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Investigation of anticonvulsant activity of *Vanda roxburghii*

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ABSTRACT

Purpose: The aim of the study was to investigate anticonvulsant effect of alcoholic extract of roots of *Vanda roxburghii* on electrically and chemically induced seizures.

Methods: The ethanolic extract of the roots of *Vanda roxburghii* (SVR) was studied for its anticonvulsant effect on maximal electroshock-induced seizures pentylenetetrazole, picrotoxin induced seizures in mice. The latency of tonic convulsions and the number of animals protected from tonic convulsions were observed.

Results: It has been observed in the present study that the ethanolic extract of roots of *Vanda roxburghii* (100 mg/kg) showed significant ($P < 0.05$) increase in latency to clonic convulsions. *Vanda roxburghii* possess anticonvulsant activity against Pentylenetetrazole (PTZ), Maximal electroshock (M.E.S.) and Picrotoxin (PTX) induced convulsions in mice.

Keywords: Epilepsy, Anticonvulsant, *Vanda roxburghii*, PTZ, MES, PTX.

1. Introduction

Vanda roxburghii R.Br. commonly called as Rasna, belongs to family Orchidaceae is widely used in Ayurvedic medicine for treatment of rheumatoid arthritis. It is also useful in dyspepsia, bronchitis, inflammations, and diseases of the abdomen, hiccough and tremors and as an antipyretic agent [1]. *Vanda roxburghii* R.Br. contain β -sitosterol, γ -sitosterol, heptacosane, octacosanol, acetyl tetracosylferulate [2], 17- β -hydroxy-14,20-epoxy-1-oxo-[22R]-3 β -[O- β -D-glucopyranosyl]-5,24-withadienolide [3] and melianin [4]. Sterols are anti-inflammatory agents. β -sitosterol has been shown to possess anti-inflammatory and anti-pyretic properties [5]. Epilepsy is one of the most common disorders of the central nervous system (CNS). Currently available treatment for the epilepsy possesses major side effects like minimal impairment of central nervous to death due to aplastic anemia or hepatic failure as result of which sufferings of patient increases. Since plants have provided many drugs in the past and they remain a rich source of novel compounds hence an attempt is made to evaluate anticonvulsant effects of alcoholic extract of *Vanda roxburghii*.

2. Materials and Methods

2.1 Preparation of extract: The roots of *Vanda roxburghii* (SVR) were purchased from commercial source. The roots (1.0 kg) were crushed to a coarse powder and extracted with ethanol using Soxhlet extractor for 24 h. The extract was concentrated under reduced pressure and then dried in air. This ethanolic extract of roots of *Vanda roxburghii* (SVR) was stored in a refrigerator and reconstituted in water for injection just before use.

2.2 Animals: Adult male Swiss albino mice (18-22 g) were purchased from National Institute of Biosciences, Pune and kept in quarantine for one week in housed at the institute animal house in groups of six animals per cage at standard laboratory conditions at a temperature of 24 ± 1 °C, relative humidity of 45–55% and 12:12 h dark and light cycle. The experiments were carried out between 10:00 am to 5:00 pm. Animals had free access to food (standard chaw pellet, Pranav Agro industries Ltd., Sangli, India) and water ad libitum. Experimental protocols and procedures were approved by the Institutional Animal Ethics Committee (CPCSEA/IAEC, 05/05/2012). Animals were brought to testing laboratory 1 h before the experimentation for adaptation purpose. The experimentation was carried out in noise free area.

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2.3 Drugs Pentylenetetrazole (PTZ) (Sigma Aldrich, India), Phenytoin (PHY) (Eptoin®, Sun Pharma Ltd., India), Diazepam (DZP) (Calmpose®, Ranbaxy Ltd., India) were used in present study. Except ethanolic extract of roots of *Vanda roxburghii* (SVR), all other reagents were purchased from S.D. Fine Chemicals, Mumbai, India.

