Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
7(1), 2018, 01-06

ISSN 2321 – 0958

Original Research Article

ANTICONVULSANT SCREENING OF HYDRO-ALCOHOLIC ROOT EXTRACT OF *BERBERIS ARISTATA* IN MICE

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Abstract: Objective: To explore anti-convulsant potential of hydro-alcoholic extract of *Berberis aristata* (*B. aristata*) from pentylenetetrazole-induced and maximal electroshock (MES) seizure test. **Material & Methods:** The test groups receive different doses (50, 100, 250 and 500 mg/kg) of the hydro-alcoholic extract; however positive control (standard) Phenobarbitone (20 mg/kg) using for Pentylenetetrazole-induced Seizure and Diazepam (1mg/kg) used as standard for maximal electroshock induced seizure. Swiss albino mice weigh about 20-25 g of either sex selected for the investigation. **Results:** Phytochemical investigation reveals that occurrence of alkaloids, flavonoids in the hydro-alcoholic root extract of *Berberis aristata*. Acute toxicity study shows no morality action up to 1000 mg/kg dose after 72 h observation. Hydro-alcoholic extract of *B. aristata* offered a concentration depending upon act of pentylenetetrazole-induced seizure (PTZ) and maximal electroshock induced seizure (MES). **Conclusions:** These finding suggest that hydro-alcoholic extract of *B. aristata* remarkable effect in the treatment in epilepsy.

Key-words: Anti-convulsant, *Berberis aristata*, MES, Pentylenetetrazole, Diazepam, Epilepsy.

Introduction: Epilepsy is a disorder pertain neurological imbalance of brain patho-physiology and effected in wide range of humankind throughout world. It is describe by impulsive and episodic occur of a transient change of behavior due to the chaotic, synchronous and recurring firing of inhabitants of brain neurons[1]. Incidence of epilepsy in developed countries is approximately 50 out of 100,000 peoples, while that of developing country is 100 out of 100,000 persons approximately[2]. It has been apparent that the presently existing of anti-epileptic drugs like phenytoin, carbamazepine and sodium valproate bring with them many serious side effects particularly neurotoxicity[3]. As mainstream of

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Received on: December 2017
Accepted after revision: February 2018

anti-epileptic drugs are spent life long, attendant management of other drugs inclines to the risk of drug interface. Although, novel anti-epileptic likes gabapentin, vigabatrin, lamotrigine will be used additional to the orthodox agents[4]. Thus, it's essential to study for an antiepileptic drug that is highly effective and safe in items of drug linked harmfulness. The goal of treating an epileptic is not only to eliminate the incidence of seizures but also principal a self-sustained life[5].

Berberis aristata commonly identified by "Indian barberry, tree turmeric "family known is berberidaceae. It is widely used in Ayurvedic system of medicines since very long time. *B. aristata* commonly phyto-constituents, yellow coloring alkaloids known as berberine, oxyberberine, berbamine, aromoline, a protoberberine alkaloid karachine, palmatine, oxycanthine and taxilamine and tannins, sugar, starch[6]. Pharmacological studies on the plants reveal the proven activity of its hypoglycemic[7-9], antibacterial[10-12], wound healing[13], anti-diarrheal [14], anti-allergic[15] and anticancer[16-18].

In scientific literature evidence there are not prove that *B. aristata* as anti-convulsant potential. Thus, it was decided to investigate, anti-convulsant screening of *B. aristata*on experimentally induced model in rats.

Materials and Methods

Plant Material: The roots of *Berbaris aristata* was collected from district Sehore, Madhya Pradesh, India. The plant were identified and authenticated by Department of Botany, Saifia College of Science & Education, Bhopal, Madhya Pradesh, India. Specimen voucher no. is 211/Bio/saifia/17. A voucher specimen of the plant is deposited at the College of Pharmacy, SSSUTMS, Sehore, Madhya Pradesh, India.

Preparation of Extract: Fresh *B. aristata* root was clean, dried in shadow and powdered by a mechanical crusher. Each dry root crumpled to a fine texture and 200 g of the dry plant was continually extracted by water: ethanol (50:50). The extract was concentrate using vacuum conditions and the filtrate were used in the experiments. The fresh extract was re-dissolved in standard saline and particular to adult albino Swiss rat fed a standard animal diet. Unless otherwise stated, hereafter, the term 'extract' means the water: ethanol (60:40) extract of root parts of *Berbaris aristata*[19].

