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PHYTOCHEMICAL SCREENING AND ANTICONVULSANT ACTIVITY OF WEDELIA CHINENSIS

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Subject index terms;

- GABA: Gama Amino Butyric Acid
- NMDA: n-methyl-D-aspartate
- PTZ: Pentylenetetrazole
- MES: Maximum electroshock
- HLTE: Hind limb tonic extension
- HLE: Hind limb extension

AEDs - Antiepileptic drugs

ABSTRACT

The aim of the present study was to carry out phytochemical screening and anticonvulsant activity of various extracts of the whole parts of Wedelia chinensis. The extracts (Petroleum ether, Ethanolic & Aqueous) were subjected to various chemical tests in order to identify the main phytoconstituents of the plant. The results of the present investigation revealed that the ethanolic extract contains glycosides and little amount of alkaloids and flavonoids, petroleum ether extract indicates the presence of steroids and aqueous extract was rich in glycosides and saponins. The anticonvulsant activity of ethanolic and aqueous extract of whole plant of Wedelia chinensis at a dose level of 250, 500, 750 mg/kg b.w, p.o. was performed in mice by using MES and PTZ methods. Phenytoin was used as a reference compound (25mg/kg b.w, i.p). Seizures were induced by delivering electroshock of 50 mA for 2 seconds by means of electro convulsiometer through a pair of ear clip electrodes whereas in PTZ model, PTZ at the dose of 80 mg/kg was injected i.p. to induce clonic-tonic convulsions in mice. It was shown that in both models, the ethanolic extract at dose level of 750 mg/kg has shown comparable activity to that of phenytoin, where as the ethanolic extract (250, 500mg/kg) and aqueous extract (250, 500, 700mg/kg) also shows potent activity but less significant than that of the standard.

INTRODUCTION: Epilepsy is one of the most common serious neurological conditions. In contemporary society, the frequency and importance of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age [1, 2]. It affects approximately 50 million people Worldwide [3]. According to several publications this can amount to 70% of the people with epilepsies, with a high prevalence of about 0.8% in children below the age of seven years. The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose-related and chronic toxicity, and teratogenic effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy [1, 4].

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles [5]. Now, various phytochemical and pharmacological studies have been carried out on these anticonvulsant plants [6]. Herbal medicines are often considered to be a gentle and safe alternative to synthetic drugs. More than half of the medically important pharmaceutical drugs are either natural products or derivatives of natural products [7, 8, 9].

Wedelia chinensis (Asteraceae), a perennial herb, is one of the most commonly occurring plants in India. It has a renowned position in Indian systems of medicine and is used in condition of inflammation, as anthelmentic and febrifuge [10, 11]. It is also used in treatment of various hepatic disorders like viral hepatitis and neuropharmacological disorders like convulsion as a traditional drug [12].

MATERIAL AND METHODS:

Plant material: The whole plant of *Wedelia chinensis* was collected from local area of New Delhi and authenticated by Dr. Anjula Pandey, National Herbarium of Cultivated Plant, National Bureau of plant genetic resource, New Delhi and the specimen voucher no. was NHCP/NBPGR/2008/5/1947.

Plant extract: The plant material was dried, powdered to moderately coarse powder and then about 200 gm materials were extracted with petroleum ether (60-80°C), ethanol (95%) and water. The extracts were dried under vacuum.

Preliminary Phytochemical Studies: The different extracts were then subjected to qualitative phytochemical screening for the identification of the phytoconstituents. Ethanolic and aqueous extracts of whole plant of *Wedelia chinensis* at a dose level of 250, 500 and 750 mg/kg body weight were used for monitoring the anti-convulsant activity.

Preparation of suspension of extracts: Dried ethanolic and aqueous extracts were suspended in a solution of normal saline (0.9% w/v) and Tween 20 (95:5) and subjected to anticonvulsant activity.

Animals: Swiss albino mice weighing 18-25 g of either sex were used for the study. The animals were procured and housed in the animal house of Teerthanker Mahaveer College of Pharmacy, Moradabad at least 2 weeks prior to the study, so that they could adapt to the new environment. Animal house was well maintained under standard hygienic conditions, at 22 ± 2° C, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water *ad libitum*. Mice were

housed in groups of 6 per cage. Cleaning and sanitation was done on alternate days. Paddy husk was provided as bedding material which was cleaned every day. The cages were maintained clean. The study was carried out as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) norms after obtaining approval from the Institutional Animal Ethical Committee of Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad.

Assessment of Anticonvulsant activity:

Maximal Electroshock seizure (MES) model: Maximal electroshock seizure model was used to evaluate the anticonvulsant activity of ethanolic extract. Seizures were induced in mice by delivering electroshock of 50 mA for 0.2 seconds by means of an electro - convulsiometer through a pair of ear clip electrodes [13]. The test animals (n=6) received 250, 500, 750 mg/kg of ethanolic and aqueous extracts orally and standard group received phenytoin (25 mg/kg) [14] injected i.p. and tested after 30 minutes for MES induced seizure response. All the experimental groups were compared with the control treated with vehicle.

PTZ-induced seizures: PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic-tonic convulsions in mice. The test animals (n=6) received 250, 500, 750 mg/kg of ethanolic and aqueous extracts orally and standard group received phenytoin (25 mg/kg) injected i.p. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected [15].

