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EVALUATION OF ANTIEPILEPTIC ACTIVITY OF ETHYL ACETATE EXTRACT OF URTICA DIOICA LEAVE IN MICE

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Abstract

The present study was aimed at evaluating the antiepileptic activity of the ethyl acetate extract of Urtica dioica leaves using experimental models in mice. Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and current antiepileptic drugs often have limitations such as side effects and drug resistance. Therefore, there is a growing interest in exploring herbal alternatives. In this study, the ethyl acetate extract of Urtica dioica leaves was prepared and subjected to phytochemical screening, which revealed the presence of flavonoids, phenolics, alkaloids, and tannins—compounds known for their neuroprotective and antioxidant properties. The Maximal Electroshock Seizure (MES) and Pentylenetetrazole (PTZ)-induced seizure models were used to assess the anticonvulsant potential. The extract significantly reduced the duration of hind limb tonic extension in the MES model and delayed the onset of seizures in the PTZ model, indicating both generalized tonic-clonic and absence seizure protection. The results suggest that the antiepileptic activity of the extract may be attributed to its antioxidant properties and its ability to modulate GABAergic and glutamatergic neurotransmission. The study supports the traditional use of Urtica dioica in epilepsy and highlights its potential as a natural antiepileptic agent.

Keywords: Urtica dioica, Antiepileptic activity, Maximal electroshock seizure (MES), Pentylenetetrazole (PTZ), Flavonoids, Swiss albino mice

Introduction

The central nervous system is one of the two principal divisions of the body's nervous system. The nervous system is the body's communication network and control center. The central nervous system (CNS), consisting of the brain and the spinal cord, is the control center for the entire nervous system. All the sensations of the body are relayed to the central nervous system. All nerve impulses that cause muscles to contract and glands to secrete come from the central nervous system. [1]

Epilepsy

Epilepsy is the condition of recurrent spontaneous seizures arising from abnormal electrical activity within the brain. While anyone can experience a seizure under the appropriate patho-physiological conditions, epilepsy suggests an enduring alteration of brain function that facilitates abnormal neuronal firing. The abnormal electrical activity that underlies epilepsy is the result of biochemical processes at the cellular level promoting neuronal hyper-excitability and neuronal hypersynchrony. A single neuron which discharging abnormally is insufficient to produce a clinical seizure but it occurs only in the context of large neuronal networks. Cortical and several key subcortical structures are involved in generating a seizure.

Seizure

A transient dysfunction of brain due to an abnormal firing of cerebral neurons which may or may not have a clinical manifestation. Seizures happen when brain cells, which communicate through electrical signals, send out abnormal signals. Having several seizures (recurrent seizures) is

considered epilepsy. Seizures are not considered to be epilepsy if they occur only once or correctable. Epilepsy can happen at any age, but it is most common in the elderly. However, even mild seizures that happen more than once should be treated, because they could cause harm if they happen while you are driving, walking, swimming [3].

Pathophysiology of epilepsy

The brain is made up of three main structures cerebrum, the brain stem and the cerebellum. The cerebrum is the largest and most recognizable of these three structures and is the one most often involved in epilepsy.

Need of the St udy

Epilepsy is a chronic neurological disorder that affects millions of people worldwide, characterized by recurrent seizures due to abnormal electrical activity in the brain. Despite the availability of numerous antiepileptic drugs (AEDs), a significant proportion of patients—approximately 30%—remain resistant to current therapies. Moreover, many AEDs are associated with adverse effects such as cognitive impairment, sedation, and hepatotoxicity, which limit their long-term use. These challenges highlight the urgent need for safer, more effective, and affordable alternatives. In this context, medicinal plants offer a promising avenue for drug discovery due to their rich phytochemical profiles and traditional use in treating neurological disorders. *Urtica dioica*, commonly known as stinging nettle, has been traditionally used for various ailments, including epilepsy, and is known to contain bioactive compounds such as flavonoids, phenolics, and coumarins that possess antioxidant and neuroprotective properties. However, the antiepileptic potential of its ethyl acetate extract remains underexplored. This study aims to scientifically evaluate the antiepileptic activity of the ethyl acetate extract of *Urtica dioica* leaves using validated animal models, thereby bridging the gap between traditional knowledge and modern pharmacological research.

