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EVALUATION OF ANTI-EPILEPTIC POTENTIAL OF *NIGELLA SATIVA* IN VALIDATED ANIMAL MODELS

Nafisa Aijaz Ahmed[1], Sampath A. Gouru[2]*, Pankaj M. Pimpalshende[3], Pradeep C. Dave[4], Amol J. Giri[5], Purushottam R Ladhha[6], Gopalkrishna R. Sitaphale[7], Gaurav J. Jogade[8]

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Abstract

Epilepsy is a neurological disorder characterized by recurrent seizures, affecting millions of people worldwide. Despite the availability of several anti-epileptic drugs, there remains a need for more effective and safer treatment options. *Nigella sativa*, commonly known as black seed or black cumin, is a medicinal plant with a long history of use in traditional medicine. This study aimed to evaluate the anti-epileptic potential of the ethanolic extract of *Nigella sativa* in validated animal models of epilepsy.

The ethanolic extract of *Nigella sativa* was prepared, and its chemical composition was analyzed to identify the presence of bioactive compounds. Validated animal models of epilepsy, including pentylenetetrazole (PTZ) and maximal electroshock (MES) models, were used to induce seizures. The animals were orally administered with different doses of the ethanolic extract of *Nigella sativa*, and seizure severity, duration, and frequency were recorded.

The results of this study demonstrated that the ethanolic extract of *Nigella sativa* significantly reduced seizure severity, duration, and frequency in both PTZ- and MES-induced seizure models. The anti-epileptic effects were found to be dose-dependent, with higher doses of the extract exhibiting greater reductions in seizure activity. These findings suggest that the ethanolic extract of *Nigella sativa* possesses anti-epileptic potential.

Keywords: *Nigella sativa*, anti convulsant, MES, Pentylenetetrazole, mice.

[1]Business development coordinator, Kuwait Saudi Pharmaceutical Company

[2]Senior Manager, QA CGMP and GDP, EQRX international Inc, Cambridge, Massachusetts, USA

[3]Hi-Tech College of Pharmacy, Chandrapur, Maharashtra.

[4]Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Navi Mumbai, Kharghar, 400614

[5,6,7,8] Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana. MS 443204

Main Author: Nafisa Aijaz Ahmed, Email : nafisadhansay@gmail.com.

Corresponding Author: Sampath A. Gouru*, Email : gsayyappa@gmail.com

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Introduction: Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons.[1] Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per100,000. [2] It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients.[3] The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity.[4] As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine, etc are used supplemental to the conventional agents. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. The aim of treating an epileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life.

Nigella sativa, commonly known as black seed or black cumin, is a medicinal plant with a long history of use in traditional medicine. (Family: Ranunculaceae) that has been regarded to possess various medicinal properties. [5]

Nigella sativa has been used as an antiepileptic agent in traditional system of medicine in India. The objective of this investigation was to ascertain the scientific basis of its use in treatment of epilepsy. The present investigation reports antiepileptic activity of the ethanolic extract of *Nigella sativa* on which no previous data available.[15-41]

MATERIALS AND METHODS

Collection and identification of plant material

Leaves of *Nigella sativa* were procured and authenticated by the renowned botanist and voucher specimen was deposited in herbarium for future reference. The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation of alcoholic extract

The powder of Leaves of *Nigella sativa* leaves was charged in to the thimble of a Soxhlet's apparatus and extracted using 95% alcohol for 18 hrs. Appearance of colorless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50^{o}c to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container till used.

Experimental animals

Albino mice of either sex weighing between 20-30g were procured from central animal house for experimental purpose. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet from. Water was allowed *ad libitum* under hygienic conditions. All animal studies were performed in accordance to guideline of CPCSEA and Institutional Animal Ethical Committee (IAEC) guidelines.

Acute toxicity study

The acute toxicity of leaf extracts of *Nigella sativa* was determined by using albino mice of either sex weight between

(20-25 g), maintained under standard conditions. The animals were be fasted for 3 hr prior to the experiments. Animals were administered with single dose of either alcoholic extract of leaves of *Nigella sativa* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. From the LD_{50} dose 1/20, 1/10 and 1/5th doses were selected and considered as low, medium and high doses respectively.

