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Pharmacology

A STUDY ON ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF CISSUS QUADRANGULARIS

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ABSTRACT

AIM & OBJECTIVE: To evaluate the anticonvulsant effect of aqueous extract of Cissus quadrangularis in MES induced seizures, in adult male albino rats in comparison with diphenyl hydantoin.

MATERIALS AND METHODS: 15 adult male albino rats weighing 250-300g, were divided into five groups of three each. Group I which served as control received 0.5 ml of 1% gum acacia; Group II (standard) – received diphenylhydantoin; Groups III, IV and V received the test compound- aqueous extract of Cissus quadrangularis at a graded dose of 100,150 and 200 mg/kg body weight respectively. After 2 hours of oral feeding, convulsions were induced 10 minutes apart in each animal and the phases of convulsion along with its duration were observed.

RESULTS : The duration of tonic extension and post ictal depression were significantly shortened in experimental groups (Groups II and V) compared to control (Group I). The reduced duration of these components are due to the anticonvulsant effect of the drug and the extract which indicates that the aqueous extract of Cissus quadrangularis possesses significant anticonvulsant effect.

KEYWORDS :

INTRODUCTION

Epilepsy is as old as mankind, described about 3000 years ago in Mesopotamia and was attributed to the God of moon. A Seizure is a paroxysmal event due to abnormal, excessive, high frequency, hypersynchronous action potential discharges from an aggregate central nervous system neurons while epilepsy is the tendency to have recurrent seizures[1]. Many people with epilepsy feel stigmatized by the society and become isolated unnecessarily[2]. Epilepsy is a common condition with an incidence of 80 cases per 1,00,000 persons per year. The conditions that may commonly result in chronic seizure disorder are severe penetrating head trauma, stroke, infections, tumors and abnormalities in CNS development. Seizures are made episodic by precipitating or provocating factors, some of them being intrinsic physiological processes like psychological or physical stress, sleep deprivation, hormonal changes, extrinsic factors like exposure to toxic substances and certain medication. The treatment of epilepsy can be considered in four parts – Removal of causative / precipitating factors, use of antiepileptic drugs, surgical excision of epileptic foci and other surgical measures and regulation of physical / mental activity. Drug treatment should be considered after more than one seizure has occurred. Antiepileptic drugs appear to act primarily by blocking the initiation and spread of seizures. Of patients whose epilepsy in controllable, only a single drug is necessary in 80%.

Even with the advent of so many antiepileptic drugs because of the commonly appearing adverse effects, the hunt for a safer antiepileptic agent is going on. Existing anti-seizure drugs provide adequate seizure control in about two thirds of patients[3]. A fraction of the epileptic population is resistant to all available drugs and this may be due to increased expression of multidrug transporter P glycoprotein 170, a product of the ABCB1 gene. And even when epilepsy is under control, special issues like adverse effects, management of epilepsy during pregnancy because of teratogenecity, a continuous search for a relatively safe remedy is always there.

Medicinal plants continue to play a significant role in modern medicine due to their inherent, distinct chemical and biological properties. In nature a plant is able to synthesize complex molecules from simple ones through highly specific reaction mechanisms. Herbal medicines are widely used in epilepsy in many parts of the world. About 150 plants are said to be recorded in traditional medicine and 10 warrant further investigation. The Chinese mixture Saiko- Keishi - to which is made up of 9 plant products, has produced reduction in seizure frequency, severity or even freedom form seizures[4].

So one such attempt has been made here to evaluate the antiepileptic effect of aqueous extract of Cissus quadrangularis and the efficacy has been compared with the standard anti epileptic drug – diphenyl hydantoin.

MATERIALS AND METHODS:

After obtaining ethical clearance from the Institutional Animal Ethical Committee, this experimental study was conducted for a period of 6 months(from September 2006 to February 2007) in the Central Animal House, Institute of Pharmacology, Madurai Medical College, Madurai.

Materials Required for the Study:

Experimental animals

In the present study, adult male albino rats have been used, because the major consideration in selecting an animal model remains on the seizure type under study. Here it is GTC seizures. As there are predictable variations during estrous cycle and drug disposing mechanism, male albino rats were selected. The convulsions induced by MES method resembles generalized tonic clonic seizures of human. 15 Healthy adult male albino rats, 10 months old, weighing around 250-300 g (mean wt – 286 gms) were used. The animals were selected from the inbred colony maintained in the Central Animal House, Madurai Medical College. They were fed with commercially available standard pellet diet obtained from AMRUT FEEDS, Pranav agro industries limited and water ad libitum.

