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Evaluation of anticonvulsant activity of Polyherbal extracts of Mucuna birdwoodiana Tutcher, Mucuna andreana Micheli and Mucuna holtonii in albino rats.

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Abstract

Epilepsy is a chronic neurological disorder. The plant materials Mucuna birdwoodiana Tutcher, Mucuna andreana Micheli and Mucuna holtonii. This species is accepted and its native range is the bank of Khadakpurna River and the specimens were authenticated of plant materials were deposited in Maharashtra, India, The extract showed no toxicity and significantly prolonged the onset and reduced the duration of the seizures induced by MES. Phenytoin (25 mg/kg, i.p.) completely inhibited the seizures in this model. Similarly, in the seizures induced by PTZ the extract also prolonged the onset and reduced the duration of the seizures though not in a dose dependent manner. Sodium valproate also inhibits the PTZ seizures. The plant extract however showed a significant anticonvulsant activity at 400 mg/kg in comparison with Sodium valproate. The extract also attenuates the chemical (PTZ) induce oxidative stress in the brain. Moreover, the extract (400 mg/kg) also significantly decreases the GABA transaminase enzyme activity in PTZ model.

Keywords: Neurological disorder, Anticonvulsant activity, epilepsy, maximal electric shock, Polyherbal formulation.

Introduction

Epilepsy is common neurological disorders which may be define as a chronic and dynamic neurological condition of an abnormal, excessive, hyper synchronous discharge of a population of cortical neurons associated with ongoing neuronal damage, particularly when uncontrolled and causes physical, psychological, and social abnormalities in patients.[3] Thus, a decrease in concentration of gamma amino butyric acid (GABA) leads to many pathological processes in the brain which may be manifests into convulsive episodes.[3] Recurrent and spontaneous seizures may enhance reactive oxygen species (RPE) in the central nervous system.[4] Due to the high levels of polyunsaturated fatty acids, high rate of oxidative metabolism and lower levels of antioxidant defenses, the brain is more susceptible to oxidative stress.[5] Although a large number of anticonvulsant agents are present in the market, still a number of cases of drug resistance increases day by day. Available antiepileptic drugs are inadequate and not capable to control seizures in many patients (failure rate 30–40%).[6] Since the treatment duration is very long for epilepsy; therefore, the

drugs produce a variety of side effects and other neuropsychological disorders.[7] In addition, these drugs cannot prevent neurodegeneration process and have a negative impact on cognitive abilities and memory. Ninety percent of epileptic patients are from developing countries [8] and approximately three-fourths of them are not receiving adequate treatment. These few factors create interest in researches to find new and effective agents and formulations from natural sources,which are safe in prolonged usage for the management of epilepsy.

Etiology:

Epilepsy has several causes. Its most likely cause in individual patients relates to age at onset.

A. Idiopathic or constitutional epilepsy: Seizures usually begin between 5 and 20 years of age but may start later in life. No specific cause can be identified and there is no other neurological abnormality.
B. Symptomatic epilepsy

Materials and Methods

Materials

The plant materials Mucuna birdwoodiana Tutcher, Mucuna andreana Micheli and Mucuna holtonii. Obtained From the bank of Khadakpurna river and the specimens were authenticated of plant materials were deposited in Maharashtra, India, for further reference.

Method

To establish the scientific basis of mechanism, we examined the effects of Polyherbal extract of PHF (100, 200, and 400 mg/kg, p.o.) on maximal electric shock (MES), pentylenetetrazol (PTZ), and convulsions as well as gamma aminobutyric acid (GABA) glutamate level in the brain tissues in PTZ induced seizure model. Phenytoin (25 mg/kg, i.p.) For MES induced seizure and Sodium valproate (300 mg/kg, i.p.) for PTZ induced epilepsy were used as reference drugs, respectively.

Animals: Male albino mice weighing 18 – 30 g are obtained from animal house, P Wadhwani College of Pharmacy Yavatmal. All test animals are allowed free access to food and water ad libitum, both being withdrawn just prior to experimentation. They are divided into 8 groups, each group consisting of 6 animals.

