Protective Effect of *Aqueous Extract of Lagenaria Siceraria* (Molina) Against Maximal Electroshock (MES) -Induced Convulsions in Albino Rats

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ABSTRACT

Background

The *Lagenaria siceraria* (Molina) belongs to family Cucurbitaceae, commonly known as bottle guard or calabash in English. All the parts of plant like root, fruit, leaves and flower has been evaluated for its various activities like antioxidant, antihelmintic, cognitive enhancer, anticancer, antianxiety, antidepressant, antihyperlipidemic, fibrinolytic cardio protective and hepatoprotective. Even though it is claimed to have antiepileptic action, no documentation is available.

Objective

To assess the anticonvulsant activity of aqueous extract of *Lagenaria siceraria* by Maximal Electroshock seizure induced seizure models on Albino rats.

Method

Albino rats were taken and divided into five groups, each consisting of five rats. One group was used as control (normal saline 10 ml/kg), one as standard (phenytoin), and three groups for the test drug (aqueous extract of Lagenaria siceraria (AELS) in the doses of 200, 400 and 800 mg/kg) treatment. In MES model, Maximal electrical shock of 150 mA was passed for 0.2 seconds through corneal electrodes after 30 minutes of giving the drugs and normal saline. Different stages of convulsions were noted down along with time spent by the animal in each phase of convulsions. Data were statistically analyzed by One way ANOVA followed by multiple Dunnett's test.

Result

The mean reduction in hind limb extension phase was 8.2±2.10 after 400 mg/kg of AELS which is highly significant ($p<0.001$) like phenytoin. AELS at 800 mg/kg exhibited a significant 17±2.64 ($p<0.05$) protection against tonic extensor phase.

Conclusion

Aqueous extract of Lagenaria siceraria has anticonvulsant activity.

KEY WORDS

Anticonvulsant, epilepsy, lagenaria siceraria, maximal electroshock

INTRODUCTION

Epilepsy is a common and disabling neurological disorder and a major health problem both in developing and developed countries.[1] An epileptic seizure is a brief episode of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.[2] It afflicts more than 50 million people worldwide; 5 million of them have seizures more than once per month.[3] A large number of newer molecules are currently under preclinical and clinical trials and several of these will undoubtedly become meaningful additions to the pharmacological armamentarium of antiepileptic drugs.[4] In spite of the vast number of drugs, there is no ideal antiepileptic agent with properties like broad spectrum activity, minimal side effects, good oral bioavailability and low cost.[5] The present day drugs exert important adverse effects, which includes central nervous system depression, ataxia, megaloblastic anemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity, etc.[6] Evidence of medicinal plants used in various traditional systems has been documented because of their safety and lack of toxic effects and are gaining popularity in most of the developing countries.[7,8] Plants and their phytoconstituents have important role in the development of a potent anti-convulsant agent.[9] Studies of many plants having anticonvulsant action has been documented in literature. One of them is *Lagenaria siceraria* also called Bottle gourd. Even though it claims to have antiepileptic activity scientific evidence is not available after extensive literary search, so the study is done to elucidate the antiepileptic potential of *Lagenaria siceraria* fruit juice on albino rats.

METHODS

Collection of plant and preparation of extract

The study was conducted during the period November 2014 to February 2015. The *Lagenaria siceraria* fruit (Bottle gourd) was collected from the local market at Khammam. The *Lagenaria siceraria* fruit was cut into smaller pieces, dried under shade for 10 days and pulverized to coarse powder using grinding machine and the AELS fruit was extracted by a soxhelts apparatus. The extractive value of AELS fruit was 18.2 gms by w/w. The extract was dried under vacuum, and stored at room temperature and protected from direct sunlight.

Drugs

Phenytoin obtained from Zydus Cadila Healthcare Limited and 0.9% normal saline (0.9% NaCl solution) was used in this study. The AELS fruit was administered orally in the dose of 200,400 and 800 mg/kg of body weight of animals.

Animals

Fifty healthy Albino rats weighing from 150-250 gms of either sex were taken from Central Animal House, Mamata Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained under normal room temperature of 23 ± 5.00C with a 12-h light/dark cycle, with free access to food (standard laboratory pellet diet) and water. Permission from Institutional Animal Ethics Committee was taken. The guidelines for the investigation of experimental seizures in conscious animals were followed in all.

Toxicity evaluation in Albino rats

The aqueous extract was tested for its acute toxicity in albino rats. Acute oral toxicity was performed as per OECD-423 guide lines[10] to determine the acute toxicity, the extract was administered orally in an ascending order and in widely spaced doses that is 0.25 g/kg, 0.5 g/kg, 0.75 g/kg and 1 g/kg in each group; the control albino rats received normal saline. The animals were observed periodically for forty eight hours. The parameters which were observed were hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions. There were no toxic effects and mortality up to 1000 mg/kg. These doses of 200, 400 and 800 mg/kg were selected and then compared with the control and standard group.

Experimental design

After acclimatization, the animals were randomly divided into five groups of five rats each (n=5).Group-I: Normal saline 25 ml/kg as control, Group-II: phenytoin 25 mg/kg, as standard, Group-III, IV and V received three graded doses of 200, 400 and 800 mg/kg of AELS respectively. The test samples were given orally 30 minutes prior to induction of convulsions.