The findings could contribute to the development of a plant-based antiepileptic agent with fewer side effects and better patient compliance. The primary aim of this study is to scientifically evaluate the antiepileptic activity of the ethyl acetate extract of Urtica dioica leaves using established experimental seizure models in mice. The study is designed to explore the potential of this plant extract as a natural alternative to conventional antiepileptic drugs, which are often associated with adverse effects and limited efficacy in drug-resistant epilepsy. To perform phytochemical screening of the ethyl acetate extract of Urtica dioica leaves to identify the presence of bioactive constituents such as flavonoids, phenolics, alkaloids, and tannins. To evaluate the antiepileptic activity of the extract using two standard animal models: Maximal Electroshock Seizure (MES) model to assess protection against generalized tonic-clonic seizures. Pentylenetetrazole (PTZ)-induced seizure model to evaluate efficacy against absence and myoclonic seizures. To investigate the possible mechanisms of action, particularly focusing on modulation of GABAergic and glutamatergic neurotransmission and oxidative stress pathways.

MATERIAL AND METHODS

Preparation of plant extract [48]

Cold maceration technique was used for the extraction of plant material and a total of 200g of Urticadioica leaves the coarse powder was used. During the process, 100g of the coarse powder was soaked in an Erlenmeyer flask with 1L of 50% of Ethyl Acetate and then placed on a shaker tuned to 120 rpm with occasional shaking for 72h at room temperature. The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding another fresh solvent. The filtrates were left overnight in a deep freezer and then lyophilized using freeze dryer. The dried plant extract was reconstituted with distilled water for oral administration.

Phytochemical Test

Preliminary phytochemical tests were done by the methods described by usual procedures mentioned in Trease and Evans (1958) and also as specified in the book of Practical Pharmacognosy (Kokate, 2000). The details of the same are provided below. Ethyl acetate extract of leaves of

urticadioica (EAUDE) was subjected to qualitative tests for the identification of various active constituents.

Maeyer's reagent
0.355g of mercuric chloride was dissolved in 60ml of distilled water. 5.0g of potassium iodide was dissolved in 20ml of distilled water. Both solutions were mixed and volume was raised to 100ml with distilled water.

Dragendorff's reagent
Solution A: 1.7g of basic bismuth nitrate and 20g of tartaric acid were dissolved in 80ml of distilled water. Solution B: 16g of potassium iodide was dissolved in 40ml of distilled water. Both solutions (A and B) were mixed in 1:1 ratio.

Test for alkaloids
About 0.5 to 0.6g of the methanolic plant extract was mixed in 8ml of 1% HCl, warmed and filtered. 2ml fo the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's).

Test for steroids
About 0.5g of the metanolic extract fraction of each plant was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid.

Test for terpenoids
An aliquot 0.5ml of methanolic extract was mixed with 2ml of $CHCl_3$ in a test tube. 3ml of concentrated $H2SO4$ was carefully added to the mixture to form a layer.

Test for flavonoids
To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

Test for tannins
The 0.5g of powdered sample of each medicinal plant leaves was boiled in 20ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Test for Phytosterol
The extract (2mg) was dissolved in 2ml of acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulfuric acid was added along the side of the test tube.

Test for Phytosterol
1. Foam Test: 5ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well.

Test for glycosides
1. Keller – Killiani test: Added 0.4ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5ml of concentrated sulfuric acid was added along the side of the test tube carefully.
2. Hydroxyanthraquinone Test: To 1ml of the extract, add a few drops of 10% potassium hydroxide solution.

Experimental methods:
Anti-epileptic activity in mice using Maximum electroshock induced seizure method.
Experimental design:
Animal divided in to 3 groups each group carries 6 animals
Group I will receive the standard – Phenytoin (25 mg/kg) i.p.
Group II will receive leaves extract of urticadioica (EAUDE) (200 mg/kg)
Group III will receive leaves extract of urticadioica (400 mg/kg)

Experimental procedure

Maximum electroshock induced seizure (MES)

For inducing convulsion by electro shock, rectangular pulse current of high voltage (150 mA; 60 Hz) is employed. The electro shock was given to each rat for 0.2 seconds with the help of convulsion meter through pinna electrodes. Drugs likely to be effective in Grandmal epilepsy usually confer protection against electrically induced convulsion in animals. The MES convulsions are divided into five phases: a. **Tonic flexion b. Tonic extension**

Clonic convulsion: stupor and recovery or death

A substance is known to possess anticonvulsant property, if it reduces or abolishes the extensor or recovery phase of MES convulsion.