Drugs and chemicals

Preparation of extract of Cissus quadrangularis

The whole plant of Cissus quadranguloris were collected and dried in shade for 10 days. It was coarsely powdered. 200g of the powdered plant was soaked in sufficient quantity of distilled water overnight. The contents were transferred to a Soxhlet apparatus and extracted for about two hours using hot water bath. The process was repeated several times with fresh powder to get sufficient quantity of the extract. The semisolid extract obtained was weighed accurately and utilized for experimental studies. The extract was suspended in 1% gum acacia in distilled water to yield the required graded concentrations i.e, 30, 45, 60mg in 0.5ml of the suspension.

Gum acacia :

This is the dried gummy exudate obtained from the stem and branches of Acacia Senegal or other African species of acacia and is used here as a suspending agent for the oral administration of the standard drugs and test compound in 1% strength.

Diphenyl Hydantoin

Tablet diphenylhydantoin 100 mg (T. Eptoin – Knoll Pharmaceuticals Ltd) was powdered and suspended in 1% gum acacia. The suspension prepared provided 30mg/ml and it was administered orally in the dose of 50mg/kg body weight[5,6,7.] Diphenylhydantoin is the prototype drug that abolishes the tonic extensor phase of MES seizure and either prolongs or unalters the clonic phase. In addition it shortens the duration of post seizure depression[8].

Appliances / Equipments

Electro Convulsiometer

For electrical stimulation of rat Techno Electro convulsiometer was used. It is provided with various pulse stimulators and an inbuilt timer with which we can select and set the amplitude of electricity to be delivered and its duration. Stimulation is given through a pair of ear clip electrodes made of stainless steel. Saline solution or commercial ECG salt paste can be used to moisten the electrodes[9].

Oral feeding tube:

A 16 gauge hypodermic needle of 3 or 4 inch length serves as an useful oral feeding tube for the rat. The needle is blunted and a small ball of solder is applied around the distal end. A gentle 20-30° bend is made about 2cm proximal to the solder. The tube thus constructed is attached to a 2ml syringe[10].

ANTI EPITEPTIC STUDY

Methodology

15 adult male albino rats were divided into 5 groups with 3 animals each. All animals were put on overnight fasting. Group I served as control received – 0.5ml of 1% gum acacia, normal feed and water. Group II received the standard drug diphenylhydantoin at the dose of 50mg/kg[11]. The three test groups received the aqueous extract of Cissus quadrangularis at the dose of 100, 150, 200 mg/kg along with normal feed and water[12]. The test was conducted after 2 hours following drug administration. Convulsions were induced using electro convul siometer which was set to deliver a current strength of 150mA for a period of 0.2 seconds. The control animal evolved though a latent phase, phase of tonic flexion, phase of tonic extension, clonic phase and post-ictal depression[13]. Convulsions were induced in all three animals in each group at a gap of ten minutes each, so that observations can be made without any difficulty.

Group	Category	Treatment
I	Control	Normal feed + water + 0.5ml of 1% gum acacia
II	Standard	Normal feed + water + Phenytoin sodium 50mg/kg oral
III	T1	Normal feed + water + extract of Cissus quadrangularis 100mg/kg oral
IV	T2	Normal feed + water + Extract of Cissus quadrangularis 150mg/kg oral
V	T3	Normal feed + water + Extract of Cissus quadrangularis 200mg/kg oral

Following recovery from postictal depression, the animals were maintained on normal feed and water after the experiment and observed for a week and also for four weeks thereafter to find out any changes in behavioural, neurological, autonomic profile and mortality. None of the animals subjected for the study expired during the study period or during post study follow up of another 28 days.

Statistical Analysis :

The results observed were tabulated, data were analyzed statistically using Student's 't' – test. Probability values less than 0.05 were considered significant.

