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Research Article

EVALUATION OF ANTICONVULSANT ACTIVITY OF *TABERNAEMONTANA DIVARICATA* (LINN) R. BR. FLOWER EXTRACT

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ABSTRACT

The present study was designed to investigate the spontaneous motor activity (SMA) and anticonvulsant potential of alcoholic extract of flowers of *Tabernaemontana divaricata* (Linn.) (ALETD) by using different paradigms. The SMA was tested by using photoactometer. The anticonvulsant property was tested by using animal models like maximal electroshock-induced convulsion (MESIC), pentalenetetrazol (PTZ)-induced convulsion (PTZIC), strychnine-induced convulsion (SIC), picrotoxin-induced convulsion (PIC), isoniazid (INH)-induced convulsion (IIC) and 4-amino pyridine (4-AP)-induced convulsion (4-APIC) in mice. Finally the GABA (Gama-amino butyric acid) level also estimated. Significant reduction in locomotor score was recorded with a diazepam (5 mg/kg) and ALETD (100, 200 & 400 mg/kg). In MESIC a phenytoin (25 mg/kg) and ALETD (100, 200 & 400 mg/kg) were possessed anticonvulsant activity by decreased duration of tonic extensor phase of the animals. In PTZIC, SIC PIC, IIC and 4-AMIC models, a diazepam and in all dose level of ALETD offered increased on set and onset of tonic convulsion. Increased GABA level in the mice serum was observed with a gabapentin (20 mg/kg) and extract. The alkaloids, flavonides and other chemical constituents present in ALETD are speculated to account for the observed pharmacological effects of the plant's extract in the experimental animal paradigms used. The findings of this experimental animal study indicated that ALETD possess anticonvulsant property and thus lend pharmacological credence to the folkloric and ethanomedical uses of the plant in the treatment of epilepsy condition.

Keywords: *T. divaricata,* GABA, Strychnine, Gabapentin.

INTRODUCTION

Epilepsy is a neurological disorder that affects a wide range of people throughout the world. It is a disorder of brain characterize by unpredictable and periodic occurrence of a transient alteration of behaviour due to the disordered, synchronous and rhythmic firing of populations of brain neurons[1]. It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients[2, 3]. The conventional antiepileptic agents like phenytoin, carbamazeipine and sodium valporate carry with them several serious side effects notably neurotoxicity[4]. As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like gabapentin, vigabatrin, lamotrigine etc are used supplemental to the conventional agents[1]. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug related toxicity. The aim of treating an epileptic is not only to abolish the occurrence of seizures but also to lead a self sustained life.

In the present study we selected a plant namely *Tabernaemontana divaricata* (Linn.) R.Br. (*T.divaricata*) belonging to the family of Apocynaceae. It is distributed in throughout India, also cultivated as an ornamental plant. The flowers are white, sweetly fragrant in 1-8 flowered cymes at the bifurcations of the branches. In traditionally the plant is used as an emmenagogue, aphrodisiac, tonic, purgative, tonic to the brain, the liver and spleen. It is useful in paralysis, weakness of the limbs, cures scorpion-sting and epilepsy. Its charcoal is good in ophthalmia. The oil is good for epilepsy (Yunani) [5].

Earlier the plant has been studied for its, anti-inflammatory[6], acetyl cholinesterase inhibitory[7], blocking of cell proliferation, inhibition of amyloida β-peptide 35-25 induced cognitive deficits[8], anti-acne[9], antioxidant, anti-inflammatory[10] and antifertility[11] activities.

Considering the varied important activities reported in traditional system of medicine with this plant. It was planned to study the effects of flowers extract of *T.divaricata* on CNS mainly for its anticonvulsant activity.

MATERIALS AND METHODS

Drugs and chemicals: Phenytoin, Gabapentin, (Sun Pharmaceutical Industries), Diazepam (Ranbaxy Laboratories Ltd.), PTZ (Sigma Aldrich Pvt.Ltd.), INH (sd. Fine chemicals Pvt.) Strychnine, Picrotoxin, 4-Amino pyridine (HiMedia Laboratories).

Animals: Albino mice weighing between 18-22 g of either sex were used for in this study. All the animals were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. After procuring, all the animals were acclimatized for 7 days and housed in groups of 06 under standard husbandry condition[12] like room temperature 26 ± 2^{0}C, relative humidity 45-55% and light/ dark cycle of 12 h.

All the animals were fed with synthetic standard diet (Pranava Agro Industries Ltd., Bangalore.) and water was provided *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethical Committee (IAEC) of T.V.M. College of Pharmacy, Bellary (Karnataka), animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA with R. No. 462/01/CPCSEA, 2001.

Plant material: The flowers of *T.divaricata* were collected from Bellary (Karnataka State) and authenticated and identified by a Botanist Dr. Govind Raju of A.S.M. College Bellary. The flowers were dried in shadow and slices of flowers were subjected to size reduction by using mixy, to coarse powder.

