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ANTICONVULSANT ACTIVITY OF EXTRACT AND FRACTIONS OF *PINUS ROXBURGII SARG.* AGAINST PENTYLENETETRAZOLE INDUCED SEIZURE AND MAXIMAL ELECTROSHOCK (MES) INDUCED SEIZURES IN ALBINO RATS.

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ABSTRACT

Objectives: The aim of the present study was to evaluate the anticonvulsant potential of extract and fractions of *Pinus roxburgii Sarg*. against Pentylenetetrazole and maximal electroshock (MES) induced seizures in albino rats. **Materials and Methods**: The ethanol extract (300 and 600 mg/kg dose) and Chloroform, n-Hexane, n-Butanol and Ethylacetate fraction (250 and 500 mg/kg dose) of *Pinus roxburgii Sarg*. were tested at two doses (minimum and maximum) for its anticonvulsant activity using pentylenetetrazole (PTZ) induced and maximal electroshock (MES) induced seizures in albino rats. **Results:** There was significant ($P<0.05$) reduction in the duration of Tonic hind limb extension (THLE), tonic limb flexion (TLF) and clonic phase observed with extract and fractions but maximum reduction was observed with chloroform and n-Hexane fractions. Against Pentylenetetrazole(PTZ) induced seizure there was significant ($P<0.05$) reduction in the duration of myoclonic jerks, duration of clonic phase seen in all treated groups but maximum change was observed with 500 mg/kg Chloroform, 500 mg/kg n-Hexane and 600 mg/kg Extract treated group. **Conclusion:** The results demonstrated that extract and fraction of *Pinus roxburgii Sarg.* poses anticonvulsant potential against Pentylenetetrazole induced seizure and maximal electroshock (MES) induced seizures.

KEYWORDS: Pentylenetetrazole induced seizure, maximal electroshock (MES) induced seizure, *Pinus Roxburgii Sarg*., extract, fractions

INTRODUCTION

A seizure (from the Latin sacire, "to take possession of") is a paroxysmal event due to abnormal excessive or synchronous neuronal activity in the brain. Depending on the distribution of discharges, this abnormal brain activity can have various manifestations, ranging from dramatic convulsive activity to non convulsive activity or experiential phenomena not readily discernible by an observer (Lowenstein, 2015). Convulsions (from the Latin convulsionema, "to tear loose,") are involuntary, violent and spasmodic or prolonged contractions of skeletal muscle. A patient may have epilepsy without convulsions and vice versa (Sharma et al., 2011). Because a convulsion is often a symptom of seizure, the term convulsion is sometimes used as a synonym for seizure, but not all seizures are characterized by convulsion. Seizures can be "non-epileptic" when evoked in a normal brain by an acute systemic or acute neurologic insults or treatments such as electroshock or chemical convulsants, and "epileptic" when recurrent seizures occurring without evident provocation. Pharmacological agents in current clinical use inhibit seizures, and thus are referred to as anti-seizure drugs. Whether any of these prevent the development of epilepsy (epileptogenesis) is uncertain. The behavioural manifestations of a seizure are determined by the functions normally served by the cortical site at which the seizure arises (Bruton et al., 2011).Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological and social consequences of this condition(Fisher et al., 2005). The credit of defining epilepsy goes to Taylor. (1964), who expressed, "Epilepsy as a phenomenon, which scarcely warrants being called a symptom and not a disease. The incidence of epilepsy is 0.3-0.5% in different populations throughout the world, and the prevalence of epilepsy has been estimated at 5–30 persons per 1000 (Lowenstein, 2015). Epilepsy is the most frequent neurological disorder after stroke (Strine et al., 2005). Approximately 60% of all epilepsies are idiopathic. Almost any type of brain pathology can cause seizures. The underlying etiology is numerous and the abnormalities may range from symptomatic seizures due to tumour, infection, and trauma to cryptogenic forms. Cerebrovascular disease is the most commonly identified cause among adults, while prenatal insults seem to be most common among children (Robinson, 2005).

