULTS

In this study, the anticonvulsant activity of volatile oil extract of *N. sativa* seeds was evaluated against MES-induced convulsions. The present study demonstrates abolition of THLE, suggesting that the drug possesses anticonvulsant property.

The results of the experiment are tabulated in Tables 2-15, Bar Diagrams 1-5, and Line Diagrams 1-5. The results were analyzed statistically, and tests of significance were found out.

Table 2: Subgroup normality assumption check SW normality tests results

Drugs	Statistic test	Flexion	Extension	Clonus	Stupor	Postictal depression
Control	SW value	0.954	0.941	0.987	0.902	0.981
	SW *P* value	0.769	0.667	0.981	0.386	0.959
Standard	SW value	0.926		0.940	0.992	0.928
	SW *P* value	0.552		0.662	0.993	0.566
TG1	SW statistic	0.960	0.905	0.949	0.940	0.924
	SW *P* value	0.820	0.406	0.729	0.660	0.532
TG2	SW statistic	0.943	0.729	0.958	0.818	0.878
	SW *P* value	0.682	0.012*	0.808	0.085	0.258
TG3	SW statistic	0.992	0.665	0.946	0.888	0.854
	SW *P* value	0.993	0.003*	0.708	0.307	0.170
TG4	SW statistic	0.913		0.875	0.915	0.965
	SW *P* value	0.458		0.247	0.473	0.854

*Indicates significant. SW: Shapiro–Wilks

Table 3: Descriptive statistics of phases of convulsion across different treatment groups

Phases of convulsion	Groups	Mean	Variance	Median
Flexion	Control	7.820	1.148	7.865
	Standard	4.167	0.638	3.990
	TG1	7.535	0.713	7.490
	TG2	5.957	0.468	6.010
	TG3	4.487	0.248	4.475
	TG4	4.120	0.423	3.895
Clonus	Control	19.545	1.627	19.605
	Standard	9.122	1.580	9.105
	TG1	14.555	3.762	14.740
	TG2	11.420	0.784	11.445
	TG3	9.407	1.160	9.385
	TG4	8.997	0.981	8.985
Stupor	Control	114.500	103.900	115.000
	Standard	60.833	71.767	60.500
	TG1	105.333	31.467	105.00
	TG2	70.833	130.967	76.500
	TG3	63.833	40.167	62.000
	TG4	62.833	24.567	64.000
Postictal depression	Control	131.167	180.167	133.000
	Standard	53.167	52.167	52.500
	TG1	127.667	154.267	131.000
	TG2	108.833	44.167	106.500
	TG3	77.000	164.400	79.500
	TG4	54.167	19.367	53.500

TG: Test group

Table 2 provides the SW value and *P* value for different phases of convulsion across different treatment groups. Only 2 significant value in extension for TG2 and TG3. The rest follow normal distribution.

Table 3 provides the information on mean, variance, and median of different phases of convulsion in seconds across different treatment groups.

Table 4 gives the information on the equality of variance across treatment groups for different phases of convulsion.

Table 5 provides the correlation among several phases of convulsion. It is very clear that positive correlations exist and most are >80% indicating strong relationship among phases of convulsion. The same is supported by the scatter plot matrix (Graph 1).

Bartlett Chi-square statistic: 195.734, df = 10 $P \leq 0.0005$.