Preparation of alcoholic extract[13,14]: The air dried plant flowers powder was extracted successively with the different solvents of their increasing polarity in a soxhlet extractor. The alcoholic extract was subjected to evaporation by using flash evaporator to dryness on the water bath in low heat, till the thick paste remained in the evaporator. However, it was kept in a refrigerator below 4ºC till the experimental study.

Preliminary phytochemical screening[13,14]-The preliminary phytochemical investigation was carried out with alcoholic extract of flowers of *T. divaricata* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods**.** All the chemicals and reagents used were of analytical grade.

Pharmacological activities

Determination of acute toxicity (LD_{50})[15]**:** The acute toxicity of ALETD was determined by using albino mice of either sex (18-22 g). The animals were fasted 3 h prior to the experiment, Acute Toxic Class method (OECD guideline No. 423) of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extract and observed for its mortality during 48 h study period (short term toxicity). Based on short-term toxicity profile of extract the dose for the next animal was determined as per as OECD guideline No.423.

Spontaneous motor activity[16, 17]**:** Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with 3% Tween 80 (10 ml/kg p.o.) only once daily for 7 days, group II received diazepam (5mg/kg, i.p.) before test. Groups III, IV, V were treated with different doses of ALETD (100, 200 & 400 mg/kg, p.o.) respectively once daily for 7 days. On 7th day, after treatment of last dose each mouse was placed individually in the photoactometer for 10 min. Number of "cut offs" i.e., locomotor scores was recorded at the prefixed time interval i.e., initial, 0.5 h, 1 h and 2 h.

The experiment was conducted in a sound attenuated room. A drug with increased or decreased CNS activity will also produce increase or decrease in locomotor activity in the animals. Photoactometer (INCO, Ambala, India) is designed on this principle consists of a cage with 30 cm. long and 30 cm. deep with a wire mesh at the bottom. Six lights and 6 photocells were placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. Technically its principle is that a photocell is activated when animals crossing the beam of light "cut off" the rays of light falling on photocells. The photocells are connected to an electronic automatic counting device, which counts the number of "cut offs".

Anticonvulsant activity

MES induced convulsios[18-20]**:** Five groups of mice each comprising 06 animals, weighing between 18-22 g were used. Group I was maintained as normal control which was given with 3% Tween 80 only (10 ml/kg p.o.) only once daily for 7 days. Group II was injected with phenytoin (25 mg/kg, i.p.) alone on 1st day and after 30 min a 60 mA current was delivered transauricularly for 0.2 sec on mice via small alligator clips attached to cornea by using electroconvulsive meter (ECM) and different phases of convulsions were recorded. Groups III, IV and V were treated with different doses of ALETD (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of 3% Tween 80 and ALETD a 60 mA current was delivered transauricularly for 0.2 sec in mice via small alligator clips attached to cornea by using ECM and different phases of convulsion were recorded.

PTZ induced convulsion in mice[21, 22]**:** Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as control which was given with 3% Tween 80 (10 ml/kg p.o.) once daily for 7 days. Group II was administered with diazepam (5 mg/kg i.p.) alone on 1st day only after 30 min treatment anticonvulsant activity was recorded. Groups III, IV and V were treated with different doses of ALETD (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days on 7th day 30 min after diazepam and 60 min after extract administration PTZ (80 mg/kg i.p.) was administered. The following parameters were recorded during test session of initial, 30min and up to 24 h.

- Latency (onset of clonus)
- Onset of tonic convulsion
- Status of animal after 30 min
- Status of animal after 24 h
- Percentage protection

Strychnine induced convulsions in mice[22-24]**:** Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as control which was given with 3% Tween 80 (10 ml/kg p.o.) once daily for 7 days on 7th day 60 min after administration (3% Tween 80) strychnine (2 mg/kg i.p.) was administered. Group II was administered with diazepam (5 mg/kg i.p.) alone on 1st day only; 30 min after administration (diazepam) strychnine was administered. Groups III, IV and V were treated with different doses of ALETD (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days on 7th day 60 min after extract administration strychnine was administered. The following parameters were recorded during test session of initial, 30min and up to 24 h.

- Latency (onset of clonus)
- Onset of tonic convulsions
- Status of animal after 30 min
- Status of animal after 24 h
- Percentage protection.

Picrotoxin induced convulsion[23, 25]**:** Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as control which was given with 3% Tween 80 (10 ml/kg p.o.) once daily for 7 days on 7th day 60 min after administration (3% Tween 80) picrotoxin (3.5 mg/kg i.p.) was administered. Group II was administered with diazepam (5 mg/kg i.p.) alone on 1st day only 30 min after administration (diazepam) picrotoxin was administered. Groups III, IV and V were treated with different doses of ALETD (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days on 7th day 60 min after extract administration the picrotoxin was administered. The following parameters were recorded during test session of initial, 30 min and up to 24 h.