Pinus roxburghii Sargent (family: Pinacae) is commonly known as "chir pine" and has a long history of medicinal use[Shah R. 2006;]. It is native to the Himalayas, and was named after William Roxburgh.The range extends from northern Pakistan (NorthWest Frontier Province, Azad Kashmir, Margalla Hills, Islamabad Capital Territory, Murree), across northern India (Jammu and Kashmir,Punjab, Himachal Pradesh, Uttarakhand, Sikkim) and Nepal to Bhutan.It generally occurs at lower altitudes than other pines in the Himalaya, from 500–2,000 m (1,600–6,600 ft), occasionally up to2,300 m (7,500ft).

Traditionally plant Leaves (needles) and bark are used to treat eye, ear and pharynx disease, foul ulcers, haemoptysis, worm infection,flatulence, liver diseases, bronchitis, inflammation, skin disease, use as nerve tonic, expectorant(Rimpu M. et al.,2009).Snake bite , scorpion sting and for painful chest (Muhammad A.,2005).

In Ayurvedic medicine and traditionally bark of P. Roxburghii is prescribed as an antiepileptic, antiseptic, antidyslipidemic, spasmolytic and Antioxidant (Khare CP., 2007).

The therapeutic potential of Pinus Roxburgii which has been mentioned in Ayurveda to possess an antiepileptic effect has been evaluated. Reports in the previous study have revealed that bark of *Pinus Roxurgii Sarg*.provides protection against MES and PTZ- induced seizures in rats (Kaushik D., 2012).

In the present study, an effort was made to assess the potential of bark of *Pinus Roxburgii Sarg*.as an anticonvulsant. The antiepileptic action of P.Roxburgii extract and fractions was evaluated in wistar albino rats. To the best of our knowledge, till date there is only one report of such a study which provides anticonvulsant effect in maximal electroshock (MES) and PTZ–induced seizures is present.

MATERIALS AND METHODS

Ethical consideration: The study protocol was approved by Institutional Animal Ethics committee (IAEC), Jawaharlal Nehru Medical College, AMU Aligarh on 20/12/2014. All the animal experiments were carried out as per the rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Animals: Healthy, adult albino wistar rats of either sex, weighing 150-200 g were procured from central animal house, Jawaharlal Nehru Medical College, AMU Aligarh. They were kept in cages under standard environmental conditions (temp. 27±2º, humidity 30-70% day-light cycle of 12hrs) in the Pharmacology section of Central Animal House. The rats were acclimatized in laboratory conditions for 7 days before experiment. They were provided with standard diet and water ad libitum.

Plant Materials: The Stems Bark of *Pinus roxburgii Sarg*.were collected from naturally growing regions of Garhi cantt, Dehradun district, state Uttarakhand in the month of January 2015. Plant material was authenticated by Dr.Sunita Garg, Head, Raw Material Herbarium and Museum, CSIR-NISCAIR, N.Delhi. (Ref.No.NISCAIR/RHMD/Consult/2014/2784/163).

Preparation of Extract: The stem bark of *Pinus roxburgii Sarg*. was thoroughly washed and dried under shade at 28± 2ºC for 10 days. The dried plant material was grounded well into a fine powder in a electric grinder. 60 g of fine powder was packed in thimble, made of filter paper sheet and 300 ml 99.9% ethanol was used as solvent for extraction using Soxhlet extractor. Solvent was heated at 30-40^{0}C.Its vapour condensed in the condenser and the condensed extractant dripped in to the thimble containing the crude substance and extracted it by contact. Extraction process was continued until solvent coming down the siphoning tube became colourless which took 40-48 hours. The extract laden solvent was putted in Petridish and evaporated with water bath apparatus. This extraction process was repeated 5 times to obtain sufficient amount of plant extract for study. A total of 48.71 g of brown black residue with semisolid in consistency and pleasant in smell was recovered with 16% yield.

Preparation of Fractions: Ethanol Extract was fractionated in to four fractions by successive maceration with 150 ml of Chloroform, 150 ml of n-Hexane, 150 ml Ethyl acetate and 150 ml of n-Butanol as follows: 40 g of extract was taken and soaked for 24 hours in 150 ml of Chloroform in flask. Supernatant part of solution was isolated from the flask, taken in a Beaker, evaporated with water bath apparatus and concentrated, giving 8.52 g (20.92%) of Chloroform fraction.