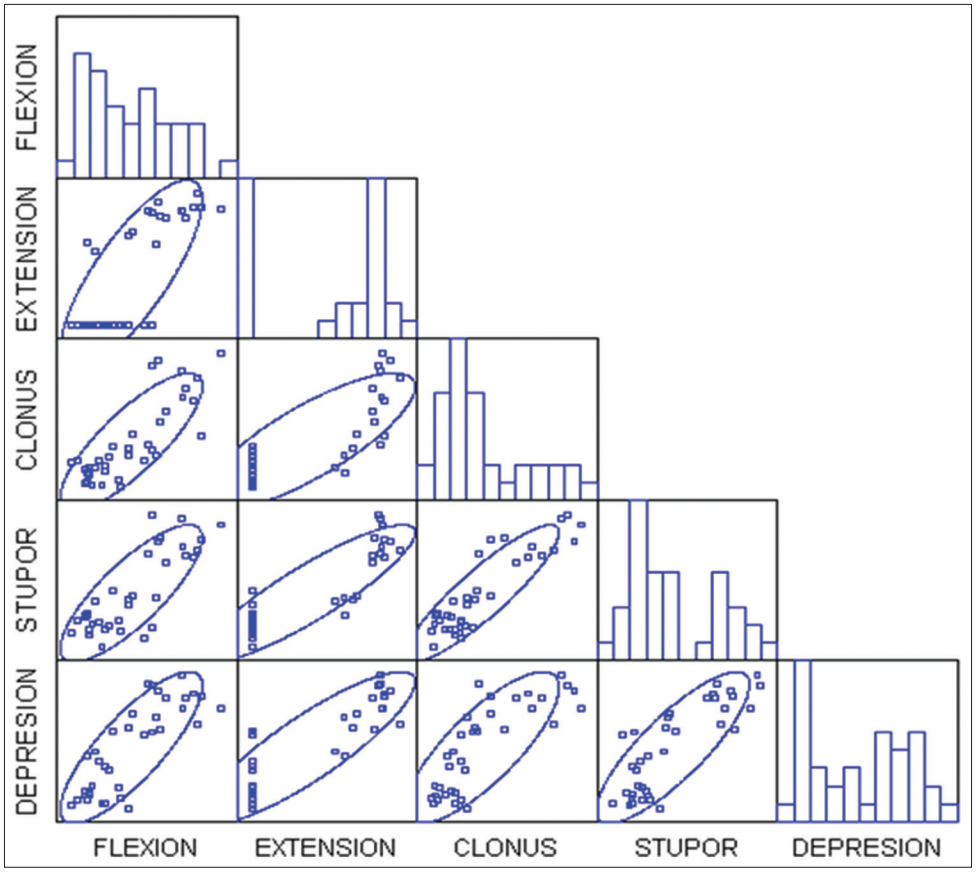
Bartlett Chi-square test for equality of several correlations shows that there is a significant difference in the equality of correlations indicating that there may be some real correlations among the variables.

Table 6 shows cell-wise correlation test, given by Bonferroni-adjusted correlation test at 5% level of significance. Indicating significance.

Table 7 is a multivariate statistical test, for testing the equality of mean vectors of different phases across treatment groups. It shows that vector means are significant.

One-way ANOVA is provided in the Table 8a. Furthermore, cross checking is been done with the nonparametric test which also supports the same and is given in Table 8b. *P* value in the last column indicates the significance.

Table 9 provides the complete detail of comparison of THLE duration in seconds among various groups in MES seizure model.



Graph 1: Scatter plot matrix (SPLOM): 68% of ellipse has been provided in SPLOM

Table 4: Barlett's Chi-square test and Levene's test for equality of several variances

Phases of convulsions	Bartlett's Chi-square test	*P* value of Chi-square test	Levene's test	*P* value of Levene's test
Flexion	3.065	0.690	0.640	0.671
Clonus	3.925	0.560	1.575	0.1971
Stupor	5.128	0.400	0.919	0.482
Depression	8.043	0.154	0.815	0.549

Table 5: Correlation analysis: Karl Pearson correlation matrix

	Flexion	Extension	Clonus	Stupor	Depression
Flexion	1.000				
Extension	0.783	1.000			
Clonus	0.810	0.811	1.000		
Stupor	0.695	0.859	0.809	1.000	
Depression	0.782	0.852	0.816	0.798	1.000

Tables 10-14 represents the *post-hoc* Fisher's test and *P* values for tonic hind limb flexion, THLE, clonus, stupor, and postictal depression, respectively, and are tabulated in the respective cells.

Table 10 (flexion) shows test Group 1 (TG1)is statistically insignificant to control group, but TG2 is significant to the control group. It is also observed from the table that TG3 and TG4 are significant with respect to control group, however, are insignificant with standard group, so comparable to standard group. Also that TG3 and TG4 are insignificant, thus comparable to each other.

Table 11 (extension) represents that TG1 is statistically insignificant to control group. It is observed from the table that TG2, TG3, and TG4 are significant with respect to control group.