Assessment of anticonvulsant activity [6]

The animals were divided into five groups of six each. Group I received 2% Gum acacia p.o., Group II received Phenytoin (25 mg/kg p.o.)/ diazepam (5.0 mg/kg p.o.), Group III received ethanolic extract of leaves of *Nigella sativa* (200 mg/kg p.o.), Group IV received ethanolic extract of leaves of *Nigella sativa* (600 mg/kg p.o.) and Group V received ethanolic extract of leaves of *Nigella sativa* (800 mg/kg p.o.).

Maximal electroshock induced seizures

60 min after drug administration maximal electro shock seizures are elicited by the application of electric shock (12 mA, 50 Hz for 0.2 sec) using corneal electrodes. Abolition of the hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity. Phenytoin (25 mg/kg p.o.) was used as reference standard.

Pentylenetetrazol-induced seizures

60 min after drug administration, seizure was induced by subcutaneous injection of PTZ (60 mg/kg) and the mice were observed for onset of myoclonic spasm and clonic convulsions. Diazepam (5mg/kg, p.o.) was included as a reference standard. The animals were observed for onset of convulsion up to 30min after PTZ administration.

Statistical analysis:

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet‟s„t‟- test. p values <0.05 were considered significant.

RESULTS AND DISCUSSION

In pentelenetetrazole induced seizure model, ethanolic extract of leaves of *Nigella sativa* 600 and 800mg/kg produced significant ($p<0.01$) reduction in duration of convulsion and was comparable to that produced by diazepam 5mg/kg, but 400mg/kg did not exhibit anticonvulsant effect. In the maximal electro shock induced seizure model, 600 and 800mg/kg and diazepam 5mg/kg showed significant ($p<0.01$) reduction in duration of convulsion, but 400mg/kg did not exhibit anticonvulsant effect. The anticonvulsant activity of at various dose levels viz, 400, 600 and 800mg/kg p.o. were studied by the pentelenetetrazole and maximal electro shock induced seizure models. The most popular and widely used animal seizure models are maximal electro shock and pentelenetetrazole induced seizure. Prevention of seizures induced by pentelenetetrazole in laboratory animals is the most commonly used preliminary screening test for characterizing potential anticonvulsant drugs. The maximal electro shock-induced seizure test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures. By contrast, the pentelenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures. Other chemoconvulsant models for primary generalized seizures include by bicuculine (GABAA receptor antagonized), strychnine (glycine receptor antagonist) and aminophylline (adenosine-receptor antagonist). The pentelenetetrazole assay has been used primarily to evaluate antiepileptic drugs. However, it has been shown that, most anxiolytic agents are also able to prevent or antagonize pentelenetetrazole-induced

convulsion. Generally, compounds with anticonvulsant activity in the petit mal epilepsy are effective in pentelenetetrazole-induced seizure model.[7]

Data from the study showed that the tonic convulsion produced by pentelenetetrazole was significantly delayed by ethanolic extract of leaves of *Nigella sativa*. The data also show that diazepam antagonize the pentelenetetrazole-induced convulsion. According to be Sarro et al, pentelenetetrazole may be exerting its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABAA receptors [8] the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively.[9-11] Phenobarbitone and diazepam have been shown to exert their antiepileptic effects by enhancing the GABA mediated inhibition in the brain.[12] It is possible that diazepam and ethanolic extract of leaves of *Nigella sativa* antagonize pentelenetetrazole convulsion in this study by enhancing GABA neurotransmission. Since the EECD delayed the occurrence of pentelenetetrazole induced convulsion, it is probable that it may be interfering with GABA-aminergic mechanism to exert its anticonvulsant effect.

The maximal electro shock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high. [13] The maximal electro shock test identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs. The pharmacology of acute maximal electroshock dose not differs from the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy, eg. audiogenic-seizure susceptible mice and rats or epileptic gerbils.[14] In addition to identifying drug activity against generalized tonic-clonic seizures, it has often been proposed that the maximal electroshock test predicts anticonvulsant drug effects against partial seizures. Further analysis of the ethanolic extract revealed the presence of several bioactive compounds, including thymoquinone, thymohydroquinone, and α-hederin, known for their neuroprotective and anticonvulsant properties. These compounds may act through modulation of neurotransmitter systems, inhibition of excitotoxicity, reduction of oxidative stress, and attenuation of neuro-inflammation, contributing to the observed anti-epileptic effects.

