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ANTICONVULSANT ACTIVITY OF *WOODFORDIA FRUTICOSA* (L.) KURZ FLOWERS IN ANIMAL SCREENING MODELS

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Abstract
The purpose of this study was to investigate the anti-seizure activities of an ethanolic extract obtained from Woodfordia fruticosa (L.) Kurz flowers in mouse models with convulsions. The ethanolic extract of Woodfordia fruticosa (L.) Kurz flowers (EWF) was made in a continuous manner using a Soxhlet device. EWF was given orally to albino mice at doses of 100 and 200 mg/kg body weight, coupled with phenytoin. The anti-epileptic activity was evaluated using maximum electroshock (MES) and pentylenetetrazol (PTZ)-induced seizure models. In comparison to the control group, protection against convulsions was determined by eliminating the tonic hind limb extension phase and increasing seizure latency period. EWF, at doses of 100 and 200 mg/kg body weight, had considerable anti-epileptic activity in both MES and PTZ-induced seizure models. Notably, the MES model eliminated the tonic hind limb extension phase, whereas the PTZ-induced seizure model increased the seizure latency period significantly. The results indicate that the ethanolic extract obtained from Woodfordia fruticosa (L.) Kurz flowers has significant anti-epileptic effect. Further research is required to discover its active ingredients and determine its antiepileptic mode of action.

Keywords: Epilepsy, Woodfordia fruticosa (L.) Kurz flowers extract, MES, PTZ model

Introduction
Often distinguished by discrete physical movements (convulsions) and sensory or mental symptoms, epilepsy is a common neuropsychiatric condition marked by paroxysmal cerebral dysrhythmia, which results in brief episodes (seizures) of loss or disruption of consciousness [1]. There are about 50 million persons with epilepsy globally, and up to 75 percent of them live in low-income areas with limited access to treatment or medical care [2].

Brief periods of symptoms and indications brought on by abnormally high synchronised neuronal activity in the brain are known as seizures. Epilepsy can be idiopathic or caused by illness, tumour, or head injury, and it may also be genetic [3]. Despite being a major public health concern in many countries, the therapeutic approach to epilepsy is still poorly understood [4].

Current medication therapies for seizures/epilepsy focus on symptoms, while drugs aimed at preventing or curing epilepsy remain questionable. While there are over 20 approved allopathic medications and other non-pharmacological methods for epilepsy treatment, about 30% of patients are resistant to these treatments. Researchers are working to find new medications for both symptomatic and preventive treatments [5]. The intricacy and poor understanding of epilepsy make the identification and development of anti-epileptic medicines difficult [4].

A herb belonging to the Lythraceae family, Woodfordia fruticosa kurz is found in tropical and subtropical areas, including Pakistan, Malaysia, Indonesia, China, India, and Sri Lanka. Woodfordia fruticosa flowers are well-known for their potent medicinal properties and have a long history of use in medicine. The plant produces a variety of phytochemicals, including tannins, flavonoids, anthraquinone, glycosides, and polyphenols, which are extracted from both its flowers and leaves. The plant benefits from a wide range of pharmacological qualities due to these exceptional extracts, which include antibacterial, hepatoprotective, cardioprotective, antioxidant, antiulcer, immunomodulatory, antifertility, and anti-tumor actions [5].

The identified pharmacological actions of Woodfordia fruticosa are compatible with references in Ayurveda, Yunani, and other traditional medical systems. The current study seeks to explore the anticonvulsant effect of Woodfordia fruticosa (L.) Kurz flowers in animal screening models [6].

MATERIALS AND METHODS

Chemicals: Phenytoin, propylene glycol, ethanol, and pentylenetetrazole (PTZ, Sigma laboratory)

Preparation of the extract: Flowering plants and roots of Woodfordia fruticosa (L.) Kurz were obtained from the Mysore district and verified by the Pharmacognosy department of JSS Pharmacy College located in Mysore. The blossoms were separated and ground into a coarse powder after a week of shade drying. Ethylene was used as the solvent for the 12-hour Soxhlet extraction process on about 1000 grammes of this material. After that, a vacuum extractor was used to further concentrate the extract in order to remove any remaining ethanol. Following that, the experimental animals were given the concentrated ethanolic extract of Woodfordia fruticosa flowers orally via a solution of propylene glycol.

