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Anticonvulsant and antioxidant effect of hydroalcoholic extract of *Valeriana wallichii* rhizomes in acute and chronic models of epilepsy in albino rats

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Oxidative stress plays an important role in the aetiology of seizure-induced neuronal death. The present study was aimed to evaluate the anticonvulsant and antioxidant activity of hydroalcoholic extract of *Valeriana wallichii* (HAEVW) rhizomes in albino rats. Flavonoids possess a potent anticonvulsant effect through modulation of GABA/BDZ receptor and neuroprotective effect by regulating oxidative stress in PTZ-induced convulsion. Total phenolics and flavonoids content of HAEVW rhizomes were measured and the anticonvulsant activity was evaluated using PTZ and MES induced convulsion models. The *in-vivo* antioxidant activity was evaluated using the PTZ-kindled model by estimating brain malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and nitric oxide (NO). It was observed that the flavonoids content of the extract was 80% of the total phenolics content and HAEVW (200 and 400 mg/kg) showed a significant (P <0.01) delay in onset, decrease in duration of tonic convulsion and hind limb tonic extension (HLTE) in PTZ and MES induced convulsion respectively. In the PTZ-kindling model, HAEVW rhizomes (400 mg/kg) restored the MDA and all antioxidant parameters to normal except NO. It was concluded that flavonoids of rhizomes of *V. wallichii* may be responsible for the anticonvulsant and antioxidant activity of the extract.

Keywords: Catalase, Glutathione, Malondialdehyde, Nitric oxide, Oxidative stress, Seizure, Superoxide dismutase, *Valeriana wallichii*.

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Introduction

Epilepsy is a common neurological disorder with a prevalence rate of 0.5-1.5 per cent varying between developed and developing countries[1]. An epileptic seizure is caused by abnormal, excessive, and synchronous neuronal activity in different areas of the brain and produces various signs and symptoms accordingly[2]. The excessive firing of neurons accompanied with epileptic discharges could result in a variety of changes in the cellular level, e.g., activation of glutamate receptors, alteration of γ-aminobutyric acid (GABA) receptor, expression of cytokine, increased oxidative stress, neurogenesis and stimulation of some late cell death pathways[3]. Prolonged seizure or status epilepticus (SE) leads to the production of reactive oxygen species (ROS) mainly by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through N-methyl-D-aspartate (NMDA) receptor activation[4]. The SE also causes activation of inducible nitric oxide synthase (iNOS) that produces nitric oxide (NO), which react with superoxide (O_2^-) to form the peroxynitrite ($ONOO^-$) and contribute the severity of oxidative stress[5]. Excessive production of the free radicals can damage macromolecules in the cells that causes lipid peroxidation, deoxyribonucleic acid (DNA) damage, enzyme inhibition, and mitochondrial damage which leads to neuronal death[6]. Azam *et al.*, concluded that the use of free radical scavengers in the treatment of epilepsy provides important steps for the design of novel antiepileptic drugs[7]. Although there have been new drugs developed for epilepsy, the failure rate on the neuroprotective effect of antiepileptic drugs (AEDs) in the clinical trials is high, also the oxidative stress was not influenced by the AEDs[7,8]. So the drugs having a protective effect against ROS/RNS induced neuronal death may have beneficial clinical implications.

Traditional herbs due to the presence of bioactive ingredients have a great potential to treat epilepsy. Among all, flavonoids are widely distributed plant secondary metabolites found in many fruits, vegetables and herbs containing dietary supplements. Experimental evidence demonstrated that flavonoids exert antiepileptic activity by modulating the $GABA_A$-Cl channel complex as they are structurally similar to

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benzodiazepines and also potentiate the neuroprotective effect of diazepam and gabapentin by regulating oxidative stress in PTZ-induced convulsion[9,10]. The *Valeriana wallichii* commonly known as Indian valerian is one of the important plant species belonging to the family Valerianaceae. It was well recommended by Ayurveda and other ethnomedicine streams of India as sedative-hypnotic and anticonvulsant. *V. wallichii* has shown various pharmacological activities like sedative-hypnotic, and antidepressant[11,12]. It contains two new flavonoids namely 6-methylapigenin (MA) and hesperidine (HN). MA possesses an anxiolytic property by binding to benzodiazepine binding site (BDZ-bs) and is also able to potentiate the sleep enhancing properties of HN[13]. Therefore, the present study was undertaken to evaluate the anticonvulsant and *in-vivo* antioxidant activity of *V. wallichii* extract in acute (PTZ and MES) and chronic models of epilepsy (PTZ-kindling) respectively in albino rats.

