



Received 28 February, 2010; received in revised form 15 April, 2010; accepted 25 April, 2010

EVALUATION OF ANTICONVULSANT ACTIVITY OF *HIBISCUS ROSA SINESIS* FLOWER EXTRACTS

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ABSTRACT

Keywords:

Anticonvulsant;
MES,
INH,
Hibiscus rosa sinensis

Objective of the study was to assess the anticonvulsant activity of extracts of *Hibiscus rosa sinensis* in albino rats. Methods: The anticonvulsant activity of extracts of flowers of *Hibiscus rosa sinensis* in rats were assessed using maximum electroshock seizure (MES) test and isoniazid (INH) using albino rats. The time taken before the onset of clonic convulsions, the duration of clonic convulsions and mortality protection were recorded. Results: The alcoholic extract of *Hibiscus rosa sinensis* flowers decreased the duration of hindlimb extension; clonus and stupor phase of MES induced convulsions as compared to control. However, all the extracts of flowers of *Hibiscus rosa sinensis* Linn. did not show any significant anticonvulsant activity against INH induced seizures. Conclusion: The alcoholic extract of *Hibiscus rosa sinensis* inhibits MES -induced convulsions but not INH-induced convulsions in animal models.

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INTRODUCTION: Epilepsy is characterized by recurrence of seizures, defined as the manifestation of paroxysmal and disordered neuronal discharges in the brain. Seizures can vary widely in their clinical presentation, depending on site, extent and mode of propagation of the paroxysmal discharge and hence now looked at as spectrum of clinically different varieties rather than a single disease^{1, 2}. In contemporary society, the frequency and importance of epilepsy can hardly be overstated from the epidemiologic studies. However, in most studies, the overall incidence of epilepsy in developed societies has been found to be around 50 cases per 100,000 persons per year, and rises steeply in older age^{3, 4}.

The current therapeutic treatment of epilepsy with modern antiepileptic drugs (AEDs) is associated with side-effects, dose-related and chronic toxicity, and teratogenic effects, and approximately 30% of the patients continue to have seizures with current AEDs therapy^{3, 5}. Antiepileptic drug (AED) therapy remains far from optimal. In many patients, the presently available AEDs such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione, etc., are unable to control seizures efficiently. Furthermore, the dose-related neurotoxicity and other side effects associated with established AEDs limit their clinical use. The newer AEDs like oxcarbazepine, vigabatrin, lamotrigine, gabapentin, felbamate, etc., represent a real progress in the treatment of nonresponders or refractory patients. However, the problem of adverse effects

has also not been circumvented completely⁶. Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested on modern bioassays for the detection of anticonvulsant activity and many such plants remain to be scientifically investigated⁷. Hence, search should continue to develop newer, more effective, and safer neuroprotective agents for treatment of epilepsy.

Hibiscus rosa sinensis, Linn. (Family Malvaceae) is a conspicuous, ornamental, evergreen, glabrous shrub native to tropical Asia⁸. A literature survey reveals that various parts (roots, flower and leaves) of *Hibiscus rosa sinensis*, Linn. are used in Ayurvedic system of medicine for its different pharmacological activities. Roots are used to treat venereal diseases, fever, cough, gonorrhoea and menorrhagia⁹. In traditional medicine, leaves are used as diuretic, emollient and laxative¹⁰. Flowers are used in folklore medicine as demulscent, emollient, refrigerant, aphrodisiac, brain tonic and cardiogenic. A decoction of flowers is useful in bronchial catarrh, menorrhagia, and fertility control¹¹.

The flowers of *H. rosa sinensis* are traditionally used in the treatment of epilepsy¹². The claim of therapeutic success of the plant in the treatment of epilepsy has not been scientifically scrutinized. The main aim of this project was, therefore, to investigate the anticonvulsant effect of *Hibiscus rosa sinensis* L. flower extracts in rats. The present study was carried out to evaluate anticonvulsant effect of *Hibiscus rosa*

sinesis, flowers by maximal electroshock seizure (MES) and isoniazid-induced seizures.

MATERIALS AND METHODS:

Plant material: *Hibiscus rosa sinensis*, flowers was collected from the Belgaum, Karnataka. The plant was authenticated by Mr. P.S.N Rao Joint Director of Botanical Survey of India, Pune India. A voucher specimen (No. RB- 104) of plant material was kept at Pharmacognosy museum of K. L. E. S's College of Pharmacy Belgaum, Karnataka, India for future reference.