Extract left in flask was 31.48 g (40-8.52). Similarly 31.48 g of extract was soaked in 150 ml of n-Hexane in flask for 24 hours. Supernatant part was isolated, evaporated and concentrated giving 2.41 g (7.65%) of n-Hexane fraction. Extract left in flask was 29 g (31.48-2.41).29 g of extract was soaked in 150 ml of Ethyl-acetate for 24 hours. Supernatant part was isolated, evaporated and concentrated giving 2.11 g (7.27%) of Ethyl-acetate fraction. Extract left in flask was 26.89 g (29-2.11).Similarly 26.89 g of extract was soaked in 150 ml of n-Butanol for 24 hours. Supernatant part was isolated, evaporated and concentrated giving 22 g (81.81%) of n-Butanol fraction.The amount of left extract in flask was 5 g.

DRUGS AND CHEMICALS

Pentylenetetrazole powder (SRL Pvt Ltd, Mumbai), Phenytoin injection (Cadila healthcare limited, Ahmadabad),Sodium valproate injection (Sun pharma Lab.Ltd,Mumbai),Distilled water,Normal saline.

INSTRUMENT
Electroconvulsiometer (orchid scientifics Ltd. Nasik, India), Electronic balance, Beakers, Syringes, Feeding cannula

EXPERIMENTAL DESIGN
After 7 days of adaptation to the laboratory conditions, the animals were randomly assigned to the twelve (12) experimental groups consisting of five (5) rats. All the tests were performed between 9:00 and 15:00 hr as per the following protocol.

Maximal electroshock induced seizures (MES)
Normal saline 1ml/kg p.o administered in normal control group rats (group-1). Phenytoin was dissolved in distilled water and 25 mg/kg was injected intraperitonially (i.p) in positive control groups rats (group-2) at volumes ranging 0.1-0.2 ml/100 g body weight.

Ethanol extract of 300 mg/kg and 600 mg/kg dose diluted in NS and given p.o to group-3 and group-4 rats. Chloroform, n-Hexane, n-Butanol and Ethylacetate fraction were diluted in NS and administered p.o at 250 mg/kg and 500 mg/kg dose in group-5, 6.7,8,9,10,11,12 respectively. The volume of test compounds was ranging from 0.1 to 0.2 ml/100 g body weight for oral administration. In each group of animals, seizure induction with MES was done 30 min after test drug administration. (Test compounds were administered 30 min before MES).

Pentylenetetrazole induced seizures (PTZ)
Normal saline 1ml/kg p.o administered in normal control group rats (group-1). Sodium valproate was dissolved in distilled water and 300 mg/kg was injected intraperitonially (i.p) in positive control groups rats (group-2) at volumes ranging 0.1-0.2 ml/100 g body weight.

Ethanol extract of 300 mg/kg and 600 mg/kg dose diluted in NS and given p.o to group 3 and group-4 rats. Chloroform, n-Hexane, n-Butanol and Ethylacetate fraction were diluted in NS and administered p.o at 250 mg/kg and 500 mg/kg dose in group-5, 6.7,8,9,10,11,12 respectively. The volume of test compounds was ranging from 0.1 to 0.2 ml/100 g body weight for oral administration.PTZ was dissolved in normal saline and injected intrapritonially at a volume of 0.1 to 0.2 ml/100 g. In each group seizure induction with PTZ was done 30 min after test drug administration.

EXPERIMENTAL PROCEDURE
Maximal electroshock induced seizures (MES)
This test was done by a modification of the method described by Castel-Branco et al. (2009). Animals were weighed and marked on a day before treatment with test compounds.

In each animal electroshock stimulation was applied through transauricular electrodes from an electroconvulsiometer at an intensity 150 mA fixed current, a 50-60 Hz pulse frequency for 0.2 duration.Tips of the electrodes were wetted with normal saline before applying over pinna.Animals were observed for 30 min and the whole seizure episode was video recorded.

Following parameters were noted in each animals
Onset of seizures.
Duration of tonic limb flexion (TLF).
Duration of tonic hind limb extension (THLE)
Duration of clonic phase.
Total duration of seizures.

All the group of animals were administered test compound 30 min before MES.

Briefly, following stimulus application an immediate severe tonic seizure with limb flexion (Tonic limb flexion) followed by maximal extension of the hind limb (Tonic hind limb extension) with stiff body was observed; at the end of this tonic phase, which usually lasts for 10-20 sec, clonic seizures started, characterized by paddling movements of the hind limbs and shaking of the body, which lasted for around up to 15-25 sec.The animal was usually able to come back to an upright position and start moving around, apparently recovering its normal behaviour.