Materials and Methods

Plant material

The hydroalcoholic extract of *V. wallichii* (HAEVW) rhizomes was procured from UNICO Pharmaceuticals, Ludhiana with batch number VW/007/18.

Experimental animals

The Wistar albino rats of both sexes weighing 150-200 g body weight were procured from Saha Enterprises, Kolkota, West Bengal, after getting approval from the IAEC of Roland institute of pharmaceutical sciences, registration number 926/PO/Re/S/06/CPCSEA and IAEC approval no 93. The animals were acclimatized in the animal house of the institution for two weeks on a 12 h light and dark cycle environment and were provided with a commercial pellet diet with *ad libitum.*

Preliminary phytochemical screening

The qualitative estimation of secondary metabolites present in HAEVW rhizomes was performed as per the standard procedure[14].

Estimation of total phenols content

The total phenols content of the HAEVW rhizomes was determined by Folin-Ciocalteu method. Briefly, 1 mL of crude extract (1 mg/mL) was mixed thoroughly with 5 mL of Folin-Ciocalteu reagent (1:10), followed by 4 mL sodium carbonate (7.5%). The mixer was allowed to stand for 20 min at 25 °C in the dark, and absorbance was measured at 760 nm using a UV-visible spectrophotometer. The total phenolic content was calculated from the calibration curve of gallic acid (Fig. 1a). The total phenol content of the extract was expressed as mg gallic acid equivalents (GAE) per g of dry extract and was calculated by using the formula: T= c×v/m, where T = total phenols content (mg GAE/g dry extract), c = concentration of gallic acid obtained from calibration curve in mg/mL, v = volume of extract in mL, and m = mass of the extract in g[15].

Estimation of total flavonoids content

The total flavonoids content of HAEVW rhizomes were estimated by the aluminium chloride complex forming assay method. Briefly, 1 mL of crude extract (1 mg/mL) was mixed thoroughly with 0.2 mL of 10% aluminium chloride solution, 0.2 mL of 1 M potassium acetate solution, and 5.6 mL of distilled water. The resultant mixture was incubated at room temperature for 30 min to complete the reaction. The absorbance of the mixture was measured at 415 nm using a UV-visible spectrophotometer. The total

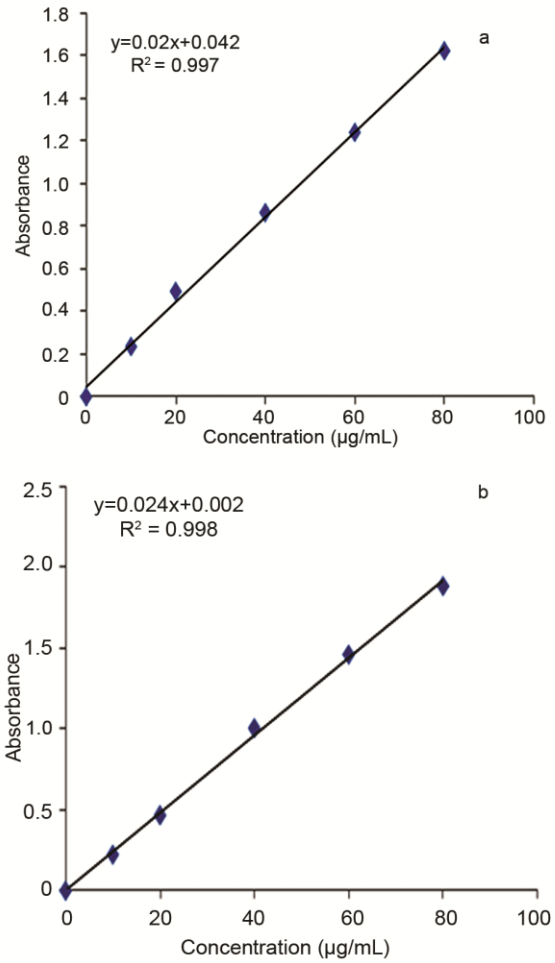

Fig. 1 — Calibration curve, a) Gallic acid and, b) Rutin.

flavonoids content of the extract was calculated from the calibration curve of the rutin (Fig. 1b). The total flavonoids content of HAEVW was expressed as mg rutin equivalents (RE) per g of dry extract and was calculated by using the formula: T= c×v/m, where T = total flavonoids content (mg RE/g dry extract), c = concentration of rutin obtain from calibration curve in mg/mL, v = volume of extract in mL, and m = mass of the extract in g[15].