Table 12 (clonus) represents that TG1 is statistically insignificant to control group, but TG2 is statistically significant to control group. It is also observed that TG3 and TG4 are significant with respect to the control group, however, are insignificant with standard group, so comparable to standard group. Furthermore, that TG3 compared to TG4 is insignificant, thus comparable to each other.

Table 13 (stupor) shows that TG1 is statistically insignificant to control group, but TG2 is statistically significant to control group. It is observed from the table that TG3 and TG4 are

Table 6: Matrix of Bonferroni probabilities

	Flexion	Extension	Clonus	Stupor	Postictal depression
Flexion	<0.0005*				
Extension	<0.0005*	<0.0005*			
Clonus	<0.0005*	<0.0005*	<0.0005*		
Stupor	<0.0005*	<0.0005*	<0.0005*	<0.0005*	
Postictal depression	<0.0005*	<0.0005*	<0.0005*	<0.0005*	<0.0005*

*Indicates the statistical significance

Table 7: Multivariate test statistics

Statistic	Value	F-statistic	df	*P* value
Wilks's Lamda	0.006	11.803	25.98	<0.0005*
Pillai trace	2.068	4.233	25.150	<0.0005*
Hotelling-Lawley trace	35.595	34.741	25.122	<0.0005*

*Indicates the statistical significance

Table 8a: Univariate ANOVA table

Phases of convulsion	SS	Df	MS	F	*P* value
Flexion					
Between groups	85.47	5	17.094	0.902	<0.0005*
Error	18.19	30	0.606	0.386	
Extension					
Between groups	842.018	5	168.404	0.992	<0.0005*
Error	202.580	30	6.753	0.993	
Clonus					
Between groups	525.838	5	105.168	0.940	<0.0005*
Error	49.470	30	1.649	0.660	
Stupor					
Between groups	17,033.47	5	3406.694	0.818	<0.0005*
Error	2014.167	30	67.139	0.085	
Postictal depression					
Between groups	37,523.33	5	7504.667	0.888	<0.0005*
Error	3072.667	30	102.422	0.307	

SS: Sum of squares, Df: Degrees of freedom, MS: Mean square, SE: Standard error, ANOVA: Analysis of variance *Indicates the statistical significance.

Table 8b: Kruskal–Wallis test

Phases of Convulsion	Kruskal–Wallis H test	*P* value
Flexion	28.135	<0.0005*
Extension	29.207	<0.0005*
Clonus	29.390	<0.0005*
Stupor	25.164	<0.0005*
Postictal depression	30.536	<0.0005*

*Indicates the statistical significance

significant with respect to control group, however, are insignificant with Standard group, so comparable to standard group. Also that TG3 compared to TG4 is insignificant, so comparable to each other.

Table 14 (postictal depression) represents that TG1 is statistically insignificant to control group. TG2 and TG3 are statistically significant to control group, but not comparable to standard. TG4 is significant with respect to control group, however, is insignificant with standard group, so comparable to standard group.

Table 15 shows percentage protection in THLE among various treatment groups in MES seizure model.

DISCUSSION

Almost 30% of epileptic patients suffer from pharmacoresistance. The treatment of pharmacoresistant patients usually requires polytherapy. The long-term use of antiepileptic drugs is limited due to their adverse effects, withdrawal symptoms, deleterious interactions with other drugs and economic burden, especially in developing countries.[2]

There is still a need for an ideal antiepileptic agent with properties such as broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability, and low cost.[5] Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of antiepileptic drugs with novel structures and better safety and efficacy profiles. Hence, the search should continue to develop newer more effective and safer neuroprotective agents for the treatment of epilepsy.[7]

In this study, the anticonvulsant activity of volatile oil extract of *N. sativa* seeds was evaluated against MES-induced convulsions. The present study demonstrates the abolition of THLE showing that drug possesses anticonvulsant activity.