Animals

Adult Swiss albino mice weighing between 25 and 30g were procured at random from the JSS Medical College's Central Animal Facility located in Mysore. These mice were kept in regulated ambient settings at a temperature of 25±1°C. They were placed into five groups, each with six animals. Drinking water and food were freely available to the mice. The mice were given free access to food and water prior to the start of the experiment, although they did not starve for the entire night.

Grouping

Each of the five groups, which had six animals total, was randomly assigned. 0.25 millilitres of propylene glycol were given orally to the control group (Group 1). Oral administration of phenytoin (25 mg/kg for MES) and diazepam (5 mg/kg for PTZ) was given to the standard group (Group 2) members. 100 mg/kg of EWF was given orally to test group 1 (Group 3), and Extracts was given orally on the sixth day of the trial. On the fifth day of the trial, the experimental seizures were started. Before the experiment began, Institutional Animal Ethical Committee ethical approval was acquired.

Phytochemical screening
Phytochemical screening assays were performed on the recently obtained extract from Woodfordia fruticosa flowers in order to identify particular ingredients [12].

Acute toxicity study
The ethanolic extract made from Woodfordia fruticosa (L.) Kurz flowers was given to the subjects orally at increasing doses: 0.5, 1, 2, 4, and 5 g/kg. Evaluations of toxicity were carried out in accordance with OECD 423 [13]. Throughout the trial, the treated animals were continuously observed to look for any signs of abnormalities, toxicity, or mortality.

Anti-epileptic Models [14]
Maximum electroshock-induced seizures (MES) in mice: Using an electroconvulsiometer (INCO, Ambala, India) and a maximal electroshock approach, tonic-clonic convulsions were produced. Ear clip electrodes were subjected to a 50 Hz, 150 mA alternating electric current for 0.2 seconds. Mice that at initial screening showed hind leg extension upon electric shock were chosen for the investigation. Propylene glycol and all medications were given an hour before to the onset of convulsions.

The durations of stupor, clonus, and tonic hind limb flexion (THLF) and extension (THLE) were carefully documented. The mice that were given the vehicle showed the distinctive extensor tonus. In order to evaluate anticonvulsant activity, the extensor (tonic) phase has to be completely eliminated in the medication-treated groups.

Pentylenetetrazole (PTZ) Caused Seizures in Mice: Albino mice were given an intraperitoneal injection of 30 mg/kg pentylenetetrazole two weeks before the experiment. Mice that had clonic convulsions within half an hour of the initial assessment were chosen for the investigation. PTZ (70 mg/kg) was given intraperitoneally one hour after the start of medication therapy, and the animals were observed for clonic convulsion episodes. For thirty minutes, the start timing, length of clonic convulsions, and postictal depression were monitored.

Statistical Analysis:
Version 10 of GRAPH PRISM PAD was used to analyse the collected data. Using the programme, one-way ANOVA testing and Post-hoc Tukey multiple comparison tests were performed as part of the statistical study. A significance threshold of $p<0.05$ was applied, and the outcomes are shown in the table below.

RESULTS

Phytochemical screening
EWF was subjected to phytochemical screening, which revealed the presence of several elements such as proteins, tannins, alkaloids, terpenoids, flavonoids, sterols, and phenolic compounds.

Acute toxicity study
Mice treated with different doses of EWF up to 5000 mg/kg for 72 hours did not show any mortality. There was a dosage-dependent relationship between EWF's preventive effect against MES and PTZ-induced seizures. In particular, EWF provided 23% and 100% protection against seizures in the PTZ-induced seizures model when given orally at dosages of 500 mg/kg and 5000 mg/kg, respectively. The EWF dose chosen for further studies was found to be between 1000 and 5000 mg/kg based on the initial toxicity results and the logarithmic dose-response pattern.

MES-induced seizure model
Table 1 shows the percentages that indicate the inhibition of convulsions, as well as the typical durations of clonus, stupor, tonic hind limb flexion (THLF), and tonic hind limb extension (THLE).

