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ANTICONVULSANT ACTIVITY OF *CLEOME VISCOSA* SEED EXTRACTS IN SWISS ALBINO MICE.

Amrita Mishra[1,2], Arun K Mishra[1], Sanjay K Jain[2]

[1]Phytochemistry laboratory, College of Pharmacy, IFTM, Lodipur-Rajput, Moradabad-244001, [2]Institute of Pharmacy, Bundelkhand University, Jhansi-284128

ABSTRACT

The aim of present study was to evaluate the anticonvulsant effect of seed extracts of *Cleome viscosa* using MES and PTZ induced seizures models. The dried seeds were subjected to extraction in ethanol and water. The extracts were subjected to phytochemical tests and the carbohydrate, flavonols, coumarins, glycosides and steroid were found to be present. The ethanolic and aqueous extracts of the seeds of *Cleome viscosa* were observed for their anticonvulsant activity by Maximal Electroshock seizures (MES) test and Pentylenetetrazole (PTZ) test using swiss albino mice. Both the extracts showed significant activity in MES and PTZ induced convulsions in comparison to control. From the literature surveys as well experiments performed, it can be said that *Cleome viscosa* does poses anticonvulsant property.

Keywords: *Cleome viscosa,* Anticonvulsant, Phenytoin, Diazepam.

INTRODUCTION

Cleome viscosa Linn (Capparidaceae) also called "Dog mustard" is a herb that grow up to 1m height in India [1]. The seeds have no dormancy and germinate readily after shedding. The plant is said to be used by poor classes as vegetables. The seed oil (yield 18-37%) contains linoleic acid up to 70%, oleic acid 14%, palmatic acid 10%, stearic acid 5% as well as some volatile components[2]. A series of Coumarino-lignans (cleomiscosins) has been isolated and exhibited anti-hepatotoxic properties in tests with rats[3,4]. Several flavonoid, saponins and fatty acids have been isolated from the *Cleome viscosa*[5]. The rural people use the fresh juice of the crushed seeds of this plant for infantile convulsions and mental disorders[6,7]. In the present study; we have evaluated anticonvulsant activity of ethanolic and aqueous extract of seeds.

MATERIAL AND METHODS

The seeds of *Cleome viscosa* were collected from the university garden, Bundelkhand University, Jhansi, India and was authenticated by Dr. T. Hussain, Scientist in-charge, Herbarium section, National Botanical Research Institute, Lucknow, India. A voucher specimen no BU/M.Ph./C.V.-1 is preserved in our research laboratory for future references. After due authentication, the capsules were dried in shade and seeds were separated. Further the seeds were crushed to obtain coarse powder. All the solvents and chemicals were of pure analytical grade.

Preparation of extracts

The powdered material (500gms) was successfully extracted with ethanol and water separately in Soxhlet apparatus[8]. The liquid extract were concentrated separately and dried under vacuum. The dried extracts (ethanolic and aqueous) were preserved in desiccators until further use.

Animals

Swiss albino mice (20-30 gm) of either sex (Bred in Central Drug Research Institute, Lucknow, India) and Central Animal Facility of the Institute were used thorough the study for keeping. The animals were maintained at constant room temperature (22-25^0 ± 2^{0}C) and 12h – light/12-h dark cycle with food and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of Control and Supervision of Experimental

Animals (CPCSEA) (Regd. No. 716/02/a/CPCSEA).

Determination of Acute Toxicity

The acute toxicity studies of ethanolic and aqueous extract were determined in female swiss albino mice. The animals were fasted overnight prior to the experiment and fixed dose as OECD guideline No. 420 (Annexure 2d) method of CPCSEA was adopted for toxicity studies[9]. The extracts were administered in doses of 50, 300, 1000 and 2000mg/kg p.o. to different groups of mice each containing 10 animals and mortality were observed after 24 hrs. The ethanolic and aqueous extract of *Cleome viscosa* seeds were devoid of mortality of animals at dose of 2000 mg/kg in female albino mice by p.o. route and hence >2000 mg/kg was taken as LD_{50} cut off value and $1/10^{th}$ of the same i.e.200 mg/kg was selected for screening dose for further studies.

Maximum electroshock induced seizures (MES)

The maximal electroshock (MES) method was performed to induce the seizures in order to evaluate the anticonvulsant activity[10]. Mice deprived of food and water (*ad libitum*) for overnight, were randomly distributed in to eight groups of six animals each.

Group I served as control (vehicle treated), Group II served as standard (received Phenytoin sodium 25mg/kg body weight); Group III, Group IV and Group V were treated with alcoholic extract as 200, 400 and 600mg/kg body weight respectively, and Group VI, Group VII and Group VIII were treated with aqueous extract dose 200, 400 and 600 mg/kg body weight respectively. The test extract were administered orally in 2%v/v Tween 80 solution, 1hr prior to induce the convulsion and standard drug (Phenytoin sodium 25mg/kg) was administered i.p. 30 min. before. Electro convulsive shock (50 mA for 0.2 sec) was delivered through corneal electrode to induce convulsions to eight groups of mice (n=6). The various phases of convulsion which were produced are Flexion, Extension, Clonus and Stupor. Prior to delivery, current output was checked by multimeter. After the electric stimulation occurrence, the duration of phases were noted and HLTE (Hind limb tonic extension) phase was compared with control group. Decrease in duration of hind limb extension was considered as protective action.