Preparation of the extract: The flowers were shade dried at room temperature and finely powdered with help of a hand-grinding mill in such a way that the powdered material passed through sieve no 40. The powdered of fruit of *Hibiscus rosa sinensis* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents successively in increasing order of polarity from petroleum ether, chloroform, alcohol and finally fresh aqueous extract (chloroform: water)¹³. Each extract was concentrated in rotary flash evaporator under vacuum and dried over anhydrous sodium sulphate. The extracts were subjected to preliminary qualitative chemical analysis¹⁴. The yield was petroleum ether (40-60°C) 1.71 g, chloroform 1.063 g, alcohol 4.12 g and aqueous extract 14.17 g. The suspensions of all the extracts were prepared by using 0.5% Tween 80 in normal saline for the experiment.

Drugs and chemicals: Phenytoin sodium (Eptoin Acme formulation Pvt. Ltd., India),

isoniazid (Isonex Pfizer Pvt. Ltd.) were dissolved in normal saline. Fresh drug solutions were prepared on the day of the experiment and administered intraperitoneally (i. p.).

Animals: Studies were carried out on *Wistar* albino rats of either sex weighing 150-200 g were obtained from animal house, Department of Livestock Production, Government Veterinary College, Hebbal, Bangalore, India. Total thirty six rats were housed in groups of six in standard laboratory conditions of temperature (23± 91°C), relative humidity (55±95%), lighting (08:00–20:00 h) with food and water freely available. They were fasted 8 h before the experiments, but allowed free access to water. The study was approved by the Institutional Animal Ethical Committee.

Acute toxicity studies (LD50): The acute oral toxicity study was carried out as per guidelines set by Organisation for Economic Cooperation and Development (OECD). The median lethal dose of the petroleum ether (40-60°C), chloroform, alcohol and aqueous was determined by orally administering the extracts in increasing dose levels of 1, 2, 3, 4 and 5 g/kg body weight to healthy adult *Wistar* albino rats of either sex. The animals will be observed continuously for 2 h under the following profiles:

- I. Behavioural profile: Alertness, restlessness, irritability and fearfulness.
- II. Neurological profile: Spontaneous activity, reactivity, touches response, pain response and gait.
- III. Autonomic profile: Defecation and urination.

After a period of 24 h they will be observed for any lethality or death (% of mortality).

Assessment of anticonvulsant activity:

Maximal electroshock seizure (MES) test:

A total of thirty six rats were divided into six groups. Group 1 received 1 ml/rat of saline, group 2 received 25 mg/kg of Phenytoin, groups 3, 4, 5 and 6 received 250 mg/kg of petroleum ether (40- 60°), chloroform, alcohol and aqueous extracts respectively of *Hibiscus rosa sinensis*. The saline and standard reference drug were administered 45 min before induction of seizure, where as the test extracts of *Hibiscus rosa sinensis* were administered 1 h before induction of seizure.

To induce convulsions in the control and drug treated animals, the maximal (tonic hind limb extension) electroshock seizure (MES) test with supra maximal stimulation was carried out via transauricular copper electrodes (introduced bilaterally into the ears) with the apparatus (Inco Electro-convulsometer model# 100-3), using a fixed current 150 mA in rats for 0.2 s. The tonic extension of the hind limbs (extensor phase) and mortality were recorded.

Isoniazid (INH)-induced seizures test: A total of thirty six female rats were divided into six groups. Group 1 received 1 ml/rat of saline, group 2 received 25 mg/kg of phenytoin, groups 3, 4, 5 and 6 received 250 mg/kg of petroleum ether (40-60°), chloroform, alcohol and aqueous extracts respectively of *Hibiscus rosa sinensis*. INH (250 mg/kg b. w.) was injected intraperitoneally to induce convulsions in

the animals of all six groups 45 min to 1 h prior to administration of saline, standard drug and different extracts of *Hibiscus rosa sinensis*.

Statistical analysis: The data on the onset of tonic convulsions were analysed using one-way analysis of variance (ANOVA) followed by Dunnett's 't' test. The analysis of the number of animals convulsing was done using the Chi-squared test.

RESULTS: The preliminary phytochemical investigation revealed the presence of carbohydrate, steroids, flavonoids, triterpenoids and glycosides in methanol and aqueous extracts.

Acute toxicity: All extracts were found to be safe in the doses used and there was no mortality up to a dose of 200 mg/kg, b. w. Because of this the LD₅₀ for pet-ether, chloroform, alcohol and aqueous extracts was considered as 2 g/kg body weight and the ED₅₀ (1/10th dose LD₅₀) for these extracts was taken as 200 mg/kg.

Maximal electroshock seizure (MES) test:

In Supra maximal electro-shock seizure test it was observed that the alcoholic extract of *Hibiscus rosa sinensis* flowers decreased the duration of hindlimb extension by (4.82 ± 0.347 s) which is most significant (p < 0.0001) when compared to effects produced by control (14.46 ± 0.826 s), pet-ether extract (16.09 ± 0.678 s), chloroform extract (14.69 ± 0.610 s) and aqueous extract (8.85 ± 0.553 s) of *Hibiscus rosa sinensis*. The alcoholic extract also decreased the duration of clonus (14.74 ± 0.961s) and stupor (89.85 ± 1.946s) phase of MES induced convulsions as compared to control (Table 1).

