ANTIEPILEPTIC ACTIVITY OF ALCOHOL AND AQUEOUS EXTRACTS OF ROOTS AND RHIZOMES OF *SMILAX ZEYLANICA* LINN.

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Summary

Antiepileptic activity of alcohol and aqueous extracts of roots and rhizomes of *Smilax zeylanica* Linn. was investigated on Pentylenetetrazole (PTZ) and Maximal electro shock (MES) induced convulsion models in swiss albino mice. Both alcohol and aqueous extracts were found to be non toxic upto 3000 mg/kg. In MES induced seizures, alcohol extract at doses 300 and 600 mg/kg significantly ($p<0.001$) reduced the duration of extensor phase and time taken for recovery. Aqueous extract at doses of 300 and 600 mg/kg significantly ($p<0.01$) and dose dependently reduced the duration of extensor phase. The time for recovery was significantly ($p<0.001$) less. In PTZ induced seizures, both extracts at the dose of 600 mg/kg, significantly ($p<0.05$) delayed the onset of convulsions. The study substantiates use of *Smilax zeylanica* Linn. as an additional botanical source for the Ayurvedic drug Chopachinee in the treatment of epilepsy.

Keywords: Antiepileptic activity; Maximal Electro Shock; Pentylenetetrazole; Smilax zeylanica.

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Introduction

Chopachinee is an important Ayurvedic drug used in treatment of several diseases like diseases of nervous system, epilepsy and psychosis; the accepted botanical source of Chopachinee is *Smilax china* Linn. which is endemic to China (1,2). The genus *Smilax* Linn. belongs to Smilacaceae, consists of about 300 species in the world, out of which 24 species are found in India (3,4,5). In South India, the genus is represented by 4 species viz., *Smilax zeylanica* Linn., *Smilax aspera* Linn., *Smilax perfoliata* Roxb. and *Smilax wightii* A. DC.(6). Different species of *Smilax* Linn. such as *S. glabra* Roxb., *S. ovalifolia* Roxb. *and S. lanceifolia* Roxb., are used as substitutes of Chopachinee (1). *Smilax zeylanica* Linn. is used in the treatment of venereal diseases (7), skin disorders, sores, swellings, abscess (8,9,10) and also applied for rheumatism and pains in lower extremities (11). Roots of *S. zeylanica* Linn. are also considered as substitute for Indian sarsaparilla (*Hemidesmus indicus* Linn.) (2). Species of *Smilax* Linn. contain dioscin (spirostanol triglycoside), smilagenin and sarsapogenin (1-3%)(12). The roots of *S. zeylanica* Linn. contain diosgenin, a steroidal saponin glycoside(13).

Traditionally Chopachinee is used for the treatment of epilepsy, but scientifically it has not been proved in any of the *Smilax* species (14,15). Hence the present investigation on roots and rhizomes of *Smialx zeylanica* Linn. was undertaken.

Materials and Methods

Plant materials

Roots and rhizomes of *Smilax zeylanica* Linn. were collected from vicinity of Tirunelveli, Tamil Nadu during Feb 2006, thoroughly washed in running water, segregated from extraneous material, identified and authenticated by Dr. S.N. Yoganarasimhan, Taxonomist and Research co-ordinator M. S. Ramaiah College of Pharmacy, using local Floras (16,17). They were shade dried, coarse powdered and stored in air tight container. Herbarium specimen **(***Hemalatha* 020) is deposited at the herbarium of PG Department of Pharmacognosy, M. S. Ramaiah College of Pharmacy, Bangalore along with a sample of tested material.

Preparation of extracts

About 50 g powder was extracted with ethanol (70% v/v) in a soxhlet apparatus by continuous heat extraction for 24 h. Ethanol extract

was concentrated to a small volume under reduced pressure and evaporated to dryness (yield 8% w/w). For aqueous extract, 50 g powder was extracted with chloroform water (0.2 %) by maceration for 24 h, filtered and concentrated to dryness (yield 4% w/w). Extracts were stored in desiccator and used for further studies. Preliminary phytochemical tests were carried out following Kokate (18).

