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ISSN: 0975-7538

Research Article

Evaluation of anti-epileptic activity of chloroform extract of *Gossypium herbaceum* leaves

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ABSTRACT

The present study was aimed to evaluate antiepileptic activity of chloroform extract of leaves of *Gossypium herbaceum* (CGH) in mice. The antiepileptic activity of CGH was evaluated in mice at 10, 30, and 100 mg/Kg, p.o. by using maximum electroshock (MES), Pentylentetrazole (PTZ) and Isoniazid (INH)-induced convulsions in mice. Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's t test. In MES and PTZ methods, CGH significantly protected mice from convulsions potent than Diazepam and Phenobarbitone sodium (PS). In INH method, CGH slightly delayed the onset of convulsions. It may be concluded that CGH exhibited significant and dose-dependent antiepileptic activity potent than Diazepam and PS.

Keywords: Epilepsy, *Gossypium herbaceum*, Pentylentetrazole, Isoniazid.

INTRODUCTION

Epilepsy is a neurological disorder, which does not have any boundaries such as age, race, social class or nationality. The incidence in developing countries is reported to be 57 per 1000 which is higher than that in developed countries.

Currently available anti-epileptic drugs (AEDs) are associated with dose-related side effects and chronic toxicity which involves every organ system virtually. There is a pressing need to find drugs with lesser adverse effects. Search for alternative anti-epileptic agents with lesser adverse effects has made man turn to exploit medicinal plants.

Literature survey reveal that plants which are rich in antioxidant principles like *Brassica nigra* *Crinum ornatum*, *Cyperus rotundus*, (Devi *et al.*, 2008; Oloyede *et al.*, 2010; Khalili *et al.*, 2011) exhibited significant protection against epilepsy. Recent reports revealed that *Gossypium herbaceum* leaves also contain antioxidants (Kumar *et al.*, 2011). Hence, present study designed to evaluate the antiepileptic activity of chloroform extract of *Gossypium herbaceum* leaves.

MATERIALS AND METHODS

Drugs and Chemicals

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Received on: 13-10-2017
Revised on: 30-10-2017
Accepted on: 06-11-2017

Diazepam (Ranbaxy), Isoniazid (S.D Fine-Chem. Ltd), Pentylentetrazole (Sigma Aldrich Chemical Co.) and Phenobarbitone sodium (Bayer AG).

Plant collection

The leaves of *Gossypium herbaceum* were collected from Nellutla, Warangal, Andhra Pradesh, India. The leaves were authenticated by Prof. V. S. Raju, department of Botany, Kakatiya University with voucher number 1865.

Preparation of the extract

The fresh leaves of *Gossypium herbaceum* were collected, shade dried and were made in to coarse powder. Then chloroform extract was prepared by following maceration method (Tekwu *et al.*, 2012).

Preliminary Phytochemical Studies

Chloroform extract of plant was screened for various phytoconstituents like alkaloids, carbohydrates, flavonoids, lipids, proteins, saponins, steroids and tannins (Kokate, 1994).

Pharmacological Investigations

Animals

Young adult male Swiss albino rats (150-180 g) and male Swiss albino mice (25-30 g) were procured from M/s Mahavir Enterprises, Hyderabad. The rats and mice were given a standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) of Talla Padmavathi College of Pharmacy, Warangal, Andhra Pradesh (CPCSEA no. 1505/PO/a/11/CPCSEA).

Acute toxicity studies

Acute toxicity study was performed as per OECD 420 guidelines. Young adult male Swiss albino rats and Swiss albino mice were used. The chloroform extract of *Gossypium herbaceum* leaves was tested in both the species upto a dose of 2000 mg/kg, body weight (Veeraraghavan *et al.*, 2000).

Evaluation of Anti-epileptic activity

Maximum Electroshock (MES) in mice

Mice were divided into five groups of six mice each. Treatment schedule is as follows.

Group I: Control (DMSO)

Group II: CGH (10 mg/kg), p.o.

Group III: CGH (30 mg/kg), p.o.

Group IV: CGH (100 mg/kg), p.o.

Group V: Diazepam (3 mg/kg), p.o.

