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Anticonvulsant activity of Shankhapuspi (Convolvulus pluricaulis Chois) on Strychnine induced seizure in experimental animals

Research article

Kshirod Kumar Ratha[1*], Sthiti Srujani Mishra[2]

1. Research Officer (Ay.), Regional Research Institute of Himalayan flora, Tarikhet, Ranikhet, Dist-Almora (U.K.)
2. Ayurvedic Medical Officer, Govt. Ayurvedic College & Hospita l, Balangir, PO/ Dist-Balangir (Odisha)

Abstract

The anticonvulsant activity of the aqueous extract of *Convolvulus pluricaulis* Chois (*Shankhapuspi*) was studied against Strychnine induced convulsions.

Seizure was induced by Strychnine in *Shankhapuspi* used animal model. The co-administration of the standard anticonvulsant drug Phenytoin sodium and aqueous extract of *Shankhapuspi* resulted in significant anticonvulsant activity when compared to the anticonvulsive activity of Phenytoin sodium.

The results of the study clearly suggested that *Shankhapuspi* can be prescribed as a co-therapeutic agent of Phenytoin for arresting seizures induced by Strychnine.

Keywords: *Shankhapuspi, Covolvulus pluricaulis, Strychnine, Anticonvulsant activity.*

Introduction:

Convulsive disorders are seen in 0.4 to 2 percent population in the world and that can occur at any age. (1) Recently it is one of the important subjects of concern especially for children and pregnant women. Being a global problem, the evaluation of patent and safe anticonvulsants is a matter of concern. The more recent studies show that the anticonvulsants used are hydantoins, valporate, barbiturates and benzodiazepines.(2) However due to their adverse effects like dependence, addiction, withdrawal symptoms and longer duration of treatment, the Ayurvedic products are preferred now a days. *Shankhapuspi* (*Convulvulus pluricaulis* Chois) belongs to Convolvulaceae family and has been mentioned in Ayurveda, as a *Rasayana* which is mainly advocated for use on rejuvenation therapy. It is said to be the best among the *medhya rasayana* (intellectual promoting drug) (3) and is frequently indicated for the treatment of various mental diseases like epilepsy, insanity, obsession etc. Anticonvulsive, hypnotic, anxiolytic and mild analgesic actions of *C. pluricaulis* have been studied (4) and found that, extract of *C. pluricaulis* antagonizing the electrically induced convulsive seizures in rats and tremorine induced tremors in mice. Further antioxidant and anticonvulsant activity of methanolic extract of *C.pluricaulis* has shown to reduce the mean recovery time in maximal electricshock seizure model.(5,6)Though various evaluation of anticonvulsive activity works were done with *Convolvulus pluricaulis*(7,8,9,10),

*Corresponding Author:
Kshirod Kumar Ratha
Research Officer (Ay.),
Regional Research Institute of Himalayan flora,
Tarikhet, Ranikhet, Dist-Almora (U.K.)
E-mail: drkkratha.ayu@rediffmail.com

but use of its aqueous extract on strychnine induced seizure is done for the first time in the present work.

Materials and Method:

The study was carried out in the Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education and Research, Kolkata and in the Department of Chemical Technology, University of Kolkata.

Object:

The main aim of the study was to evaluate the anticonvulsant activity of *Shankhapuspi* (*Convolvulus pluricaulis* Chois) on Strychnine induced convulsive seizures in animal models. This assay was used to evaluate the anticonvulsant activity of Sankhapuspi in convulsion, spasm of Tetanus etc. The interval of occurrence of tonic extensor convulsions and death following strychnine injection was noted. (11)

Collection of Chemical and Material:

The analytical grade of Strychnine Hydrochloride (S) and Phenytoin sodium (Epsolin) (12) was used for the present study were locally purchased.

Collection of Plant Material:

Whole part of the plant of *Convolvulus pluricaulis* was purchased from the local supplier and was authenticated by the institute as per usual norms.

Preparation of Aqueous Extract:

The fine powder of the herb *Shankhapuspi* was taken in a round bottle. Distilled water was added and extraction procedure was performed by decoction method for 48 hrs. After complete extraction, filtration method was adopted for getting clear extract solution. Filtrate clear extract solution was dried under rotary vacuum driers for complete removal of moisture and was placed in steam bath for few minutes.

Dose Selection:

Five random doses were selected for 6 separate groups of animals. The lowest dose was 100 mg/kg bw and the highest dose was 500 mg/kg bw. Drugs were measured accordingly and dissolved in distilled water. A stock was prepared and amount required for each rat was calculated.

Selection of Standard Drug:

Phenytoin sodium (epsolin) was choosen as standard drug for inhibiting seizures in the dose of 135 mg/kg bw, intraperitoneally. (13)

Collection of Animals:

Albino mice of either sex were collected and their weight varied between 20 gm to 25 gm. They were maintained on standard laboratory diet and provided free access to water.

