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Original Research Paper

ANTICONVULSANT ACTIVITY ETHANOLIC EXTRACT OF *PICRORHIZA KURROA*

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ABSTRACT

The aim of the present study was to investigate anticonvulsant effect of ethanolic extract of *Picrorhiza kurroa* on electrically and chemically induced seizures. The ethanolic extract of the roots of *Picrorhiza kurroa* was studied for its anticonvulsant effect on maximal electroshock-induced seizures and pentylenetetrazole, picrotoxin induced seizures in mice. The latency of tonic convulsions and the number of animals protected from tonic convulsions were observed. It has been observed in the present study that SPK (100 mg/kg) showed significant increase in latency to clonic convulsions and reduced mortality, however SPK (25 and 50 mg/kg) failed to show anticonvulsant activity against PTZ induced convulsions in mice. Further animals treated with SPK (25, 50 and 100 mg/kg) did not show any significant effect on the latency to THLE in MES induced convulsion. Thus we can conclude that *Picrorhiza kurroa* possess anticonvulsant activity against Pentylenetetrazole (PTZ), Maximal electroshock (M.E.S.) and Picrotoxin (PTX) induced convulsions in mice.

Keywords: Epilepsy, Anticonvulsant, *Picrorhiza kurroa,* PTZ, MES, PTX.

INTRODUCTION

Epilepsy is common and frequently devastating neurological disorder-affecting millions of people. Epileptic convulsions often cause transient impairment of consciousness, leave the individual at the risk of bodily harm. Epilepsy affects an estimated 7 million people in India, and 50 million worldwide. *Picrorhiza kurroa* is an important medicinal plant used in traditional as well as modern medicines. The genus *Picrorhiza* and its species *Picrorhiza kurroa* Royle appeared first time on a drawing published by Royle on August 24, 1835 in his Illustration of botany and still known by names *Picrorhiza kurroa* Bentham or alternatively *Picrorhiza kurroa* Royle ex Bentham.[1] In Greek, *Picros* means bitter and *rhiza* means root. The specific name derived from Karu, the Punjabi name of the plant, which means bitter as well.[2] It is used in treatment of liver disorder, fever, asthma, jaundice caused by environmental pollution, industrial toxicants, food adulteration, malnutrition, excessive consumption of alcohol and certain infections. It is also used in gastrointestinal, urinary disorder, leukoderma, snake bite, scorpion sting and inflammatory affections.[3-6] The root has a very bitter and sharp taste. It is also used as brain tonic and in epilepsy.[7] It contains bitter principle constituent Picrorhizin. It is soluble in water and alcohol.[8] In the present study our objective is to confirm the antiepileptic/anticonvulsant action of selected plant from traditional system. These studies will help for development of new drug for treatment of epilepsy and anxiety with fewer side effects.

MATERIALS AND METHODS

Preparation of Extract

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

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 $24^0C \pm 1^0C$, 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.

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 *Picrorhiza kurroa* (SPK), all other reagents were purchased from S.D. Fine Chemicals, Mumbai, India.

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 (10mg/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.

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.

Pentylenetetrazole (PTZ) Induced Convulsions

Swiss albino male mice (25 ± 2 g) were used. Vehicle, extracts of *Picrorhiza kurroa* (SPK) 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.[9]

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 (Swinyard *et al.*, 1952). To enhance electro-conductivity 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.

Picrotoxin (PTX) Induced Convulsions

Swiss albino mice (25 ± 2 g) of either sex were used. Vehicle, extracts of *Picrorhiza kurroa* (SPK) 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.[10]

RESULT AND DISCUSSION

Acute Toxicity Study

Oral administration of extract of *Picrorhiza kurroa* was found to be safe and no mortality was observed up to 2000 mg/kg.

Effect of Extract on Pentylenetetrazole Induced Convulsions

Single dose, intraperitoneal administration of pentylenetetrazole (PTZ; 80 mg/kg i.p.) caused clonic convulsions as well as lethality in mice. Mice pretreated with SPK (100 mg/kg) showed significant ($P<0.05$) increase in latency to clonic convulsions. SPK (100 mg/kg) reduced mortality to 83.33%, whereas SPK (25 and 50 mg/kg) failed to show anticonvulsant activity against PTZ induced convulsions in mice and mortality was reduced. Diazepam (5 mg/kg) showed significant ($P<0.001$) increase in latency to clonic convulsions and mortality induced by pentylenetetrazole in mice. (Table 1 & Figure 1)

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 SPK (25, 50 and 100 mg/kg) did not show significant effect on the latency to THLE. SPK (100 mg/kg) reduced mortality to 83.33%, whereas SPK (25 and 50 mg/kg) failed to show any protection against mortality. Phenytoin (20 mg/kg i.p.) showed significant ($P<0.001$) effect against MES induced THLE and mortality. (Table 2 & Figure 2)

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. SPK (100 mg/kg) significantly ($P<0.05$) increased latency to clonic convulsions and reduced incidence of THLE without significant effect on mortality. SPK (25 and 50 mg/kg) did not showed significant effect on latency to clonic convulsions and incidence of THLE with significant effect on mortality ($P<0.05$ and $P<0.01$) respectively. 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. (Table 3 & Figure 3, 4)

CONCLUSION

It can be conclude that *Picrorhiza kurroa* possess anticonvulsant activity against Pentylenetetrazole (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.

