ANTIEPILEPTIC ACTIVITY OF INDIAN FILBERT FRUIT EXTRACT IN EXPERIMENTAL ANIMAL MODELS

ABSTRACT

Aim: To screen the antiepileptic activity of ethanolic fruit extract of Indian filbert.

Methodology: The cleaned, dried and powdered fruits of Indian filbert were subjected to extraction.Preliminary Phytochemical studies were carried out for the presence secondary metabolites.Acute toxicity and anticonvulsant effect on maximal electroshock induced seizures and metrazol (pentylenetetrazole) induced seizures carried out in experimental animals.

Results: Preliminary phytochemical studies showed the presence of steroids, triterpenoids, flavonoids, carbohydrates. Acute toxicity of ethanolic extract of fruits were found to be safe upto 2000 mg/kg body weight Ethanolic extract of Indian (100,200 and 400 mg/kg) significantly reduced the duration of seizures induced by maximal electroshock (MES) as well as protected animals from metrazol induced tonic seizures and the results were found to be significant (P=0.05) when compared to control

Conclusions: The presence of triterpenoids, steroids and flavonoid might be responsible for the antiepileptic activity of the fruit extract of Indian filbert.

Keywords: Indian filbert,antiepileptic activity,Sapindaceae,metrozol, maximal electroshock, pentylene tetrazole,phenytoin.

1. INTRODUCTION

Herbal medicine, also known as phyto-therapy is the science of using herbal remedies to treat various sicknesses. It covers all the aspects of herbal medicine, that is, plants with powerful actions to those with gentle actions. Currently, people have come to know of the side effects of synthetic drugs, which lead to an increased development of natural products as treatment for diseases [1]. This piqued the interest of scientists and the pharmaceutical industry to produce new herbal medicines with fewer adverse effects for a variety of ailments [2]. India, which has the greatest number of medicinal and therapeutic plants, has become a target for pharmaceutical companies looking to export such plants for the creation of new products. As a result, India's export business is on the rise [3].

Epilepsy, a prevalent CNS illness, has been reported to impact 5% of the world's population [1]. It is a major neurological condition characterized by recurrent unprovoked seizures. Seizures are the most common sign of epilepsy which are caused by the unusual and rhythmic high release of impulses by a group of nerve cells. Current epilepsy treatments with modern synthetic antiepileptic drugs were shown to have dose-related adverse effects and toxicity [4]. Herbs were claimed on having better antiepileptic effects. As a result, phytochemicals from a plethora of different medicinal plants have been found, giving an intriguing possibility for the creation of novel medicines.

Antiepileptic drugs (AED's) protect the body against seizures by interaction with various cellular targets. They suppress the abnormal hyperactivity of the brain cells. Nevertheless, several antiepileptic medicines have been proven to be ineffective in controlling epileptic seizures. This has stimulated researchers to develop drugs with better activity. Hence, the medicinal plants were also considered to be an important source in the development of drugs with this biological activity. As a result, medical folklore has been regarded as a valuable resource in the discovery of medications having this biological action.

Indian filbert is known as *Sapindus trifoliatus* Linn. (Family: *Sapindaceae*), a handsome tree that grows on the Indian Peninsula, primarily in South India, and is recognized as the soap nut tree [5] Various pharmacological activities possessed by the fruits of this plant includes anthelminthic, emetic, astringent properties and are used in the treatment of diarrhoea, cholera, asthma, colic due to indigestion, tubercular glands, paralysis of the limbs and lumbago. Externally it is a detergent, also used to destroy pediculi and to wash and cleanse the hairs of the head. The roots are utilised in gout, rheumatism, and paralysis, while the aromatic leaves are used in baths for aching joints [6]. The present study was undertaken for screening of in vivo antiepileptic activity of the ethanolic extract of fruits of Indian filbert.