Pentylenetetrazole (PTZ) Induced Seizures

For this protocol, the mice were divided in to eight groups of six animals each. Group I served as control (vehicle treated i.e. Tween 80, 2%), Group II served as standard received Diazepam 4mg/kg body weight (i.p.), Group III, Group IV and Group V were treated with alcoholic extract as 200, 400 and 600mg/kg body weight (p.o.) respectively and Group VI, Group VII and Group VIII were treated with aqueous extract dose 200, 400 and 600 mg/kg body weight (p.o.). 30 min after i.p. injection of Diazpam and 60 min after oral administration of extracts, 60 mg/kg PTZ was injected subcutaneously. The anticonvulsant properties of different extracts were accessed by its ability to delay the onset of myoclonic spasms and clonic convulsions.

Statistical Analysis

The results expressed as mean ± SEM and statistical analysis was carried out using one way ANOVA followed by Dunnett multiple test[11], where a

difference of P<0.01was considered significant in all cases.

RESULTS AND DISCUSSION

Maximal electroshock test

MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependant Na+ channels such as Phenytoin, Valproate, Felbamate and Lamotrigine or by drugs that block glutaminergic excitation mediated by the n-methyl-D-aspartate (NMDA) receptor, such as Felbmate[12]. *Cleome viscosa* aqueous and alcoholic extract may follow any one of the above mechanism. The result of anticonvulsant effect of *Cleome viscosa* seeds against MES and PTZ induced convulsions are shown in table1 and table 2 respectively.

The one way ANOVA analysis of the data observed indicated that both alcoholic and aqueous extract exhibited significant anti-seizure effect against MES and PTZ induced seizures. Control group animals exhibited hind limb tonic extension (HLTE) of 11.42±0.020 sec. after the delivery of an electroshock. Ethanolic extract at dose of 200 mg/kg body weight shown very less effect on total duration of HLTE while at the dose of 400 and 600 mg/kg, it reduced the duration of HLTE to 5.19±0.043 and 3.38±0.141 sec. respectively. Aqueous extract dose of 200, 400 and 600 mg/kg reduced HLTE to 11.48±0.086, 4.38±0.023 and 2.65±0.308 sec. respectively. Statistically significant results were observed with alcoholic and aqueous extract at the dose of 400 and 600mg/kg with P<0.01.

Table1. It shows effect of aqueous and alcoholic extract against MES induced convulsions

Groups	Time (sec.) in various phases of convulsions (mean±SEM)				
	Flexion	Extension(HLTE)	Clonus	Stupor	Recovery
Control	3.81±0.016	11.42±0.020	14.07±0.577	92.28±0.106	120.8±1.40
Standard	1.323±0.218**	0.00 sec**	7.77±0.169**	50.69±0.134**	30.32±0.811
[1]CV-200	3.77±0.026	11.48±0.086	13.83±0.205	90.87±0.855	118.23±0.700
[1]CV-400	2.67±0.020**	4.38±0.023**	13.49±0.410	89.84±0.622*	98.53±0.232
[1]CV-600	1.62±0.030**	2.65±0.308**	9.29±0.014**	58.05±0.226**	96.78±0.410
[2]CV-200	3.82±0.115	11.22±0.185*	13.45.±0.657	91.45±0.854	119.43±0.872
[2]CV-400	2.94±0.025**	5.19±0.043**	12.77±0.162*	71.62±0.859**	109.20±0.663
[2]CV-600	1.84±0.0118**	3.38±0.141**	8.90±0.021**	51.03±0.266**	97.13±0.989

[1]CV-200, [1]CV-400, [1]CV-600 –*Cleome viscosa* aqueous extract dose 200mg/kg, 400mg/kg and 600mg/kg body weight. [2]CV-200, [2]CV-400 and [2]CV-600 - *Cleome viscosa* alcoholic extract dose 200 mg/kg, 400 mg/kg and 600 mg/kg body weight.

Pentylenetetrazole Induced Seizures

In PTZ induced seizures , aqueous extract dose 400 and 600 mg/Kg b.w. exhibited delayed onset of clonus 84.11±0.220 and 93.66±0.545 sec. respectively and the alcoholic extracts dose 400 and 600 mg/Kg b.w. exhibited delayed onset of clonus 79.05±0.115 and 80.57±0.425 sec. respectively in comparison to control 77.12±0.540 sec. For the extensor phase (aqueous extract dose 600mg/K.g showed 321.79±0.505 sec. and alcoholic extract dose 400 and 600mg/K.g exhibited 298.51±0.815 and 317.22±0.030 sec. respectively as significant anticonvulsant activity in comparison to control extensor (278.11±0.220sec.). Further more study are however necessary to

elucidate the exact mechanism of action and the active principle responsible for above activity.

Table 2: It shows effect of alcoholic and aqueous and extract on ptz induced convulsions

Drug	Dose (mg /Kg b.w.)	Onset time in seconds (mean±SEM)		
		Jerks	Clonus	Extensor
Vehicle	-	48.72±0.085	77.12±0.540	278.11±0.220
Standard(Diazepam)	4	0.00±0.000**	0.00±0.000**	0.00±0.000**
Aqueous extract	200	49.48±0.268	78.52±0.095	280.21±0.050
	400	65.34±0.233**	84.11±0.220**	280.50±0.380
	600	71.99±0.175**	93.66±0.545**	321.79±0.505**
Alcoholic extract	200	47.89±0.227	77.97±0.490	280.28±1.045
	400	49.62±0.248	79.05±0.115*	298.51±0.815**
	600	49.78±0332*	80.57±0.425**	317.22±0.030**

Values are mean ± SEM, n=6. ** P<0.01,* P<0.05 significant when compared to control

CONCLUSION

It was found that alcoholic and aqueous extract of *Cleome viscosa* seeds (dose 400 and 600 mg/kg body weight) was having significant activity against MES and PTZ convulsions when compared to control groups.

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