Table 1: Effect of extract on pentylenetetrazole (PTZ) - induced convulsion

Sr. No.	Group	No. of animals convulsed/ No. used	Animals protected (%)	Mortality (%)
1	Control	6/6	0%	100%
2	SPK 25 mg/kg i.p.	6/6	0%	100%
3	SPK 50 mg/kg i.p.	6/6	0%	100%
4	SPK 100 mg/kg i.p.	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.

Table 2: Effect of extract on maximal electroshock (M.E.S.) - induced convulsion

Sr. No.	Group	No. of animals convulsed/ No. used	Animals protected (%)	Mortality (%)
1	Control	6/6	0%	100%
2	SPK 25 mg/kg i.p.	6/6	0%	100%
3	SPK 50 mg/kg i.p.	6/6	0%	100%
4	SPK 100 mg/kg i.p.	6/6	16.67%	83.33%
5	Phenytoin 20 mg/kg i.p.	0/6***	100%***	0%

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

Table 3: Effect of extract on picrotoxin induced convulsion:

Sr. No.	Group	Episodes of clonic convulsion	Incidence (clonic convulsion)	Protected %	Incidence (THLE)	Mortality (%)
1	Control	4.17 ± 0.48	6/6	0%	6/6	100%
2	SPK 2mg/kg i.p.	3.05 ± 0.73	6/6	0%	2/6*	100%*
3	SPK 50 mg/kg i.p.	1.23± 0.31ee	6/6	0%	0/6**	0%**
4	SPK 100 mg/kg i.p.	1.30 ± 0.22ee	6/6	0%	5/6	83.33%
5	Diazepam 5 mg/kg i.p.	--	0/6**	100%	0/6**	0%**

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

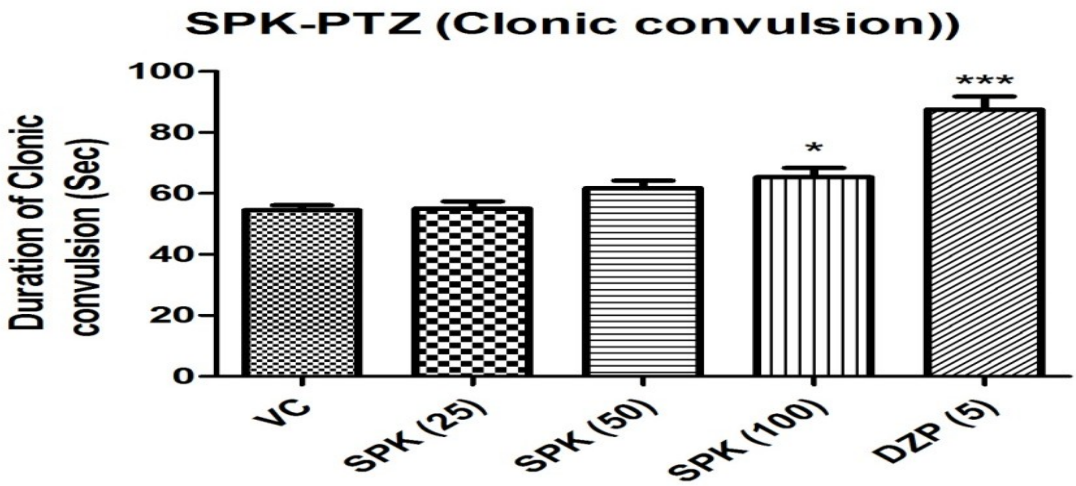

Figure1: SPK-PTZ (Clonic convulsion)

