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ANTI-CONVULSANT ACTIVITY OF SEEDS OF GOSSYPIUM HERBACEUM LINN.

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ABSTRACT

To investigate the phytochemicl constituents,Anticonvulsant activity and toxicity studies on the seed extract of *Gossypium herbaceum* Linn. Identification of major chemical constituents such as glycosides, flavonoides and tannins in methanolic and aqueous extracts of seeds of *Gossipier herbaceum linn was* carried out to confirm the presence of those in the extract. Extract contains Glycosides, carbohydrates, flavonoides, oils & fats and tannins. Particularly flavonoides and phenol compounds as a major chemical constituent. Therefore an attempt was made to separate and isolate the flavonoids from the methanolic extract of seeds of *Gossypium Herbaceous.* The authenticated seeds of *Gossypium herbaceum linn* were air dried the uniform powder of seeds of *Gossypium herbaceum* were subjected to physicochemical characterization parameters and quality control tests of various parameters which are prescribed in the pharmacopoeia literature. The methanolic extract of seeds of *Gossypium herbaceum linn* was used for separation and isolation of flavonoids by preparative thin layer chromatography. PTZ induced the median lethal dose (LD_{50}) of *Gossypium herbaceum* was 3000 mg/kg oral administration, respectively. *Gossypium herbaceum* methanol and aqueous extract produced 83% and 68% protection against convulsion respectively, compared with 100% protection with Diazepam.

KEYWORDS: Gossypium herbaceum Linn, Pentylenetetrazole, flumazenil, GABAA-benzodiazepine, Diazepam, Epilepsy.

INTRODUCTION

It is native to Indian subcontinent and the adjacent areas such as Pakistan, Nepal and the South-West of China. It is a common shrub found through out India. (The wealth of India. 1988) It is a small shrub 60 cm to 2.5meter in height with rigid sparsely pilose stems and branches; fruits are rounded beaked capsules 3-4 locular seeds usually with two coats of hairs white grey or reddish brown in colour(Arya vaidya, 2002). The chief constituents of seeds of *Gossypium herbaceum*. are gossypetin, gossypol, quercetin(Sala AV. 2005), betaine, choline and salicylic acid.(Taterson AH. 2010).

In Ayurveda, the seeds of *Gossypium herbaceum* are used in vitiated conditions of inflammations, gout, amentia, dizziness, dysentery(Nadkarni AK. 2002), orchitis, wounds, ulcers, bronchitis, agalactia, strangury, intermitent fevers and general debility(Lee H. 1988). The seeds are mainly useful in epilepsy and as an antidote to snake poison. Review of literature reveals that *Gossypium herbaceum* seeds have shown Antitumor(Lee WY), Antimicrobial (Miranda. D,) and Antifertility (Nath D Sethi), Antifungal activity(Piotr P. 2009), antioxidant activities (Lee CG 2001), antibacterial, *antihelmentic* & cytotoxic activity (LJ McGaw. 2005), Antibacterial(Reddy UM. 1981).

Epileptic activity will be carried out on *Gossypium herbaceum* because of its easily availability & popularity, exploring such drugs for their medicinal properties helps the society for a noble cause. So the present work is proposed for its antiepileptic properties, which has not been investigated or studied.

MATERIALS AND METHODS

Plant materials and preparation of the extract

In the present study, the seeds of *Gossypium herbaceum* were collected from the local areas of Dharward, Karnataka. The seeds were authenticated by Dr Harsha Hegde, Research Scientist, ICMR, Belgaum.(voucher-RMRC-488).

The seeds were then shade dried and subjected to physical evaluation. The dried seeds were subjected to size reduction to get coarse powder and Coarsely powdered seeds subjected to successive solvent extraction in a soxhlet using petroleum ether, chloroform and methanol as solvent. Finally the drug was macerated with distilled-water separately. Each time before extracting with the next solvent the powdered material will be air dried in hot air oven below

50^0C. After the effective extraction, the solvent was distilled off, the extract was then concentrated on water bath.

Animals

Swiss Albino rats of either sex (weighing 150–200 g) were obtained from Shri. Venkateshwara Enterprises, Bangalore. animals were maintained in a well-ventilated room, fed on Excel Feeds and water *ad libitum*. All studies using rats were approved by the Animal ethical Committee.

Drugs/chemicals and equipments

Pentylenetetrazole was purchased from Sigma–Aldrich Chemical Co (USA), diazepam was purchased from local market.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and empowerment, Govt. of India.

Identification of major chemical constituents such as steroids, flavonoides, triterpenoids, amino acids and tannins in extracts of seeds of *Gossypium herbaceum* was carried out to confirm the presence of those in the extract.

