ANTICONVULSANT AND MYORELAXATION ACTIVITY OF *ANACYCLUS PYRETHRUM* DC. (AKARKARA) ROOT EXTRACT

***O.P.Gautam, **Savita Verma, S. K. Jain,**

*Department Of Pharmacognosy, Institute Of Pharmacy, Bundelkhand University,
Jhansi - 284128 (U.P.) INDIA
** Department Of Pharmacy, Roorkee College Of Pharmacy, Kishanpur, Roorkee (U.K.) INDIA

**e-mail: ***om.pharma26@gmail.com

******SAVITA4PHARM@gmail.com

Corresponding author:
Savita Verma
Department of Pharmacy,
Roorkee College of Pharmacy,
Kishanpur,
Roorkee (U.K.), India.

Summary

Objective: To study the anticonvulsant activity of ethanolic extract of Anacyclus pyrethrum in albino mice.
Materials and methods: The anticonvulsant and myorelaxation activity of ethanolic extract of roots of Anacyclus pyrethrum (200, 400 & 600mg/kg., i.p.) was assessed using albino mice against maximum electroshock seizure (MES) test and rotarod test respectively.
Result: The ethanolic extract of Anacyclus pyrethrum reduced the duration of hind limb tonic extension (HLTE) in a dose dependent manner against MES model.
Conclusion: The ethanolic extract of Anacyclus pyrethrum inhibits MES-induced convulsions.

Key Words: Anticonvulsant, Anacyclus pyrethrum, MES, Rotarod.

Introduction

Akarkara called as an akarkarabh, akallaka, in Sanskrit, akararaha in Arabik, In binomial nomenclature belong to Compositae. It is native of North Africa, from where it entered into Europ. This drug entered in to India during Muslim regime. It has for the first time described in Godo Nigraha as akallaka, Letter in Sargadhaara samhita and Bhava Prakash Nigantu it is described as akarakarabha.[1]

This perennial plant, in habit and appearance like the chamomile, has stems that lie on the ground for part of their length, before rising erect. The root is almost cylindrical, very slightly twisted and tapering and often crowned with a tuft of grey hairs. Externally it is brown and wrinkled, with bright black spots. The fracture is short, and the transverse section, magnified, presents a beautiful radiate structure and many oleoresin glands. The taste is pungent and odour slight.[2]

Anacyclus pyrethrum contains an alkaloid, namely "*pellitorin*" called as *pyrethrin.* Root contains alkyl amides, which active constituent's *pyrethrin* .Alkyl amide fraction of roots of *Anacyclus pyrethrum* is made up of the following isobutylamides and tyramine amides. *Anacyclus pyrethrum* arial parts contain active constituent is *Anacyclin*, N-*methylanacyclin*, N-*methyl-N-(2-methyl propyl) 2, 8-decadiene 4, 6-diynamide.* The root contain Anacyclin, Pellitorine enetriyne alcohol , hydrocarolin, inulin (50%), traces of volatile oil and (+)-sesamin. They also contain N-(2-P-hydroxy phenylethyl) deca,dodeca, and tetradeca- trans-2,a new series of tyra mine amides corresponding to the isobutylamides**.**[3]

A. pyrethrum is used as stimulant, cordial, rubefacient. A gargle of infusion is prescribed for relaxed vulva. Root used for toothache, rheumatic and neuralgic affections and rhinitis. Roots, along with the root of *Withania somnifera* and *Vitis vinifera*, are used in epilepsy. Along with other therapeutic applications, *Ayurvedic Pharmacopoeia of India* indicates the use of the root in sciatica, paralysis, hemiplegia and amenorrhoea. The root contains anacycline, isobutylamide, inulin and a trace of essential oil. Use of the drug in patients with insulin dependent diabetes mellitus reduces the dose of insulin. It decreased the plasma glucose and serum cholesterol levels after oral administration for 3–6 weeks.[4]

The aim of this work is to evaluate the anticonvulsant activity of ethanolic extract of roots of *A. pyrethrum* in order to provide a basis for the folkloric use of the plant.

Materials And Methods

Plant material

The fresh roots were collected from the Bhagawantpura Botanical Nursery, Jhansi, U.P., India in the month of August, 2008. The plant materials was taxonomically identified and authenticated by Dr. Gaurav Nigam, Department of Botany, Bundelkhand University, Jhansi, India, and a voucher specimen (No: BU/BOT/369/ 17-01-09) was deposited in the herbarium.

Preparation of extract

The roots of Anacyclus pyrethrum were dried in air, crushed in coarse powder and subjected to successive extraction using ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure using rotatory evaporator at temperature not exceeding 40°C and then dried in vacuum oven. The extract was stored in desiccators at cool place and reconstituted in water for injection just before use.

Animals used

Male albino mice (20-30 g) of either sex were procured from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi, India. The animals were housed in standard cages with free access of food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3.0°C with a 12-h light/dark cycle. The Institutional Animal Ethics Committee approved the protocol of the study.