Chemicals and solutions

Phenytoin sodium (Ciron pharmaceuticals) – standard drug for MES method sodium valproate (sun pharmaceuticals) – standard drug for PTZ method Pentylene tetrazole (PTZ) [Himedia labs] – chemoconvulsant.
Aqueous extract of Mucuna birdwoodiana Tutcher, Mucuna andreana Micheli and Mucuna holtonii linn – test compound Distilled water – vehicle

Equipments:

a. Electro-convulsometer with accessories:
This instrument provides an alternating current stimulus of 50 cycles per second. The electronic timing circuit contained in the apparatus automatically passes stimulus current for a preset period, which may be varied from 0.1 to 1 second in steps of
0.1 second. The current, variable from 0.25 to 350 milliamperes (mA), is suitable for producing minimal and supramaximal seizures required in the assay of anticonvulsant drugs.

b. 1 ml syringes
c. Measuring jars
d. Chemical weighing balance
e. Animal weighing balance
f. Stop watch
g. Animal cages

Preparation of Extract:
Fresh leaves of selected plants was cleaned, dried under shade and crushed by a mechanical grinder. The powdered of leaves of plants (1kg) was collected and taken as alcoholic extract by Sohxlet extraction method. The extracts were successively separate by filtration using Whatman filter paper and concentrated it at an appropriate temperature (40 °C) on a rotary evaporator and dried under freeze drier. The percentage yield was found between 25-30% (w/w) respectively, and the final dried powder was stored in a closed container at a cool place.

Preliminary Phytochemical Screening of PHE: The PHE was tested qualitatively for the presence of various phytochemicals like alkaloids, flavonoids, terpenoids, tannins and phenols. Phytochemical tests were carried out by Standard.

Methods

Maximal electroshock seizure (MES) model

Swiss albino rats weighing 150-200 g were used. Seizures were evoked by supramaximal electroshock stimulation of 50 mA, 50 Hz for 0.2 s using electro-convulsiometer through transauricular electrodes. Seizures pass through phases of tonic flexion and extension of limbs, clonus period. The abolition of tonic hind limb extension (THLE) is taken as an index of anticonvulsant activity. PE along with control and standard drugs were administered to respective groups of rats 30 min before application of electroshock. The duration of THLEs was noted.

PTZ model

PTZ is a CNS stimulant. PTZ (80 mg/kg) dissolved in normal saline was given 30 min after injecting the control, standard and test drug for a respective group of rats IP, which produces excitement, myoclonic jerks, and clonic seizures. The onset of convulsions was observed until 30 min after administering PTZ. Prolongation of the duration of seizure latency was taken as an index of protection indicating the anticonvulsant activity of the test compound.

Statistical Analysis

The effect of PE in different doses in both MES and PTZ seizure induction models was expressed as mean ± standard error mean. Data were analyzed using ANOVA followed by post-hoc turkey's test. $P < 0.05$ was considered significant.

Table 1: The animals were divided into five groups with six animals in each group for both models

Groups (N=4)	Treatment	Dose
I	Control - normal saline	0.35 ml/100 g
II	Sodium valproate	300 mg/kg
III	PE	100 mg/kg
IV	PE	200 mg/kg
V	PE	400 mg/kg

N=Number of animals in each group. PE : Polyherbal Extract

RESULTS

MES Model (Table 2)

The THLE phase was abolished in the standard group. When control group is compared with PE-100 mg/kg, the difference between the mean of THLE is not significant statistically, but when compared with PE (200 mg/kg and 400 mg/kg), the difference between the mean of THLE is significant statistically ($P < 0.05$). This shows that the PE extract at doses 200 and 400 mg/kg has significantly reduced the mean duration of THLE when compared to control group indicating that it possesses anticonvulsant property.

PTZ Model (Table 3)

When control group is compared with standard, there was significant prolongation in mean duration of seizure latency. When control group is compared with a test drug, there was significant prolongation in mean duration of seizure latency ($P < 0.05$) at two doses of PE (200 and 400 mg/kg) but not at the dose of 100 mg/kg. This shows that PE at doses of 200 and 400 mg/kg, prolong the mean duration of seizure latency significantly indication that it possesses anticonvulsant property.

