



SUPRAADDITIVE EFFECT OF HYDROETHANOLIC EXTRACT OF VALERIANA WALLICHII (INDIAN VALERIAN) ROOT AND PHENOBARBITONE AGAINST MAXIMAL ELECTROSHOCK SEIZURE IN MICE

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Abstract: There was a drastic increase in use of herbal preparations in recent years which raise concerns about their toxic and interactive propensity with concomitant diseases and other drugs consumed. Valerian is one of the most widely used herbal medicines in the world. This study analyzed the interaction of *Valeriana wallichii* (Indian valerian) and Phenobarbitone on anticonvulsant activity using maximal electroshock seizures (MES) in Swiss albino mice. *Valeriana wallichii* hydroethanolic extract reduced the duration of HLTE with a dose dependant increase in potency at 450 and 900 mg/kg given as intra-peritoneal injections. Combination analysis show synergistic interaction between VwHE extract and Phenobarbitone at 50:50, and 25:75 (Vw: PBT) dose ratios, and antagonistic interaction at 75:25 (Vw: PBT) dose ratio in anticonvulsant activity, as indicated by the reduction in duration of HLTE phase of Maximal electroshock seizure model in mice. Phytochemical profile of *Valeriana wallichii* suggests many active constituents with more than one possible mechanism of action. The interaction could be pharmacokinetic or pharmacodynamic. Large scale studies at various dose ratios and with more parameters including the adverse effect profile of the combination at preclinical and clinical level are required to explore potential therapeutic applications of the combination.

Keywords: Anticonvulsant activity, electroshock seizures, *Valeriana wallichii*, Phenobarbitone.

INTRODUCTION

With multinational companies turning into herbal business, online availability and regulatory bodies all over the world licensing them as dietary supplements without enough toxicological and interaction analysis data, there was drastic increase in use of herbal preparations which raise concern about their toxic and interactive propensity. This study analyzes the interaction of *Valeriana wallichii* (Indian valerian) and Phenobarbitone on anticonvulsant activity in maximal electroshock seizures.

Valerian is one of the most widely used herbal medicines in the world. [1-3] It was first used as a treatment for epilepsy in the late 16th century reportedly by Fabius columna, who related a personal cure, but subsequently was also reported to have relapsed. Fifty years later, additional reports of its effectiveness in three cases of epilepsy were reported by Dominicus Panarolus. [4] Numerous reports by a wide variety of writers followed, and valerian subsequently became routinely used for the treatment of various nervous disorders [4-6]. Respected American medical botanist William Woodville reported that various European authorities ascribed antispasmodic, anthelminthic, diuretic, diaphoretic, and emmenagogue actions to valerian and related its usefulness for hysteria. However, Woodville himself did not consider it to be as effectual as proclaimed by other writers, an opinion reportedly supported by the *Edinburgh Dispensary*. [7] The data related to potential

drug interactions with valerian is very limited at this time. [8] Valerian and some valerian preparations have been shown to potentiate the effects of barbiturates. [9-11] Recently a study in mice reported protective effect of *Valeriana wallichii* in maximal electroshock induced seizure. [12]

MATERIALS AND METHODS

Plant Materials:

The roots of *Valeriana wallichii* were purchased from M. Subhash Chand and Sons, Karyana Merchants, Ghuh Bazar, Paprola-Dist, Himachal Pradesh. 176115. Identified and confirmed the botanical name of the plant as *Valeriana wallichii* DC (Family: Valerianaceae) by Dr. Radhakrishna Rao, Rtd. Prof. In Botany, Alvas Ayurvedic College, Mangalore, Karnataka

Valeriana wallichii root hydro ethanolic extract (VWHE) preparation:

The VW root was shade dried and exposed to exhaustive extraction using Soxhlet apparatus using distilled water and ethanol (10:90) as solvent system. [4] Yield of the Hydroethanolic extract was 25.3%, after drying on a water bath at 50°C.

Experimental animals:

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Yenepoya University and care of laboratory animals was taken as per CPCSEA guidelines. Animals were

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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. [13]

Experimental procedures:

Screening Anticonvulsant activity-Maximal electroshock seizure test: Maximal electroshock seizures test is 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. [14] Interaction of *Valeriana wallichii* root hydroethanolic extract with Standard drugs in maximal electroshock seizure models in mice

Experimental model: 30 minutes after intraperitoneal administration of the drugs, animals were exposed to Maximal electroshock seizure.

Inclusion criteria for Animals: Male Swiss albino mice of 25-30g, 3-4 months old in good health.