Drugs used

Phenytoin (Samarth Life Sciences Pvt. Ltd., Baddi, H.P., India) and Diazepam (Helios Pharmaceutical Pvt. Ltd., Baddi, H. P., India) were used in this study. The plant extract was dissolved in normal saline and subjected for anticonvulsant activity and muscle relaxant activity using MES and Rota rod models respectively. Phenytoin and Diazepam were dissolved in normal saline (0.9% NaCl solution).

Acute toxicity study

The acute toxicity for the ethanolic extract of roots of Anacyclus pyrethrum was determined in female albino mice (20-25 g). The animals were fasted overnight prior to the experiment and fixed dose OECD guideline No.420 (Annexure 2d) method of CPCSEA was adopted for acute toxicity studies. [5] The ethanolic extract was administered in doses of 300, 2000, 5000 mg/kg. p. o. to group of mice, each containing ten animals and mortality was observed after 24 h.

Evaluation of anticonvulsant activity: Maximum electroshock-induced seizures
Electro-convulsive shock, inducing Hind Limb Tonic Extension (HLTE) in 99% of the animals, was previously determined.[6] Corneal electrodes were used for bilateral delivery of electrical stimulus. Electro-convulsive shock (50 mA for 0.2 Sec.) was delivered through corneal electrode to induce HLTE phase in mice. The electrical stimulus was applied using a stimulator apparatus for five groups of six each.

Group I served as control (vehicle treated, i.p.); Group II served as standard (received Phenytoin sodium 25mg/kg body weight, i.p.), Group III, Group IV and Group V were treated with ethanolic extract as 200, 400 and 600mg/kg body weight, i.p. respectively. The current was delivered after 30 min. of intraperitonial administration of control and standard. The incidence and duration of HLTE was noted.

Myorelaxation activity: Rota rod performance
The effect on motor co-ordination was assessed using Rotarod apparatus (Biocraft Scientific System Pvt. Ltd., Agra, India) Pre-selected mice (animal that stayed for at least 2 min. on the rotating bar, 24 hrs. before testing) were placed on the horizontal rotating bar (diameter 2.5 cm, 12 r.p.m.).The test was conducted on five groups of 6mice each, 30 min. after the administration of ethanolic extract (200, 400 & 600 mg/kg,i.p.) and diazepam(1mg/kg i.p.) and normal saline (10ml/kg, i.p..).[7]

Statistical analysis
The data was presented as mean ±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test.[8] A difference of $P<0.001$ was considered significant in all cases.

Results

Acute toxicity
In the acute toxicity study, the ethanolic extract of *A. pyrethrum* was found to be safe in the doses used and there was no mortality in a dose of 2 g/kg, i.p.

Evaluation of anticonvulsant activity: Maximum electroshock-induced seizures
The ethanolic extract of Anacyclus pyrethrum exhibited almost dose dependent anticonvulsant activity. The extract significantly decreased the duration of HLTE phase in MES-induced seizures. The result is shown in table 1.

Table 1. Effect of Anacyclus pyrethrum extract on MES induced seizures in mice

S.N.	Treatment	Duration Of HLTE	Mortality (%)	Recovery (%)
1.	Vehicle	15.73±0.35	80	--
2.	Phenytoin	3.71±0.09	0	100%***
3.	APE-200	12.96±0.69	0	71.37%***
4.	APE-400	10.52±0.19	40	64.28%***
5.	APE-600	8.42±0.51	0	56.05%***

APE -200, APE -400 and APE -600 - Anacyclus pyrethrum extract dose 200, 400 and 600mg/kg body weight.
Values are mean ± SEM, n=6, ***=$P<0.001$ compared with control.

Myorelaxation activity: Rota rod performance

A significant dose dependent muscle relaxant effect of Anacyclus pyrethrum was observed in rotarod appararus compared to that produced by diazepam. The result is shown in table 2.

Table 2. Effect of ethanolic extract of Anacyclus pyrethrum on Rota rod test in mice.

S.N.	Treatment	Time Of Fall (Sec.)	Myorelaxation %
1	Vehicle	290±2.303	--
2	Diazepam	18.0±1.20	100
3	APE-200	197.56±4.38	90.86
4	APE-400	140.87±2.75	87.14
5	APE-600	84.91±1.96	78.80

Values are mean ±SEM mice were pretreated with Vehicle and APE i.p. 30 min. before Rota rod model. ***=P<0.001 (n=5).

Discussion

The observation emanated in the present study indicated that the Anacyclus pyrethrum was without any lethal effect in a dose upto 2 g/kg and possessed anticonvulsant activity against seizures induced by MES in a dose dependent way.

The most popular and widely used animal seizures model is the traditional MES test. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures.[9] MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependent Na^+ channels, such as phenytoin, valproate and lamotrigine.[10,11] or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor such as felbamate.[12] The study showed that ethanolic extract from roots of Anacyclus pyrethrum can inhibit voltage dependent Na^+ channels as phenytoin in MES induced tonic seizures.

Thus, in conclusion, Anacyclus pyrethrum possesses anticonvulsant activity against the MES induced seizures. Further research is in progress to isolate the compound responsible for the activity.

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