Standard drug – Phenytoin 0.5% CMC solution in a dose of 25mg/kg body weight i.p.

Test drug – 50% ethyl acetate leaves extract of urticadioica EAUDE in 0.5% CMC solution in a dose of 200 mg/kg body weight and 400 mg/kg body weight p.o.

Swiss albino mice of either sex weighing between 20-25 gm were weighed, marked and divided into 3 groups containing 6 mice each. The animals were fasted for 18 hours prior to the experiment with water ad libitum.

Group I (Control) 0.2ml of 0.5% CMC solution p.o

Group II (Standard) Phenytoin sodium 0.5% CMC solution at a dose of 25 mg/kg body weighti.p.

Group III (Test 1) 50% ethyl acetate extract of urticadioica leaves (EAUDE) in 0.5% CMC solution at a dose of 200 mg/kg body weight p.o.

Group IV (Test 2) 50% ethyl acetate extract of urticadioica leaves (EAUDE) in 0.5% CMC solution at a dose of 400 mg/kg body weight p.o.

Thirty min after i.p. administration of the standard drug and sixty min after p.o. administration of the test drug, the electro shock was given to each mice for 0.2seconds (150 mA; 60 Hz) with the help of Electro convulsiometer through pinna electrode and the effects were observed.

Evaluation

The parameters such as duration of hind limb flexing, hind limb extensor, stupor, and death/survival were recorded during the observation.

Rotarod test

Mice were preselected based on their ability to withstand a horizontal bar (2.5 cm diameter) revolving at a speed of 15 rpm for 120 s. After drug treatment, each animal was evaluated for time permanence on the rotating bar. Motor toxicity drugs generally reduce the time permanence on the rotarod.

Phenobarbitone sodium-induced sleeping time

Different groups of mice received phenobarbitone sodium (45 mg/kg i.p) thirty min prior to the administration of extract fractions, vehicle (control), or diazepam (1 mg/kg i.p). The animals were observed, and the latent period (time between the phenobarbitone administration and the onset of sleep) and the duration of sleep (time between the loss and recovery of the righting reflex) were recorded

RESULT AND DISCUSSION

Appearance and percentage yield of extract

Urticadioica were a light semisolid brownish color extract and the percentage yield was found to be 16.35%

Phytochemical Analysis

The phytochemical screening results revealed that the alkaloids were present by the indication of turbidity and/or precipitate formation. The color changed from violet to blue or green in some samples indicated the presence of steroids. An interface with a reddish-brown coloration was not formed in the absence of terpenoids, as positive result. Red coloration identifies the flavonoids

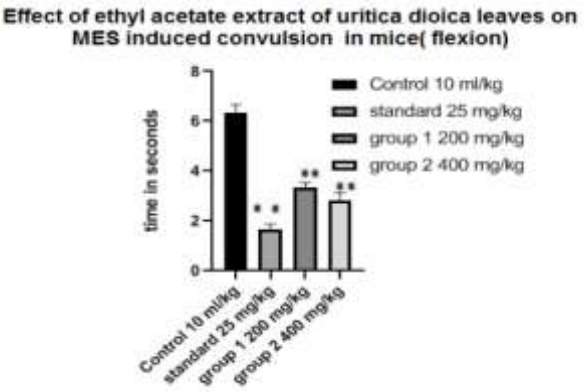
(Shinado's test). A color change was observed in the test tube, which indicated in the presence of tannins. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols. Below two observation indicated presence of saponins formation stable foam confirmed the test. The formation of blue color in acetic acid layer confirmed the test. The formation of red color confirmed the test. Above two observation indicated presence of glycosides

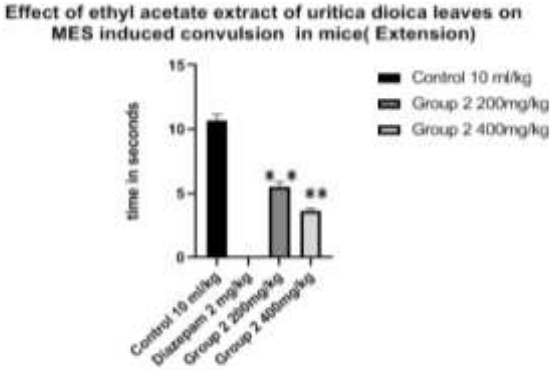
Table No 1: Effect of ethyl acetate extract of *Uriticadioica* leaves (EAUDE) on Maximal electroshock induced convulstion in mice.

