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Antiepileptic activities of ethanolic Extract of Leaves of *Chromolaenaodorata*

[1]Akash Kumar Shetty, [1] Vinutha N, [1] Kalleshappa C.M, [2] Akshara, [2*] Krupanidhi A.M

[1]*Bapuji Institute of Engineering and Technology-Davangere-577004, India*
[2] *Bapuji Pharmacy College, Davangere-577004, India*

Abstract: Seizure is a characteristic feature in epilepsy and it is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. Epilepsy is a neurological disorder characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction. The ethanol extract of the leaves of plant *Chromolaena odorata* (C.OD) was evaluated for its antiepileptic activity in Swiss Albino Mice at the dose of 50mg/kg.Antiepileptic activity was assessed by using Maximal Electroshock induced Seizures [MES] method. Extract showed reduction in the duration of all the phases of epilepsy such as Flexion, extensor, convulsion, stupor phases. The results are promising for further investigation for efficient anticonvulsant activity.

Keywords:*Chromolaena odorata*,Maximal Electric Shock, epilepsy,Seizure

I. INTRODUCTION

India is Emporium of Medicinal Plants. The 17% of Indian population is dependent on herbal remedies. Indigenous plants widely used for folk medicinal purposes are numerous and diverse. A medicinal use of plant is the oldest healthcare known to mankind. For a long period of time plants has been a clinical valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies.

Chromolaena odorata (C.OD) is a species of flowering shrub in the sunflower family Asteraceae. It is native of North America, from Florida and Texasto Mexico and the Caribbean [1], and has been introduced to tropical Asia, West Africa, and parts of Australia. The young leaves are crushed, and the resulting liquid can be used treat skin wounds [2,3,4]. The C.OD contains carcinogenic pyrolizidine alkoloids[5,6,7]. The therapeutic uses of C.OD includes antispasmodic, antiprotozoal, antitrypanosomal, antibacterial, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic agent cardiotonic[8,9,10]. The ayurvedic and Hakkims pandits were using this plant for various psychological disorders.

Around 0.5-1% of the world's population is affected with epilepsy and 30,000 people develop epilepsy every year [11, 12, 13]. According to National Institute of Neurological Disorders and Stroke (NINDS) about half of all the seizures have no known cause but may result from either brain damage or diseases. As per many researches, the cell membrane surrounding the neuron, which is crucial in generating electrical or nerve impulses, plays an important role in epilepsy [12]. As present several antiepileptic drugs (AEDs) are available to treat epilepsy. By using these antiepileptic drugs, it may leads to many side effects like chronic toxicity, teratogenic effects [13]. Plants may serve as the alternative sources for the development of new anti-convulsing agents due to their biological activities. Several plants used for the treatment of epilepsy in different system of traditional medicine have shown anti-epileptic activity when tested on animal models with fewer side effects [14]. Based on ethno-pharmacological information of the plant *Chromolaena odorata* (C.OD) was selected for investigation in the present study. The study may help in the development of cheap, effective and safe antiepileptic drugs.

II. MATERIALS AND METHODS

1.1 Plant material:

Chromolaena odorata (C.OD) of fresh dried leaves were procured from young matured plants in local areas of Davangere district, Karnataka, India.The plant was authenticated (Voucher specimen: 108/C.OD)by taxonomist Dr P.M Shivkumar, department of botany DRM Science college, KuvempuUniversity,Davanagere,India,Dried leaves were powdered to get a coarse powder.

1.2 Preparation of extract:

The Coarse powdered material was subjected to Soxhlet extraction with various solvents like petroleum ether (60-80^{0}C), chloroform, ethanol (95%) and distilled water[8,9].The ethanolic extract were dried and preserved in desiccators for further screening. Further crude ethanolic extract was subjected for animal testing.

2.3 Phytochemical screening

Phytochemical investigation on leaf extracts of *Chromolaena odorata*was carried out For the presence of alkaloids, carbohydrates, glycosides, steroids, flavonoids (Quercetin, quercetol, catechol, kaempferoletc)coumarins, saponins, fatty acids, tannins, protein, amino acids, gum, mucilage, terpenoids,fixed oil, anthroquinones and phenols were estimated[20-32]. The results are indicated in Table 1.

Isolation

Isolation of pure components involved the following steps:Chromatographic separation using silica gel (100 – 200 mesh).The extract (10 g) was chromatographed over silica gel (100 – 200) mesh on column 55 cm length and 6 cm diameter. Elution was carried out with solvent mixture of increasing polaraties. Fractions were collected in 100 ml portions and monitored by TLC (silica gel 'G' as adsorbent) and the fraction showing similar spots are pooled together. Elution with ethyl acetate: ethanol (EA:ET-OH :(40:60) gave brown crystalline solid (150 mg) and named as C.OD1. Similarly, elution with EA:ET-OH (50:50) yielded shiny brown coloured gel (90 mg) and was designed as C.OD2.

