



SCREENING FOR ANTIEPILEPTIC ACTIVITY OF *TAMILNADIA ULIGINOSA* ROOT EXTRACT

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Article Info

Received 02/01/2014

Revised 05/01/2014

Accepted 07/01/2014

Key words:

Anti epileptic activity,
Maximal Electro Shock,
Herbal extract,
Anticonvulsant.

ABSTRACT

Epilepsy is a neuropsychological disorder. It is the consequence of a paroxysmal uncontrolled discharge of neuron within the central nervous system. Epilepsy is a collection of diverse disorders that together affect approximately 1% of the general population. Around 75-80% of epileptic patients may be provided with adequate seizure control with the help of conventional antiepileptic drugs. Current therapies are largely symptomatic and are aimed at controlling seizures in affected individuals. There are number of drugs available for treatment of epilepsy in modern therapy such as Felbatol, Gabitril, Lyrica, Tomax, zonegran, trilepta. But the major disadvantage being faced is their chronic side effect which necessitates the use of herbal extract. Extract of *Tamilnadia uliginosa* (Rubiaceae) roots, a medicinal plant were used in neuro protective Ayurvedic preparations and were evaluated for its protective effect against seizures induced by Maximal Electro shock (MES) method in Male Wistar albino mice. Herbal drugs are acting at site having same mechanism of action as that of synthetic drugs. A daily dose of 250 and 500 mg/kg of the extract was administered to the animals for 15 days, after which seizures were induced by Maximum electro shock method and the duration of various phases of epileptic attacks were recorded and compared with the control animals. A significant reduction in the time taken for recovery was noted in the experimental animals. The levels of biogenic amines such as dopamine, serotonin and nor-adrenaline in the forebrain region were also estimated and a significant level of restoration was observed in the extract treated animals.

INTRODUCTION

Tamilnadia uliginosa also known as *Randia uliginosa* (Retz), a member of the family Rubiaceae. *Tamilnadia*- Genus based on name of the state of Tamilnadu in India. The plant is grown in dry deciduous forests, native to Bangladesh, India, Sri Lanka, and Thailand. The different parts of the plant are used for various ailments like Cholera, diarrhea,

dysentery, eye complaints, pimples, diuretic, tonic properties, biliousness, aphrodisiac etc [1].

Epilepsy is a syndrome of episodic brain dysfunction characterized by recurrent unpredictable spontaneous seizures. Epilepsy is a common neurological disorder. The term Seizure refers to transient alteration of behavior due to abnormal, synchronized, and repetitive burst firing of neuronal populations in the central nervous system (CNS).

Antiepileptic drugs control seizures in many patients, but patients can be kept seizure-free in less than half of the partial epilepsies. Majority of antiepileptic drugs possesses more than one mechanism of

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action [2]. They are classified based on their mechanism of action. First group consists of antiepileptic's (eg. carbamazepine, gabapentin, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, topiramate, and valproate) which block sustained repetitive firing in individual neurons, this effect being mainly due to the blockade of voltage-dependent sodium or calcium channels. These drugs are effective against generalized tonic-clonic and partial seizures. The second group includes drugs enhancing inhibitory events mediated by γ -aminobutyric acid (GABA): benzodiazepines, gabapentin, phenobarbital, tiagabine, topiramate, vigabatrin, and valproate. Some of these drugs may be used in all seizure types (absence, generalized tonic-clonic and partial seizures). The third group practically consists of one drug—ethosuximide which blocks T-type calcium channels and is active against absences. Recent evidence suggests that also zonisamide may be a T-type calcium channel inhibitor [3]. A separate category of drugs may be also suggested – these antiepileptic drugs reduce events mediated by excitatory amino acids (eg. glutamate) and at present three antiepileptics meet these criteria: felbamate, phenobarbital, and topiramate [4].

MATERIAL AND METHODS

Plant material

The fresh roots of *Tamilnadia uliginosa* (Rubiaceae) used for the preparation of the extract were collected from Tirumala hills, Tirupati. The roots collected were checked for purity and were authenticated by Dr. Madhava Chetty, Department of Botany, SV University, Tirupati. The roots were air dried under shade & coarse powdered and used for extraction.

Preparation of the Herbal extracts

Coarsely powdered, shade dried roots of *Tamilnadia uliginosa* (Rubiaceae) (1000 g), and were extracted with distilled water for 72 hrs in a soxhlet extractor. The extract obtained was cooled, filtered and the solvent recovered by distillation in vacuum.

The residue so obtained was used for subsequent experiments. The w/w yield in terms of dry material was 19.4%. The dried extract was suspended in 5% Carboxyl methyl cellulose (CMC) for animal administration.

Animals

Healthy adult male Wistar albino mice weighing about 200-250 g were used for the study. The animals were housed in polypropylene cages, maintained under standard conditions (12 h light: 12 h dark cycle; $27 \pm 1^\circ\text{C}$; 60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*.

Drugs and chemicals

Serotonin, dopamine and nor adrenaline used in the standard readings for the estimation of bio amines were obtained from Sigma (USA) and other chemicals used were of analytical reagent grade.

Acute toxicity studies

Male Wistar albino mice weighing 200-250g selected by random sampling technique was performed as per OECD-423 guidelines (acute class method). The animals were fasted 12 hrs, provided only water, after which the root extract was administered to the respective groups orally at the dose level of 50 mg/kg body weight by gastric intubation and the groups, observed for 15 days. The mortality was observed in 2 or 3 animals, and the dose administered was assigned as a toxic dose. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hrs.