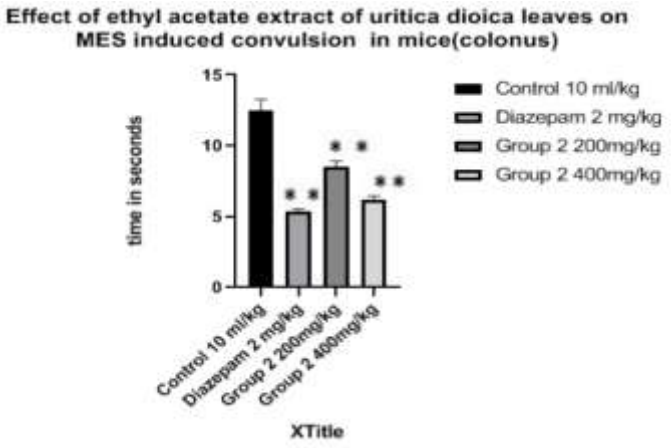
Group	Treatment	Flexion	Extension	Colonus	Stuper	% recovery
Group 1	Control (0.5%CMC)	6.33±0.33	10.67±0.49	12.5±0.76	102±1.00	33.33
Group 2	Phenytoin (25mg/kg)	1.67±0.21**	_	5.33±0.21**	70.33±2.53**	100
Group 3	EAUDE (200mg/kg)	3.33±0.21**	5.5±0.42**	8.5±0.42**	92.5±1.43	50
Group 4	EAUDE (200mg/kg)	2.83±0.30**	3.67±0.21**	6.16±0.30**	81.17±1.57**	83.33

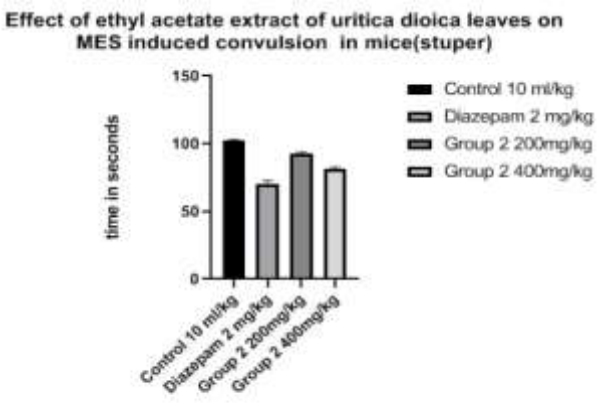
Values expressed as means ± SEM. n=6, ns= not significant, **p<0.001 when treated groups compared with control group analyzed by one-way ANOVA followed by Dunnett's test

Evaluation was made by electroshock with the help of Electroconvulsio meter through pinna electrode after 1 hr of administration of vehicle, standard and test drug to the respective group of animals. The ethyl acetate extract of *Urticadioica* leaves exhibited a dose dependent significant **(p<0.001) reduction in various phases of epileptic seizure on comparison with the control group. It was observed that the EAUDE 200mg/kg and 400 mg/kg were showed 50% **(p<0.001) and 83.33% **(p<0.001) inhibition of convulsion produced by MES, respectively. The phenytoin used as a standard drug inhibited 100% of convulsion. Extract at both the doses significantly prolonged the onset of convulsions in the extract treated group compared to vehicle treated control group.