2.4 Acute toxicity study

Extract in doses of 30, 100, 300, 1000, 2000 and 5000 mg/kg was administered intraperitoneally to mice for toxicity study. Mice were then observed for incidence of mortality or any sign of toxicity up to 24 h after injection. The dosing schedule was followed as per the OECD guideline 425. Only one mouse received a dose at a particular time. First animal received a dose of 30 mg/kg, i.p or p.o. Animal was observed for 3 hours after injection for any toxicity signs, survival or death. If the first animal died or appeared moribund, the second animal received a lower dose (10 mg/kg). The dose progression or reduction factor was 3.2 times of the previous dose. If no mortality was observed in the first animal then the second animal received a higher dose (100 mg/kg). Dosing of the next animal was continued depending on the outcome of the previously dosed animal for a fixed time interval (3 hour). Survived animals were observed for outcomes for a period of 24 hr.

2.5 Treatment schedule

Animals were divided into groups as per the requirement of each experiment. In PTZ, MES, PTX, induced convulsion model each group contained 6 mice. The pretreatment time for vehicle and extract was 45 min while that for standard drug was 30 minutes before the start of session. All the experiments were conducted from 10:00 h to 17:00 h.

2.6 Pentylenetetrazole (PTZ) induced convulsions

Swiss albino male mice (25 ± 2 g) were used. Vehicle, extracts of *Vanda roxburghii* (SVR) or the standard drug

(diazepam 5 mg/kg) were administered by intraperitoneal route. PTZ 80 mg/kg was injected intraperitoneally to all mice after 45 minutes of vehicle or extracts and 30 min after the standard drug. Immediately after PTZ administration mice were placed individually and observed for: ^[1] Latency to clonic convulsions ^[2] Incidence (no. of mice showing convulsions) and ^[3] Mortality for the duration of 30 min ^[6].

2.7 Maximal Electroshock (MES) induced convulsions

Swiss albino mice (25 ± 2 g) of either sex were used. Test was started 45 min after intraperitoneal administration of vehicle or extracts and 30 min after standard drug (phenytoin 20 mg/kg i.p). To start session a 60 Hz alternate current of 45 mA for 0.2 sec was applied to the animal through corneal electrodes ^[6]. To enhance electroconductivity two drops of 0.9% NaCl were applied on each eye before applying current. After electric stimuli, latency and incidence of tonic hind limb extension (THLE) and mortality was observed for duration of 15 min.

2.8 Picrotoxin (PTX) induced convulsions

Swiss albino mice (25 ± 2 g) of either sex were used. Vehicle, extracts of *Vanda roxburghii* (SVR) or the standard drug (diazepam 5 mg/kg) were administered by intraperitoneal route. Forty-five minutes after administration of vehicle or extract and 30 min after diazepam all mice were treated with 3.5 mg/kg picrotoxin by subcutaneous route. Immediately after picrotoxin injection mice were observed for following symptoms during next 45 min: ^[1] Latency to tonic convulsions, ^[2] Latency to clonic convulsions ^[3] Incidence (no. of mice showing convulsions) and ^[4] Mortality ^[7].

3. Result and Discussion

3.1 Acute toxicity study

Oral administration of extract of *Vanda roxburghii* (SVR) was found to be safe and no mortality was observed up to 2000 mg/kg.

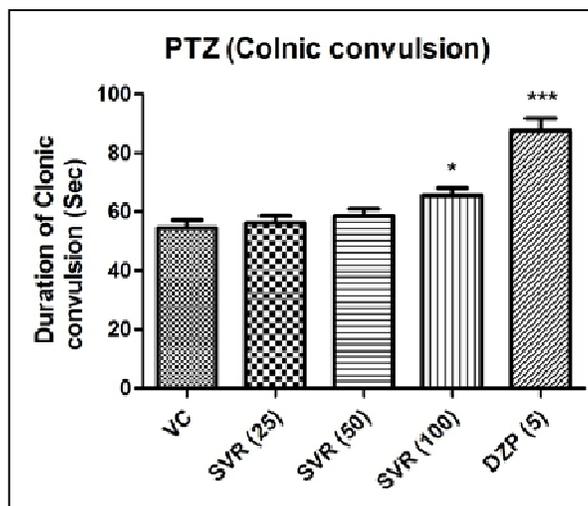


Fig 1: Effect of extract of on Pentylenetetrazole induced convulsions.