Experimental animals

Swiss albino mice weighing 18-25 g of either sex were procured and housed in animal house of M. S. Ramaiah College of Pharmacy, Bangalore at least 2 weeks prior to study, so that animals could adapt to new environment. Animal house was well maintained under standard hygienic conditions, at a temperature (22 ± 2^0C), room humidity (60 ± 10%) with 12 h day and night cycle, with food and water *ad libitum*. All pharmacological work was carried out as per CPCSEA norms, after obtaining approval from Institutional Animal Ethical Committee of M. S. Ramaiah College of Pharmacy, Bangalore, India.

Acute toxicity studies

Acute toxicity studies were carried out to study acute toxic effects and to determine minimum lethal dose of drug extracts. Alcohol and aqueous extracts were administered orally to overnight fasted animals at doses of 30, 100, 300, 1000 and 3000 mg/kg of body weight. After administration of extracts, animals were observed continuously for first three hours, for any toxic manifestation like increased motor activity, salivation, acute convulsion, coma and death (19). Thereafter, observations were made at regular intervals for 24 h. Further, animals were under investigation up to a period of 1 week.

Antiepileptic activity

Maximal electroshock (MES) induced convulsion method.

Each group comprised of 6 mice.
Group 1: Control (Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).
Group II: Standard (Phenytoin 25 mg/kg i.p. + Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).
Group III: Alcohol extract (300 mg/kg orally + Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).
Group IV: Alcohol extract (600 mg/kg orally + Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).

Group V: Aqueous extract (300 mg/kg orally + Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).
Group VI: Aqueous extract (600 mg/kg orally + Electro-convulsive shock 150mA, 0.2 sec, using ear electrode).

Alcohol and aqueous extracts were orally administered to respective groups at doses of 300 and 600 mg/kg, followed by electro convulsive shock after 1h. The standard group was induced electro-convulsive shock, 30 min after i.p. injection of phenytoin (25 mg/kg). Animals were individually observed for various parameters such as tonic flexion, tonic extensor phase, clonic convulsions, and stupor. The time taken for recovery or death after electro-convulsive shock was also recorded (20-26).

Pentylenetetrazole (PTZ) induced convulsion method:

Each group comprised of 6 mice.
Group 1: Control (Pentylenetetrazole 70 mg/kg i.p.)
Group II: Standard (Diazepam 4 mg/kg i.p. + Pentylenetetrazole 70 mg/kg i.p.)
Group III: Alcohol extract (300 mg/kg orally + Pentylenetetrazole 70 mg/kg i.p.)
Group IV: Alcohol extract (600 mg/kg orally + Pentylenetetrazole 70 mg/kg i.p.)
Group V: Aqueous extract (300 mg/kg orally + Pentylenetetrazole 70 mg/kg i.p.)
Group VI: Aqueous extract (600 mg/kg orally + Pentylenetetrazole 70 mg/kg i.p.)

Alcohol and aqueous extracts were orally administered to respective groups at doses of 300 and 600 mg/kg, followed by PTZ (70 mg/kg i.p.) after 1h. The standard group was injected PTZ (70 mg/kg i.p.), 30 min after i.p. injection of diazepam (4 mg/kg). Animals were individually placed in trays and observed. Latency and duration of myoclonic jerks as well as incidence of seizures, time taken for death/recovery was recorded (20-26).

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparison test. All values were expressed as Mean ± SEM.