TABLE 1: EFFECT OF *HIBISCUS ROSA SINESIS* EXTRACTS AGAINST MES INDUCED SEIZURES IN RATS

Group	Treatment (mg/kg b.w.) (Dose)	Time (s) in Various Phases of Convulsions (Mean ± SEM)				Recovery/Death
		Flexon	Extensor	Clonus	Stupor	
1	Control (Saline 1 ml/rat)	3.01 ± 0.24	14.46 ± 0.82	18.81 ± 1.94	105.4 ± 1.109	Recovery
2	Standard Phenytoin (25)	1.57 ± 0.17**	0.00 ± 0.00***	31.10 ± 1.371***	66.80 ± 1.16**	Recovery
3	Pet. Ether (40-60°C) Extract (200)	2.44 ± 0.23	16.09 ± 0.67	13.90 ± 1.30	101.21 ± 1.29	Recovery
4	Chloroform Extract (200)	2.35 ± 0.25	14.69 ± 0.61	32.42 ± 1.03***	97.44 ± 0.87***	Recovery
5	Alcohol Extract (200)	2.47 ± 0.16	4.82 ± 0.34***	14.74 ± 0.96	89.85 ± 1.96***	Recovery
6	Aqueous Extract (200)	3.71 ± 0.19	8.85 ± 0.55***	31.14 ± 1.15***	61.08 ± 1.156***	Recovery

n=6, **p<0.01 significant, ***p<0.0001 most significant, (Compared with the respective control) One way Anova, followed by Dunnett's multiple comparison tests

Isoniazid (INH)-induced seizures test:

From the statical data obtained from the anticonvulsant effect of flowers of *Hibiscus rosa sinensis* Linn. against INH induced seizure, it was revealed that all the extracts of flowers of *Hibiscus rosa – sinensis* Linn did not show any significant anticonvulsant activity against INH induced seizures and there was incidence of 100% mortality in all treated and untreated groups except standard group.

TABLE 2. EFFECT OF *HIBISCUS ROSA SINESIS* EXTRACTS AGAINST INH INDUCED SEIZURES IN RATS

GROUP	TREATMENT (MG/KG B.W.) (DOSE)	No. of rats in each group(n=6) showing		% of Mortality
		Clonus	Tonus	
1	Control (Saline 1 ml/rat)	6	6	100%
2	Standard Phenytoin (25)	2	1	0%
3	Pet. Ether (40-60°C) Extract (200)	6	6	100%
4	Chloroform Extract (200)	4	6	100%
5	Alcohol Extract (200)	6	4	100%
6	Aqueous Extract (200)	6	5	100%

Data analysed by Chi square test; Note: n=6 in each group. P value summery not significant

DISCUSSION: The preliminary phytochemical investigation of different extracts of *Hibiscus rosa sinensis* revealed the presence of carbohydrate, glycosides, flavonoid, triterpenoid and steroids. The result of the current study indicate that the ethanol extract of *Hibiscus rosa sinensis* has delayed the onset of convulsions induction, and was able to decreased the durations of hind limb extension, clonus and stupor phases of MES induced convulsion as compared to control and the effects produced by petroleum-ether, chloroform and aqueous extract of the *Hibiscus rosa sinensis*. The standard drug phenytoin in a dose of 25 mg/kg body weight provided 100 % protection and also significantly reduced the duration of stupor when compared to control. In other words the alcoholic extract is able to decreased the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, which indicate the alcoholic extract does

posses potent anticonvulsant activity against generalized tonic clonic seizure (grand mal) while other extracts viz. Petroleum ether extract, chloroform and aqueous extracts of *Hibiscus rosa sinensis* did not showed statistically an significant effect in extensor phase as compared to control. In the present study, all the extracts of flowers of *Hibiscus rosa sinensis* Linn. did not show any significant anticonvulsant activity against INH-induced seizures and there was incidence of 100% mortality in all treated and untreated groups except standard group.

In conclusion, the data obtained in present study indicated that alcohol extract of *Hibiscus rosa sinensis* may be said to exert its anticonvulsant effect against MES- induced seizures via non-specific mechanisms and no anticonvulsant effect against INH-induced seizures. However more extensive study on mechanism of action and safety of the plant as medicinal remedy has to be carried out.

ACKNOWLEDGEMENT: We thank KLES's College of Pharmacy Belgaum Karnataka.for providing all the facilities to conduct this work.

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