Hygeia:: journal for drugs and medicines
October 2013 – March 2014
OPEN ACCESS
A half yearly scientific, international, open access journal for drugs and medicines
Research article section: Pharmacology
Rid: F-8586-2013 , Article ID- Hygeia.J.D.Med/105/13

Evaluation of Anticonvulsant Activity of the Seed Oil Extract of *Nigella sativa*: an Experimental study.

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Article history: Received: 27May 2013, revised: 19June 2013, accepted: 12 July 2013, Available online: 3 October 2013

ABSTRACT

Plan: *The present study was carried out to evaluate anticonvulsant activity of Nigella sativa oil in Pentylenetetrazol (PTZ) and Maximal electroshock (MES) induced seizures in mice.*
Methodology: *The anticonvulsant activity of Nigella sativa oil at dose of 10 mg/kg/p.o. was evaluated in mice by using electroshock and PTZ seizure methods. The standard was taken as Phenytoin for electroshock method and Diazepam for PTZ method.*
Outcome: *In PTZ model Nigella sativa showed statistically significant protection in increasing the latency of convulsions (p value 0.030). While in MES model though there was decrease in the duration of tonic hind limb extension but it was not statistically significant.*
Conclusions: *Nigella sativa has shown anticonvulsant activity in PTZ model, which is suggestive of its potential benefit in petit mal type of epilepsy and hence there is need to test it in various other animal models.*
Keywords: *Nigella sativa, Maximal electroshock (MES), Pentylenetetrazol (PTZ), Seizures.*

1. INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. Though there is an intensive research, the pathophysiology, etiology and manifestation of disease are poorly understood. All the drugs currently available to treat epilepsy provide satisfactory seizure control in 60-70 % patients. However, these drugs are associated both with notable adverse effects, such as drowsiness, ataxia, hepatotoxicity and megaloblastic anemia, as well as potentially life threatening conditions.[1] Therefore, the continued search for safer and more effective new antiepileptic drugs is both an imperative and a challenge.

Recently it has been found that Black Seed (*Nigella sativa*) has an anti epileptic activity. [2] *Nigella sativa* is an annual herbaceous plant which belongs to the family Ranunculaceae.

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Hygeia journal for drugs and medicines, 2229 3590, 0975 6221
Researcher id: F-8586-2013

The seeds are small and black in colour and possess aromatic odour and taste. *Nigella sativa* (NS) seeds (black seeds) contain two types of oils, i.e. Fixed oil and volatile oil. Volatile oil of NS seed is composed mainly of thymoquinone (2-isopropyl-5- methyl- 1,4- benzoquinone) and monoterpenes[2]. Interestingly, *Nigella sativa* has shown protection against epilepsy and convulsions in few animal studies.[3] It is being used in indigenous system of medicine since many years for various indications such as analgesic, anti-inflammatory, diuretic and anti cancer agent. [4]

Furthermore few studies have demonstrated that Black Seed oil is devoid of toxicities associated with drugs currently used in the treatment of epilepsy and convulsions.[5] Hence the present study was carried out to clarify whether, as proposed, *Nigella sativa* leads to inhibition of seizure discharges induced by maximal electroshock and pentylenetetrazol in experimental animals/models.

2. MATERIALS AND METHODS

Albino mice of either sex weighing 25 to 30 gm were used. **S**eizures were introduced by Maximal electroshock induced seizures [6] and Pentylenetetrazol induced seizures.[7] Study was carried out after obtaining approval from the Institutional Animal Ethics Committee, S.R.T.R. Government Medical College, Ambajogai.

2.1. Drugs and Chemicals

a. *Nigella sativa*: Seed extracted oil was obtained from Manish Herbals, M.P., India. Phytochemical analysis showed, it was standardized, fixed oil with dark brown color, specific gravity of 1.096, Saponification value of 195.35, Refractive index of 1.4666, Acid value: 23.74, identified by presence of Carvons (2.74%) and was given as a test drug in a dose of 10 ml/kg/p.o.
b. Phenytoin: Pure powder form of Phenytoin was dissolved in normal saline and administered at the dose of 25 mg/kg/p.o.
c. Diazepam: Pure powder form of Diazepam was dissolved in normal saline and administered at the dose of 2.5 mg/kg/p.o.

2.2. Groups and Sample size

The animals were divided in following groups with n= 6.

1. Control: Normal saline.
2. Phenytoin(MES)
3. Diazepam (PTZ induced seizures).
4. Nigella sativa Fixed Seed Oil Extract.