2. MATERIAL AND METHODS

2.1. Preparation of ethanolic extract: The riped fruits of Indian filbert were obtained from Mangaluru, Karnataka. The plant was authenticated by botanist Dr.Noeline J. Pinto, Head of botany Department, St. Agnes College,Mangaluru. The fruits were cleaned, dried and subjected for size reduction into a coarse powder using mechanical grinder. Obtained powder was then passed through sieve no. 40 and extracted using ethanol by process of maceration. Resulting extract was dried using flash evaporator under controlled temperature and reduced pressure.

2.2. Preliminary Phytochemical Screening

To know the existence of secondary metabolites related to antiepileptic activity of Indian filbert, standard phytochemical examination was performed [7].

2.3. Acute Toxicity Studies

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD50 of the drug extract. The acute toxicity study was carried out in adult female albino rats by "up and down" method [8] (OECD guidelines 425)[9]. The animals were fasted overnight and next day ethanolic extracts of fruits of the plant Indian filbert suspended in 0.5% CMC was administered orally at different dose level. Then the animals were observed continuously for 3 h for general behavioral, neurological, autonomic profiles and then every 30 min for next 3 h and finally death after 24 h [10]. Wistar albino rats of either sex weighing between 150 – 200 g and albino mice of either sex weighing between 20-30 g were obtained from KSHEMA, Deralakatte Mangalore. These animals were used for the acute toxicity and antiepileptic studies.

The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 60 ± 5 % relative humidity and 12 h light dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co. Mumbai and water ad libitum throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output. Guidelines of IAEC of KSHEMA, Deralakatte, Mangaluru (KSHEMA/AEC/01/2002) was followed during experimentation.

2.3.1. Selection of doses

For the assessment of all the biological activities, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50 % of the one tenth dose, and a high dose, which was twice that of one tenth dose. (200 mg/kg, 100 mg/kg, 400 mg/kg).

2.4. Assessment of Antiepileptic Activity

2.4.1. Maximal Electroshock (MES) Induced Convulsions

The convulsive effect of MES [10, 11] was considered to be analogues to grandmal type of convulsion in man. Five groups of 6 rats of body weight 200-250 mg were selected for the study. Group III, IV, V received the test drug (100,200,400 mg/kg body weight) of fruit extract of Indian filbert respectively. Group II received the standard drug

(Phenytoin 25 mg/kg) and group I served as control. A supramaximal electrical stimulus of 150 mA was given to the animals for 0.2 sec through ear clip electrodes. Animals were observed and various phases of maximal electroshock seizures viz; tonic hind limb flexion, tonic hind limb extension and tonic clonic phases were noted. Abolition or decrease in the duration of extension phase was taken as an index of anticonvulsant activity. For statistical analysis of the data E/F (extensor / flexor) was used.

2.4.2. Metrazol Induced Convulsions [12]

Five groups of adult albino rats, each groups comprising of 6 animals were selected. For the group III, IV, V (test group) the test substance (100, 200,400mg/kg body weight) respectively was administered by oral route. Group II received standard drug Na valproiate, group V served as control. After 45 min, metrazol was administered by ip route in a dose of 80 mg/ kg of the body weight to all the groups and the animals were observed for onset of clonic convulsions upto 30 min after metrazol administration.

2.5. Statistical Analysis:

The results were expressed as mean ± SEM. The total variation present in the data was analysed by one way analysis of variance (ANOVA) followed by Post hoc test (Dunnett's test).

3. RESULTS AND DISCUSSION

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening, the fruits of Indian filbert showed and confirmed the presence of steroids, flavanoids, glycosides, triterpenoids and saponins as Secondary Metabolites

3.2. Acute toxicity studies

The ethanolic extract was found to be safe upto 2000mg/kg body weight.