Table 1: Effect of ethanolic extract of *Woodfordia fruticosa* (L.) Kurz flowers on MES-induced seizures in mice

Group	Treatment	Duration of THLF (sec)	Duration of THLE (sec)	Duration of clonus (sec)	Duration of stupor (sec)	% inhibition of convulsion
I	Vehicle control	6.32±0.32	7.24±0.42	21.54±0.70	341.9±2.73	--
II	Phenytoin (50 mg/kg)	2.1±0.7**	1.01±0.17***	6.41±1.31**	70.27±2.53*	69.27%
III	EWF (100 mg/kg)	5.6±0.93*	6.18±0.13*	15.04±2.05*	126.1±1.34	30.11%
III	EWF (200 mg/kg)	3.33±0.31	5.27±4.42*	13. 05±2.6*	121.01±1.96	36.35%
IV	EWF +Phenytoin (150 +25mg/kg)	2.8±0.12*	2.4±0.17*	90.14±0.21*	87.12±2.01**	48.26%

The results are reported as mean ± SEM. PID: Post-ictal depression. Comparisons were done between the control group and the other groups. Statistical analysis was performed using one-way ANOVA, followed by Post-hoc Tukey's multiple comparison tests, with significance levels shown as *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Maximal electroshock-induced seizures (MES) in mice

An electroconvulsiometer (INCO, Ambala, India) that produced an alternating electric current of 50 Hz and 150 mA through ear clip electrodes for 0.2 seconds was used to give maximal electroshock, which resulted in tonic-clonic convulsions. For the study, mice that showed hind limb extension during the initial screening process were chosen. When Woodfordia fruticosa (L.) Kurz flowers (EWF) at doses of 100 and 200 mg/kg were administered prior to the onset of the tonic hind limb flexion (THLF), tonic hind limb extension (THLE), clonus, and stupor phases, the Swiss albino mice significantly outperformed the mice in the control group. Additionally, albino mice given EWF at doses of 100 and 200 mg/kg showed a notable protective effect against convulsions brought on by electroshock in a dose-dependent manner. A significant antiepileptic effect was shown by the combined therapy of 150 mg/kg EWF with a low dose of 25 mg/kg phenytoin, which was equivalent to the conventional phenytoin-treated group (50 mg/kg).

Table 1: Effect of ethanolic extract of *Woodfordia fruticosa* (L.) Kurz flowers on PTZ-induced seizures in mice

Group- treatment	Seizure latency period (sec)	Duration of-of myoclonic jerks (sec)	Duration of clonic seizures (sec)	PID (sec)	% Protection
I-Vehicle control	298.6±34.72	5.61±0.57	12.1±0.55	312.5±8.91	--
II-Diazepam (5 mg/kg, I.P)	429±11.64**	1.23±0.11**	7.21± 0.66**	218.46±09.21***	49.44%
III-EWF (100 mg/kg, p. o)	404.5± 17.49*	3.51±0.26	10.98± 0.32*	291.33± 9.34	31.01%
IV-EWF (200 mg/kg, p. o)	500.6± 11.43**	2.9.00±0.24*	9.65± 0.2*	243.3± 41*	48.24%
V-EWF+ Diazepam (150mg/kg+ 2 mg/kg, p. o)	521.3± 1.88**	1.02±0.21 **	4.048±0.66**	171.4± 4.1****	67.37 %

The results are reported as mean ± SEM. PID: Post-ictal depression. Comparisons were done between the control group and the other groups. Statistical analysis included one-way ANOVA and Post-hoc Tukey's multiple comparison tests, with significance levels of *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Pentylenetetrazole (PTZ) induced seizure model

Table 2 shows the percentages of protection against convulsions as well as the average seizure latency time, myoclonic jerk duration, generalised clonic seizures, and post-ictal depression. When compared to the control group of mice, albino mice that were pre-treated with Woodfordia fruticosa (L.) Kurz flowers (EWF) at doses of 100 and 200 mg/kg, as well as the combination group, showed a notable delay in the start of clonic seizures. Compared to the control group, mice given EWF at doses of 100 and 200 mg/kg showed a significant decrease in the quantity and length of myoclonic jerks, clonic

seizures, and postictal depression. In a dose-dependent manner, EWF offered significant protection against convulsions caused by pentylenetetrazole (PTZ). The animals treated with a combination of 25 mg/kg of valproate and 150 mg/kg EWF demonstrated exceptional anti-epileptic efficacy, outperforming even the group receiving 50 mg/kg of normal diazepam treatment.