Dose 1 - 200 mg/kg Body Weight

Analysis of results of TG1 animals in MES model (Tables 10-14 and Line Diagrams 1-5) that received 200 mg/kg of test compound showed a reduction in all phases, namely, tonic hind limb flexion, THLE, clonus, stupor, and postictal depression. When compared to control group, reduction in

Table 9: Comparison of THLE duration in seconds among various groups in MES seizure model

Groups	Number of rats						Mean±SE
	1	2	3	4	5	6	
Control group	11.32	13.17	12.23	11.56	10.67	11.13	11.68±0.365
Standard group	0	0	0	0	0	0	0±0
TG1	10.87	11.44	11.68	11.37	10.63	11.75	11.29±0.183
TG2	0	9.34	0	8.13	0	8.86	4.38±1.969
TG3	0	0	8.23	0	7.35	0	2.59±1.646
TG4	0	0	0	0	0	0	0±0

THLE: Tonic hind limb extension, MES: Maximal electroshock, SE: Standard error, TG: Test group

Table 10: Flexion matrix of pair-wise comparison probabilities

	Control	Standard	TG1	TG2	TG3	TG4
Control	1.000					
Standard	<0.0005*	1.000				
TG1	0.531	<0.0005*	1.000			
TG2	<0.0005*	<0.0005*	0.001*	1.000		
TG3	<0.0005*	0.482	<0.0005*	0.003*	1.000	
TG4	<0.0005*	0.918	<0.0005*	<0.0005*	0.421	1.000

*Indicates the statistical significance. TG: Test group

Table 11: Extension matrix of pair-wise comparison probabilities

	Control	Standard	TG1	TG2	TG3	TG4
Control	1.000					
Standard	<0.0005*	1.000				
TG1	0.797	<0.0005*	1.000			
TG2	<0.0005*	0.007*	<0.0005*	1.000		
TG3	<0.0005*	0.094	<0.0005*	0.242	1.000	
TG4	<0.0005*	1.000	<0.0005*	0.007*	0.094	1.000

*Indicates the statistical significance. TG: Test group

Table 12: Clonus matrix of pair-wise comparison probabilities

	Control	Standard	TG1	TG2	TG3	TG4
Control	1.000					
Standard	<0.0005*	1.000				
TG1	<0.0005*	<0.0005*	1.000			
TG2	<0.0005*	0.004*	<0.0005*	1.000		
TG3	<0.0005*	0.703	<0.0005*	0.011*	1.000	
TG4	<0.0005*	0.867	<0.0005*	0.003*	0.584	1.000

*Indicates the statistical significance. TG: Test group

the duration of clonus was statistically significant, whereas remaining phases were not statistically significant.

Dose 2 - 400 mg/kg Body Weight

Analysis of results of TG2 animals in MES model (Tables 9-14 and Line Diagrams 1-5), which received 400 mg/kg of the test compound, showed a reduction in all phases, namely, tonic hind limb flexion, THLE, clonus, stupor, and postictal depression which were statistically significant when compared to control group. Reduction in all phases of convulsion was statistically not comparable to standard; that means the reduction in the duration of phases of convulsion was less when compared to standard. There was complete abolition of THLE in 3 animals out of 6 animals. 50% abolition in THLE at this dose was observed.

Table 13: Stupor matrix of pair-wise comparison probabilities

	Control	Standard	TG1	TG2	TG3	TG4
Control	1.000					
Standard	<0.0005*	1.000				
TG1	0.062	<0.0005*	1.000			
TG2	<0.0005*	0.043*	<0.0005*	1.000		
TG3	<0.0005*	0.531	<0.0005*	0.149	1.000	
TG4	<0.0005*	0.675	<0.0005*	0.101	0.834	1.000

*Indicates the statistical significance. TG: Test group

Dose 3 – 600 mg/kg Body Weight

Analysis of results of TG3 animals in MES model (Tables 10-14 and Line Diagrams 1-5) that received 600 mg/kg of test compound showed a reduction in all phases, namely, tonic hind limb flexion, THLE, clonus, stupor, and postictal depression. Reduction in duration of tonic hind limb flexion, extension, clonus, and stupor was statistically significant when compared to control group

Table 14: Postictal depression matrix of pair-wise comparison probabilities

	Control	Standard	TG1	TG2	TG3	TG4
Control	1.000					
Standard	<0.0005*	1.000				
TG1	0.554	<0.0005*	1.000			
TG2	0.001*	<0.0005*	0.003*	1.000		
TG3	<0.0005*	<0.0005*	<0.0005*	<0.0005*	1.000	
TG4	<0.0005*	0.865	<0.0005*	<0.0005*	<0.0005*	1.000

TG: Test group, *Indicates the statistical significance.