RESULTS :

15 adult male albino rats weighing 250-300g of either sex were divided into five groups of three each. Efforts were made to follow animals everyday in the Central Animal House. Group I which served as control received 0.5 ml of 1% gum acacia; Group II - standard – received diphenylhydantoin; Groups III, IV and V received the test compound - aqueous extract of Cissus quadrangularis at a graded dose of 100,150 and 200 mg/kg body weight respectively. All animals were fed orally using oral feeding tube. After 2 hours of oral feeding convulsions were induced 10 minutes apart in each animal and the phases of convulsion along with its duration were observed. The observations were tabulated and compared for their significance in Tables 1– 5. On close follow up the animals which received diphenylhydantion (or) the aqueous extract of Cissus quadrangularis (or) placebo, did not show any behavioural abnormalities (or) weight loss. Similarly animals belonging to the Groups III, IV and V didn't show any evidence of bowel disturbances (or) change in eating or drinking habits. These indicated that the extract did not have any systemic toxicity as well.None of the rats considered for the present study expired either during the study period or during post study follow up of another 28 days. The guidelines provided by the Ethical Committee for the animals were adhered strictly.

Anticonvulsant effect

Latent Phase:

The mean latent phase in Groups I, II, III, IV and V were 2.67 ± 0.5574, 2.33 ± 0.5774, 2.33 ± 0.5774 2.0 ± 0.5774 and 1.33 ± 0.5774 respectively with no significant change in the duration of latent phase (Table 1).

TABLE – 1 LATENT PHASE

Group		Duration in secs	Mean ± SD	Remarks
I	Control	3 2 3	2.67 0.5774	**p > 0.05 Not Significant**
II	Standard	2 2 3	2.33 0.5774	
III	Test – 1	3 2 2	2.33 0.5774	
IV	Test – 2	2 2 2	2 0.5774	
V	Test – 3	1 1 2	1.33 0.5774	

Phase of tonic flexion

The mean duration of phase of tonic flexion in Groups I, II, III, IV and V were 4.33 ± 0.5774, 3.33 ± 1.1547, 2.67 ± 0.5774, 3.33 ± 0.5774 and 2.33 ± 0.5774 respectively with no significant change in the duration of phase of tonic flexion (Table 2).

TABLE – 2 PHASE OF TONIC FLEXION

Group		Duration in secs	Mean ± SD	Remarks
I	Control	5 4 4	4.33 ±0.5774	p > 0.05 Not Significant
II	Standard	2 4 4	3.33 ± 0.5774	
III	Test – 1	2 3 3	2.67 ± 0.5774	
IV	Test – 2	4 3 3	3.33 ± 0.5774	
V	Test – 3	3 2 2	2.33 ± 0.5774	

Phase of tonic extension

The mean duration of phase of tonic extension of Groups I, II, III, IV and V were 19.33 ± 3.0557, 1.33 ± 1.1547, 11.67 ± 0.5774, 11.0 ± 1.0 and 6.67 ± 1.1547 respectively with a significant reduction in Group II ($p < 0.001$) and Group V ($p < 0.01$) when compared to the control (Table 3).

TABLE – 3 PHASE OF TONIC EXTENSION

Group		Duration in secs	Mean ± SD	Remarks
I	Control	22 16 20	19.33 ± 3.0551	
II	Standard	2 0 2	1.33 ± 1.1547	p< 0.001
III	Test – 1	12 11 12	11.67 ± 0.5774	p< 0.05
IV	Test – 2	10 12 11	11.0 ± 1.0	p< 0.05
V	Test – 3	6 8 6	6.67 ± 1.1547	p< 0.01

Clonic Phase

The mean duration of clonic phase in Groups I, II, III, IV and V were 2.67 ± 0.5774, 28.0 ± 5.2915, 11.67 ± 1.5275, 22.67 ± 9.0185 and 21.67 ± 1.5275 with a significant prolongation of the clonic phase in Group II ($p < 0.01$), IV ($p < 0.05$) and V ($p < 0.001$) when compared to control (Table 4).

TABLE –4 PHASE OF CLONUS

Group		Duration in secs	Mean ± SD	Remarks
I	Control	3 3 2	2.67 ± 0.5774	
II	Standard	30 22 32	28.0 ± 5.2915	p< 0.01
III	Test – 1	12 13 10	11.67 ± 1.5275	p<0.001
IV	Test – 2	14 22 32	22.67 ± 9.0185	p< 0.05
V	Test – 3	20 23 22	21.67 ± 1.5275	p< 0.001

Phase of post ictal depression

The mean duration of post ictal depression in Groups I, II, III, IV and V were 4.87 ± 0.3683, 1.18 ± 0.1277, 3.69 ± 0.3630, 3.39 ± 0.0503 and 2.40 ± 0.0950 with a significant reduction in the duration in Group II ($p < 0.001$) and Group V ($p < 0.01$) when compared to control (Table 5).