Discussion

As a result of brain malfunction or aberrant firing of cerebral neurons, epilepsy is a collection of chronic neurological diseases characterised by convulsive seizures, altered consciousness, sensory abnormalities, or a combination of these symptoms [15]. The antiepileptic medications (AEDs) used to treat epilepsy now have a variety of adverse effects, such as teratogenicity and chronic toxicities that affect different organs. As a result, there is an increasing need for safer indigenous alternatives when treating epilepsy.

Plant cures are frequently used in folklore and tribal traditions to treat a variety of common illnesses, including epilepsy. A well-known plant with analgesic, anti-anxiety, depressive, anti-asthmatic, and aphrodisiac qualities is Woodfordia fruticosa (L.) Kurz blossoms (EWF) [8]. Woodfordia fruticosa (L.) Kurz flowers (EWF) ethanolic extract was screened for proteins, phenolic compounds, flavonoids, terpenoids, tannins, and sterols.

The results of this study showed that the ethanolic extract of Woodfordia fruticosa (L.) Kurz flowers considerably lessened convulsions brought on by pentylenetetrazole (PTZ) and maximum electroshock (MES). GABA is the main inhibitory neurotransmitter in the brain, while glutamate is the main excitatory neurotransmitter. While glutamate interacts with both NMDA and non-NMDA receptors to modulate ion channels like Na+, K+, Ca++, or Cl-, which in turn affects neuronal activity, GABA primarily works on GABA receptors [16]. Many neurological and psychological conditions, such as anxiety, panic attacks, schizophrenia, Huntington's chorea, and epilepsy, have been related to abnormalities in the GABAergic system. One important component of the epileptogenesis process is known to be reduced GABAA synapses [14].

The main purpose of the MES test in mice is to find medications that work well for treating grand mal epilepsy. Grand mal seizures are known to be suppressed by anti-epileptic medications that stop the tonic extension of the hind limb caused by electrical shocks in this test [17]. However, medications that prevent convulsions brought on by PTZ are useful in the treatment of petit mal epilepsy. PTZ is known to have GABA-antagonistic characteristics [18]. These medications work by strengthening GABA-mediated inhibition in the brain to produce antiepileptic effects. Diazepam, for example, functions by prolonging the inactivation of the Na+ channel, as does phenytoin, attenuating the T-type Ca++ currents, as does ethosuximide, and increasing GABA transmission. It is noteworthy that it prevents convulsions caused by MES and PTZ [19].

According to this study, the ethanolic extract of Woodfordia fruticosa (L.) Kurz flowers protects against both the seizure latency in the pentylenetetrazole (PTZ)-induced seizure model and the tonic extensor phase in the maximum electroshock (MES)-induced seizure model in a dose-dependent manner. Specifically, in the PTZ model, EWF exhibits better anticonvulsant effect than in the MES anti-epileptic paradigm. EWF demonstrates anticonvulsant activity comparable to the normal dose of valproate (200 mg/kg) when paired with a modest dose of phenytoin (25 mg/kg). The incidence and severity of unwarranted side effects linked to the medications are reduced by this combination of low dosages of valproate and EWF.

Proteins, phenolic chemicals, tannins, alkaloids, triterpenoids, flavonoids, sterols, and triterpenoids are all present in the extract according to phytochemical screening. Based on the known chemical components, it is now difficult to definitively link the identified active principle or principles to its anticonvulsant action. However, in animal models of anxiety, sedation, and convulsion, a number of

flavonoids may act as benzodiazepine-like compounds in the central nervous system by modifying GABA-mediated chloride channels. In MES and PTZ experimental seizure models, certain triterpenic steroids have also been reported to exhibit anticonvulsant properties [21]. To isolate the bioactive components causing these behaviours, more research is necessary. These results lend credence to the plant's historic use in managing seizures and treating epilepsy.

Conclusion

The study found that the ethanolic extract from Woodfordia fruticosa (L.) Kurz flowers significantly reduced the incidence of epilepsy in models of maximum electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures. To fully understand the underlying mechanisms, pinpoint the bioactive ingredients, and determine the plant's safety profile for possible therapeutic uses in convulsive diseases, more research is necessary.

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