Table 15: Percentage protection in THLE among various treatment groups in MES seizure model

Treatment groups	Percentage protection
Standard group	100
TG1	Nil
TG2	50
TG3	66.66
TG4	100

THLE: Tonic hind limb extension, MES: Maximal electroshock

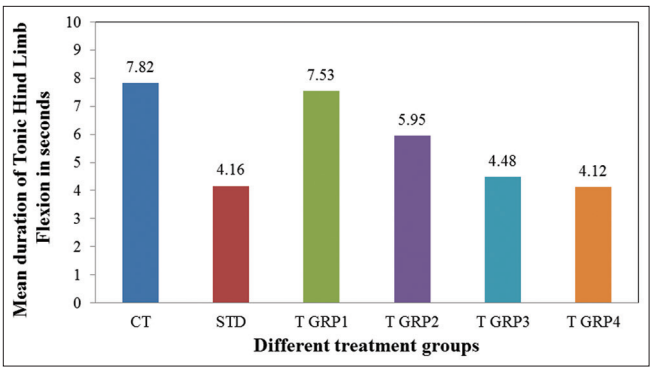
and comparable to standard group. However, the reduction duration of postictal depression was statistically significant when compared to control but not comparable to the standard. There was the complete abolition of THLE in four animals out of six animals. 66.66% abolition in THLE at this dose was observed.

Dose 4 – Volatile Oil of *N. sativa* 200 mg/kg + Sodium Valproate 150 mg/kg Body Weight

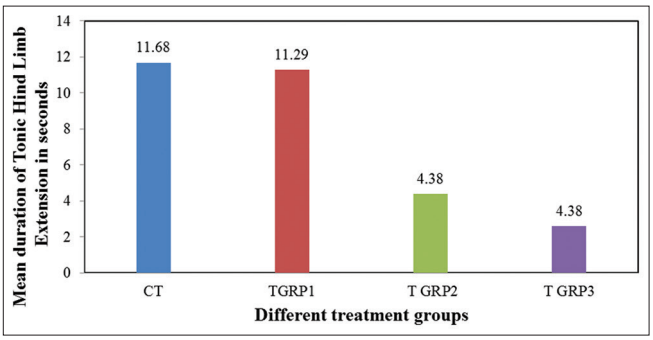
Analysis of results of TG4 animals in MES model (Table 10-14 and Line Diagrams 1-5) that received volatile oil of *N. sativa* 200 mg/kg body weight and sodium valproate 150 mg/kg body weight (sub anticonvulsant dose) showed a reduction in all phases, namely, tonic hind limb flexion, THLE, clonus, stupor, and postictal depression. Reduction in duration of tonic hind limb flexion, clonus, stupor, and postictal depression was statistically significant when compared to control group and comparable of standard group. There was complete abolition of THLE in all six animals. 100% abolition in THLE at this dose was observed. Furthermore, reduction in duration of tonic hind limb flexion, clonus, and stupor but not postictal depression were statistically insignificant and comparable to TG3.

Swinyard et al. have considered abolition of hind limb tonic extension as the protective end point against MES-induced seizures.[16]

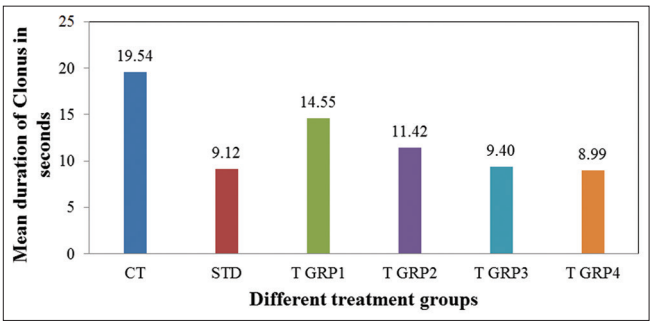
According to this study, sodium valproate showed 100% protection against hind limb extension. At doses of 200 mg/kg of volatile oil of *N. sativa,* there was no significant anticonvulsant activity. At the dose of 400 and



Bar diagram 1: Comparison of mean duration of tonic hind limb flexion across different treatment groups

