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ANTICONVULSANT EFFECT OF AQUEOUS EXTRACT OF AERIAL ROOT OF *FICUS RELIGIOSA* IN ANIMAL MODELS

Smita Singh[1*], Md. Parwez Ahmad[1], Deependra Prasad Sarraf[2], Chandan Mishra[1], Prabin Kumar Singh[3],

[1]Department of Pharmacology, National Medical College Teaching Hospital, Birgunj, Nepal
[2]Department of Pharmacology, BPKIHS, Dharan, Nepal
[3]Department of Paediatrics, Narayani Sub-regional Hospital, Birgunj, Nepal

ABSTRACT

Ficus religiosa commonly found tree in Indian sub-continent has numerous neuro-pharmacological effects including epilepsy in traditional medicine. Therefore, anticonvulsant effect of aqueous aerial root extract of F. *religiosa* at oral doses 25, 50 and 100 mg/kg was studied using Maximum electroshock (MES) and Pentylenetetrazole (PTZ) induced seizure models in mice. F. *religiosa* showed anticonvulsant effect dose dependently in MES & PTZ test. In MES model F. *religiosa* 100mg/kg significantly ($p < 0.05$) lowered duration of Tonic hind limb extension. In PTZ model, all three doses of F. *religiosa* significantly ($p < 0.05$) increased latency to convulsion. These findings thus provide scientific evidence in support of the folkloric use of this plant in the management of epilepsy.

Keywords: Anticonvulsant, *Ficus religiosa*, Maximum electroshock, Pentylenetetrazole, Root

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***Address for Correspondence**

Smita Singh, Department of Pharmacology, National Medical College Teaching Hospital, Birgunj, Nepal, Email: ssktm3@gmail.com

INTRODUCTION

Epilepsy is a disorder of the brain characterized by tendency to have recurrent seizure, affecting at least 50 million people worldwide, among which 80% are in developing countries[1]. Despite of development of newer anti-epileptic drugs like Leviteracetam, Lamotrigine, Topiramate etc. Satisfactory seizure control has not yet been established. In addition anticonvulsant drugs have serious side effects which necessitates development of new drugs or lead molecules with more suitable margins of safety, efficacy, cost effectiveness and tolerability [2] . Hence search for the ideal natural anticonvulsant drug still continues as Global estimates suggest that about three- fourth of human population have been relying especially on plant derived traditional medicine as they are easily assesible, cheaper and people have belief that they have lesser side effects than allopathic medicine. Therefore these days the World Health Organization is encouraging, promoting and facilitating effective use of herbal medicine in developing countries[3].

F. *religiosa* commonly known as peepal, belongs to family Moraceae and is widely distributed in Indian subcontinent. It has mythological, religious and medicinal importance[4]. F. *religiosa* different parts have been used in traditional medicine to treat different disorders like tooth ache, sexual disorders, arthritis, elephantiasis, stomatitis, malarial fever, respiratory disorders including epilepsy [5]. Many of this traditional uses have been validated by scientific researches such as the Methanolic bark extract of F. *religiosa* has analgesic and anti-inflammatory effect[6], Anti-oxidant effect of aqueous bark extract of F. *religiosa* [7], Anti- ulcer activity of of ethanolic extract of stem bark [8], the Methanolic extracts of Fig possesses Anti-amnesic[9] and Anticonvulsant property of fig of F. *religiosa*[10]. Despite

of wide ethno-medicinal use of this plant only few experimentally validated results are available in use of adventitious roots as an anticonvulsant in Nepal. In the present study aqueous extract of F. *religiosa* has been prepared using soxhlet apparatus and anticonvulsant effect has been evaluated using maximal electrical shock (MES) and Pentylenetetrazole (PTZ) induced convulsion models in mice. This study might open a pathway for the development of plant derived new lead molecule for the treatment of Epilepsy.

MATERIAL AND METHODS

Design of study

Quantitative experimental study conducted in mice

Drugs and chemicals

Pentylenetetrazole (Sigma chemicals, USA), Phenytoin (M-Toin, Medopharm, India), Diazepam (Valium, Piramal Healthcare, India) were used in the experiment.

