

Drug interaction analysis-*Nigella sativa* L. seed (Black Cumin) ethanolic extract on anti-seizure activity of Phenobarbitone sodium



Lovelyn Joseph¹, Rejeesh Edavan Puthallath², Sudarshanram Narayan Rao³

¹Associate Professor, Department of Pharmacology, PK Das Medical College, Vaniyankulam, Kerala, India

²Associate Professor, Department of Pharmacology, Mount Zion Medical College, Adoor, Kerala, India

³Senior Professor, Pharmacology (Rtd), Independent Researcher

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ABSTRACT

Background: Multidisciplinary therapy is gaining popularity and drug herb interactions is one of the biggest risk factor contributing to therapeutic failures or toxicities. Apart from analysis of one of such Drug-Herb combinations, this work also aims at emphasizing need for ethnopharmacovigilance practices. **Aims and Objectives:** Herb-Drug Interaction analysis between *Nigella sativa* seed ethanolic extract and Phenobarbitone sodium in maximal electroshock seizure in Swiss albino mice with a modified isobolographic analysis. **Materials and Methods:** Experiment model was Maximal electroshock seizure in Swiss albino mice. Dose ratios of the combination tested were NsEE: Phenobarbitone; 3:1(NsEE75%: Pbt25%), 1:1, 1:3. Analysis was done with modified isobolographic analysis. **Results:** *Nigella sativa* seed ethanolic extract exhibit synergistic interaction with Phenobarbitone sodium at 1:1 and 1:3 dose ratios and antagonistic interaction at 3:1 combination. The parameter measured was Hind limb tonic extensor phase in maximal electroshock seizure test in Swiss albino mice. **Conclusion:** The study showed that interaction profile of *Nigella sativa* extract-phenobarbitone combination is dose dependent and requires well designed posological studies in epileptic patients to formulate dose adjustment guidelines for multidisciplinary therapy with the herb and Phenobarbitone, also to avoid food drug interactions.

Key words: *Nigella Sativa*; Maximal electroshock seizures; Epilepsy; Phenobarbitone; Ethnopharmacovigilance

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INTRODUCTION

Majority of epileptic patients from developing world depend on herbal medicines. Insufficient supply of drugs, unavoidable adverse effects and incomplete seizure control are major issues. Standardization of herbal medicines as per pharmacological principles and therefore analysis of drug interactions is the resolution for the scenario.

This research work is part of such an effort to validate and standardize antiepileptic application of black cumin (*Nigella sativa* seed), which has been part of agriculture since ages, both for their culinary uses and therapeutic values.¹ These herbs exhibited potent antiseizure property

in mice models which are published earlier.² In current study of drug interaction between Phenobarbitone sodium with *Nigella sativa* seed ethanolic extract in Maximal electroshock induced seizures was studied with a modified isobolographic analysis.

MATERIALS AND METHODS

Plant materials

The seeds of *Nigella sativa* (English: Small Fennel, Black Cumin) were procured locally from Mangalore. Identified and confirmed the botanical name of the plant as *Nigella sativa* by Taxonomist, Department of Applied Botany, Mangalore University, Karnataka.

Address for Correspondence:

Dr. Rejeesh Edavan Puthallath, Department of Pharmacology, Mount Zion Medical College, Adoor, Kerala, India. **Mobile:** +91-7902625570.

E-mail: rejeesh83@gmail.com

Extraction

Nigella sativa shade dried seeds were powdered and extracted with Soxhlet apparatus, in portions of 200g, with 99% ethanol at 60°C temperature for 3 days. After exhaustive extraction, the collected ethanolic extract (NsEE) was dried in a water bath at 50°C and stored under refrigeration. The final yield of the extraction was 25%.²

Experimental animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC), Yenepoya University and care of laboratory animals was taken as per CPCSEA guidelines. Animals were housed (Animal house, Yenepoya University, Reg.no 347/CPCSEA) in polypropylene cages and maintained at temperature (25 ±2°C) and light (light period, 06.00– 18.00) in a controlled room with relative humidity of 50–55%. Food and water were provided ad libitum. Experiments were carried out between 15:00 and 19:00 h.³

Experimental procedures

Screening Anticonvulsant Activity-Maximal electroshock seizure test: Maximal electroshock seizures test was performed in mice with 0.2-second series alternating current with 50Hz frequency and an intensity of 60 mA, applied through ear electrode primed with an electrolyte solution. Reduction in duration of tonic hind limb extension was taken as a measure of efficacy in this test.^{4,5}

Interaction with standard drugs

Thirty minutes after intraperitoneal administration of the drugs, animals were exposed to maximal electroshock seizure.

Inclusion criteria for animals

Male Swiss albino mice 25-30g, 3-4 months old in good health.

ROA - intraperitoneal (i.p)

Method of analysis of drug interactions^{6,7,8,9}

A. Tabulated Duration of HLTE(Hind Limb Tonic Extensor Phase of maximal electroshock seizure in mice) of various experimental groups (HLTEExp), Statistical analysis was done with ANOVA followed with Dunnett’s multiple comparison tests.