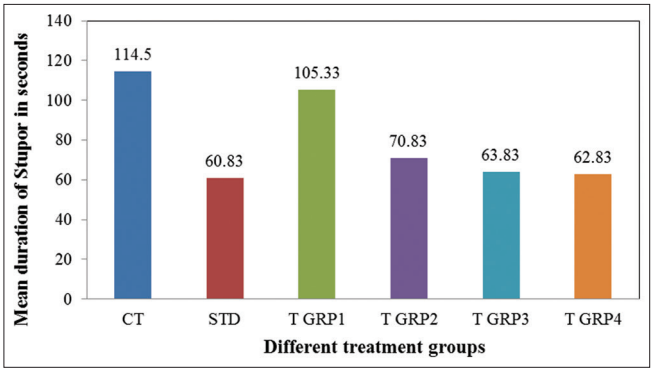


Bar diagram 2: Comparison of mean duration of tonic hind limb extension across different treatment groups

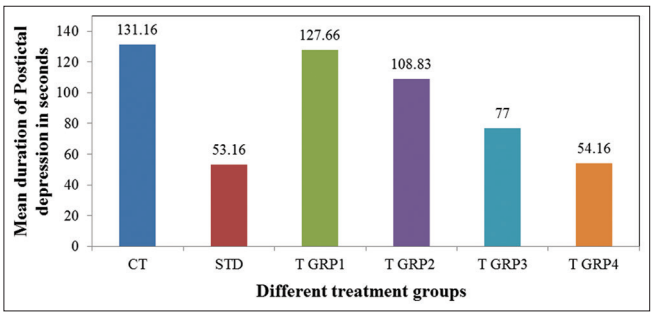


Bar diagram 3: Comparison of mean duration of clonus across different treatment groups

600 mg/kg, THLE was abolished in 50% and 66.66% of animals, respectively, when compared to standard. This indicates that the anticonvulsant activity of volatile oil of *N. sativa* is less when compared to the standard drug,



Bar diagram 4: Comparison of mean duration of stupor across different treatment groups



Bar diagram 5: Comparison of mean duration of posticta depression across different treatment groups

sodium valproate. The combination of volatile oil of *N. sativa* 200 mg/kg body weight with sodium valproate (150 mg/kg) showed statistically significant reduction in all phases of convulsion when compared to control group and were comparable of standard group. The combination of volatile oil of *N. sativa* 200 mg/kg body weight with sodium valproate (150 mg/kg) showed complete abolition of THLE in all 6 animals, thus showed 100% protection against THLE.

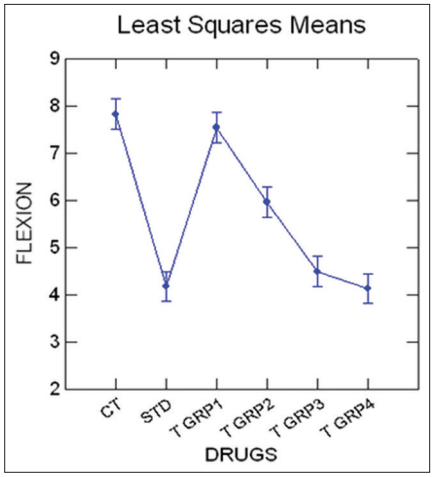
The above analysis gives us the result that:

- Volatile oil of *N. sativa* by itself has significant anticonvulsant activity in the dose of 400 and 600 mg/kg body weight. However, anticonvulsant activity produced by it is less when compared to the standard drug sodium valproate in the dose of 300 mg/kg
- The combination of volatile oil of *N. sativa* with sodium valproate at their sub anticonvulsant doses showed significant anticonvulsant activity. This suggests that volatile oil of *N. sativa* has potentiated the effect of sodium valproate.

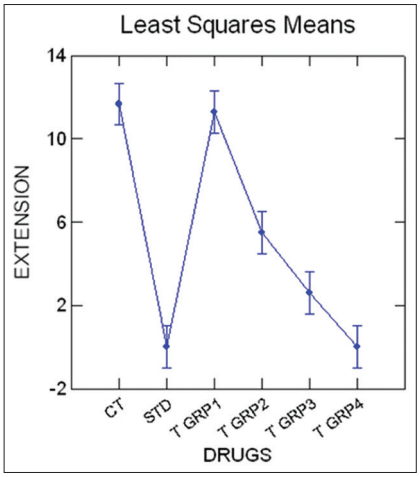
Hence, the test compound may be useful in generalized tonic-clonic seizures (grand mal) and partial epilepsy.