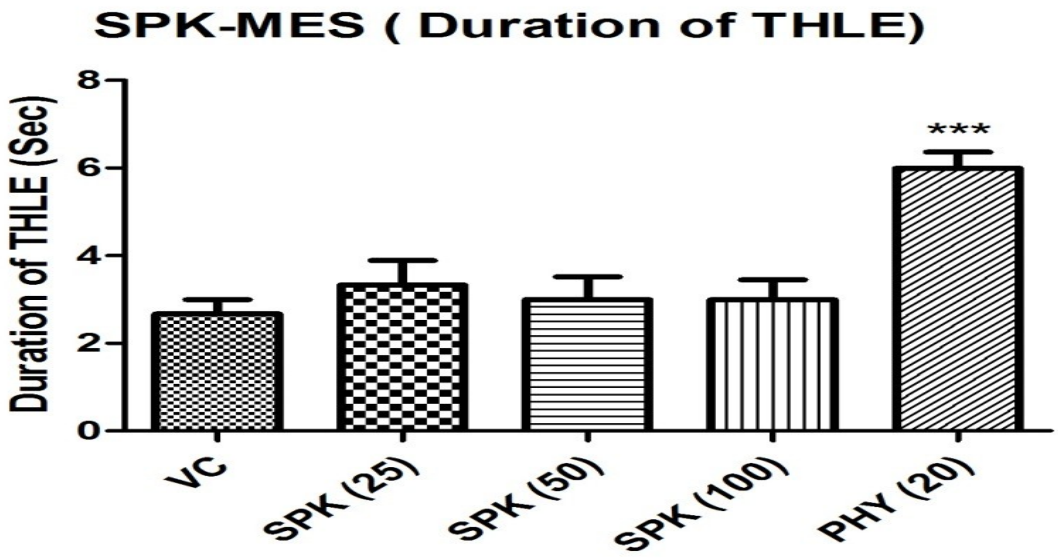

Figure2: SPK-MES (Duration of THLE)

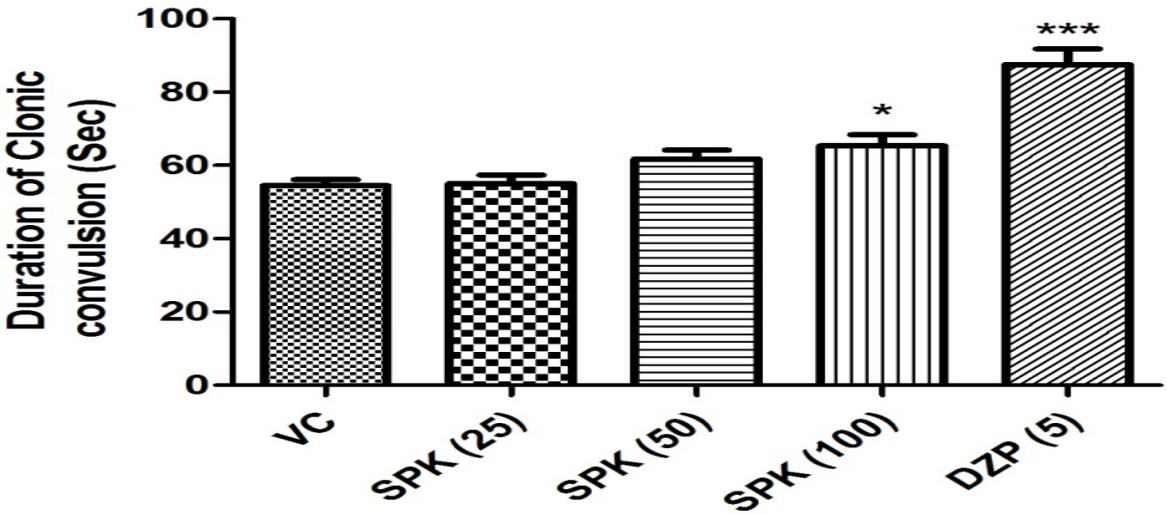

Figure3: SPK-PTX (Clonic convulsion)

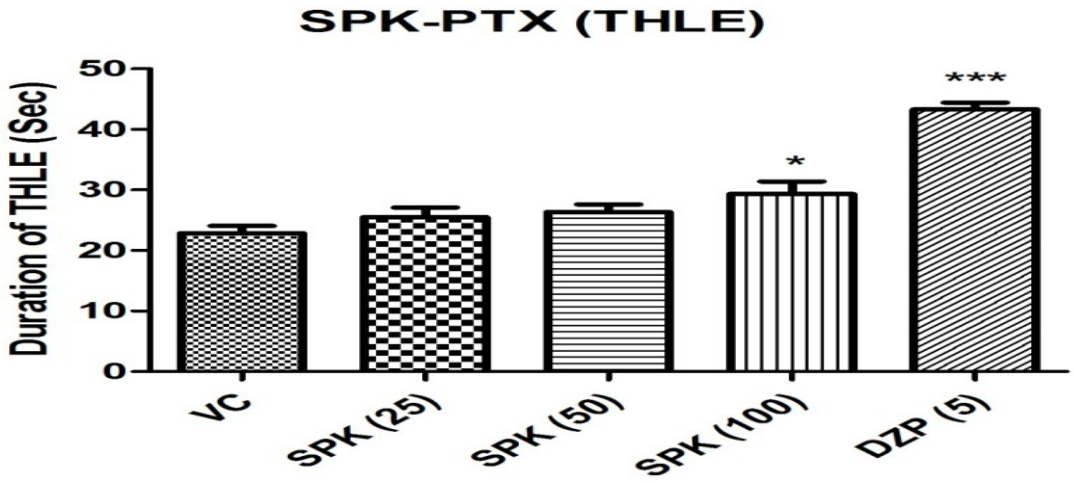

Figure4: SPK-PTX (THLE)

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