Screening for anticonvulsant activity

***Pentylenetetrazole (PTZ)-induced convulsion in* rats**

The anticonvulsant property of the drug in this model was assessed by its ability to protect against PTZ induced convulsions as per reference. The animals were weighed and selected for the experiment. Rats of either sex were used. The rats are then divided into six groups of six rats each. Penteletetrazole (70mg/kg b.w.) was administered intra-peritoneally to induce convulsions in the control and drug treated animals. Group -1 received saline, Group- 2 received 70mg/kg b.w. of PTZ & 4mg/kg of b.w. Diazepam. Groups-3,4, 5 & 6 received 300 mg/kg of b.w. petroleum ether, chloroform, methanolic and aqueous extracts respectively.

The reagents were prepared fresh; the concentration, dose, route and the duration before induction of convulsion.

Maximal electroshock-induced convulsion in rats

The anticonvulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions. The animals were first weighed and selected for the experiment depending on weight. Rats of either sex were used. The rats were then divided into six groups of six rats each. Group -1 received saline, Group- 2 received 25 mg/kg of Phenytoin, Groups-3, 4, 5 & 6 received 300 mg/kg of b.w. of petroleum ether, chloroform, methanol, aqueous extracts respectively. Maximal electroshock of 150 mA current for 0.2seconds administered through ear electrodes to induce convulsions in the control and drug treated animals. The reagents were prepared fresh; the concentration, dose and duration before induction of convulsion were observed.

Statistical analysis

Results were expressed as percentage (%) protection and mean±SEM where applicable. Statistical significance was tested using Student's *t*-test. The difference was taken to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity studies

The LD_{50} values were obtained for various extracts is 3000mg/kg. 1/10th of this lethal dose was taken as effective dose (therapeutic dose) for subsequent-anticonvulsant activity i.e 300 mg/kg b.w for all extracts.

Screening for anticonvulsant activity

The results of models used to evaluate the effectiveness of various extracts of seeds of *Gossypium herbaceum* were (1) Maximal Electroshock (MES) Seizure and (2) Pentylenetetrazole (PTZ) Seizure. (shown in Table no-1 and 2 respectively).

The MES model is generally used to evaluate the anticonvulsant drugs against generalized tonic-clonic seizure (grand mal) in rodents which is related to intensity of current stimulus and the dose. MES produced various phases of convulsion i.e. Flexion Extensor, Clonus and Stupor. The duration of tonic extension of the hind limb was used as end point i.e. prevention or decrease in the duration of hind limb extension was considered as a protective action.

The various extracts of seeds of *Gossypium herbaceum* were given orally with the help of gastric tube to rats. The result of all the extracts is compared with the result produced by

control. The data resulted from anticonvulsant effect of different extracts of seeds of *Gossypium herbaceum* showed that the methanolic extract of seeds of *Gossypium herbaceum* decreased the duration of hind limb extension by (9.898±0.2651**sec) which is most significant ($p<0.01$) when compared to control (15.33 0.3344sec) and the effects produced by petroleum-ether (40^0-60^0 C) ($14.87±0.2418^{NS}$ sec), chloroform extract (14.19±0.1414*sec) and aqueous extract (10.85±0.3583**sec) of the seeds of *Gossypium herbaceum linn.*

The methanolic extract of seeds of *Gossypium herbaceum* also decreases the duration of clonus (15.88±1.401sec) and stupor (63.25±1.546**sec) phase of MES induced convulsion as compared to control, clonus (25.31±0.6939 sec) and stupor (106.5±4.425sec).

In other words the methanolic extract is able to decrease the duration of hind limb extension (extensor phase), clonus and also the duration of stupor phase, which indicate the methanolic 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 seeds of *Gossypium herbaceum* did not show statistically any significant effect in extensor phase as compared to control.

The result of anticonvulsant effect of seeds of *Gossypium herbaceum* against MES and PTZ induced convulsions are shown in Table-1and 2.

From the statistical data obtained from the anticonvulsant effect of seeds of *Gossypium herbaceum* against PTZ induced seizure it was revealed that methanolic extract of seeds of *Gossypium herbaceum* showed highly significant anticonvulsant activity against PTZ induced seizures as compared to the effect produced by control group and other groups treated with extracts of seeds of Gossypium herbaceum methanolic extracts of seeds of *Gossypium herbaceum* not only delayed the onset action of seizures but all animals in the group were recovered. There was no incident of mortality in all the groups of animals treated with extracts of seeds of Gossypium herbaceum except in pet-ether and chloroform extract which shows 50% mortality.

The methanolic extract was also able to protect the all the animals in the group since there was no mortality was observed.