Results and Discussion

Acute toxicity studies did not exhibit any toxic symptoms or mortality till the end of study; both alcohol and aqueous extracts were non toxic upto to 3000 mg/kg. In MES induced seizures, alcohol extract at doses of 300 and 600 mg/kg significantly ($p<0.001$) reduced duration of extensor phase. The effect of alcohol extract at 300 and 600 mg/kg was comparable with standard drug phenytoin. At the same time, aqueous extract at doses of 300 and 600 mg/kg also reduced duration of extensor phase significantly ($p<0.01$ and $p<0.001$ respectively). The effect of aqueous extract on extensor phase was dose dependent. Both extracts and the standard drug significantly reduced duration of recovery period ($p<0.001$), when compared with control. No mortality was observed in any group (Table 1, Figs. 1,2). In PTZ induced seizures, the administration of alcohol and aqueous extracts of *Smilax zeylanica* Linn. at the dose of 600 mg/kg, 1 h prior to the injection of PTZ, significantly ($p<0.05$) delayed the onset of convulsions. Either extracts at the dose of 300 mg/kg, did not delay latency period to significant extent. However there was an extremely significant decrease ($p<0.001$) in the duration of recovery period in all extract treated groups. Diazepam at the dose of 4 mg/kg totally abolished convulsions (Table 2, Figs. 3,4).

Table 1: Extensor phase and Time taken for recovery (MES induced convulsions)

Group	Dose (mg/kg)	Extensor phase (Sec.)	Time taken for recovery (Sec.)
Control	_	18.16±1.5	568.73±30.15
Standard drug (Phenytoin)	25	0.66±0.42***	28.33±3.80***
Alcohol extract-1	300	5.33±1.85***	177.5±42.92***
Alcohol extract-2	600	5.0±2.7***	65±23.87***
Aqueous extract-1	300	6±2.1**	140±37.7***
Aqueous extract-2	600	5.6±2.04***	117.5±35.95***

Values expressed as mean ±SEM
Extensor phase: One way ANOVA (One way analysis of variance) $p<0.0001$ Tukey Kramer Multiple Comparison Test ** $p<0.01$; *** $p<0.001$, when compared with control
Time taken for recovery: One way ANOVA (One way analysis of variance) $P< 0.0001$. Tukey Kramer Multiple Comparison Test *** $p<0.001$, when compared with control.

Fig. 1. Extensor phase (MES induced convulsion)

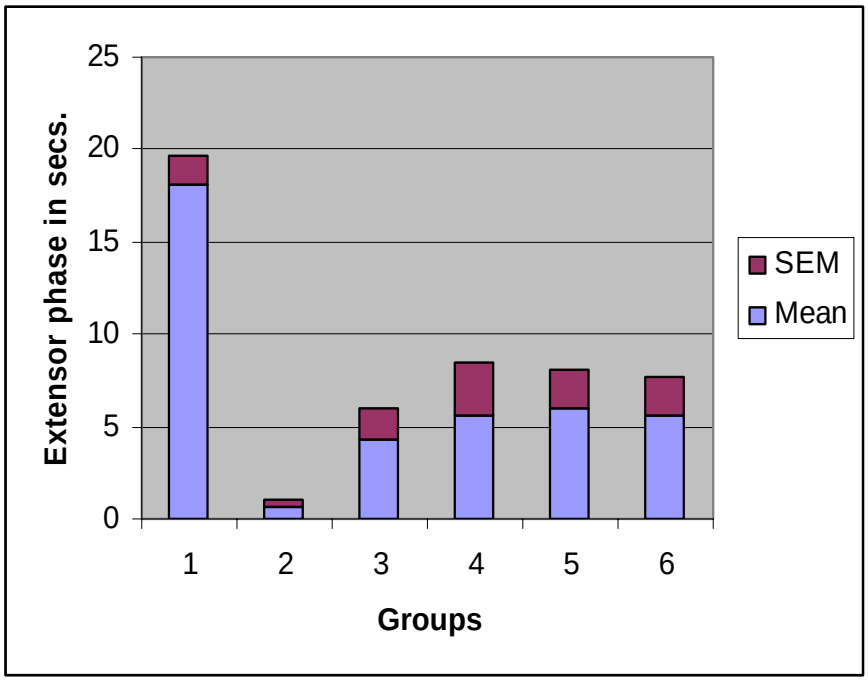


Fig. 2. Time taken for recovery (MES induced convulsion)