Grouping:

Group 1 Vw 100% (n=6):	<i>Valeriana wallichii</i> hydroethanolic extract(VwHE) 900 mg/kg/i.p
Group 2 Vw 50% (n=6):	VwHE – 450 mg/kg/i.p
Group 3 Vw:PBT ; 75:25 (n=6):	VwHE 675mg/kg/i.p + PBT 2.5mg/kg/i.p
Group 4 Vw:PBT ; 50:50(n=6):	VwHE 450mg/kg/i.p + PBT 5mg/kg/i.p
Group 5 (n=6): Vw:PBT ; 25:75	VwHE 225mg/kg/i.p + PBT 7.5mg/kg/i.p
Group 6 PBT 100% (n=6):	Phenobarbitone 10mg/kg/i.p
Group 7 Control (n=6):	10% DMSO 0.1/10 body wt/i.p

Method of Analysis of Drug Interactions

- Tabulated Duration of HLTE of various experimental groups (HLTE_{Exp}), Statistical analysis was done with ANOVA followed with Dunnett’s multiple comparison tests.
- Simulated the additive HLTE (HLTE_{Add}) duration for 75:25, 50:50, 25:75 combinations from the HLTE values of 100% Vw and 100 % Standard drug using the formula,

$$[(\text{mean duration of HLTE Vw } 100\%/2) + (\text{mean duration of HLTE PBT } 100\%/2)]$$
- 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).
- If HLTE_{Exp} fall below the HLTE_{Add} curve the combination is synergistic. If HLTE_{Exp} fall on HLTE_{Add} Curve the combination is additive. If HLTE_{Exp} fall above HLTE_{Add} the combination is antagonistic.

RESULTS

- Valeriana wallichii* hydroethanolic extract reduced the duration of HLTE with a dose dependant increase in potency at 450 and 900mg/kg given as intraperitoneal injections.(Figure: 1)
- At 50:50, and 25:75 dose ratio (Vw: PBT) the combination showed synergistic interactions (Figure: 2).
- At 75:25 dose ratio (Vw: PBT) the combination showed antagonistic interaction (Figure: 2).

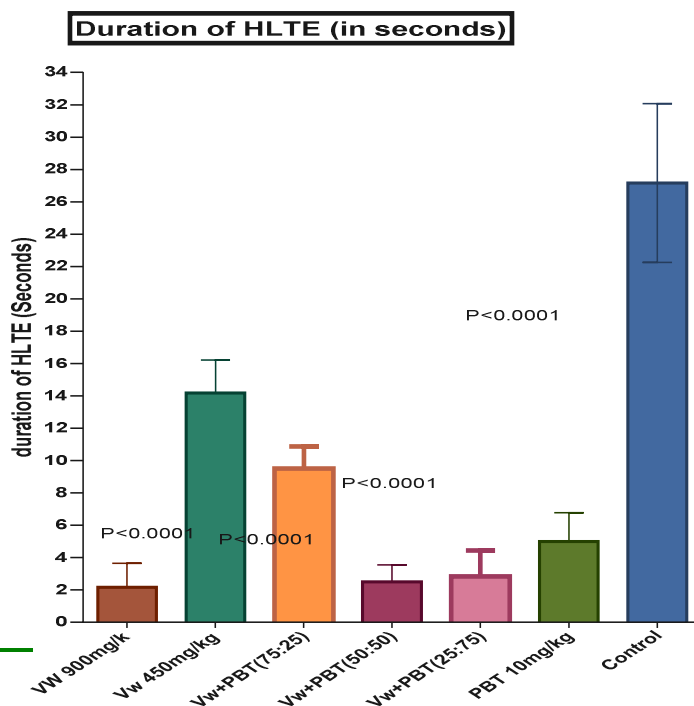


Figure.1: The bar diagram shows duration of Hind Limb Tonic Extensor Phase (Seconds) among different treatment groups. A dose dependant reduction in duration of HLTE was observed among treated groups compared to control group, ANOVA and Dunnett’s multiple comparison test were done and were found to be statistically significant (P< 0.001).

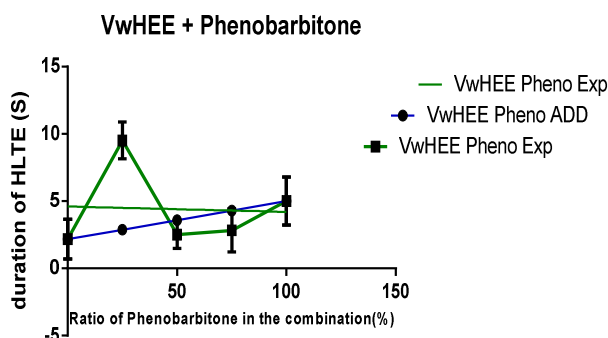


Figure.2: Analysis of interactions of *Valeriana wallichii* hydroethanolic extract (VwHE) with Phenobarbitone (PBT) with maximal electroshock seizure models in mice: At 50:50, and 25:75 dose ratio (Vw: PBT) the combination showed synergistic interaction. i.e., in combination the duration of HLTE (HLTE_{Exp}) in MES was less than the mathematical prediction (HLTE_{Add}) based

on the principles of additive interactions, whereas 75:25 dose ratio (Vw: PBT) was antagonistic.