Grouping of Animals:

All the animals (mice) were divided into 9 groups each containing 5 in number. The groups of animal were kept in their respective cages marked on them. The 9 cages were marked with the sign. C, C_{100}, C_{200}, C_{300}, C_{400}, C_{500}, Strychnine, Phenytoin and Phenytoin + C_{400} to indicate control (normal saline), aqueous extract of *Shankhapuspi* in 100 mg, 200 mg, 300 mg, 400 mg and 500 mg/kg bw, respectively. S indicates Strychnine Hcl (S) administrated in the dose of 2 mg/kg bw, intraperitoneally. (14)

Number of Experiments:

5 sets of experiment were performed for evaluating anticonvulsant property of *Convolvulus pluricaulis* and the result obtained from the experiment were verified by various statistical analyses.

Procedure of Drug Administration:

The aqueous extract of *Convolvulus pluricaulis* was administered orally using feeding cannula of 2.5 cm

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long made up of Silver (AG) metal. It was introduced safely direct into the stomach. In this way the drug was administrated in accurate dose without useless mechanical loss. During administration, mice were carefully handled by which traumatic injury was avoided. Aqueous extract of *Convolvulus pluricaulis* was administered daily for14 consecutive days.

Experimental Procedure Strychnine Hcl (S) induced convulsion:

Aqueous extract of *C. pluricaulis* was administrated to the experimental animal for 14 days prior to induction of convulsion in mice. Before 12 hours of induction of convulsion the food was withdrawn. In the morning the last dose of trial drug was administrated orally 1 hr prior to induce convulsion with strychnine. Each mouse was placed in to an individual plastic cage for observation for 1 hr. The delay of onset was calculated in comparison with the control and standard groups.

Observation:

ED_{50} value was calculated. Further more, the time interval between Strychnine Hcl (S) injection and occurrence of seizure was measured. The delay onset was calculated in comparison with the control and standard groups.

Statistical Analysis:

The percentage changes due to effect of *C. pluricaulis* and Phenytoin sodium were calculated with respect to control. Various statistical analysis such as student't' test, analysis of variance (ANOVA) and various multiple comparison analysis were performed to verify the level of significance.

Results and Discussion:

Different studies on *Convolvulus pluricaulis* were studied by the scholars from time to time. V.N. Sharma (1965) *et al* studied anti-convulsive effect of *C.pluricaulis* against electrically induced seizures, which showed the inhibitory effect of the drug. Present study showed significant anticonvulsant effect of the drug at the dosage of 400 mg/kg bw along with 135 mg/kg bw of Phenytoin sodium.

The results, verified by statistical analysis (*t*-test and confidence level), are shown in appropriate tables. The analysis of variance (ANOVA) in two ways between the samples and between the animal sets and rank means have been shown.

The methods of evaluating anticonvulsive activity of *Convolvulus pluricaulis* in different doses along with Phenytoin are listed in Tab. – 1. The relative percent changes with respect to control along with statistical data have been listed in Tab. – 1 and 2 & Fig. 1.

Table-1: **Effect of *Convolvulus pluricaulis* (C) on Strychnine (S) induced convulsion.**

Sample	A1		A2		A3		A4		A5	
	Average onset of convulsion (minute) ± S.E.	Relative percent change	Average onset of convulsion (minute) ± S.E.	Relative percent change	Average onset of convulsion (minute) ± S.E.	Relative percent change	Average onset of convulsion (minute) ± S.E.	Relative percent change	Average onset of convulsion (minute) ± S.E.	Relative percent change
Control	58.8 ± 0.80	-	57.60±1.666	-	57.6±1.666	-	56.8±1.067	-	58.2±0.916	-

C_{100}	6.884±0.22	88.171[a]	6.276±0.331	89.104[a]	6.816±0.34	89.260[a]	7.008±0.36	87.661[a]	6.784±0.377	88.343[a]
C_{200}	7.848±0.376	86.515[a]	7.156±0.349	87.576[a]	8.04±0.351	86.041[a]	7.528±0.358	86.746[a]	8.238±0.302	85.845[a]
C_{300}	9.896±0.576	82.996[a]	9.056±0.813	84.277[a]	9.12±0.357	84.166[a]	8.424±0.38	85.169[a]	8.68±0.407	85.085[a]
C_{400}	11.91±1.069	79.536[a]	10.622±0.901	81.559[a]	11.014±0.764	80.878[a]	9.516±0.93	83.246[a]	12.78±1.655	78.041[a]
C_{500}	13.12±0.905	77.457[a]	13.692±0.63	76.229[a]	12.28±0.559	78.680[a]	10.344±1.367	81.788[a]	13.306±1.720	77.137[a]
Strychnine (S)	1.876±0.152	96.776[a]	2.162±0.30	96.246[a]	2.028±0.295	96.479[a]	2.58±0.34	95.457[a]	2.148±0.294	96.309[a]
Phenytoin	50.984±1.822	12.398[a]	49.796±0.927	1.548[a]	47.758±2.791	17.086[b]	47.616±1.858	16.169[b]	49.426±1.741	15.075[b]
Phenytoin + C_{400}	56.316±1.395	3.237[d]	54.8±1.019	4.861[d]	50.102±2.750	13.017[b]	53.146±1.466	6.433[c]	54.012±1.761	7.195[c]

A1-A5 indicate experimental sets
≠ Average (minute) of five observations
S.E. = Standard error (n = 5)
C_{100}, C_{200}, C_{300}, C_{400} and C_{500} indicate *C.pluricaulis* in the doses of 100 mg, 200mg, 300mg, 400mg and 500mg per kg body wt. respectively. Control indicates administration of normal saline. Phenytoin administered as standard drug in the dose of 135 mg per kg I.P.