3.3. Maximal Electroshock (MES) Induced Convulsions:

The duration of hind leg extension in rats with vehicle (control) was11.80±0.30 sec. Whereas rats treated with the ethanolic extract of fruits 100,200,400mg/kg.body weight exhibited hind leg extension for 8.49±0.24, 8.17±0.16, 3.84±0.15 sec respectively. The test groups of Indian filbert treated 100,200 & 400 mg/kg body weight protected animals from seizures and duration of hind leg extension was reduced. (**Table 1**)

Table 1. Seizures and duration of hind leg extension

Groups	Treatment (mg/kg body weight)	Duration in seconds(Mean±S.E.M)			E/F
		Flexor (F)	Extensor (E)	Tonic-clonic	
Control	5ml/kg	2.38±0.1	11.80±0.30	2.34±0.17	4.96±0.42
Phenytoin	25	2.06±0.20*	2.19±0.23*	2.64±0.25*	1.06±0.02*
Ethanolic extract of Indian filbert	100	2.53±0.15*	8.49±0.24*	2.59±0.13*	3.35±0.18*
	200	2.55±0.19*	8.17±0.16*	2.17±0.21*	3.21±0.25*
	400	2.52±0.22*	3.84±0.15*	8.69±0.14*	1.52±0.08*

* The mean difference is significant at the P< 0.001 level, when compared to the control group

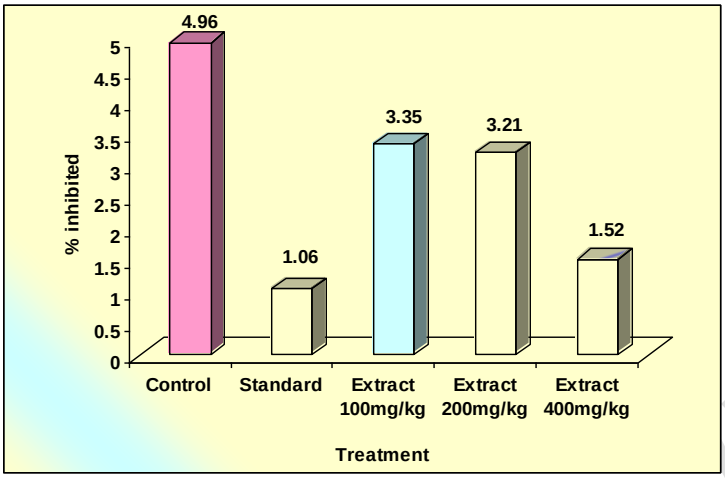

Figure1: Effect of Ethanolic Fruit Extract of Indian filbert On Maximal Electroshock Induced Convulsions

The ethanolic extract has protected the animals from the seizures and reduced the duration of hind leg extension at the doses of 100,200,400 mg/kg body weight, whereas the control did not show any protection, indicating that the ethanolic extract is effective against seizures induced by MES. Comparison of control with standard and test groups by one way ANOVA,where P<0.001 indicating the activity is very highly significant. The activity of a compound to prevent maximal electroshock seizures is believed to correlate with its activity to prevent the spread of seizure discharge through neural tissue. Activity against maximal electroshock seizures is thought to indicate potential efficacy in the treatment of major motor (grandmal) seizures.Phenytoin is the antiepileptic drug best known for its selective action in preventing maximal seizures [13].

3.4. Metrazol Induced Convulsions:

In animals treated with vehicle, clonic convulsions appeared 80.6±13.14 sec after metrazol administration and all the animals dead after seizures. The ethanolic extract of fruit extract at doses 100,200,400 mg/kg body weight delayed the onset of clonic convulsions at 603.1±67.76, 922.2±85, 1285±151.81 secs respectively. The ethanolic extract of fruits significantly and dose dependently delayed the onset of convulsions at doses 100,200,400 mg/kg body weight.(table 2)

Table 2: Effect of ethanolic fruit extract of Indian filbert on metrazol induced convulsions

Groups	Treatment (mg/kg body weight)	Onset of clonic convulsions (seconds)
Control	5ml/kg	80.6±13.14
Sodium valproate	80	2179.7±42.34*
Ethanolic extract of Indian filbert	100	603.1±67.76*
	200	922.2±85*
	400	1285±151.81

The mean difference is significant at the P <0.001 level, when compared to the control group