TABLE – 5 PHASE OF POST ICTAL DEPRESSION

Group		Duration of secs	Mean ± SD	Remarks
I	Control	300 245 312	2.85.67 ± 35.73	
II	Standard	92 67 75	78 ± 12.77	p< 0.001
III	Test – 1	234 222 250	235 ± 14.05	p< 0.1
IV	Test – 2	214 224 220	219.33 ± 5.03	p< 0.05
V	Test – 3	160 150 169	159.67 ± 9.50	p< 0.01

DISCUSSION:

An ideal antiseizure drug should suppress all seizures without any unwanted effects. Unfortunately the available antiseizure drugs provide symptomatic relief in two thirds of patients only. In addition they cause adverse effects ranging from minimal impairment of CNS to death from aplastic anemia and hepatic failure. Hence researchers are making efforts to identify molecules to prevent generation of seizures or to abort the abnormal electrical activity. The observations emanated in the present study indicated that the duration of tonic extension and post ictal depression were significantly shortened in experimental groups (Groups II and V) compared to control (Group I). The reduced duration of these components are due to the anticonvulsant effect of the drug and the extract which indicates that the aqueous extract of Cissus quadrangularis possesses significant anticonvulsant effect. Statistical analysis also revealed that Group V had a significant anticonvulsant effect almost comparable to that of Group II. It is likely that plant extracts are safer and as they are already components of dietary supplements in day to day life, further evaluation is required to understand the exact molecular mechanism. Hopefully the data so obtained will form the nucleus for further research in the treatment of seizures.

REFERENCES :

1. Lowenstein DH, Seizures and Epilepsy; In Kasper DL et al (eds), Harrison's Principles of Internal Medicine, 16th Edition, Volume II, Mc Graw Hill, New York, USA, 2005; Chapter 348; Pages 2357-2372.
2. Allen CMC et al, Neurological Diseases. In Haslett. C et al (eds) Davidson's Principles of Practice of Medicine, 19th Edition, Churchill Livingstone Elsevier Limited, Philadelphia, USA, 2002; Chapter 22, Pages 1123-1134.
3. Porter BJ et al, Anti seizure drugs. In Katzung BG (eds), Basic and Clinical Pharmacology, 10th Edition, Mc Graw Hill, New York, USA, 2007; Chapter 24, Pages 374-393.
4. Shorvon SD, Principles of Drug Treatment. In Handbook of Epilepsy Treatment, 2nd Edition, Blackwell Publishing, Massachusetts, USA, 2005; Chapter 2, Page 111, 105.
5. Mc Leod LJ. Action on the central Nervous system. In Pharmacological Experiments on intact preparations. E &S Livingstone, Edinburgh. and London, 1970; Chapter V, Page 87.
6. Ghosh.MN, Statistical analysis. In Fundamentals of Experimental pharmacology, 3rd Edition, Hilton Company, Kolkata, 2005; Chapter 34, Pages 218-229, 241.
7. Farris EJ, Griffith JQ, Dosages of drugs for Rat. The Rat in Laboratory Investigations, 2nd Edition, J.B. Lippincott company, Philadelphia 1949; Page 342.
8. Mc Namara JO. Pharmacotherapy of the Epilepsies. Laurence BL et al (eds) Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition, Mc Graw Hill Medical Publishing, New York, USA, 2006; Chapter 19, pages 501-510.
9. Tomen James EP et al. Anticonvulsants. In Laurence DR et al (eds) Evaluation of Drug activities: Pharmacometries, Volume 1, Academic Press, London and New York, 1964; Chapter 13, Pages 287-299.
10. Gay WI, Infusion Techniques. In Methods of Animal Experimentation, Volume I, Academic Press, New York & London, 1965; Page 33.
11. Vidhya SR et al. Anticonvulsant and neurotoxicity profile in rats. In journal of Ethnopharmacology Volume 102, Issue 3, December 2005, Pages 351-356.
12. Viswanathaswamy AHM et al . Some neuro pharmacological effects of Extract of Cissus quadrangularis in mice & rat. In Bioline International – African Journal of Biomedical Research, Volume 9, Number 2, 2006. Pages 69-75.
13. James Toman EP et al, Properties of maximal seizures and their alteration by anticonvulsant drugs and other agents, Marshal Merkin et al (eds) Journal of Neurophysiology 1946, Volume 9, Page 231-239.