Reproducibility values are measured by 't' test and the values are significant at $^{a}p < 0.01$; $^{b}p>0.1$.

Table-2: Statistical analysis of variance (ANOVA) and multiple comparision analysis of *C.pluricaulis* of different doses on Strychnine (S) induced convulsion.

ANOVA (Two ways)	**Multiple comparisons**		
	Least significant different procedure	**Student zed range procedure**	**Duncan's new multiple range procedure**
F_1=6411.189[a] (df=7,28) F_2=1.383[b] (df=4,28)	Critical difference=1.4491 (C_{100})(C_{200}) (C_{300})(C_{400})(C_{500}) (Phenytoin) (Phenytoin+C_{400})	Critical difference=3.9195 (C_{100})(C_{200}) (C_{300})(C_{400})(C_{500}) (Phenytoin) (Phenytoin+C_{400})	Critical difference=3.0538 (C_{100})(C_{200}) (C_{300})(C_{400})(C_{500}) (Phenytoin) (Phenytoin+C_{400})

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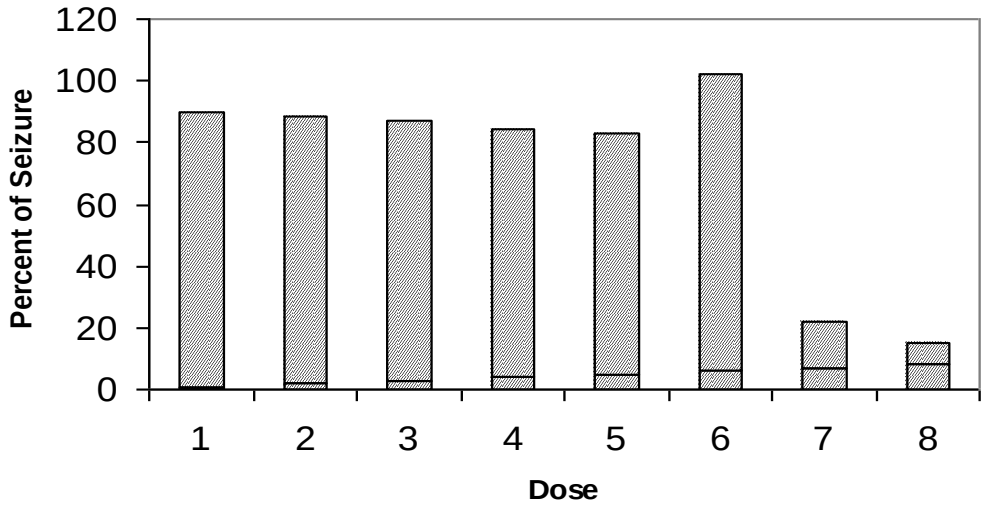
C_{100}, C_{200}, C_{300}, C_{400} and C_{500} indicate *C.pluricaulis* in the doses of 100 mg, 200mg, 300mg, 400mg and 500mg per kg body wt. respectively. Phenytoin administered as standard drug in the dose of 135 mg per kg I.P.

F_1 and F_2 indicate analysis between samples and between animal sets respectively

Significance levels of F values: $^{a}p<0.001$; $^{b}p<0.10$

Two means not included in the same parenthesis are statistically significantly different at $p<0.05$.

Figure 1: Relative percentage changes of *C.pluricaulis* activity on Strychnine (S) induced Convulsion.



'1-5' indicate *C.pluricaulis* in the doses of 100-500 mg/ kg bodyweight respectively, '6' indicates Strychnine administered in the dose of 2 mg/ kg bodyweight , '7' indicates Phenytoin sodium administered in the dose of 135 mg/ kg bodyweight and '8' indicates Phenytoin administered along with *C.pluricaulis* in the doses of 400 mg/ kg bodyweight.

From the observations it appeared that *Convolvulus pluricaulis* in the dosage of (100-500 mg/kg) has no significant inhibition effect on Strychnine induced convulsion, however *Convolvulus pluricaulis* in the dose of 400 mg/kg bw is administrated in combination with Phenytoin sodium (135 mg/kg bw) showed significant inhibitory effect on Strychnine induced convulsion. Thus the therapeutic index of Phenytoin sodium is enhanced by increasing LD_{50} value without altering ED_{50} value.

Conclusion

It can be concluded that only the aqueous extract of *Shankhapuspi* (*Convolvulus pluricaulis*) (500 mg/kg bw) could not arrest seizure induced by Strychnine where as aqueous extract of *Convolvulus pluricaulis* in the dosage of 400 mg/kg bw acted as co-therapeutic agent of Phenytoin sodium for arresting seizure induced by Strychnine Hcl (S).

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