Statistical analysis: The data obtained were analyzed by student's t test using SPSS. P values less than 0.05 were considered statistically significant.

RESULTS: Details of various phytochemical constituents present or absent in different extracts of whole plant of *Wedelia chinensis* is being shown in **table 1**, in which the ethanolic extract was found to contain glycosides and alkaloids, petroleum ether extract indicates the presence of steroids and aqueous extract was found to be rich in glycosides and saponins.

TABLE 1: PHYTOCHEMICAL CONSTITUENTS PRESENT IN WEDELIA CHINENSIS

Phytoconstituents	Petroleum ether (60-80˚C)	Ethanolic extract	Aqueous extract
Alkaloids	-	+	-
Flavonoids	-	+	-
Saponins	-	-	+
Steroids	+	-	-
Glycosides	-	+	+

The anticonvulsant activity of ethanolic and aqueous extracts at various dose levels viz., 250, 500 and 750 mg/kg p. o. were studied by the maximum electroshock-induced and PTZ seizure models. The anticonvulsant activity induced by MES model of the ethanolic and aqueous extracts of *Wedelia chinesis* has been shown in **table 2**, in which the ethanolic extract at dose level of 750 mg/kg has shown comparable activity to that of Phenytoin (standard) whereas the ethanolic extract (250 and 500 mg/kg) and aqueous extract (250, 500, 750 mg/kg) has also shown potent activity but less significant than standard.

In PTZ induced seizures, the administration of *Wedelia chinensis* ethanolic and aqueous extracts at doses of 250, 500 & 750 mg/kg body weight 1 hr prior to the injection of PTZ, significantly ($p<0.05$) delayed the onset of convulsions as shown in **table 3**. Phenytoin in a dose of 25mg/kg totally abolished the episodes of convulsions.

TABLE 2: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF *WEDELIA CHINENSIS* ON HIND LIMB EXTENSION INDUCED BY MES IN MICE

Group	Dose (mg/kg)	Hind limb extension (Mean ± SE)
Control	-	11.30±1.43
Phenytoin	25	00.50±0.28*
WCEE	250	05.16±0.65*
WCEE	500	04.50±0.80*
WCEE	750	04.16±0.47*
WCAE	250	06.33±0.49*
WCAE	500	06.16±0.30*
WCAE	750	05.33±0.61*

Values are expressed as mean ± SE (n=6); * p<0.05, as compared to control

TABLE 3: EFFECT OF ETHANOLIC AND AQUEOUS EXTRACTS OF *WEDELIA CHINENSIS* ON PTZ INDUCED SEIZURES IN MICE

Group	Dose (mg/kg)	Onset Time (Sec)	Duration of HLTE (Sec)
Control	-	51.81±0.11	36.70±0.49
Phenytoin	25	0*	0*
WCEE	250	54.85±0.11*	32.33±0.89*
WCEE	500	59.40±0.13*	25.25±0.59*
WCEE	750	64.70±0.12*	20.81±0.75*
WCAE	250	53.85±0.10*	35.08±0.56*
WCAE	500	55.58±0.12*	32.96±0.60*
WCAE	750	57.85±0.16*	30.28±0.54*

Values are expressed as mean ± SE (n=6); * p<0.05, as compared to control

DISCUSSION: Pharmacological evaluation of the anticonvulsant properties of the aqueous and ethanolic extracts of *Wedelia chinensis* against PTZ induced seizure revealed that both the ethanolic and aqueous extracts exhibited statistically significant and dose dependent delay in the onset of seizure; both extracts showed significant and dose dependent reduction in the duration of HLTE. However, the ethanolic extract was more active against PTZ induced convulsion than the aqueous extract. On the other hand, both extracts showed significant and dose dependent reduction of the HLE induced by Maximum Electro Shock (MES).

Pentylenetetrazole is an antagonist of Gama Amino Butyric Acid (GABA) at $GABA_A$ receptor which has been widely implicated in epilepsy[16] furthermore; drugs which protect animals against the generalized clonic seizure induced by PTZ are effective in protection and management of petit mal epilepsy [17].

MES induced seizure can be prevented either by drugs that inhibit voltage gated sodium channel such as phenytoin or by drugs that inhibit glutaminergic excitation mediated by NMDA receptors such as felbmate [18]. In addition, drugs that are effective in protecting animals against the tonic clonic extensor spasm induced by MES are effective in the management of and/or protecting against grand mal epilepsy [17] This implies that *Wedelia chinensis* may be effective as an anticonvulsant medicinal plant and its anticonvulsant effect my involve Gabergic inhibitory and Glutaminergic excitatory mechanisms or inhibition of the voltage gated sodium channel.

Phytochemical screening of the plant showed that the plant contains alkaloids, flavonoids, sterols, glycosides and saponins, to which the anticonvulsant activity of the plant extracts may be attributed.

CONCLUSION: In conclusion, *Wedelia chinensis* extracts may have potential anticonvulsant activity which may be due to the presence of certain active phytoconstituent. The anticonvulsant activity of *Wedelia chinensis* may involve Gabergic transmission and Glutaminergic transmission or sodium channel blockage. Further studies are however needed to isolate the active principle(s) of the plant and to enlighten the mechanism underlying its anticonvulsant effect.

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