The test was started one hour after oral treatment with the extract or the vehicle or the standard. Electroconvulsimeter with corneal electrodes (45 mA, 50Hz for 0.2 sec) was used to induce Tonic hind limb extensions (THLE). Percentage of inhibition of convulsions relative to control was calculated (Vogel, 1997).

$$\text{Percentage of Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Pentylenetetrazole (PTZ)-induced convulsions

Mice were randomly allotted to five different groups of six each. Treatment schedule is as follows.

Group I: Control (DMSO)

Group II: CGH (10 mg/kg), p.o.

Group III: CGH (30 mg/kg), p.o.

Group IV: CGH (100 mg/kg), p.o.

Group V: Phenobarbitone sodium (40 mg/kg), i.p.

Mice belonging to Group I, II, III and IV were administered with pentylenetetrazole (PTZ) (75 mg/kg, i.p.) one hour after vehicle and extract treatments. Mice belonging to Group V received PTZ, 15 min after phenobarbitone sodium (40 mg/kg, i.p.). Onset time as well as duration of convulsions were recorded (Dhanasekaran *et al.*, 2010).

Isoniazid (INH)-induced convulsions

Five Groups of six Swiss albino mice were used. Treatment schedule is as follows.

Group I: Control (DMSO)

Group II: CGH (10 mg/kg), p.o.

Group III: CGH (30 mg/kg), p.o.

Group IV: CGH (100 mg/kg), p.o.

Group V: Diazepam (4 mg/kg), i.p.

One hour after the administration of vehicle or chloroform extract of *Gossypium herbaceum* leaves, isoniazid at a dose of 300mg/kg, s.c. was administered to mice belonging to Group I, II, III, IV and 15 min after administration of diazepam to mice belonging to Group V. The latency of convulsions was recorded (Madhu *et al.*, 2009).

Statistical analysis

The data was analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test and p<0.05 was considered as statistically significant. The data was expressed as mean ± Standard deviation (SD).

RESULTS

Preliminary Phytochemical Studies

Preliminary phytochemical studies revealed the presence of steroids, flavanoids and lipids (Table 1).

Pharmacological Investigations

Acute toxicity studies

In acute toxicity study, the chloroform extract was found to be safe upto 600 mg/kg, p.o. So, the doses of 10, 30, and 100 mg/kg, p.o. were selected to evaluate antiepileptic activity.

Evaluation of Anti-epileptic activity

Maximum Electroshock (MES) in mice

The average onset, duration of THLE and percentages of inhibition of convulsions were given in Table 2. The time of onset of THLE in extract and standard treated animals was more and duration was less when compared to the control group animals.

Pentylenetetrazole (PTZ)-induced convulsions

The average onset, duration of convulsions and percentages of inhibition of convulsions were given in Table 3. The time of onset of convulsions in control group animals was very less and duration when compared to the extract and standard group animals.

Isoniazid (INH)-induced convulsions

The average latency of convulsions was given in Table 4. The latency of convulsions in control group animals was very less when compared to the extract and standard group animals.

DISCUSSION

Epilepsy is a serious neurological disorder, which does not have any boundaries such as age, race, social class or nationality. Different types of epileptic seizures have varied susceptibility to currently available AED and on the whole approximately two thirds of the patients

Table 1: Preliminary phytochemical studies

Phytoconstituents	CGH
Alkaloids	- ve
Steroids	+ ve
Carbohydrates	- ve
Tannins	- ve
Flavonoids	+ ve
Saponins	- ve
Lipids	+ ve
Proteins	- ve

Table 2: Effect of CGH on MES-induced convulsions

Group (n=6)=6	Treatment	Dose	Onset of THLE (sec)	Duration of THLE (sec)	Percentage inhibition of convulsions
I	DMSO	-	1.35±0.04	118.91±1.99	-
II	CGH	10 mg/kg	1.89±0.06**	49.24±0.85**	58.59**
III	CGH	30 mg/kg	2.61±0.19**	43.78±0.56**	63.18**
IV	CGH	100 mg/kg	3.76±0.05**	37.64±0.73**	68.35**
V	Diazepam	3 mg/kg	2.46±0.08**	49.37±0.74**	58.48**

CGH: Chloroform extract of *Gossypium herbaceum*; Values were mean±SD. ** $p < 0.01$ when compared to Group I (control).

