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. These herbs exhibited potent antiseizure property in mice models which are published earlier. 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.
**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%.

**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.

**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.

**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**

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.

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,

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

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 HLTEExp and HLTEAdd (Y axis) was plotted against different dose percentage of standard drug (X axis).

**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 | 675 mg/kg | 2.5 mg/kg |
| Group 3 – NsEE: PBT; 50:50 | 450 mg/kg | 5 mg/kg |
| Group 4 – NsEE: PBT; 25:75 NsEE | 225 mg/kg | 7.5 mg/kg |
| Group 5 – PBT 100% | 0 | 10 mg/kg |

**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 HLTEExpr fell on HLTEAdd Curve. At 3:1 dose ratio HLTEExpr 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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