Acute toxicity study

The acute toxicity study of the extract was performed as per OECD guideline 423. Healthy young adult albino nulliparous, non-pregnant female rats weighing 150-200 g were administered with 300, 1000, and 2000 mg/kg of HAEVW orally. Animals were observed individually for the first 30 min, then for the first 24 h, with special attention given in the first 4 h, and daily thereafter for a total of 14 days to observe toxicity sign like changes in skin and fur, eyes, mucous membrane, respiratory, autonomic and central nervous system and behavioural pattern[16].

Experimental design

The rats were fasted overnight for 5-6 h but had free access to water to prevent dehydration and were randomly divided into five groups of six rats each (n=6). The rats were treated as per the following i.e. group-I: distilled water (10 mL/kg, p.o.), group-II: standard (diazepam, 4 mg/kg, i.p. and phenytoin sodium, 25 mg/kg, i.p. for PTZ and MES induced convulsion respectively), group-III-V: HAEVW rhizomes (100, 200, and 400 mg/kg, p.o. respectively).

PTZ-induced convulsion

One hour after the treatment, each group received a convulsive dose of PTZ (80 mg/kg) subcutaneously. Then the animals were observed for 30 min for the onset and duration of myoclonic seizure and no of deaths after 24 h[17].

MES-induced convulsion

One hour after the treatment, each group received an electric shock of 45 mA for 0.2 s duration through an ear clip electrode by using an electro-convulsiometer (Inco, India). The onset and duration of hind limb tonic extension (HLTE) was observed for 2 min[17].

PTZ-kindling

The rats were divided into four groups of six rats each (n=6) i.e. Normal control (NC), PTZ-kindled (PTZ-k), Diazepam (DZP), and Extract (HAEVW). The treatment was given daily but PTZ (35 mg/kg, s.c.) was administered on every alternative day to all groups except NC. Rats were observed for 30 min after administration of PTZ and seizure score were recorded as follows: stage 0 (no response); 1 (myoclonic jerk); 2 (straub tail); 3 (clonic jerk without loss of righting reflex); 4 (clonic seizure with loss of righting reflex); 5 (clonic-tonic seizure). The animals were considered as kindled after attaining a seizure score of 4 on three consecutive days. The rats acquired kindling after 12 injections of sub-convulsive dose of PTZ. On the 26th day, a convulsive dose of PTZ (75 mg/kg, s.c.) was administered and observed for 30 min. Then the rats were sacrificed by cervical dislocation and the brain was quickly removed and washed two times with cold normal saline solution and stored in a deep freezer (-30 °C) for a maximum of 10 hours[18].

Biochemical estimation of oxidative stress parameters

The brains were removed, weighed, and 10% w/v tissue homogenate was prepared in 0.1 M Phosphate buffer (pH 7.4) using a Teflon tissue homogenizer (Memi, India) for 2 min after cutting the brains into small pieces with a scissor. The homogenate was centrifuged at 10,000 × g for 15 min in a cooling centrifuge (Eltek, India) and the supernatant was separated and used for estimation of lipid peroxidation and antioxidant enzymes levels. All the procedures were performed at +4º C. The lipid peroxidation marker MDA[19], SOD[20], CAT[21], GSH[22], total nitrite as an indicator of NO[23] and protein[24] were estimated according to the standard procedure.

Statistical analysis

Statistical analysis was carried out using one way ANOVA followed by Dunnett's post hoc test. Data were expressed as mean±SD. All analysis was made using graph pad prism statistical software (version 8.01) and *P* value less than 0.05 was considered statistically significant.

Results

Preliminary phytochemical tests

The preliminary phytochemical tests of the HAEVW rhizomes showed the presence of secondary metabolites like alkaloids, saponins, steroids, terpenoids, flavonoids, and tannins.