Table 1: Grouping and dose selection

Grouping (n=6)	NsEE	Phenobarbitone sodium
Group 1 – NsEE 100%	900 mg/kg	0
Group 2 – NsEE: PBT; 75:25	675mg/kg	2.5mg/kg
Group 3 – NsEE: PBT; 50:50	450mg/kg	5mg/kg
Group 4 – NsEE: PBT; 25:75 NsEE	225mg/kg	7.5mg/kg
Group 5– PBT 100%	0	10mg/kg

B. Simulated the additive HLTE (HLTEAdd) duration for 75:25, 50:50, 25:75 combinations from the HLTE values of 100% NsEE and 100 % Standard drug using the formula,

$$[(\text{mean duration of HLTE NsEE } 100\% * N\%) + (\text{mean duration of HLTE PBT } 100\%*P\%)]$$

N%= Fraction of NsEE

P%= Fraction of PBT

C. Dose response curve of the duration of the tonic hind limb extensor phase HLTEExp and HLTEAdd (Y axis) was plotted against different dose percentage of standard drug (X axis).

D. If HLTEExp fall below the HLTEAdd curve the combination is synergistic. If HLTEExp fall on HLTEAdd Curve the combination is additive. If HLTEExp fall above HLTEAdd the combination is antagonistic.

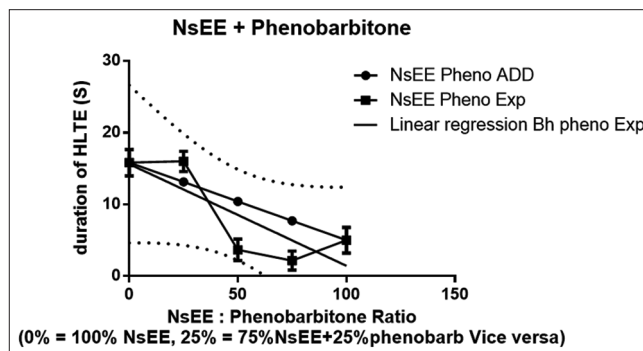


Figure 1: Dose response curve of the duration of the tonic hind limb extensor phase HLTE Exp and HLTE Add (Y axis) was plotted against different dose percentage of standard drug (X axis).

Table 2: Duration of HLTE (Hind Limb Tonic Extensor Phase of maximal electroshock seizure in mice) of various experimental groups (HLTE Exp)

Group	Mean HLTE (seconds)	SD
Group 1 – NsEE100%	15.8	1.8
Group 2 – NsEE: PBT; 75:25	16	1.4
Group 3 – NsEE: PBT; 50:50	3.7	1.5
Group 4 – NsEE: PBT; 25:75 NsEE	2.2	1.3
Group 5 – PBT 100%	5	1.8

Table 3: Simulated the additive HLTE (HLTE Add) duration for 75:25, 50:50, 25:75 combinations from the HLTE values of 100% NsEE and 100% standard drug using formula

Dose Ratio	HLTE Add (seconds)
NsEE: PBT; 75:25	13.12
NsEE: PBT; 50:50	10.41
NsEE: PBT; 25:75	7.7

RESULTS

Inference: NsEE: Phenobarbitone combination exhibited Synergism at 1:1 and 3:1 since HLTEExp fell on HLTEAdd Curve. At 3:1 dose ratio HLTEExp fell above HLTEAdd and the combination is antagonistic (Figure 1).

DISCUSSION

Herb-Drug Interaction profile of *Nigella sativa* ethanolic extract on antiseizure activity of Phenobarbitone in mice maximal electroshock model is significant and dose dependent as seen with the results and analysis using modified isobolographic method. Wide spread use of the herb for culinary and medicinal purposes increases the risk of accidental drug interactions, as in most cases the doctor are not informed about the use of the herb as both the patient and doctor may not know the potential for such herb drug or food drug interaction between black cumin(*Nigella sativa* seeds) and Phenobarbitone.

Avoiding concomitant use of the herb during treatment with phenobarbitone is advisable to avoid undesirable drug interactions; until we elucidate dose response relationship of the combination in patients and prepare a dose adjustment table for the herb and Phenobarbitone.

The interaction profiling of the combination at various dose ratios in epileptic patients is essential for the application of a multidisciplinary therapy, especially to address treatment gap due to insufficient supply and cost of the drugs.

Phenobarbitone like most of the antiepileptic agents pose high risk of drug interactions and a narrow safety margin, requiring target level (plasma therapeutic concentration) guided dosing strategy aided with therapeutic drug monitoring (TDM). Most of the parts of the world TDM facilities are not available or are not affordable leading to unnecessary incidents of therapeutic failures and toxic effects.

These findings and reports from isobolographic analysis indicate detailed, well designed clinical studies with the herb and tailor the doses of the combination. Until then the combined use of *Nigella sativa* products with Phenobarbitone sodium in seizure patients should be considered contraindicated.

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Authors Contribution:

RJ - Concept and design of the study, reviewed the literature, conducted experiment, data collection and analysis, manuscript preparation and critical revision of the manuscript; **LJ** - reviewed the literature, manuscript preparation and critical revision of the manuscript; **SNR**- Critical revision of the manuscript.

Work attributed to:

Analyze Drug-Herb Interaction analysis and also to emphasize need for mandating Ethnopharmacovigilance practices.

Orcid ID:

Dr. Rejeesh Edavan Puthallath - <https://orcid.org/0000-0001-9927-9221>
Dr. Lovelyn Joseph - <https://orcid.org/0000-0003-2148-4107>

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