Pentylenetetrazole (PTZ) – induced seizures
PTZ was freshly prepared and dissolved in normal saline and administered in a dose of 60 mg/kg i.p. This dose of PTZ has been standardized as 100% convulsant dose with minimal mortality in rats (Malhotra and Gupta, 1997).Animals were weighed and marked on a day before treatment with test drug.Each animal was administered with PTZ at a volume of 0.1-0.2 ml/100mg intraperitonially.Animal was observed for 60 min.Whole seizure episode was video recorded.Following parameters were noted:Onset of myoclonic jerks,Duration of myoclonic jerks,Duration of clonic phase,Total duration of seizures.All the group of animals were administered test compound 30 min before PTZ injection.

Percentage protection
Percentage protection was calculated in both the models as follows

% Abolition of THLE
= No of animals with THLE abolition/Total No. Of animals × 100

% Abolition of clonic phase
= No of animals with clonic phase Abolition/Total No. Of animals × 100

Statistical analysis: The data were analyzed with the SPSS version20 (Statistical Package for Social Sciences) software package. All the data is presented as mean ± standard error of mean. The data was analyzed by

ANOVA followed by Post hoc–Tukey test. A P-value of < 0.05 was considered significant.

Acute toxicity study

Toxicity studies were conducted as per internationally accepted protocol drawn under Organization for Economic Co-operation and Development (OECD) guidelines 425 in healthy young female albino rats. After 7 days of acclimatization to the laboratory condition and overnight fasting prior to dosing, a fixed test dose of 2000 mg/kg was administered by oral gavage using feeding cannula. Animals were observed once during the first 30 min after dosing, periodically during the first 24 hours and daily thereafter for a period of 14days.All observations were systemically recorded.

RESULT

Acute toxicity study of test compounds (Fractions) was found safe at the dose of 2000 mg/kg according to the OECD guidelines 425. And did not show any mortality up to 2000 mg/kg.Limit test was done for estimation of LD 50 (Median lethal oral dose). LD 50 > 2000 mg/kg was found for each fraction and extract.

Effect of extract and fractions of P.Roxburgii Sarg. against maximal electroshock (MES) induced seizures

Group	Onset of seizures	Duration of TLF	Duration of THLE	Duration of Clonic phase	Total duration of seizure	Recovery
NC(Normal saline)	2.20±0.200	5.60±0.245	10.40±0.245	11.20±0.374	27.20±0.200	3.40±0.244
PC(Phenytoin)	4.40±0.245	1.20±0.374	0.000	3.40±0.245	4.60±0.509	1.60±0.244
Extract 300	2.60±0.245	4.80±0.200	9.80±0.200	10.20±0.374	24.80±0.374	2.20±0.200
Extract 600	3.40±0.400*	4.60±0.245	9.40±0.245*	9.60±0.245**	23.60±0.244	2.20±0.200
CHL 250	3.40±0.245*	4.00±0.316**	9.40±0.245*	9.80±0.200*	23.20±0.374	1.80±0.200
CHL 500	3.80±0.200**	3.40±0.245***	8.20±0.200***	8.40±0.245***	20.00±0.316	1.60±0.244
HEX 250	3.20±0.374	5.20±0.374	9.40±0.245*	10.60±0.400	25.20±0.447	2.20±0.200
HEX 500	3.60±0.245*	4.40±0.245*	8.80±0.200***	10.00±0.316*	23.20±0.374	1.80±0.200
BUTA 250	2.40±0.245	5.60±0.245	9.40±0.245*	11.20±0.490	26.40±0.600	2.20±0.200
BUTA 500	3.20±0.374	4.20±0.200**	9.20±0.200**	10.20±0.200	23.60±0.400	2.00±0.000
EE 250	2.60±0.245	5.40±0.245	10.40±0.245	11.60±0.316	26.80±0.374	2.40±0.244
EE 500	3.20±0.374	5.40±0.245	10.20±0.374	10.60±0.245	26.20±0.489	2.20±0.200

TLF-Tonic limb flextion, THLE-Tonic hind limb extension. CHL-Chloroform, HEX-Hexane, BUT-Butanol, EE-Ethylacetate, NC-Normal control, PC-Positive control.
Value indicate mean ± SEM, *P<0.05, **P<0.01, ***P<0.001.