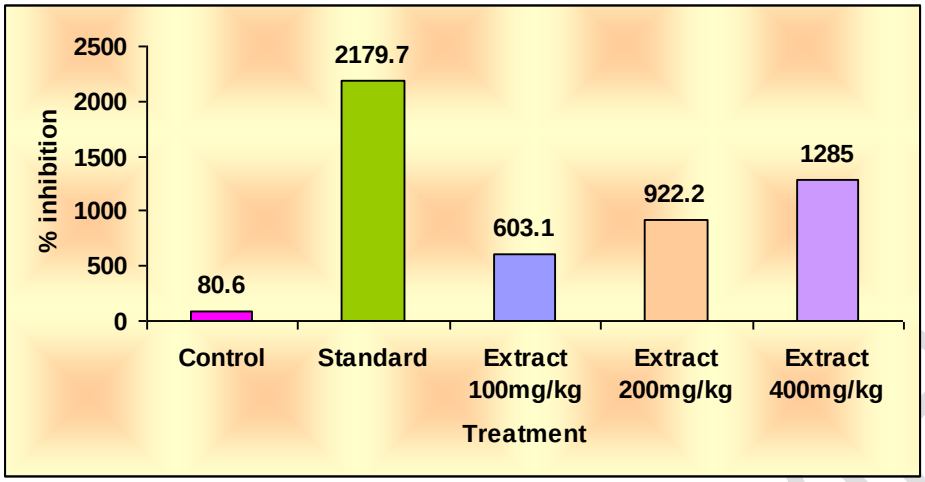

Figure2: Effect of ethanolic fruit extract of Indian filbert on metrazol induced convulsions

In case of metrazol induced convulsions, the ethanolic extract of fruits at doses of 100,200,400 mg/kg body weight delayed the onset of clonic convulsions, whereas control group convulsions appeared in a short duration. As the test group is compared with control group $P<0.001$, indicating the results are very highly significant. The ability of a compound to prevent threshold seizures induced by subcutaneous metrazol injection has been correlated with the ability to raise the threshold excitation of neural tissue. Selective action of this is believed to indicate potential efficacy against absence (petit mal) seizures [13].In both the test group animals as the dose increased, the protection from seizures was also increased.

Grandmal epilepsy is comparable to MES-induced seizures. In both experimental and clinical settings, evidence suggests that a mismatch between excitatory and inhibitory neurotransmission in the brain is a major contributor to seizure onset. In the CNS, gamma-amino butyric acid (GABA) is the most prominent inhibitory neurotransmitter. GABA functioning impairment is well known to cause seizures, whereas facilitation has an anticonvulsant effect. GABA is synthesized from glutamate by the enzyme glutamic acid decarboxylase, which is only found in GABAergic neurons. GABA activates on three distinct receptors, GABAA, GABAB, and the newly characterized GABAC, when it is released synaptically. Specific membrane-bound transport molecules carry GABA from the synaptic cleft into targeted neuron terminals and glial cells. GABA is either recycled to the readily releasable neurotransmitter pool (GABAergic nerve terminals exclusively) or converted by the mitochondrial enzyme GABA-transaminase [14, 15] to the inert compound succinic acid semialdehyde (neurons and glial cells). The convulsion in the MES method is caused by a disturbance in GABA activity in the brain.

Metrazol/PTZ is a commonly utilized chemical as well as an acute experimental model for testing prospective anticonvulsant medicines in preliminary screening. It works by acting as an antagonist at the GABAA receptor complex, which is thought to be how it works. Several biochemical explanations involving the inhibitory GABAergic system and the excitatory amino acid glutamate and aspartate system have been proposed. The mechanism by which Metrazol /PTZ is believed to exert its action is by acting as an antagonist at the GABAA receptor complex [16].

4. CONCLUSION

These findings suggest that the ethanolic fruit extract of Indian filbert possesses significant antiepileptic property, further one of the possible mechanism/s behind the antiepileptic activity of Indian filbert fruit extract may be due to enhanced GABA levels in the brain. Indeed, there is a scope for further studies to explore molecular mechanism and identify the phytoconstituents responsible for the anticonvulsant activity.

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" (KSHEMA/AEC/01/2002)

NOTE:

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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