DISCUSSION

The name valerian is said to be derived either from Valerius, who was reported to have first utilized its medicinal properties, or from the Latin term *valere*, meaning health or well-being (4, 15). It has been used medicinally for at least 2000 years. Various species of valerian continue to be included in the pharmacopoeiae of many nations such as Belgium, France, Germany, Italy, Switzerland, and the United Kingdom, and a valerian monograph has been accepted by the United States Pharmacopeial Convention for inclusion in the *National Formulary* (Pharmacopeial Forum 1998).[4] Various species of valerian are used as sedative and antispasmodic worldwide.

Valeriana wallichii (Indian valerian) possess anticonvulsant activity in maximal electroshock seizures in mice after intraperitoneal administration as indicated by reduction in duration of tonic hind limb extensor phase with a dose dependant increase in potency (450mg/kg, 900mg/kg). Maximal electroshock seizure model is one of the gatekeeper tests for screening anticonvulsant programs because of,

1. Broad spectrum: Its broad sensitivity to drugs with diverse mechanisms of action including increase in GABAergic, decrease in Na⁺/ Ca²⁺/ Glutamergic activities in brain etc.
2. Predictability : Close correlation of anticonvulsant activity in MES model and Grandmal epilepsy in human (e.g.: Phenytoin, Phenobarbitone, Sodium valproate etc)
3. Precise and relatively consistent end point – Duration of Tonic Hind Limb Extensor (HLTE) Phase

Phytochemical profile of *Valeriana wallichii* suggest many active constituents with more than one mechanism of action, Valipotriates (which decompose to form Baldrinals), Sesquiterpenes, Sesquiterpenes carboxylic acids (valerenic acid and others), GABA (gamma- amino-butyrac acid), Lignans (hydroxypinoresinol), Monoterpenes (pinenes and camphene), Flavonoids. [16, 17, 18, 19] Novel flavonoids with CNS activity, such as 6-methylapigenin and 2S (-)-hesperidin have been isolated from *Valeriana wallichii* and have been proven to possess a benzodiazepine binding site ligand. These compounds have sedative, sleep-enhancing and anxiolytic properties. [19] One of valerian's active ingredients is isovaleric acid, a substance similar to valproic acid, which could likely possess anticonvulsant properties, as isovaleramide does. For this reason, large doses of valerian could theoretically provide potentially effective quantities of the anticonvulsant compounds for the treatment of

epilepsy patients, but more controlled research is needed in human subjects in order to reach a conclusion as to its efficacy for this purpose.[20] Lovelyn et al reported protective effect for *Valeriana wallichii* hydroethanolic extract after oral administration in MES models. Considering the fact that Valipotriates (which decompose to form Baldrinals) showed a very low bioavailability after oral administration, we decided to choose intra peritoneal route of administration in this study. VwHE showed protection after intraperitoneal administration (450mg/kg, 900mg/kg) at comparable dose range to the oral administration (400mg/kg, 600mg/kg). Since the intraperitoneal administration didn't show much difference in anticonvulsant potency, Valipotriates & baldrinals may not contribute for anticonvulsant properties of the VwHE.

There are recommendations to avoid taking preparations containing valerian along with sedative drugs, as this may potentiate the effects of Central Nervous System (CNS) depressants [8,21] and not to combine valerian preparations with anti-seizure medications, unless under medical supervision [22, 23]. The combination analysis show synergistic interaction in anticonvulsant activity between VwHE and Phenobarbitone at the given dose ratio as indicated by the reduction in duration of HLTE phase.

Implications of the finding:

- a) The combination could reduce the dose of Phenobarbitone, if combination proves to have a better adverse effect profile than Phenobarbitone alone.
- b) The combination is a potential candidate for trial in treatment resistant cases of Grandmal epilepsy.
- c) Mechanism of synergistic interaction could be pharmacodynamic because GABAergic and Calcium channel blocker effects are attributed to Valeriana species by many authors and GABAergic drugs and CCBs are known to have synergy with Phenobarbitone.
- d) In addition antagonistic interaction at 75:25; Vw: PBT dose combination indicates that the Phenobarbitone is not potentiating VwHE, so the interaction may not be mutual.

Large scale studies at various dose ratios and with more parameters including the adverse effect profile of the combination with either preclinical or clinical trials are required to explore potential therapeutic applications of the combination.

CONCLUSION

Combination analysis show synergistic interaction between VwHE and Phenobarbitone at the given dose ratios (VwHE 450mg/kg/ip + PBT5mg/kg/ip, VwHE 25mg/kg/i.p + PBT7.5mg/kg/ip), and antagonistic interaction at VwHE 675mg/kg/i.p + Phenobarbitone

(PBT) 2.5mg/kg/i.p in anticonvulsant activity, as indicated by the reduction in duration of HLTE phase of Maximal electroshock seizure model in mice.

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