Selected dose of Extract and Fractions were tested against maximal electroshock induced seizures in rats.

Significant (P<0.05) delayed in **Onset of seizures** was observed with Extract 600 mg/kg, chloroform 250 mg/kg and n-Hexane at 500 mg/kg treated group. Delayed onset was found very significant (P<0.01) with chloroform 500 mg/kg treated group. Although not significant but delayed onset was also observed with others test compound treated group.Duration of tonic limb flexion (TLF) was reduced significantly (P<0.05) in group treated with n-Hexane 500 mg/kg and very significant (P<0.01) reduction was seen in chloroform 250 mg/kg and n-Butanol 500 mg/kg treated group. Highly significant (P<0.001) reduction was seen in chloroform 500 mg/kg group.There was significant (P<0.05) reduction in the duration of Tonic hind limb extension (THLE) in group treated with Extract 600 mg/kg, chloroform 250 mg/kg, n-Hexane 250 mg/kg and n-Butanol 250 mg/kg and very significant (P<0.01) reduction was observed with n-Butanol 500 mg/kg treated group. Reduction was highly significant (P<0.001) in chloroform 500 mg/kg and n-Hexane at 500 mg/kg treated group. Reduction was also observed in other treated group but was not significant.There was significant (P<0.05) reduction in the duration of clonic phase with chloroform 250 mg/kg and n-Hexane 250 mg/kg treated group. Very significant (P<0.01) reduction was observed with Extract 600 mg/kg treated group and highly significant (P<0.001) reduction was observed with chloroform 500 mg/kg group.

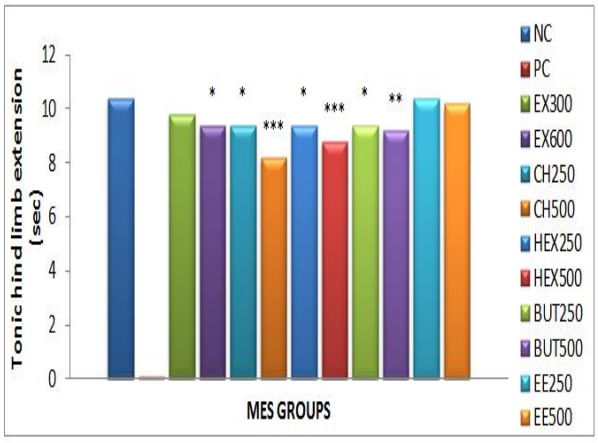


Figure 1: Effect of extract and fractions on duration of THLE in maximal electroshock induced seizures.
Value indicates Mean ± SEM. Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001.

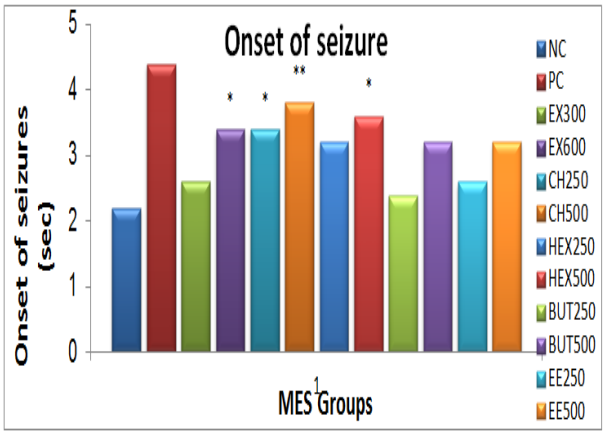


Figure 2: Effect of extract and fractions on onset of seizures in maximal electroshock induced seizures in rats.

Percentage protection offered by test compound in MES induced seizure model: Percentage protection was calculated by a formula described in the section of material and methods, and it was observed that there was 60% protection at 500 mg/kg Chloroform treated group, 40% protection was observed with 250 mg/kg Chloroform, 250 & 500 mg/kg n-Hexane and 500 mg/kg n- Butanol treated group and 20% protection was observed with 250 mg/kg n-Butanol, 300 & 600 mg/kg Extract and 500 mg/kg ethylactate treated group.