Total phenolics and flavonoids content

The total phenolics and flavonoids contents of the HAEVW rhizome were found to be 0.10 mg GAE/g of extract and 0.08 mg RE/g of extract, respectively.

The total flavonoids content of the extract was 80% of the total phenolics content.

Acute toxicity study

The results of the acute toxicity study highlight that rhizomes of HAEVW in all the tested doses showed a characteristic CNS depressant like effect in all the animals. There was no mortality observed after 24 h up to the dose of 2000 mg/kg body weight.

Effect of HAEVW rhizomes in PTZ-induced convulsion

HAEVW rhizome (100, 200, and 400 mg/kg, p.o.,) delayed the onset of PTZ-induced tonic convulsion and duration of tonic convulsion dose-dependently but 200 and 400 mg/kg, p.o. showed significant (P <0.01) delay in onset of tonic convulsion and duration of tonic convulsion as compared to distilled water administered group. Diazepam (4 mg/kg, i.p.) showed 100% protection whereas HAEVW rhizome (400 mg/kg, p.o.,) showed 83.3% protection against mortality and were statistically significant (P <0.01) compared to control (Table 1).

Effect of HAEVW rhizomes in MES-induced convulsion

HAEVW showed a dose-dependent delay in onset and decrease in duration of HLTE. The mean onset and duration of HLTE of HAEVW at doses 200 and 400 mg/kg (p.o.) showed a statistically significant (P <0.01) difference, compared to distilled water administered groups (Table 2).

Effect of HAEVW rhizomes on oxidative stress markers

The MDA was significantly (P <0.001) increased in the PTZ-k group as compared to the NC group, indicating lipid peroxidation during PTZ-kindling. In DZP treated group there was a significant (P <0.01) increase in MDA and NO and a significant (P <0.01) decrease in all the antioxidant enzymes were observed as compared to NC. The mean MDA level was decreased in HAEVW rhizomes treated group as compared to PTZ-k group (Fig. 2a), whereas SOD, CAT, and GSH were increased almost up to the level of the NC group (Fig. 2b-d). The brain NO content was decreased in HAEVW rhizomes treated group as compared to PTZ-k group but the value was still

Table 1 — Effect of HAEVW rhizomes in PTZ induced convulsion in rats

Groups	Treatment (Dose)	Onset of tonic convulsion (s) Mean±SD	Duration of tonic convulsion (min) Mean±SD	No of animals alive after 24 h	% Protection against mortality
I	Distilled water (10 mL/kg, p.o.,)	62.66±2.16	23.66±3.07	0/6	0
II	Diazepam (4 mg/kg, i.p.,)	0 ± 0**	0±0**	6/6	100**
III	HAEVW (100 mg/kg, p.o.)	96.33±31.43	28.66±2.33*	1/6	16.67
IV	HAEVW (200 mg/kg, p.o.,)	134.3±43.53**	23.16±5.07	2/6	33.3
V	HAEVW (400 mg/kg, p.o.,)	243.83±39.67**	15±2.75**	5/6	83.3**

Values are expressed as mean±SD (n=6); Comparison between Distilled water treated vs all the treatment groups; Statistical significant test for comparison was done by one way ANOVA, followed by Dunnett's post hoc test **P <0.01 compared to distilled water treated group

Table 2 — Effect of HAEVW rhizomes in MES induced convulsion in rats

Groups	Treatment (Dose)	Onset of HLTE (s)	Duration of HLTE (s)
I	Distilled water (10 mL/kg, p.o.,)	1.65±0.52	95.83±9.82
II	Phenytoin sodium (25 mg/kg, i.p.,)	120±0**	0±0**
III	HAEVW (100 mg/kg, p.o.,)	2.13±0.44	69.5±10.09
IV	HAEVW (200 mg/kg, p.o.,)	3.23±0.56**	46±5.86**
V	HAEVW (400 mg/kg, p.o.,)	5.34±0.98**	21.16±5.84**

Values are expressed as mean±SD (n=6); Comparison between distilled water treated vs all the treatment group; Statistical significant test for comparison was done by one way ANOVA, followed by Dunnett's post hoc test **p<0.01 compared to distilled water treated group.