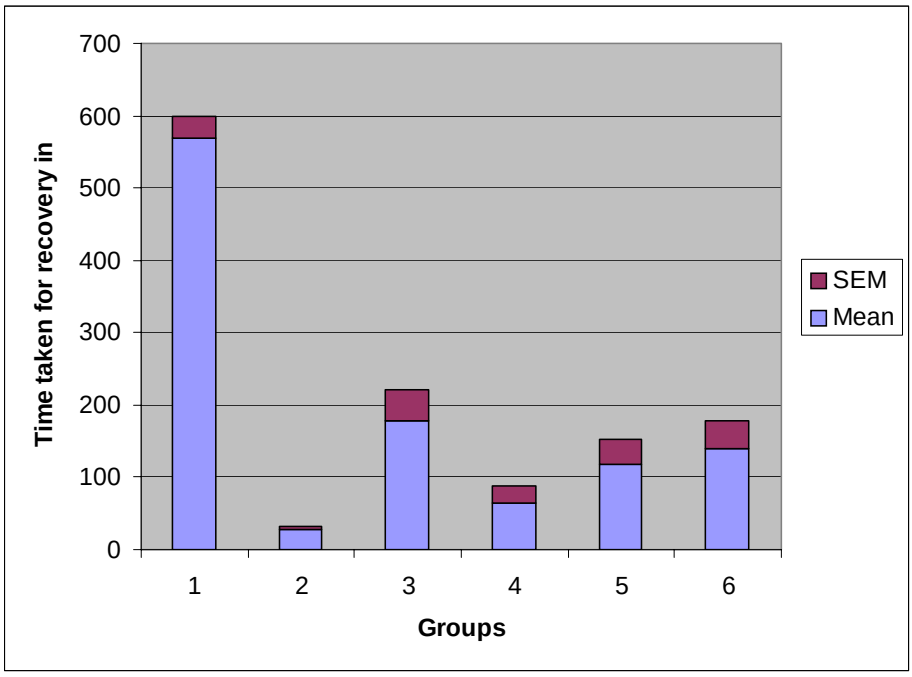


Table 2: Onset of convulsion and time taken for recovery (PTZ induced convulsion)

Treatment in mg/kg	**Onset of convulsion (sec.)**	**Time taken for recovery (sec.)**	**Lethality**
Control PTZ (70)	81±14.92	1455.5±67.03	2/6
Standard (Diazepam) (4) + PTZ (70)	0±0***	0±0***	0/6
Alcohol extract (300) + PTZ (70)	121±3.38	760±81.48***	3/6
Alcohol extract (600) + PTZ (70)	135.83±10.19*	708.6±12.16***	1/6
Aqueous extract (300) + PTZ (70)	118.33±17.78	748±85.22***	1/6
Aqueous extract (600) + PTZ (70)	139.83±11.25*	680±80.0***	0/6

Values expressed as mean ±SEM

Onset of convulsion: One way ANOVA (One way analysis of variance) $p<0.0001$

Tukey Kramer Multiple Comparison Test * $p<0.05$, *** $p<0.001$, when compared with control

Time taken for recovery: One way ANOVA (One way analysis of variance) $P< 0.0001$.

Tukey Kramer Multiple Comparison Test *** $p<0.001$, when compared with control

Fig. 3. Onset of convulsions (PTZ induced convulsion)