Conclusion: The ethanolic extract of leaves of *Nigella sativa* exhibited significant anti-epileptic potential in validated animal models of epilepsy. The presence of bioactive compounds with known anticonvulsant properties further supports the efficacy of the extract. Future studies should focus on elucidating the precise mechanisms of action and evaluating the safety and efficacy of the extract in human clinical trials. If these results are confirmed, the ethanolic extract of leaves of *Nigella sativa* could emerge as a promising natural alternative for the treatment of epilepsy, offering potential benefits for patients in terms of improved seizure control and reduced side effects associated with conventional anti-epileptic drugs.

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Table 1: Effect of ethanolic extract of leaves of *Nigella sativa* on MES induced convulsions in mice.

Treatment	Dose	Onset of clonus convulsion (sec)	Duration of extensor convulsion (sec)
Control	2% gum acacia	26 ± 0.57	24 ± 0.57
Phenytoin	25mg/kg p.o.	6.53 ± 0.60	5.9 ± 0.60
EENS	400mg/kg p.o.	23.16 ± 0.60	23.16 ± 0.60
EENS	600mg/kg p.o.	17.33 ± 0.66**	13.33 ± 0.66**
EENS	800mg/kg p.o.	14.84 ± 0.60**	12.83 ± 0.60**

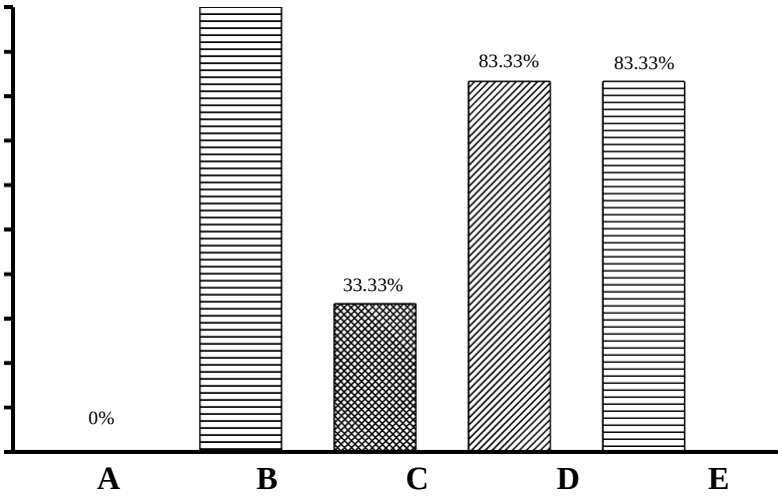
Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett‟s„t‟ test. Where, **represents highly significant at p<0.01, EENS: ethanolic extract of leaves of *Nigella sativa* MES: Maximal electro shock.

Table 2: Effect of EENS leaves on PTZ (60mg/kg) induced convulsion in mice

Treatment	Dose	Onset of convulsion (sec)	Duration of convulsion (sec)
Control	2% gum acacia	670.5 ±10.135	39.66 ± 0.66
Diazepam	5mg/kg p.o.	908.83 ±15.39	6.83 ± 0.71
EENS	400mg/kg p.o.	724.5 ±15.23*	23 ± 0.96
EENS	600mg/kg p.o.	791.16 ±9.02**	19.33 ± 0.91**
EENS	800mg/kg p.o.	823.33 ±7.81**	15.33 ± 0.49**

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnett‟s„t‟ test. Where, **represents highly significant at p<0.01, EENS: ethanolic extract of leaves of *Nigella sativa* MES: Maximal electro shock.

Fig. 01 Percentage Convulsion Protection Activity of EECD Against PTZ Induced Convulsions In Mice



A: 2% gum acacia p.o., B: Diazepam 5mg/kg p.o., C: EENS 400mg/kg p.o.. D: EENS 600mg/kg p.o. E: EENS 800mg/kg p.o