MES-Induced Seizures

Corneal electrodes were used for bilateral delivery of electrical stimulus (Maximal Electroshock Seizures, MES-150mA; 50Hz; 0.2 Sec) using electro convulsiometer. Duration of tonic hind leg extension and of stupor was observed. Suppression of tonic hind limb extension was taken as a measure of efficacy and compared with the control and phenytoin group. All precautions were taken to minimize animals suffering. The animals were observed for the following 30 min, in a separate cage for latency of clonic convulsion, tonic extension, time of stupor and death.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out by using Analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests using Primer of Biostatistics (Stanton A, Glantz; Primer for Windows. McGraw-Hill, Inc., Version 5.0) (2011). P values < 0.05 were considered significant.

RESULTS

Evaluation was done by electro-shock using corneal electrodes after 30 minutes of administration of extract.

Table 1. Effect of aqueous extract of Lagenaria Siceraria in MES induced seizures in Albino Rats

Treatment Groups n=5	Duration of tonic Extension in sec. (Mean± SEM)	Stupor in sec.	Recovery/ Death
Group I (NS-25ml/kg)	23.8± 1.49	187.4 ±6.86	Recovered
Group II (Phenytoin-25mg/kg)	5±1.14**	62.4±12.09**	Recovered
Group III (AELS-200mg/kg)	24.2±2.43	148.8±4.85*	Recovered
Group IV (AELS -400mg/kg)	8.2±2.10**	79.6±10.33**	Recovered
Group V (AELS -800mg/kg)	17±2.64*	181.4±15.57	Recovered

AELS: Aqueous Extract of Lagenaria Siceraria **p<0.000-Highly significant * p<0.05- significant, n-No of animals

The duration of extensor phase in the control group was 23.8 ± 1.49 seconds. (Table 1) The standard drug phenytoin exhibited highly significant 5±1.14 seconds (p<0.001) reduction in hind limb extension phase. AELS in the dose of 200 mg/kg showed no effect in hind limb extension phase.

AELS at 400 mg/kg exhibited highly significant (p< 0.001) reduction in the onset of stupor just like phenytoin. AELS at 200 mg/kg also exhibited significant (p< 0.05) reduction in the onset of stupor. But higher dose of AELS (800 mg/kg) showed no effect on the stupor phase of seizures. There was no morbidity or mortality seen in any of the treated groups.

DISCUSSION

Epilepsy is one of the most common chronic serious neurological disorders of the central nervous system manifested by recurrent unprovoked seizures. Seizures are discrete; time limited alteration in brain function including changes in motor activity, autonomic function, consciousness, or sensation that results from an abnormal and excessive electrical discharge of a group of neurons within the brain. It affects approximately 50 million people worldwide.[2] MES-induced convulsion model is applied to screen drugs for generalized tonic-clonic seizures. The tonic extensor phase is selectively abolished by the drugs effective in generalized tonic clonic convulsions. MES causes several changes at the cellular level, disrupting the signal transduction in the neurons. MES causes cellular damage by facilitating the entry of Ca^{2+} into the cells in large amounts, prolonging the duration of convulsions.[11] Apart from Ca^{2+} ions, MES may also facilitate the entry of other positive ions like Na+, blockade of which, can prevent the MES-induced tonic extension.[12] Commonly prescribed anticonvulsant drugs like sodium valproate and phenytoin act by modulation of these ion channels.[13] The other drugs that antagonize NMDA receptors or potentiate opioids and GABA receptors are also reported to protect against MES-induced seizures.[14] Many drugs that increase the brain content of Gama amino butyric acid (GABA) like Phenytoin, sodium valproate etc have exhibited anticonvulsant activity against seizures induced by MES.[15] The current drugs used in treatment of epilepsy are associated with dose-related side effects, and chronic toxicity, and teratogenicity effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy.[16] Folk remedies are the basis for the discovery of many modern medicines. Many of the plants have been studied for their anticonvulsant properties.

The photochemical investigation of Lagenaria Siceraria ethyl acetate fraction revealed the presence of flavonoids. Flavonoids have been reported to process significant anticonvulsant activity in various plants.[17] Leaf extract of Globimetula braunii, Carissa carandas Linn.[18] Root Extract,[19] Methanolic extract of Holoptelea Integrifolia leaves,[20] Whole plant extracts of Melissa parviflora,[21] Leaf extract of panama pinnata,[22] all of them exhibited anticonvulsant activity in experimental animals. Phytochemical screening of all the above plants showed that the plants contains alkaloids, flavonoids, sterols, glycosides and saponins, to which the anticonvulsant activity of the plant extracts may be attributed.

Flavonoids, sterols and terpenoids have been implicated in various pharmacological actions on central nervous system including anticonvulsant and anxiolytic activity.[23,24] Triterpenic steroids and triterpenoidal saponins are reported to possess anticonvulsant activity in some experimental seizure models such as MES and PTZ.[25] The *Lagenaria siceraria* seeds contain steroidal moieties like avenasterol, codisterol, elesterol, isofucasterol, stigmasterol, sitosterol, compesterol, spinasterol; and sugar moieties including rhamnose, fructose, glucose, sucrose, raffinose and saponin.[26,27] The flavonoids in LS fruits are mainly isovitexin, isoorientin, saponarin, and saponarin 4'-O-glucoside.[28] All the above references confirm our study that *Lagenaria siceraria* has anticonvulsant activity in experimental animals. Extensive research should be done to discover the molecule in *Lagenaria siceraria* that has antiepileptic activity which may heap as an adjuvant to other anti-epileptic drugs.

CONCLUSION

This study provides pharmacological evidence for its use as anticonvulsant in folk medicine. The study concludes that aqueous extract of *Lagenaria siceraria* had exhibited significant anticonvulsant activity against electroshock-induced Seizure (MES) in albino rats.

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