*2.3. Statistical test***:**

Results were expressed as Mean± SEM. Group mean differences were ascertained by one way ANOVA followed by post hoc test and unpaired t test.

2.4. Animals and experimental conditions

The animals were randomly allocated to different experimental groups (n=6). They were placed in plastic cages with husk bedding and at controlled temperature of 25 ± 2^0 C and 12 h: 12 h light dark cycle. They were given access to food and water *ad labitum* except during the experimental test period. The animals were allowed to adjust to the laboratory conditions such as light, temperature and noise for half hour before being subjected to the experiments. All experiments were carried out at the same time of the day i.e. between 10.30 am to 5 pm to minimize circadian influences on seizure susceptibility**.**

2.5. Electro convulsion [6]

For the evaluation of anticonvulsant activity of drugs by electroconvulsion, the maximal electroshock seizure (MES) method was used. Ear electrodes were used for induction of seizures. Alternating current of 50 mA was given for 0.2 sec duration.

Drugs (n=6)	*Dose*	*Latency To Convulse (Sec) Mean ± S.E.M*	*Duration Of T.H.E. (Sec) Mean ± S.E.M.*	*% Seizure Protection*	*% Mortality*
Control (Normal Saline)	0.5 ml	1.70 ± 0.0365	13.86 ± 0.055	0	33.33
Phenytoin	25mg/kg	1.90± 0.036	0	0	0
Nigella sativa	10 ml/kg	1.71± 0.149	12.93 ± 0.049	0	0

Table: 1 Effect of Nigella sativa on maximal electroshock induced seizures in albino mice:

T.H.E. Tonic Hind limb Extension, Phenytoin, Nigella sativa oil and normal saline were administered 60 min before the induction of seizures. Values are expressed as the Mean ± SEM.
*ANOVA followed by post hoc test and unpaired t test, ***p < 0.001, **p < 0.01, *p < 0.05, as compared to control.*

The phases of maximal seizure shown by mice typically consists of

a) Phase of tonic limb flexion.
b) Full extension of limbs.
c) Clonic interval.
d) Asphyxial death (in some animals).

Failure to extend the hind limbs to an angle with the trunk greater than 90^0 is defined as protection. The effect of Nigella sativa was studied on MES induced seizure by giving drug per orally 60 minutes prior to electroshock. The effect of drugs was observed and results were compared statistically with standard drug Phenytoin and control in terms of prolongation of latent phase, reduction in the duration of tonic phase.

2.6. Chemo convulsion:

In this method[7], the anticonvulsant activity of drugs was studied according to the method described by Brown and Goodman (1952). PTZ dissolved in 0.9 % normal saline was given intra-peritoneally in a dose of 75 mg/kg in a volume not exceeding 0.01 ml/gm of body weight.
Three distinct phases constituted the PTZ seizure sequence i.e.

1. Myoclonus : defined as whole body twitch with straub tail phenomenon,
2. Clonus : manifested by clonic spasms often followed by stupor or unusual positioning and a unusual lethal component,
3. Clonic- tonic hind limb extension.

Animals were tested for PTZ induced convulsions 60 minutes after the administration of the Nigella sativa & Diazepam and were observed for 30 minutes. The protective effect of Nigella sativa was judged on prolongation of latent phase and reduction in the total number of episodes of clonic spasms.

Table: 2.Effect of drugs on Pentylenetetrazol induced convulsions in mice.

Drug (n=6)	*Dose (mg/kg)*	*Latency to convulse (sec) Mean ± S.E.M.*	*Number of clonic convulsions Mean ± S.E.M.*	*% Mortality*	*% Seizure protection*
Control (Normal Saline)	0.5ml	63.5 ± 4.105	3.3 ± 0.421	83.33	0
Diazepam	2.5 mg/kg	No convulsions	0	0 %	100 %
Nigella sativa	10 ml/ kg	75.5 ± 1.117*	1.03 ± 1.674	33.33	0

Diazepam, Nigella sativa oil and normal saline were administered 60 min before the induction of seizures.
*Values are expressed as the Mean ± SEM., ANOVA followed by post hoc test and unpaired t test ***p < 0.001, **p < 0.01, *p < 0.05, as compared to control.*

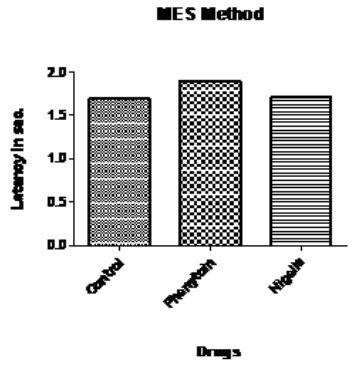

Figure 1: Latency of drugs in MES method.