100% protection was observed with positive control phenytoin treated group and no protection was observed with normal control normal saline treated group.

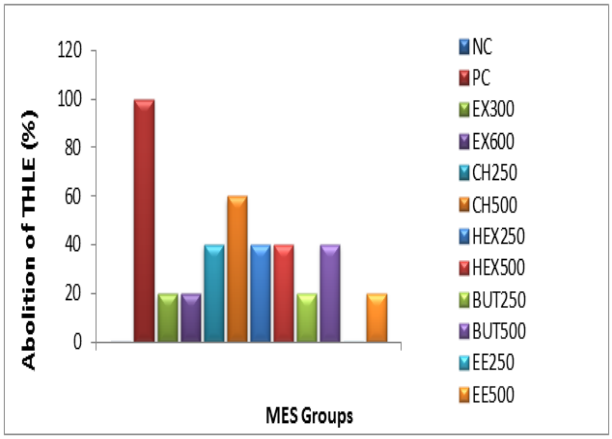


Figure 3: Percentage protection in rats treated with different doses of extract and fractions.

Tab 2: Effect of extract and fractions of P.Roxburgii Sarg. against Pentylenetetrazole (PTZ)-induced seizures.

group	Onset of myoclonic jerks	Duration of myoclonic jerks	Duration of Clonic phase	Total duration of seizure
NC (Normal saline)	65.20±1.715	57.80±0.663	25.20±0.374	83.00±0.837
PC (Sodium Valproate)	179.20±0.583	3.20±1.319	2.00±0.316	5.20±1.356
Extract300	71.80±1.594	55.40±0.837*	24.00±0.316	79.00±0.894
Extract600	72.80±1.594*	54.60±0.510*	23.40±0.400	78.40±0.600*
CHL250	75.60±1.435**	54.40±0.748**	24.00±0.632	78.40±0.980*
CHL500	78.40±1.122***	50.60±0.245***	19.80±0.374***	70.40±0.245**
HEX250	71.80±1.594	55.00±0.548*	24.20±0.800	79.40±0.678
HEX500	73.40±1.749*	55.00±0.548*	22.60±0.510**	77.60±0.510*
BUTA250	71.80±1.594	55.40±0.510	24.00±0.548	79.40±.030
BUTA500	72.80±1.594*	55.40±0.400	23.80±0.374	79.20±0.663
EE250	69.40±1.631	57.40±0.510	25.00±0.316	82.40±0.678
EE500	69.80±1.855	56.80±0.374	24.40±0.245	81.20±0.490

Value indicate mean ± SEM, *P<0.05, **P<0.01, ***P<0.001. CHL-Chloroform, HEX-Hexane, BUT-Butanol, EE-Ethylacetate, NC-Normal control, PC-Positive control.

Same doses of test compounds as in MES model were used to evaluate their effect against Pentylenetetrazole (PTZ) - induced seizures.

Effect of ethanol extract and their Chloroform, n-Hexane, n-Butanol, and Ethyl acetate fraction against pentylenetetrazole induced seizures in rats

Delayed onset of myoclonic jerks were seen in all treated groups but significant (P<0.01) change was observed with 500 mg/kg Chloroform, 500 mg/kg n-Hexane and 600 mg/kg Extract treated group.There was significant (P<0.05) reduction in the duration of myoclonic jerks was observed with 300 & 600 mg/kg Extract and 250 & 500 mg/kg n-Hexane treated group. Very significant (P<0.01) reduction in the duration of myoclonic jerks was observed with 250 mg/kg chloroform. Highly significant (P<0.001) reduction was observed with 500 mg/kg chloroform treated group. Although not significant but reduction in the duration of myoclonic jerks was also observed with others test compounds. There was significant (P<0.05) reduction in the duration of clonic phase was observed with 250 mg/kg chloroform and n-Hexane 250 mg/kg treated group whereas very significant (P<0.01) reduction was observed with 500 mg/kg chloroform and 500 mg/kg n-Hexane treated group. Total duration of seizures was reduced in all treated groups but significant (P<0.05) reduction was observed with n-Hexane 250 mg/kg and n-

Butanol 500mg/kg treated group whereas very significant (P<0.01) reduction was observed with chloroform 250,500 mg/kg and n-Hexane 500 mg/kg treated group.