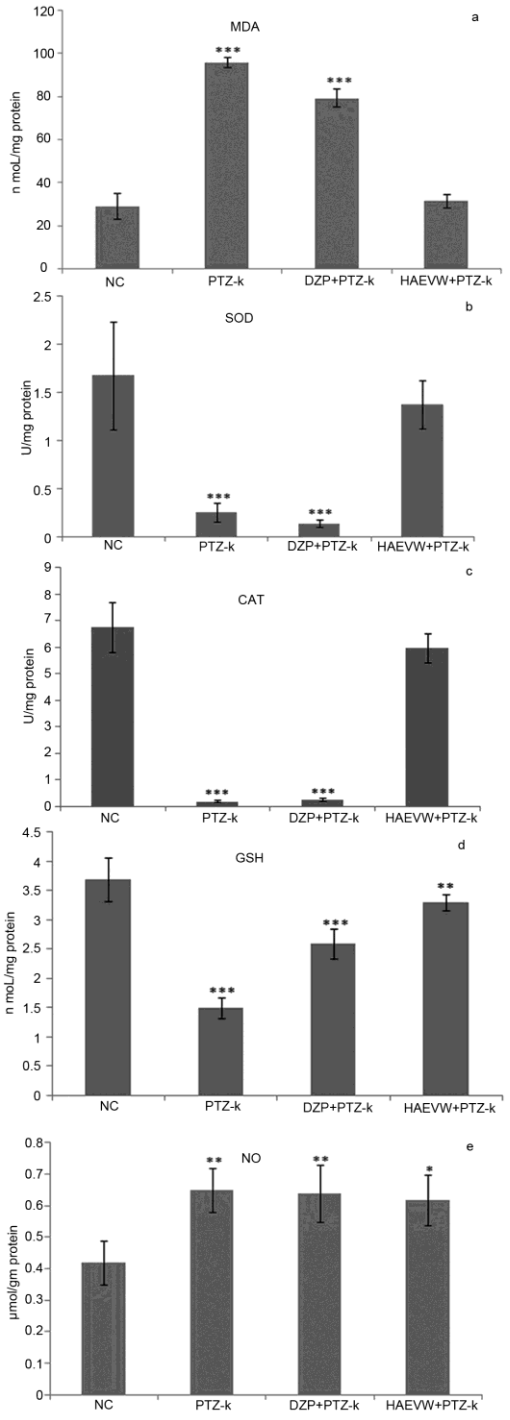

Fig. 2(a-e) — Effect of HAEVW rhizomes on brain MDA, SOD, CAT, GSH and NO level in normal control and PTZ-kindling rats. All the values were expressed as mean±SD, (n=6); Comparison between: NC vs PTZ-kindled groups;**P* <0.05, ***P* <0.01,****P* <0.001 compared to NC group. NC-Normal control; PTZ-k-Pentelentetrazole kindling; DZP-Diazepam; HAEVW-Hydroalcoholic extract of *Valeriana wallichii*; MDA-Malondialdehyde; SOD-Superoxide dismutase; CAT-Catalase; GSH-Glutathione; NO-Nitric oxide.

significantly (*P<0.05*) higher as compared to NC group (Fig. 2e).

Discussion

PTZ induced convulsion by inhibiting γ-aminobutyric acid (GABA) level in the cortex[25]. It was observed that the delayed in onset and decreased in duration of PTZ-induced convulsion by HAEVW rhizomes might be eliciting its effect on the GABAergic system. Antiepileptic drugs that block MES induced seizures are known to block voltage-gated Na^+ channels or blocks N-methyl D-aspartate (NMDA) receptors and prevent the spreading of seizure[26]. In the present study, the onset and duration of HLTE by MES induced seizure was significantly decreased by HAEVW rhizomes, indicating that the extract might produce its anticonvulsant activity by blocking the Na^+ channel or blocking the NMDA receptor, which requires further study.