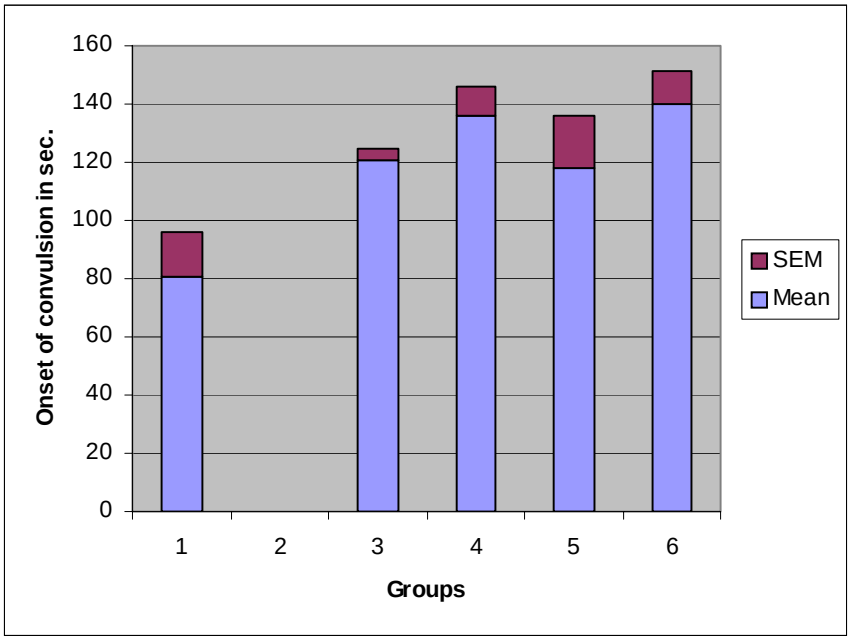
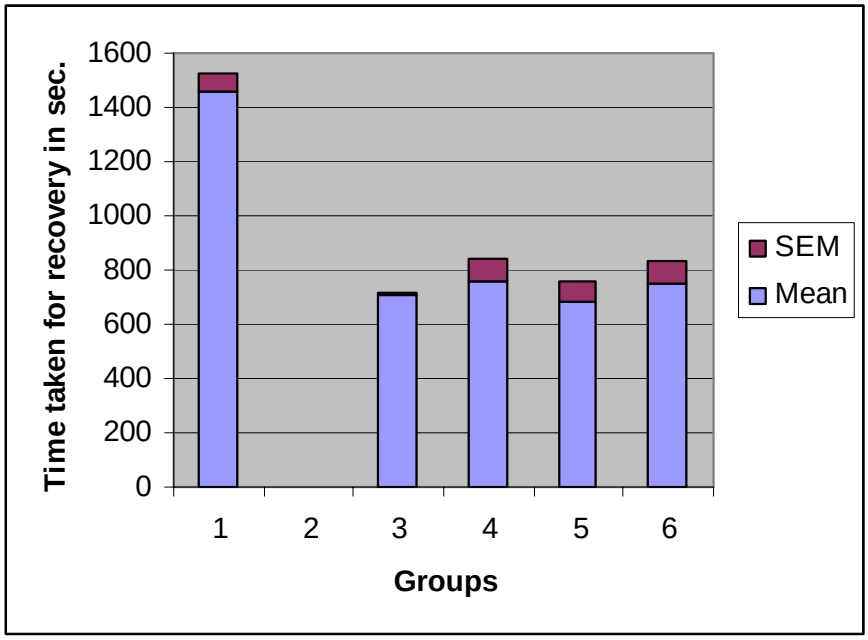


Fig. 4. Time taken for recovery (PTZ induced convulsion)



Preliminary phytochemical analysis revealed presence of glycosides, alkaloids, carbohydrates, fixed oils, fats, saponins, phytosterols, tannins, gums and mucilage. Some medicinal plants such as *Glycyrrhiza glabra* Linn.(24), *Calotrpis gigantea* Linn.(25), *Nymphoides macrospermum* Vasudevan (26), *Cotyledon orbiculata* Linn.(27), containing steroids, phenols, tannins, saponins, glycosides are reported to possess antiepileptic activity. *S. zeylanica* also revealed presence of glycosides, saponins phytosterols and tannins, which may account for its potential antiepileptic activity. However, further work can be undertaken to isolate and identify the bioactive constituent(s) responsible for antiepileptic activity.

Conclusion

Present study revealed antiepileptic activity of alcohol and aqueous extracts of roots and rhizomes of *Smilax zeylanica* Linn. by PTZ and MES induced convulsion models. Both extracts at the dose levels 300 and 600 mg/kg showed significant antiepileptic activity. The study substantiates use of *Smilax zeylanica* Linn. as an additional botanical source for the Ayurvedic drug Chopachinee in the treatment of epilepsy.

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