Percentage protection offered by test compound in PTZ induced seizure model: Percentage protection was calculated by a formula described in the section of material and methods, and it was observed that there was 60% protection at 500 mg/kg chloroform, 40% protection with 250 mg/kg chloroform and 500 mg/kg n-hexane. 20% protection with 600 mg/kg Extract, 250 mg/kg n-Hexane and 500 mg/kg n-Butanol. 100% protection was observed with positive control (Sodium valproate) treated group and no protection was observed in Extract 300 mg/kg, n-Butanol 250 mg/kg, Ethylacetate 250,500 mg/kg and normal saline treated groups.

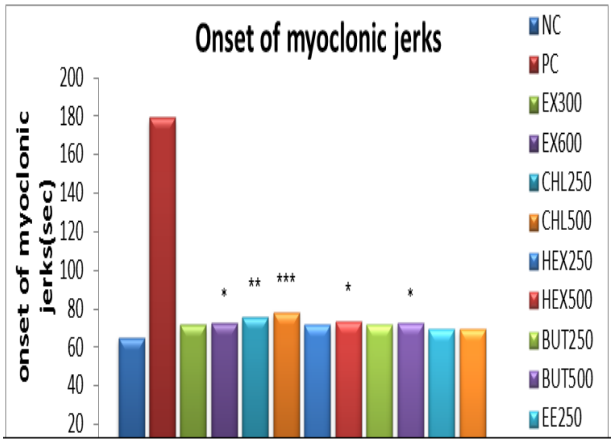
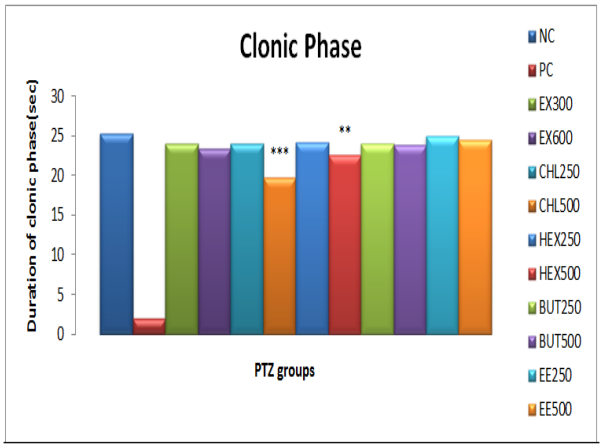


Figure 4: Effect of extract and fractions of *P.Roxburgii Sarg*. on the onset of seizures in PTZ model.

Value indicates Mean ± SEM. Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001.



Value indicates Mean ± SEM. Values are expressed as Mean ± SEM (n = 5) *P<0.05, **P<0.01, ***P<0.001.

Figure 5: Effect of extract and fractions of P.Roxburgii Sarg. on duration of clonic phase in PTZ model.

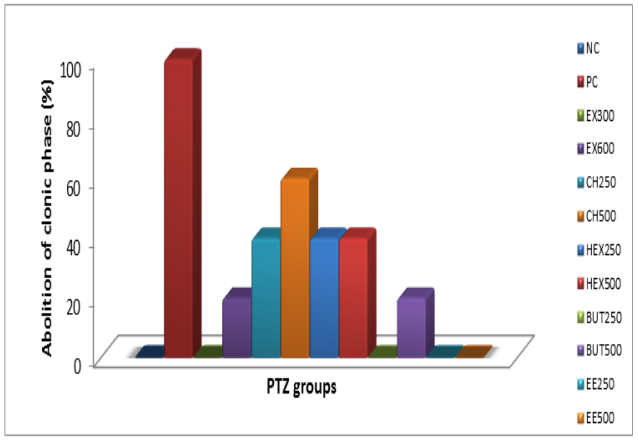


Figure 6: Percentage protection in rats treated with different doses of extract and fractions.

DISCUSSION

Nowadays, herbal medicines are being increasingly used to supplement the conventional medicines worldwide and epilepsy is no exception. This, however, necessitates the scientific scrutiny of these herbal medicines (Barrett et al., 1999 and Tindle et al., 2005).