Both PTZ and MES induced seizure models remain 'Gold Standards' for the evaluation of anticonvulsant activity and are used as a tool in the screening of compounds for early drug discovery[27]. It has been stated that anticonvulsant drugs that prevent tonic extension of MES act by blocking the spread of seizure whereas drug that either prevents or delays seizure of PTZ act by elevating the threshold. The present study revealed that the HAEVW rhizomes attenuated both PTZ and MES induced convulsion indicates that it has the property of elevating the seizure threshold and blocking seizure spread. M. Marder *et al.*, reported that *V. wallichii* contains two new flavonoids such as 6-methylapigenin (MA) and hesperidin (HN). MA is acting as ligand for BDZ-bs of $GABA_A$ receptor which potentiates the sedative and sleeping properties of HN[13]. The preliminary phytochemical study also revealed that the HAEVW rhizomes contain flavonoids. The estimation of total flavonoids content showed that the HAEVW rhizomes contain 0.08 mg of flavonoids per g of extract. Thus the suppression of convulsion by HAEVW rhizomes might be attributed to the flavonoid content of the extract through modulating the effect of GABA in the brain. Further study is required to evaluate the effect of MA and HN on PTZ and MES induce convulsion.

The nervous system is more susceptible to the damaging effect of oxidative stress, due to the high content of polyunsaturated fatty acids and relatively low antioxidant enzymes[28]. Oxidative stress in CNS has been precipitated in the PTZ kindled models

of epilepsy[29]. PTZ kindling results in altered glutamatergic function, increased liberation of glutamate causes an increased influx of calcium leading to excitotoxicity. Overproduction of free radicals due to excitotoxicity has a significant role in causing neuronal death in PTZ kindled animals[30]. Under normal physiological conditions, tissue injury caused by free radicals is controlled by antioxidant enzyme systems.

In the present study, it was observed that MDA and NO were increased in the brain of the PTZ-k rats as compared to NC rats. There was a significant reduction in the activity of SOD and CAT was observed in PTZ-k rats. An increase in oxidative stress and mitochondrial dysfunction plays a crucial role in the seizure induced neuronal death and seizure[31]. Administrations of HAEVW rhizomes significantly increased antioxidant enzymes in PTZ-k rats and protect the neurons from oxidative stress-induced neuronal damage. GSH is a natural antioxidant involved in the cellular detoxification of reactive oxygen species[32]. The level of GSH is reduced in a chronic model of epilepsy as well as the brains of the patient with chronic epilepsy[33]. In the present study, the level of GSH was reduced in PTZ-k rats due to the generation of free radicals but the pretreatment with HAEVW rhizomes increased the level of GSH. NO acts both as a proconvulsant or anticonvulsant, the dual role of NO depends on the epilepsy models used[34]. PTZ-kindling stimulates NMDA receptors which leads to activation of nNOS activation and mitochondrial superoxide anion production[35]. In the present study, it was observed that NO was increased by PTZ-k and administration of HAEVW decreased the NO. Phenols and polyphenolic compounds, such as flavonoids are widely found in plants have been shown to possess significant antioxidant activities[36]. The preliminary phytochemical test of HAEVW showed the presence of phenolics and flavonoids and the total flavonoids content of the extract was 80% of the total phenolic content. So the reduction in NO due to pretreatment with HAEVW in PTZ-k rats may be due to the presence of phenolics and flavonoids content, which required further study. The results of these studies are summarized in Fig. 2 (a-e).

Overall, HAEVW rhizomes ameliorate oxidative stress by decreasing lipid peroxidation, restoring GSH level, increasing SOD and CAT levels as well as decreasing NO by the virtue of its antioxidant properties. The extract also decreased the production of NO leads to decreased production of peroxynitrite. Pretreatment with HAEVW rhizomes prevents convulsion as well as free radical-induced neuronal damage due to its antioxidant properties. Zeng *et al*. reported that flavonoids extracted from licorice have a protective effect on seizure-induced neuronal cell death and cognitive impairment through their antioxidative properties[37]. The preliminary phytochemical studies also confirmed that the HAEVW rhizomes contain flavonoids. So the anticonvulsant and antioxidant effect of HAEVW rhizomes might be due to the presence of flavonoids like 6-methylapigenin and hesperidine and it also require further investigation.

Conclusion

The hydroalcoholic extract of *V. wallichii* rhizomes has both anticonvulsant and antioxidant properties in acute and chronic models of epilepsy. The anticonvulsant and antioxidant properties of the extract might be due to the presence of flavonoids. However, further study should be done to explore the anticonvulsant and antioxidant effect of flavonoids of the *V. wallichi* rhizome in the treatment of epilepsy and epilepsy-induced neuronal damage due to oxidative stress.

Conflict of interest

The authors declare that there is no conflict of interest between the authors on any financial or personal interest.

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