Drugs: Pentylenetetrazole and Diazepam were procured from Sigma Chem. Company (Hyderabad, India). Some concentration of the drug was setrecently by suspending in Gum-acacia in water. The solvents using were of analytical grade. Ethanol, Petroleum Ether (BDH, Mumbai, India) used as solvent and vehicle separately.

Animals: Swiss albino male mice (20-25 g) were divided into five group in housed (n=6) under a standard 12 hours light and dark cycle and controlled conditions of temperature as well humidity (25±2°C, 55-65%). Mice usual standard rodent grub and water *ad libitum*. The experiment protocol was approved by Institutional Animals Ethics Committee (IAEC) of College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Madhya Pradesh, India under CPCSEA, New-delhi. Animals were adapted to laboratory circumstances for seven days before bringing out the experiments.

Acute Toxicity: The acute oral toxicity study was carried out for hydro-alcoholic extract of *B. aristata* root by fixed dose methanol as per OECD (1993) guideline no. 420. Healthy adult female Swiss albino mice chosen for this study have been weighing among 25 to 35 g. Animals were separated into four individual groups of three animals each and fasted overnight 5, 50, 300 and 2000 mg/kg *b. w.* doses was managed to the Group I, II, III, and IV correspondingly.

After direction of extracts several parameters like body temperature, CNS activity, maturation, defecation *etc.* were experiential for 24 h. Four groups of mice of both sexes (six animals per group) were accomplished oral a single dose of 5, 10 or 15 times of actual dose of aqueous root extract of *B. aristata*. The rats were perceived for gross behavioral, neurologic, autonomic and toxic outcome unceasingly. Food consumption, feces and urine were also investigated after 2 hours also at 6 hours intervals for 24 h[20].

Phytochemical Screening: In phytochemical investigation of the hydro-alcoholic extract of *B. aristata* were performed using reagents and chemicals like alkaloids using Dragendorff's reagent, flavonoids by using of Mg and HCl, tannins with 1% gelatin and 10% NaCl solutions, and saponin that can produce suds and induce hemolysis reaction[21].

Anticonvulsant Activity

Pentylenetetrazole-induced Seizure Test: Rats of either sex (20±2 g) were divided into five sets of ten animals each(1) negative control group with usual saline (10 mL/kg), (2) positive control group with phenobarbitone sodium (20 mg/kg), (3, 4, 5 and 6) hydro-alcoholic extract treated groups (50, 100, 250 and 500 mg/kg separately). The mice were given hydro-alcoholic extract, and control, intraperitoneally, 60 minutes before to the administration of 90 mg/kg pentylenetetrazole (PTZ). Every animal is located into adistinct plastic crate for thought fasting 1 hour. Seizures and tonic-clonic convulsions are recorded. At least 80% of the animals in the control group have to show convulsions[22].

Maximal Electroshock Seizure Test: Electroconvulsive shock (50 mA for 0.2 sec) was brought done ear electrodes to persuade hind limb tonic extensions (HLTEs) in mice. The extract was administered orally by doses of 50, 100, 250 and 500 mg/kg into test groups. Gum acacia in water and Diazepam (4mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. Electroconvulsive shock was delivered 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted closely for 2 min. The animals that did not exhibit HLTE were careful secure. Percentage of hang-up of annexations comparative to controls was calculated[23].

Statistical Analysis

The data was analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test using ρ values <0.05 were reflected important.

RESULTS

Acute Toxicity Study: There was no death between the grouped doses of mice up to a dose of 1000 mg/kg for duration of 72 h. This result perhaps suggests that the hydro-alcoholic extract is comparatively safe or non-toxic in mice at the doses used for this study.

Phytochemical Investigation: Phytochemical investigation of the hydro-alcoholic extract showed the presence of alkaloids and tannins.

Pentylenetetrazole-induced Seizure Test: Pentylenetetrazole (PTZ, 90 mg/kg, *i.p.*) produced hind-limb tonic seizures in all the 10 mice used. *B. aristata* root hydro-alcoholic extract (BHE, 50-500 mg/kg, *i.p.*) produced a dose related, significantly (ρ < 0.05-0.001) protection of the mice against BHE-induced seizures **(Table 1)**. The plant extracts (BHE, 50-500 mg/kg *i.p.*) significantly delayed (ρ < 0.05-0.001) the onset of, and antagonized PTZ-induced seizures. Phenobarbitone which have using as reference anticonvulsant drugs, (PBT, 20 mg/kg, *i.p.*) also greatly delayed the onset of, and significantly antagonized (ρ <0.001), PTZ-induced seizures.