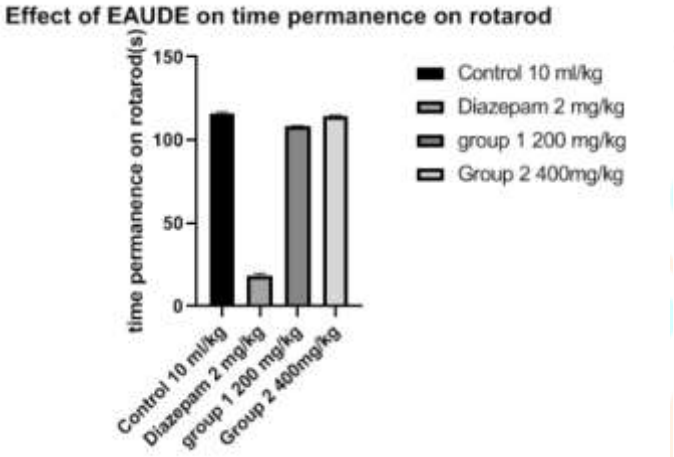


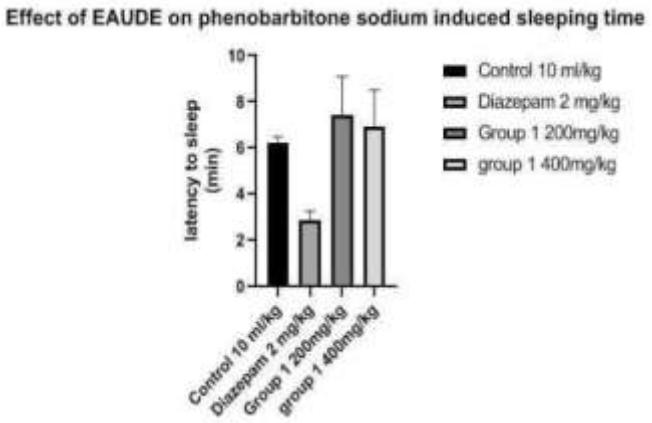
Effect of EAUDE on rotarod behavior in mice:
Treatment of EAUDE showed no significant ($p>0.05$) change in time permanence on the rotating bar when compared to control group.

Table 2: Effect of EAUDE on time permanence on rota-rod

Group	Treatment	Time permanence (s)
Control	10ml/kg p.o	116.21±0.58
Diazepam	2mg/kg i.p	18.55± 1.21
Group I	200mg/kg p.o	108.16±0.47
Group II	400mg/kg p.o	114.16±0.74

Each value represent mean± SEM (n=6) * $p<0.01$ compared to control. One way ANOVA and Dunnett's test as post hoc test were performed.





Effect of EAUDE on phenobarbitone sodium induced sleeping time:
EAUDE did not exhibit sedative properties at the selected doses examined in this experiment as there was no significant ($p>0.05$) reduction in the latency to loss ofrighting and the duration of sleep caused by phenobarbitone

Table 3:.Effect of EAUDE on phenobarbitone sodium induced sleeping time:

Group	Treatment	Latency to sleep (min)	Duration of sleep (min)
Control	10ml/kg,po	6.21±0.28	82.78±1.49
Diazepam	2mg/kg,ip	2.86±0.41	189.51±2.66
Group I	200mg/kg,po	7.44±1.66	85.65±3.38
Group II	400mg/kg,po	6.92±1.59	83.10±1.96

Each value represent mean± SEM (n=6) * $p<0.01$ compared to control. Oneway ANOVA and Dunnett's test as post hoc test were performed

DISCUSSION
Epilepsy is one of the most common serious disorders of the brain, affecting at least 50 million people worldwide. It knows no geographical, racial or social boundaries. Epilepsy accounts for 1% of the global burden of disease, determined by the number of productive life years lost as a result of disability or premature death. Among primary disorders of the brain, this burden ranks with depression and other affective disorder. Eighty per cent of the burden of epilepsy is in the developing world, where 80–90% of people with epilepsy receive no treatment at all. It is also necessary to recognize that epilepsy consists of more than seizures for the affected individual and effects on his or her family. Epilepsy leads to multiple interacting medical, psychological, economic and social repercussions, all of which need to be considered. For measuring the anticonvulsant activity in mice has been mostly undertaken using a few classical animal models such as the MES induced convulsions. The ethyl acetate extract of *urticadioica* which have been not studied so far for its antiepileptic activity. Anticonvulsant activity was studied by using MES induced convulsions and pretreatment with ethyl acetate extract of *urticadioica*. In MES induced convulsions model ethyl

acetate extract of *urticadioica*(200 and 400 mg/kg) has significantly **($p<0.001$) decreased the duration in various phases of epileptic seizure and increased the percentage protection when compare to the control group. Previous research reports have stated that the drugs having anxiolytic, anti convulsant at low doses such as benzodiazepines produce sedative or myorelaxant activity at high doses. considering this, the present study also evaluated the effect of EAUDE on phenobarbitone induced sleeping time and time permanence on rota rod and the results showed that, the EAUDE at the doses used in this study didn't significantly ($p>0.05$) alter motor coordination and also it didn't potentiate the phenobarbitone induced sleeping time ($p>0.05$). This indicates that action produced by EAUDE in this study is neither through peripheral neuromuscular blockade and nor by altering locomotor activity.

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