Thus, many plants were known for their anticonvulsant activity. Various physiochemical and pharmacological studies have been carried out on these anticonvulsant plants (Loscher W and Schmidt D, 1988). Currently available anticonvulsant drugs are able to efficiently control epileptic seizures in about 50% of the patients; another 25% may show improvement where as the remainder does not benefit significantly (Nadkarni A K, 1982). Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult, so, a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compound, which may belong to new classes.The MES test is the most frequently used test as an animal model for identification of anticonvulsant activity of drugs for the generalized tonic-clonic seizures “grand mal”. (Loscher W and Schmidt D, 1988; Oliveira FA et al., 2001).This model is based on observation of the stimulation by repeated electrical pulses. (QuintansJúnior LJ et al.,2002).The MES model is used to identify compounds which prevent seizure spread, corresponding to generalized tonic-clonic seizures in humans (Kupferberg HJ.1989 ; Kupferberg HJ, Schmutz M,1998).Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor, such as felbamate (Sayyah M et al.,2002). Currently used anticonvulsant drugs such as phenytoin and carbamazepines which are effective in treatment of generalized tonic-clonic and partial seizures have been found to show strong anticonvulsant action in MES test (White HS, 1997 and Macdonald RL, 1995).

PTZ-induced seizure is analogous to petit mal seizure and human generalized seizure (Loscher W and Schmidt D, 1988). Compounds effective against this experimentally induced seizure models are effective against petit mal type of epilepsy (Vida JA.,1995). Drugs that are effective against petit mal seizures reduce T-type calcium currents and these types of seizures can also be prevented by drugs that enhance γ-aminobutyric acid (GABA) or benzodiazepine receptor which mediated neurotransmission such as benzodiazepines and phenobarbitone (Macdonald RL,1995 and Swinyard EA,1952).Studies have shown that activation of receptor are also involved in the initiation and generalization of PTZ-induced seizures (Nevins ME, Arnolde SM.,1989 and Velísek L,1990).Drugs that block glutamatergic excitation mediated by NMDA receptors, such as felbamate, have anticonvulsant property against PTZ-induced seizures (Macdonald RL,1995).

In Ayurvedic medicine and traditionally bark of P. Roxburghii is prescribed as an antiepileptic (Khare CP., 2007).

The therapeutic potential of Pinus Roxburgii which has been mentioned in Ayurveda to possess an antiepileptic and antioxidant effect has been evaluated. Reports in the previous study have revealed that bark of Pinus Roxurgii Sarg.provides protection against MES and PTZ- induced seizures in rats (Kaushik D., 2012).

In the present study, an effort was made to assess the potential of bark of Pinus Roxburgii as an anticonvulsant and antioxidant. The antiepileptic action of P.Roxburgii extract and fractions was evaluated in wistar albino rats. To the best of our knowledge, till date there is only one report of such a study which provides anticonvulsant effect in maximal electroshock (MES) and PTZ–induced seizures is present.

In our study Ethanol extract and Chloroform,n-Hexane,n-Butanol and Ethylacetate fraction of Pinus Roxurgii has shown protection against Maximum Electroshock Seizures(MES) and PTZ- induced seizures in rats.

In MES induced seizures, the extract and fractions of bark of P. Roxburghii reduced the all phases of seizures and shortens the recovery time effectively when compared with normal control group. There was significant reduction in the duration of Tonic hind limb extension (THLE) in group treated with n-hexane 500 mg/kg and n-Butanol 500 mg/kg and reduction was highly significant in chloroform 500 mg/kg treated group. Reduction was also seen in other treated group but was not significant.

In PTZ induced seizures there was delayed in the onset of myoclonic jerks, duration of myoclonic jerks,clonic phase and total duration of seizures in all treated groups but delayed is highly significant ($P<0.01$) in Chloroform 250,500 mg/kg and n-Hexane 500 mg/kg treated group.

CONCLUSION

Based on present study we found that, bark of Pinus Roxburgii Sarg. has protective effect against both MES and PTZ- induced seizures. Maximum protection was seen in Chloroform and n-Hexane treated groups. Result of the present study suggests the therapeutic utility of Pinus Roxburgii Sarg. supplementation in the case of intractable seizures and in the management of GTCS and petit mal seizures as an adjunct with the standard antiepileptic drugs. This can lead to better seizure control and reduce the problem of intractable seizures which is associated with one third cases of epilepsy.

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