2.4 Experimental Animals

Swiss Albino Mice of either sex were used for the study. The animals were kept at 27º±2ºC, Relative humidity 44-56% and light and dark cycles of 10 and 14 hr, respectively, for 1 week before and during the experiments. Animals were provided with water ad libitum and standard diet and the food was withdrawn 18-24 hr before the start of the experiment.

2.5 Acute Toxicity Study

Acute toxicity study was performed on Swiss Albino Mice and the animal were kept fasting for overnight providing water and libitum, after which the extracts were administered orally and observed the mortality of animals.

2.6 **Statistical Significance**

The results of the study were expressed as mean ± SEM, n = 6.Statistical analysis was done by using one way analysis variance (ANOVA) followed byTukey-Kramer Multiple Comparisons.

Antiepileptic Activity

Maximal Electroshock induced Seizures method [Table-2]

The seizure was induced by maximal electroshock in Swiss Albino Mice with the help of electro-convulsometer by passing current of 45 mA for 0.2 second using ear clip electrodes. The animals were divided into six groups each containing 6 animals (n = 6). The test samples were given 1 hr prior to induction of convulsions.

Group I (Control): Received normal saline (1 ml/kg body weight).

Group II (Standard): Received diazepam (4mg/kg body weight).

Group III Received Ethanol extract of *Chromolaena odorata*(EEC.OD) (50 mg/kg body weight).

III. RESULTS AND DISCUSSION

3.1 Tabel I: showing phytochemical constituents

SL.No	Extract	Consistence	Yield(gm)	Constituent
1.	Petroleum ether	Solid	10	Saponin glycosides, fixed oil, fat
2.	Chloroform	gel	7	protein, amino acids, fatty acids
3.	Ethanol	gel	15	Phenol,tanins,Phenols,flavonides, Alkolodes

3.2 Acute Toxicity Study

During acute toxicity studies, ehanolic extract at (1000 mg/kg body weight) neither produced any abnormal effect - nor moribund stages no death was observed.The activity was performed as per CPCSEA guide lines.

3.3 Table 2. Showing Antiepileptic activity

SL No	Treatment Group	Response in Sec Mean±SEM				Remarks
		Flexion	Extensor	Clonic Convulsion	Stupor	Recovery/ Death
1.	Control	17.5±0.763	15.5±0.980	26.16±0.980	17.166±0.833	Recovery
2.	Phenytoin(4mg/kg., i.p)	1.5±0.341	1.5±0.341	1.5±0.223	1.666±0.333	Recovery
3.	COD(50mg/kg.,p.o)	4±0.577	4.833±0.307	5.8±0.307	3.166±0.654	Recovery

One Way ANOVA by Tukey-Kramer Multiple Comparisons Test P<0.001 shows Highly Significant.

IV. DISCUSSION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is the second most common chronic neurological condition seen by neurologists. Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing country is 100 per 100,000 populations. India is home to about 10 million people with epilepsy (prevalence of about 1%) [33]. The number of epilepsy specialists and neurologists being very small in India, most people with epilepsy are being diagnosed and treated by non-specialists at both primary and secondary care levels. It is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness [34]. Several different types of human epilepsies have been characterized based on the classification of International League against Epilepsy (ILAE). According to this classification, epilepsy has been divided into partial epilepsy (simple and complex), generalized symptomatic epilepsy and unclassified epilepsy. Imbalances between the excitatory and inhibitory neurotransmitters are responsible for seizures. At neuronal level, seizures activity often occurs when glutamic acid excitatory neuro-transmitters over rides gamma amino butyric acid (GABA) mediated inhibition [35]. In the assessment of antiepileptic study, several models have been developed. Many drugs that increase the brain contents of GABA [36] have exhibited the antiepileptic against seizures induced by MES induced [37].

As per table II the drug ethanolic extract of leaves of *Chromolaena odorata*shows significant Anticonvulsant activity at 50mg/kg Body weightagainst Maximal electroshock induced convulsion test animals [38].The test compound has significantly abolished the various convulsion phase.

V. CONCLUSION

It can be concluded from the study that the anticonvulsant effects of the ethanol extract of *Chromolaena odorata* (C.OD) may be via non-specific mechanisms [40]. However, extensive studies are needed to evaluate the precise mechanism(s), active principles, and the safety profile of the plant as a medicinal remedy for convulsive disorders.

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