Estimation of duration of epileptic seizures [5]

The animals were divided into four groups (n=6) and Group I animals served as control receiving 1 ml of 5 % CMC p.o, Group II served as drug control receiving oxcarbazepine 20 mg/kg, p.o and Group III and IV animals were administered with the herbal extract at doses of 250 and 500 mg/kg, p.o for 15 days respectively. On the 15th day, seizures were induced to all the groups of animals using electro convulso meter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electro shock (MES) seizures for 0.2 second. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the mice. This increases the contact and reduces the incidence of fatalities. The observed duration of various phases of epilepsy was tabulated in table 1.

Estimation of biogenic amines

The animals were divided into five groups (n=6) and Group I animals served as control for reference standards, Group II animals served as negative control receiving 1 ml of 5 % CMC p.o, Group III served as drug control receiving oxcarbazepine 20 mg/kg p.o, Group IV animals were administered with the root extract at a dose of 250 mg/kg p.o and Group V animals received herbal extract at a dose of 500 mg/kg p.o for 15 days. On the 15th day, seizures were induced to all the groups except Group I animals using electro convulsimeter and biogenic amines in the fore brain of the mice were estimated. The mice were sacrificed by cervical dislocation, since sacrificing by over dose of anesthesia may alter the brain monoamine levels. After sacrificing, the brain was rapidly removed and the fore brain was dissected on a cooled microtome at 20°C .



The fore brain region was weighed and fore brain of two mice of the same group were pooled and homogenized with 6 ml of cold acidified butanol. Each homogenate pool served as a tissue sample for the respective groups. Internal standards were prepared by the addition of known amounts of mixed standards, (150 µg each of noradrenaline, dopamine and serotonin). The readings were limited to the neither excitation maxima 395-485 nm for noradrenaline, 330-375 nm for dopamine and 360- 470 nm for serotonin. The results were expressed as ng/g of wet brain tissue in table 2.

RESULTS AND DISCUSSION

The root extract exhibited a dose dependent significant reduction in various phases of epileptic seizure on comparison with the reference standard oxcarbazepine 20 mg/kg, p.o. A significant increase in the dopamine,

serotonin and noradrenaline level was noted in the fore brain region for extract treated animals. A significant reduction in the time required for the recovery was observed in this study (Table 1), which proves that herbal root extract provides a beneficial effect in controlling MES induced seizures. Serotonin agonists, precursors and neuronal uptake inhibitors are reported to enhance narcoleptic catalepsy. The increase in the brain monoamine level by inhibiting the monoamine oxidase (MAO), an enzyme responsible for destruction of biogenic amines tends to raise the seizure threshold. A low concentration of dopamine in cerebellum also has an inhibitory effect on glutamate. Inhibition of prostaglandin synthesis is reported to increase the brain levels of dopamine and noradrenaline, which also causes an inhibition of seizure activity.

Table 1. Effect of the extract of *Tamilnadia uliginosa* roots on MES induced convulsions in Male Wistar albino mice

Group	Drug	Flexion (sec)	Extension (sec)	Clonus (sec)	Stupor (sec)	Recovery (sec)
I	Control	5.2±0.11	14.2±0.21	14.3±2.4	5.2±1.1	181.1
II	oxcarbazepine 20 mg/kg	4.1±0.31	0	8.2±3.1	2.1±0.3	176.1
III	Plant extract 250 mg/kg	3.91±0.17	1.42±0.18	7.31±0.41	25±1.1	134.72
IV	Plant extract 500 mg/kg	4.21±0.21	1.21±0.12	6.15±0.21	20±0.2	124.22

Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test.

Table 2. Effect of the extract of *Tamilnadia uliginosa* roots on the levels of biogenic amines in forebrain of epilepsy induced Male Wistar albino mice

Group	Drug	Serotonin (ng/g of wet tissue)	Dopamine (ng/g of wet tissue)	Noradrenaline (ng/g of wet tissue)
I	CONTROL	168.61±1.11	416.31±1.60	80.27±0.21
II	MES	70.01±0.51	131.27±0.21	30.22±0.11
III	Oxcarbazepine (20mg/kg)	86.11±0.21	270.17±0.11	51.12±0.22
IV	Plant extract (250mg/kg)	95.54±0.21	312.04±0.81	69.71±1.01
V	Plant extract (500mg/kg)	117.23±0.21	332.20±0.11	61.72±2.21

MES = Maximal Electro Shock Induced Group. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test.

CONCLUSION

We have found that administration of the extract of *Tamilnadia uliginosa* roots for 15 days increased the seizure threshold in MES induced mice

and its possible mechanisms may be due to the inhibition of Prostaglandin synthesis and Monoamine Oxidase Enzyme.

REFERENCES

1. Srinivasulu C and Indraneil Das. (2008). The Herpetofauna of Nallamala Hills, Eastern Ghats, India: An Annotated Checklist, With Remarks on Nomenclature, Taxonomy, Habitat Use, Adaptive Types and Biogeography. *Asiatic Herpetological Research*, 3(11), 110–131.
2. Cheolsu Shin. (1994). Mechanism of epilepsy. *Annual Review of Medicine*, 45, 379- 389.
3. Biton V. (2007). *Clin Neuropharmacol*. 30(4), 230-40.
4. Piotr Czapiński, Barbara Blaszczyk, Stanislaw J Czuczwar. (2005). Mechanisms of action of antiepileptic drugs. *current topics in medicinal chemistry*, 5(1), 3-14.
5. Muralidharan P, Srikanth J. (1990). Antiepileptic Activity of *Morinda Citrifolia* Linn Fruit Extract. *E-Journal of Chemist*, 7, 612-616.