3.2 Effect of extract of on Pentylentetrazole induced convulsions

Single dose, intraperitoneal administration of pentylentetrazole (PTZ; 80 mg/kg i.p.) caused clonic convulsions as well as lethality in mice. Mice pretreated with SVR (100 mg/kg) showed significant ($P<0.05$) increase in latency to clonic convulsions. SVR (50 and

100 mg/kg) reduced mortality to 50% and 83.33% respectively, whereas SVR (25) failed to show anticonvulsant activity against PTZ induced convulsions in mice and protection of animals against mortality. (Table 1 & Fig. 1). Diazepam (5 mg/kg) showed significant ($P<0.001$) increase in latency to clonic convulsions and mortality induced by pentylentetrazole in mice.

Table 1: Effect of extract of on Pentylentetrazole induced convulsions.

Sr. No.	Group	No. of animals convulsed/ No. used	Animals protected (%)	Mortality (%)
1	Vehicle 1	6/6	0%	100%
2	SVR (25)	6/6	0%	100%
3	SVR (50)	6/6	50%	50%
4	SVR (100)	5/6	16.67%	83.33%
5	Diazepam 5 mg/kg i.p.	0/6***	100% ***	0%***

*** $P<0.001$ Data of clonic convulsion was analyzed by one way ANOVA followed by Dunnett's test. Data of incidence and mortality was analyzed by Fisher's exact test.

3.3 Effect of extract on Maximal electroshock (MES) induced convulsions

Corneal electroshock of 45 mA for 0.2 Sec. induced tonic hind limb extension (THLE) and mortality in all mice. Animals treated with SVR (25, 50 and 100 mg/kg) did not show significant effect on the latency to THLE. SVR (50

and 100 mg/kg) reduced mortality to 66.67 and 33.33%, whereas SPK (25 mg/kg) failed to show any protection against mortality. (Table 2 & Fig. 2). Phenytoin (20 mg/kg i.p) showed significant ($P<0.001$) effect against MES induced THLE and mortality.

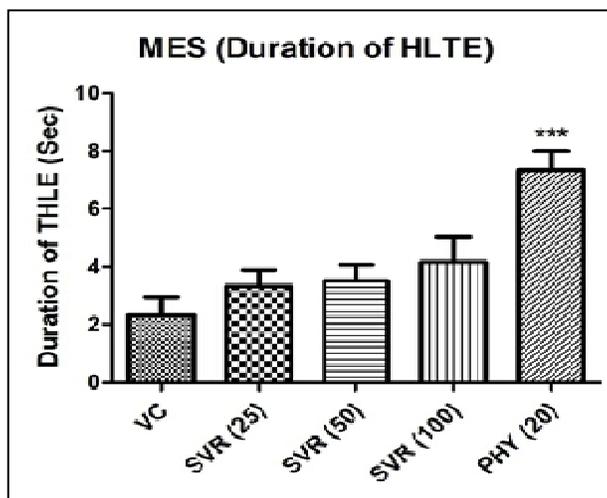


Fig 2: Incidence of tonic hind limb extension (THLE) PTZ.

Table 2: Effect of extract on Maximal electroshock (MES) induced convulsions.

Sr. No.	Group	No. of animals convulsed/ No. used	Animals protected (%)	Mortality (%)
1	Control	6/6	0%	100%
2	SVR (25)	6/6	33.33%	66.67%
3	SVR (50)	6/6	100%***	0%
4	SVR (100)	6/6	66.67%	33.33%
5	Phenytoin 20 mg/kg i.p.	0/6***	100% ***	0%

3.4 Effect of extract on Picrotoxin (PTX) induced convulsions

Single dose, subcutaneous administration of PTX (3.5 mg/kg s.c.) induced clonic convulsions followed by THLE (after some clonic episodes) and mortality in mice. SVR (100 mg/kg) significantly ($P<0.01$) increased latency to clonic convulsions and reduced significantly ($P<0.05$) incidence of THLE without significant effect on mortality.