Collection of plant material and preparation of extract

The aerial root of F. *religiosa* was collected from garden of BPKIHS, Eastern part of Nepal, with Latitude and Longitude 26° 49′ 0″ N and 87° 17′ 0″ E respectively. The specimen of test drug was deposited in National Herbarium and Plant Laboratories, Kathmandu (The voucher no is 5021). The material was washed, shade dried for seven days and then grinded to fine powder. About 10 gm of fine powder was taken in clean sterile Soxhlet apparatus (Jain Scientific Glass Works Ambala Cantt; Extraction Pot: 250 ml; Soxhlet chamber size: 100 ml; Heater: DICA India) and extracted with 150 ml of distilled water continuously for 6 hrs. The extract obtained was filtered with Whatman filter paper 1. The filtrate was evaporated at 50ºC for a brief time interval, stopped just before the apparently saturated solution precipitated and left in room temperature till the moisture dried. Finally percentage yield was 20 %(w/w) which were used depending upon the experiment.

On the day of experiment, 2.5mg/ml, 5mg/ml and 10mg/ml solution in distilled water were prepared such that 1ml/100gm mouse body weight could be given in test drug groups for desired test dose of 25mg/kg, 50mg/kg and100mg/kg respectively. The test drug F. *religiosa* and vehicle control were given through oral route with the help of oro-gastric tube whereas standard control route varied with the experiment [11].

Animals

Healthy, twelve weeks old, Swiss albino mice (25-30gm) of either sex bred in the breeding house of Bisheshwar Prasad Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal were used. The study was conducted in the Laboratory of Department of Clinical Pharmacology and Therapeutics of BPKIHS. Experiments were performed between 8:00 and 16:00 h in March-May 2014 when the average temperature was between 11 to 21°C. The animals were maintained under controlled room temperature (22±3°C) light and dark (12:12hour) conditions. They were given food pellets and water ad libitum but fasted overnight before the experiment. Animals were randomly selected and six mice were used per group in all experiments. All experimental protocols were approved by the Institutional Ethical Review Board of BPKIHS. (No: 898/070/071).

Phytochemical screening

It was done qualitatively to identify the presence of various chemical constituents. Glycoside was detected by water and sodium hydroxide solution, flavonoids with Mg and HCl, tannins with ferric chloride solution, steroids with chloroform and sulphuric acid whereas saponins by the capability of extract to produce suds. These were identified by characteristic color changes using standard procedures [12].

Acute toxicity study

A toxicity study of the aqueous extract of F. *religiosa* was performed according to OECD guideline no. 425 using Swiss albino mice (25 -30g). Six mice were serially administered with the aqueous extract of F. *religiosa* upto dose limit of 2000 mg/kg as recommended in the guideline. After administration of the dose, each animal was observed every hour for signs of toxicity and abnormality in behavior up to 48 hours. Subsequently daily observations were made for toxicity and mortality up to 14 days[13].

Experimental design

The animals were divided into six in each group of control, standard control and test drug in each experiment. The experimental animals were given drugs as following.

Group 1: Distilled water/ PTZ 40mg/kg IP

Group 2: F. *religiosa* 25 mg/kg

Group 3: F. *religiosa* 50 mg/kg

Group 4: F. *religiosa* 100mg/kg

Group 5: The standard control varied with the experiment

Maximal Electroshock Seizure (MES) test

One hour after oral drug administration mice were subjected to alternating current of 150 mA from a convulsiometer (Techno, India) for 0.2 sec through a pair of electrodes attached to each ear[14]. Each animal was observed for 2 complete minutes. Parameters observed and documented a. Duration of tonic hind limb extension (THLE).

b. Percentage of animals protected against seizure (PAS) in one hour.

Phenytoin (20mg/kg per oral) was the standard control for this test [15].

Pentylenetetrazole (PTZ) induced seizure

After forty five mins of post dosing with the test drug (F. *religiosa*) mice were given PTZ 40mg/kgIP [14]. Diazepam (4 mg/kg; IP) served as standard control[16] which was administered fifteen mins prior to experiment. Parameters observed and documented

a) Latency to seizure onset.

b) Percentage of animals protected against seizure (PAS) in one hour.