Table 3: Effect of CGH on PTZ-induced convulsions

Group (n=6))	Treatment	Dose	Onset of Convulsions (min)	Duration of convulsions (min)	Percentage inhibition of convulsions
I	DMSO	-	7.43±0.11	18.57±0.40	-
II	CGH	10 mg/kg	14.47±0.08**	11.05±0.24**	40.53**
III	CGH	30 mg/kg	19.34±0.07**	7.04±0.03**	62.09**
IV	CGH	100 mg/kg	24.46±0.07**	2.51±0.05**	86.49**
V	Phenobarbitone sodium	40 mg/kg	4.51±0.09**	9.24±0.09**	50.23**

CGH: Chloroform extract of *Gossypium herbaceum*; Values were mean±SD. ** $p < 0.01$ when compared to Group I (control)

Table 4: Effect of CGH on INH-induced convulsions

Group(n=6)	Treatment	Dose	Latency of convulsions (min)
I	DMSO	-	25.21±0.35
II	CGH	10 mg/kg	32.03±0.24**
III	CGH	30 mg/kg	37.55±0.04**
IV	CGH	100 mg/kg	43.12±0.03**
V	Diazepam	4 mg/kg	63.27±0.13**

CGH: Chloroform extract of *Gossypium herbaceum*; Values were mean±SD. ** $p < 0.01$ when compared to Group I (control)

with epilepsy can have remission of seizures (Samrjn et al., 1997). Hence there is a pressing need to find drugs with lesser adverse effects.

Gossypium herbaceum leaves are enriched with antioxidant principles (Velmurugan et al., 2012; Velmurugan et al., 2013). Hence, present study designed to evaluate the antiepileptic activity of chloroform extract of leaves of *Gossypium herbaceum*.

Chloroform extract of leaves was prepared to predict which phytoconstituents are responsible for the remarkable antiepileptic activity. Preliminary phytochemical studies revealed the presence of flavonoids, lipids and steroids.

In acute toxicity study, the extract was found to be safe upto 600 mg/kg, p.o. So, the doses of 10, 30, and 100 mg/kg, p.o. were selected for the study. Antiepileptic activity of the extract was evaluated by three animal models in which GABAergic neurotransmission is involved viz. MES, PTZ and INH models.

A MES-induced convulsion is a suitable model for identifying compounds/extracts effective in grand mal epilepsy (Vogel, 1997). CGH treated mice exhibited significant and dose-dependent antiepileptic activity and more percentage inhibition at higher doses when compared to diazepam treated mice (58.48%, $p < 0.01$). As the extract was protecting mice from MES-induced

convulsions, it might become useful in treating grand mal epilepsy.

PTZ is a predictive model for identifying compounds/extracts effective in treating petit mal epilepsy (Vogel, 1997). Animals which received CGH exhibited significant and dose-dependent antiepileptic activity and more percentage inhibition of convulsions at higher doses when compared to Phenobarbitone sodium. As the extract was effectively inhibiting PTZ-induced convulsions, it might become useful in the treatment of petit mal epilepsy.

Isoniazid lowers the brain GABA levels in humans to approximately the same extent in rats and mice (Pieri *et al.*, 1985). It has been found that all the three doses of CGH significantly delayed the latency of convulsions in mice but failed to protect the mice against mortality.

CONCLUSION

Steroids were proved to be involved in neuromodulatory effects and many flavonoids are reported to act as benzodiazepine-like molecules in the central nervous system and modulate GABA-generated chloride currents in animal models of convulsion (Hernandez *et al.*, 2007; Asl *et al.*, 2007; Chauhan *et al.*, 1988). In addition to steroids, flavonoids were also present in CGH which might be responsible for the activity of CGH. This may be because of involvement of flavonoids and sterols in central inhibitory and neuromodulatory effects (Rocha *et al.*, 2002). So they might be responsible for the maximal activity of CGH.

Present study demonstrated that the chloroform extract showed maximum activity against PTZ-induced convulsions because CGH might increase the seizure threshold and antagonize the action of PTZ. Further the presence of flavonoids may partially contribute the significant activity of chloroform extract of leaves of *Gossypium herbaceum*. Present study evidences that plants rich in antioxidant principles can be used in the treatment of epilepsy.

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