Table 1. Effects of *Berbarisaristata*root aqueous extract (BHE), phenobarbitone on pentylenetetrazole (PTZ)-induced seizures in mice.

	Treatment dose (mg/kg, *i.p.*)		No. convulsed/ No. used	% Animals not convulsed (*i.e.* % animals protected)	Latency of tonic convulsion (min) (mean ± SEM)
PTZ	BHE	Phenobarbitone			
90	-	-	10/10	0	8.01±0.35
90	50	-	7/10	30	11.30±1.44[a]
90	100	-	6/10	40	14.52±2.23[b]
90	250	-	4/10	60	17.48±2.23[c]
90	500	-	2/10[d]	80	20.39±2.41
90	-	20	0/10[d]	100	0.00[c]

[a]ρ< 0.05; [b]ρ< 0.01; [c]ρ< 0.001 vs. pentylenetetrazole control (PTZ, 90 mg/kg, *i.p.*).
[d]ρ< 0.001 *vs.* pentylenetetrazole control (PTZ, 90 mg/kg, *i.p.*).

MES-Induced Seizures: Albino mice pre-treated with the hydro-alcoholic extract has been suggestively endangered from convulsions persuaded by electroshock one hour post-doing. The duration of HLTE achieved at the doses 250 and 500 mg/kg were 13.3±0.18 (ρ<0.001) and 12.1± 0.28 (ρ<0.001) respectively. Extract at both the doses, protracted the onset off convulsions in the extract preserved group compared to vehicle treated control group **(Table 2)**.

Table 2. Effect of hydro-alcoholic extract of *B. aristata* on tonic seizures persuaded by maximal electroshock in mice.

Treatment	Duration of HLTE (s)	Protection against seizure	Mortality Protection after 30 min (convulsion survivors/ animals tested)	Mortality protection after 24 h (convulsion survivors/animals tested)
Normal saline (10 mL/kg)	15.6 ± 0.2	0/8	6/8	6/8
Diazepam (1 mg/kg)	10.6 ± 0.25	4/8*	8/8	8/8
Hydro-alcoholic extract (50 mg/kg)	15.3±0.18	0/8	6/8	6/8
Hydro-alcoholic extract (100 mg/kg)	14.8±0.27	0/8	7/8	7/8
Hydro-alcoholic extract (250 mg/kg)	13.3±0.18**	1/8	7/8	7/8
Hydro-alcoholic extract (500 mg/kg)	12.1± 0.28***	2/8*	8/8	7/8

The hydro-alcoholic extract and diazepam were administered 30 minutes prior to induction of MES seizures. Values are the mean ± SEM for eight mice.
***ρ< 0.001 and *ρ< 0.05, as compared to control (normal saline).
HLTE Hind Limb Tonic Extension, *MES* Maximal Electroshock, *SEM* Standard Error of Mean

Discussion: In present study, anti-convulsing screening of hydro-alcoholic root extract of *Berberis aristata* from pentylenetetrazole-induced (PTZ) and maximal electro-shock model. The statistical data of these study finding that, significantly rises the onset time and reductions the duration of seizures by electroconvulsive shock at optimum doses as compare with standard drug using phenobarbitone. The study such as reveals that the beginning of tonic convulsion shaped by Pentylenetetrazole was knowingly behind and also duration of seizures was protracted.

MES and PTZ should be using their convulsant effects by inhibiting the action of gamma amino butyric acid (GABA) at GABA-A receptors. Gamma amino butyric acid is the main inhibitory neurotransmitter which is disturbed in epilepsy.

Diazepam is standard anti-epileptic drugs have been exposed to use their anti-epileptic properties by improving GABA-mediated reserve in the brain. It is likely that Diazepam antagonize MES and PTZ convulsions in that study by increasing GABA neuro-transmission.

Since the hydro-alcoholic extract of *Berbaris aristata* stuck the incidence of MES and PTZ convulsions, it is likely that it may be nosy with gabaergic mechanism(s) to use their anticonvulsant result.

Conclusion: In concluded that, *B. aristata* possess anti-convulsant potential against the PTZ and MES induced seizures. However extensive studies are needed to screening precise mechanism(s), isolation of active constituents, and establishment of its pharmacokinetics profile.

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