Table 2: Effect of Polyherbal extract on MES-induced seizures in albino rats

Groups	Treatment	THLF	THLE	Clonus	Stupor	Postictal depression
I	Control (NS-0.35 ml/100 g)	3.83±0.17	7.20±0.61	10.26±0.51	273.50±39.01	244.33 ± 28.33
II	Standard (phenytoin - 25mg/kg)	8.23±0.4	0.00±0.00	11.68±0.40	280.87±28.73	279.16±26.24
III	PE (100 mg/kg)	4.16±0.25	6.76±0.80	18.20±0.48**	115.0±16.36**	229.83±36.86
IV	PE (200 mg/kg)	4.40±0.21	5.88±0.6	16.51±0.21**	103.5±24.64	182.0±27.67
V	PE (400 mg/kg)	5.18±0.34	3.86±0.3	11.95±0.41	84.16±12.60	105.5±25.11

All values are mean± SEM, statistical analysis by one-way ANOVA followed by Turkey's post-hoc test, *P<0.05, **P<0.01. SEM: Standard error mean, THLF: Total hind limb flexion, MES: Maximal electroshock seizure, THLE: Total hind limb extension

Table 3: Effect of Polyherbal extract on PTZ induces seizures in albino

Group	Treatment	Seizure latency or onset (seconds)	Duration of myoclonic jerks (seconds)
I	Control (NS - 0.35 ml/100 g)	211.16±15.10	703.3±20.24
II	Standard (sodium valproate - 300 mg/kg)	323.83±29.44**	441.83±28.56**
III	PE (100 mg/kg)	219.0±27.80	665.16±34.73
IV	PE (200 mg/kg)	274.16±25.19**	523.66±31.65**
V	PE (400 mg/kg)	298.33±25.84**	125.0±29.26**

All values are mean± SEM, statistical analysis by one-way ANOVA followed by Turkey's post-hoc test, **P<0.01. SEM: Standard error mean, PTZ: Pentylenetetrazole, PE: Polyherbal Extract

DISCUSSION

Epilepsy is a very common disorder affecting 1% of the world's population.[15] The anticonvulsants available are neither effective universally nor invariably safe. Due to long-term therapy with unwanted effects of many drugs the compliance with medication is very minimal. This study is to evaluate the anticonvulsant activity of Polyherbal extract of in MES and PTZ seizure-induced Mice. MES and PTZ tests are the best-validated method for assessment of AED in human generalized tonic–clonic seizures and absence seizures, respectively, among the tests used for evaluation of anticonvulsant activity.

In our study, it was found that treatment with PE extracts (200 mg/kg and 400 mg/kg) in mice significantly reduced THLE in MES- induced seizure model. MES-induced seizures are abolished by the drugs that block voltage-gated Na^+ channels such as Phenytoin and carbamazepine or by the drugs that block N-methyl-D-aspartate receptors like felbamate. Protection of PE extract against THLE indicates that the drug the ability to inhibit or abolish the spread of seizures within the brain suggesting the presence of an anticonvulsant compound in the extract. Similarly, it was found that treatment with PE extracts (200 and 400 mg/kg) significantly prolong the mean duration of seizure latency in PTZ seizure model. PTZ induced convulsions are prevented by the drugs that block T-type Ca^{2+} current in thalamus like sodium valproate or the drugs which gamma-amino butyric acid ($GABA_A$) agonistic like diazepam. Protection of PE extract against PTZ induced seizure suggests a interaction with GABA-ergic neurotransmission indicating the presence ofan anticonvulsant compound in the extract.

CONCLUSION

In conclusion, the results suggest that the Polyherbal extract of Mucuna birdwoodiana Tutcher, Mucuna andreana Micheli and Mucuna holtani of anticonvulsant activity which can be compared with the standard sodium valproate in electrically and chemically induced epileptic animal models. Further studies are required to elucidate the exact mechanism by which this plant acts as an anticonvulsant agent.

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