Seizure was defined as jerky movements of whole body or convulsion [17]. Each mouse was observed for one whole hour for the occurrence of seizure.

Statistical analysis

All data were presented as Mean ± Standard Error of Mean (SEM). Statistical differences between the test drug and standard control groups were calculated using Mann – Whitney U test. Results were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

In the acute toxicity study, neither death nor any observable neurobehavioral effects were observed. Due to lack of observable toxicity, LD50 wasn't determined. In the preliminary dose determining study, we arbitrarily selected dose of 200 mg/kg p.o. of F. *religiosa* and evaluated its activity in the PTZ (40 mg/kg, i.p.) induced convulsions in mice. However, at this dose of the extract, PTZ-induced convulsions were completely inhibited. Owing to the high potency, a subsequent study was conducted at reduced doses of 100, 50 and 25 mg/kg.

The anticonvulsant effect of aerial root of F. *religiosa* was determined using Maximal Electroshock seizure and Pentylenetetrazole induced seizure models.

MES is a valid model of screening potential anticonvulsant drugs as well as to identify compounds which prevent seizure spread[18]. The drugs which inhibit MES induced seizures mostly inhibit the sodium current and block the repetitive firing of neurons[19].

PTZ induces clonic seizures[18] by acting as an antagonist at the γ-aminobutyric acid type A ($GABA_A$) receptor. So, convulsions produced by PTZ can be effectively blocked by Diazepam which increases frequency of opening of $GABA_A$ receptor [20].

In our study aqueous aerial root extract of F. *religiosa* dose dependently decreased the duration of tonic hind limb extension and latency to onset of convulsion in MES and PTZ induced seizure models respectively. In MES test, F. *religiosa* 100 mg/kg showed 100% protection against seizure in 1 hr similar to that of Phenytoin. Similar to Diazepam, FR 100 mg/kg completely inhibited the onset of PTZ induced seizure. In both the test models, the extract demonstrated a significant increase in latency of onset of convulsion ($p < 0.05$) compared with the vehicle control in unprotected animals. (Table 1&2)

Table 1: Comparison of mean duration of tonic hind limb extension in MES test

Experimental Groups	Mean duration of THLE± SEM	PAS(%) in 1 hr
Vehicle control	13.50 ± 0.42	0
Phenytoin	-	100
F. *religiosa* 25mg/kg	4.50 ± 2.84[a]	66.67
F. *religiosa* 50mg/kg	2.33 ± 2.33[a]	83.34
F. *religiosa* 100mg/kg	-	100

n=6, Values are expressed as Mean ± SEM, Mann – Whitney U test, [a]P<0.05v/s vehicle control.

Table 2: Comparison of mean latency of convulsion in PTZ induced seizure

Experimental Groups	Mean latency of convulsion± SEM	PAS(%) in 1 hr
Vehicle control	65.33 ± 0.71	0
Phenytoin	-	100
FR 25mg/kg	134.66 ± 19.42[a]	0
FR 50mg/kg	152.50 ± 24.37[a]	0
FR100mg/kg	-	100

n=6, Values are expressed as Mean ± SEM, Mann – Whitney U test, [a]P<0.05v/s vehicle control

The preliminary phyto-chemical analysis revealed the presence of flavonoids, glycosides, tannin and saponin. Different literatures have shown that saponin has its anticonvulsant effect due to blockade of voltage dependent Na+ channels[15], Modulation of GABAergic functions[16], blockade of NMDA receptor[17]. Thus our study scientifically proves the use of root of F. *religiosa* in epilepsy which might be due to presence of saponin.

CONCLUSION

Results of the present study clearly indicate that oral administration of aqueous aerial root extract of F. *religiosa* at different doses of 25, 50 and 100 mg/kg in MES and PTZ induced seizure model produces a significant dose dependent anticonvulsant activity. Observations of the present study could justify, the folkloric use of this plant in the management of epilepsy. However further studies are required to establish its exact mode of action, isolation and characterization of constituents responsible for activity.

DECLARATION OF INTEREST:

The author reports no conflict of interests**.**

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