CONCLUSION

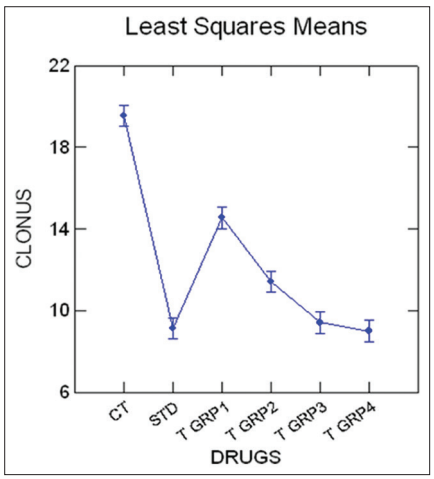
The anticonvulsant activity of volatile oil of *N. sativa* in different dosage profiles - 200, 400, and 600 mg/kg body weight and combination of volatile oil of *N. sativa* 200 mg/kg with sodium valproate 150 mg/kg were evaluated by MES model, and the results were compared with that of respective control groups and respective standard groups. The volatile oil of *N. sativa* has shown the significant anticonvulsant activity at the dose of 400



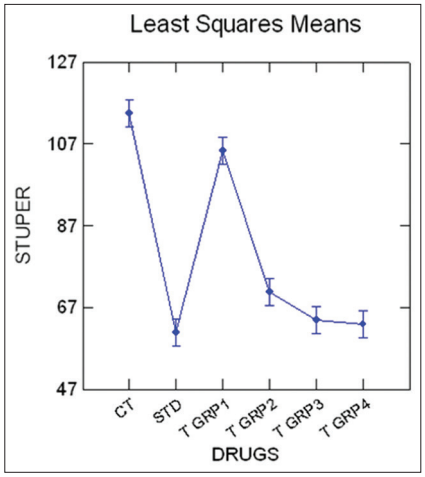
Line diagram 1: Comparison of least square means of flexion



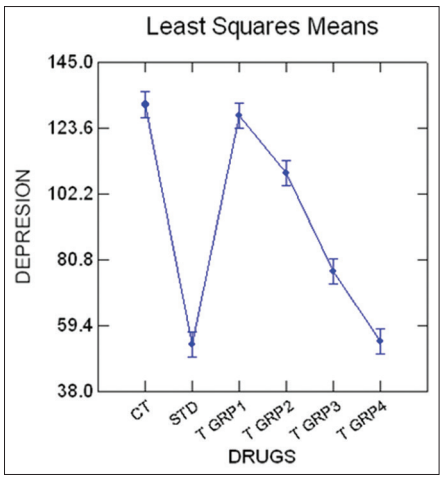
Line diagram 2: Comparison of least square means of extension



Line diagram 3: Comparison of least squares means of clonus



Line diagram 4: Comparison of least square means of stupor



Line diagram 5: Comparison of least square means of postictal depression

and 600 mg/kg body weight. Further, the combination of volatile oil of *N. sativa* 200 mg/kg with sodium valproate 150 mg/kg has shown significant anticonvulsant activity. The anticonvulsant activity of volatile oil of *N. sativa* was less when compared to sodium valproate. The anticonvulsant activity of combination, volatile oil of *N. sativa* 200 mg/kg with sodium valproate 150 mg/kg (in their sub anticonvulsive dose) was comparable to standard drug. From this, it may be predicted that volatile oil of *N. sativa* may be useful either alone or in combination with sodium valproate in the treatment of generalized tonic-clonic seizures (grand mal) epilepsy and partial seizures. The combination of subtherapeutic doses of valproate volatile oil of *N. sativa* resulted in the potentiation of sodium valproate. Thus, the reduction in the valproate dose required for anticonvulsant activity may be valuable in suppressing its unwanted effects such as hepatotoxicity and teratogenic implications. However, further studies are required to confirm the same.

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