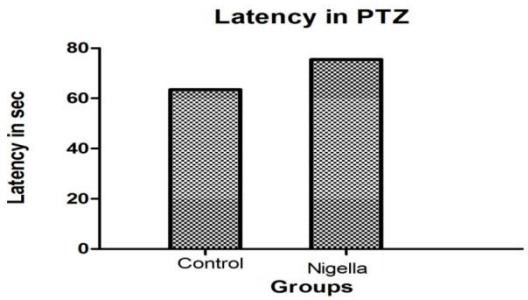

Figure 2: Latency of drugs in PTZ method.

3. RESULTS

3.1. Effects of Nigella sativa on Maximal Electroshock induced seizures:

As shown in table 1 Nigella sativa had reduced the duration of tonic hind limb extension in mice but there was no effect as far as the latency to generation of seizures is concerned, when compared to control group. Though these findings were statistically not significant when compared with latency of control and standard group using one way ANOVA test (*p* value *0.068*), but literature suggests that Nigella sativa has anticonvulsant activity in various animal models. At the same time though there was reduction in duration of tonic hind limb extension, the difference again was not statistically significant when compared with control group using unpaired't' test (*p* value 0.648) . Also the percentage mortality and percentage seizure protection was comparable with standard group and better than control group animals. Nigella sativa showed 100 % protection as far as mortality is concerned.

3.2. Effects of Nigella sativa on PTZ induced seizures:

As shown in table 2, *Nigella sativa* showed increased latency as far as seizure generation is concerned when PTZ was given. When compared statistically with control group using unpaired't' test, it was significant (*p* value 0.030). Also the numbers of clonic convulsion were less compared to control group; however the values are statistically insignificant when compared by unpaired't' test (*p* value 0.052). In PTZ induced convulsions also the percentage mortality and percentage seizure protection was comparable with standard group and better than control group animals. So there is significant protection in latency for generation of seizure in PTZ induced seizure model.

4. DISCUSSION

Various studies conducted have shown that *Nigella sativa* possesses anticonvulsant activity. Study conducted by *H. Hosseinzadeh and S. Parvardeh*, to evaluate the Anticonvulsant effects of *thymoquinone*, the major constituent of *Nigella sativa* seeds; indicate that thymoquinone exhibits anticonvulsant activity in the PTZ-induced seizure model. Whereas thymoquinone did not exhibit any anticonvulsant property in MES model.[8] These findings conclude that thymoquinone may be as useful in petit mal epilepsy as the compounds showing protection in PTZ-induced seizure model are protective in petit mal epilepsy. On the other hand it may not be useful in grand mal epilepsy as MES model is representative of grand mal epilepsy. Similarly *Ilhan A, Gurel A and Armutcu F et al* conducted a study[8] to evaluate antiepileptogenic and antioxidant effects of Nigella sativa oil against pentylenetetrazol-induced kindling in mice. Their study[9] clearly demonstrated a potent anticonvulsant property of Nigella sativa Oil against the development of kindling consequences in PTZ-kindled mice. This showed that Nigella sativa Oil was a very effective neuroprotective agent against PTZ kindled seizures via its potent antioxidant actions.

In our study, the results were not so promising. Though Nigella sativa showed increased latency as far as seizure generation is concerned and also the numbers of clonic convulsion were less compared to control group in PTZ induced seizure methods, but protection was statistically significant only in increasing the latency of seizures while reduction in numbers of clonic convulsion were statistically insignificant . Similarly in Maximal Electroshock Seizure method results were statistically insignificant. In view of these conflicting results, further research is warranted by means of various other models of epilepsy in order to establish the exact mechanism of this seed extract, thereby strengthening the hypothesis.

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Swapnil C Jaykare , Vijay M Motghare , Sudhir L Padwal, Vinod S Deshmukh , Jyoti R Patil , Harshal N Pise , and Rakesh R Pore .Evaluation of Anticonvulsant Activity of the Seed Oil Extract of Nigella sativa: an Experimental study Hygeia.J.D.Med.* **2013**; 5(2): page.21-26. Available from http://www.hygeiajournal.com / Article ID- Hygeia.J.D.Med/105/13