SVR (50 mg/kg) showed significant ($P<0.05$) effect on incidence of THLE without significant effect on clonic convulsions and mortality. SVR (25 mg/kg) did not show significant effect on latency to clonic convulsions and incidence of THLE and mortality. (Table 3 & Fig. 3,4). Diazepam (5 mg/kg) showed significant ($P<0.001$) effect on clonic convulsions as well as THLE and mortality ($P<0.01$) induced by the picrotoxin.

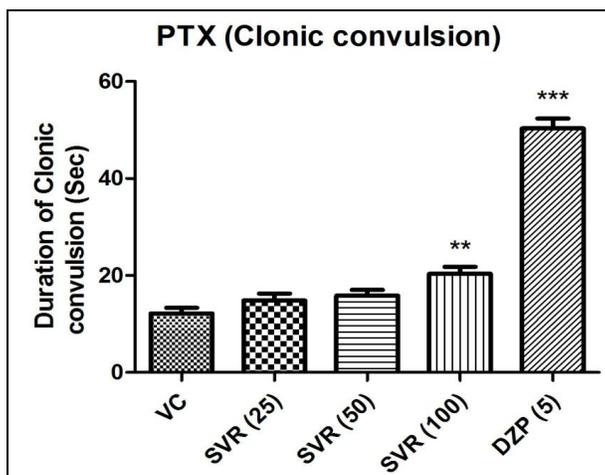


Fig 3: Effect of extract on Picrotoxin (PTX) induced convulsions.

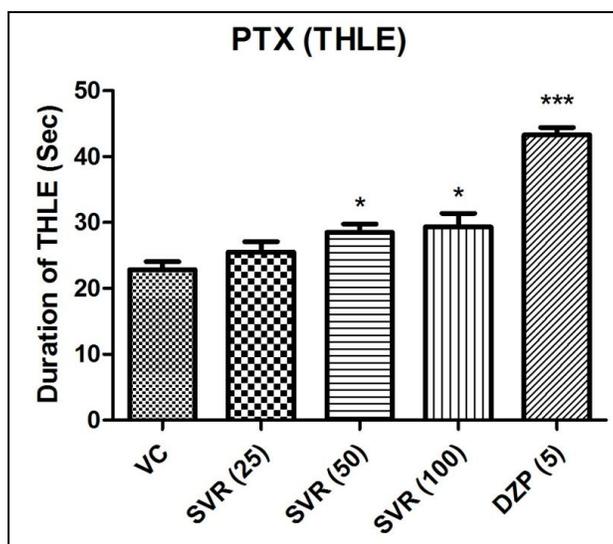


Fig 4: Incidence of tonic hind limb extension (THLE) PTX.

Table 3: Effect of extract on Picrotoxin (PTX) induced convulsions.

Treatment (mg/kg i.p.)	Episodes of clonic	Incidence (clonic conv.)	Protected %	Incidence (THLE)	Mortality dead/used (%)
Vehicle	4.27 ±0.18	6/6	0%	6/6	83.33%
SVR (25)	1.13 ±0.41**	4/6	33.33%	2/6*	33.33%
SVR (50)	1.20 ±0.25**	5/6	16.67%	2/6*	33.33%
SVR (100)	1.23 ±0.51**	5/6	16.67%	2/6*	33.33%
Diazepam (5)	--	0/6**	100%	0/6**	0%**

4. Conclusion

It can be concluded that *Vanda roxburghii* possess anticonvulsant activity against Pentylentetrazole (PTZ), Maximal electroshock (M.E.S.) and Picrotoxin (PTX) induced convulsions in mice. Further extensive studies are needed to evaluate the precise mechanism(s), active principles, and the safety profile of the plant as a medicinal remedy for convulsion.

5. Conflict of interest

The authors declare that they have no conflict of interest.

6. Acknowledgement

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