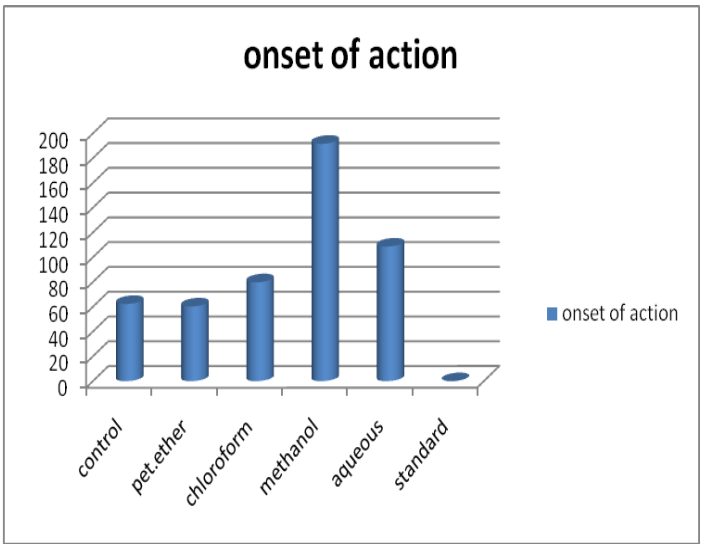
Overall the methanolic extract of seeds of *Gossypium herbaceum* plant able to show the most significant activity against MES and PTZ induced seizures.

Table No. 1: Effect of seeds of *Gossypium herbaceum*against MES Induced convulsions.

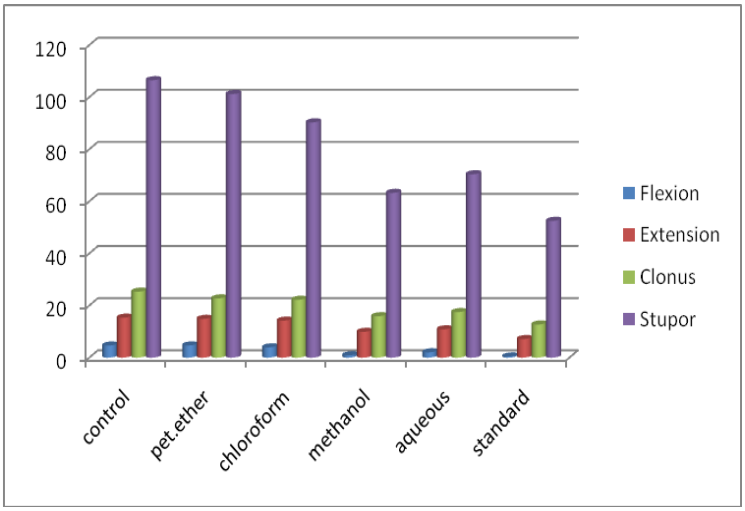
Drug	Dose mg/Kg b.w.	Time (Sec) in various phases of convulsions (Mean±SEM)				
		Flexion	Extension	Clonus	Stupor	Recovery
Control (Saline1ml/rat)	-	4.625±0.3237	15.33±0.3344	25.31±0.6939	106.5±4.425	Recovery
Pet. Ether Extract	300	4.667±0.1647 NS	14.87±0.2418 NS	22.72±0.6498 NS	101.2±1.627 NS	Recovery
Chloroform Extract	300	3.935±0.1285*	14.19±0.1414*	22.2±0.496*	90.32±1.61**	Recovery
Methanol Extract	300	0.865±0.072**	9.898±0.2651**	15.88±1.401**	63.25±1.546**	Recovery
Aqueous Extract	300	2.025±0.1063**	10.85±0.3583**	17.46±0.4989**	70.4±4.524**	Recovery
Standard phenytoin	25	0.4367±0.1333**	7.137±0.2267**	12.7±0.5107	52.51±1.842**	Recovery

Table No.2: Effect of seeds of *Gossypium herbaceum*against PTZ Induced Convulsions

Drug	Dose (mg/kg)	Onset time in Seconds (Mean±SEM)	Recovery/ Mortality
Control (Saline 1ml/rat)	-	62.33±0.7601	Mortality
Pet. Ether Extract +PTZ	300	60.33±6.163	50%Recovery
Chloroform Extract +PTZ	300	79.83±2.33*	50% Recovery
Methanol Extract +PTZ	300	191.7±7.149**	83% Recovery
Aqueous Extract +PTZ	300	108.7±2.836**	68% Recovery
Diazepam + PTZ	4	0	Recovery



Graph 1: Effect on PTZ induced seizers (delayed onset).



Graph 2: Effect on MES induced seizers (abolishing extensor phase).

CONCLUSION

The activity of *Gossypium herbaceum*may be due to natural phytochemicals such as flavonoids, tannins, steroids derivatives. In another study the plant was found to increase learning and memory performance in experimental animals.

It is concluded that seeds mainly contain phenolic compound which may be responsible for anticonvulsant activity, however other mechanisms may not be ruled out.

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