- Latency (onset of clonus)
- Onset of tonic convulsion
- Status of animal after 30min
- Status of animal after 24 h
- Percentage protection.

INH induced convulsion[22, 26]**:** Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as control which was given with 3% Tween 80 (10 ml/kg p.o.) once daily for 7 days on 7th day 60 min after administration (3% Tween 80) INH (300 mg/kg i.p.) was administered. Group II was administered with diazepam (5 mg/kg i.p.) alone on 1st day only; 30 min after administration (diazepam) INH was administered. Groups III, IV and V were treated with different doses of ALETD (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days on 7th day 60 min after extract administration the INH was administered. The following parameters were recorded during test session of initial, 30min and up to 24 h.

- Latency (onset of clonus)
- Onset of tonic convulsion
- Status of animal after 30 min
- Status of animal after 24 h.
- Percentage protection.

4-AP induced convulsions[22, 27, 28]**:** Animals in group I served as control were treated with vehicle (3% Tween 80 p.o.) once daily for 7 days. Group II served as standard received diazepam (5 mg/kg i.p.) alone on 1st day only; 30 min after administration (diazepam) 4-AP was administered. Groups III, IV and V received ALETD at the dose level of 100, 200 & 400 mg/kg p.o. respectively once daily for 7 days. On 7th day 60 min after administration of vehicle and extract 4-AP (13.3 mg/kg i.p) was administered. The following parameters were recorded during test session of initial, 30 min and up to 24 h.

- Latency (onset of clonus)

- Onset of tonic convulsion
- Status of animal after 30 min
- Status of animal after 24 h.
- Percentage protection.

Estimation of GABA by spectrophotometer method [29, 30]**:** Animals group I maintained as control were treated with vehicle (3% Tween 80 p.o.) once daily for 7 days. Group II was administered with gabapentin (20 mg/kg i.p.) alone on 1st day only 30 min after administration (gabapentin) blood sample was collected through caudal vein. Groups III, IV and V were treated ALETD at different dose level (100, 200 & 400 mg/kg p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of vehicle and extract, blood sample was collected through caudal vein of the each animal. The serum was separated and transferred into plastic tubes and serum samples were stored at -20°C until analysis. All the glass and polypropylene equipments used for trace elements analysis were soaked in 10 % (V/V) nitric acid for 12 h and then rinsed with double distilled deionised water.

The serum sample (0.1ml) was added to 1.5 ml of absolute alcohol and centrifuged at 3000 g for 15 min. The upper layer was aspirated and 0.3 ml was put on Whatman's filter paper which was dipped in phenol solution for 24 h and subsequently dried in air. There after ninhydrin salt solution was sprayed on chromatographic paper and heated at 65°C for 10 min. The spot developed due to chromatographic mobility of GABA was cut and put in 3 ml solution of absolute alcohol for elution. The optical density was taken on a spectrophometer at wavelength of 509 nm and compared with standard GABA solution.

Statistical analysis: The results obtained with various experiments were subjected to statistical analysis by using **One-way** ANOVA followed by ***Dunnett*** test to assess the significance difference if any among the groups and P<0.05 was considered as significant.

RESULTS

Acute toxicity studies: An acute toxicity study of ALETD was determined in mice, as per OECD guidelines No. 423. The extract was administered orally to different groups of mice at different dose levels and extract produced no mortality up to 2000 mg/kg. So 1/5th, 1/10th, and 1/20th of LD_{50} doses were selected for the present study.

Effect of extract on SMA in mice: A standard diazepam as well as ALETD treated groups exhibited a significant sedative effect by decreasing locomotor activity in a test period when compared to normal control group. (Table No.1)

Table 1: Effect of ALETD on SMA (Photoactometer) in mice

Groups No.	Treatment	Dose (Per kg)	Number of movements			
			Initial Mean ± SEM	0.5 h Mean ± SEM	1h Mean ± SEM	2 h Mean ± SEM
Group I	Control (3% Tween 80)	10 ml p.o.	326.66 ± 19.46	289.66 ± 5.99	286 ± 4.35	271.33 ± 7.37
Group II	Diazepam	5 mg p.o.	280.33 ± 2.89 ns	72.16 ± 3.84 **	63.16 ± 2.30**	49.83 ± 5.89**
Group III	ALETD	100 p.o.	327.16 ± 3.42 ns	258.33 ± 17.69 ns	183 ± 2.87**	117.16 ± 3.36**
Group IV	ALETD	200 p.o.	333.83 ± 13.65 ns	266 ± 25.56 ns	177.83 ± 2.17**	138.5 ± 6.88**
Group V	ALETD	400 p.o.	305.67 ± 15.35 ns	240.33 ± 11.49 ns	171.33 ± 4.55**	111.16 ± 3.16**

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Anticonvulsant activity

Effect of extract on MESIC in mice: A standard drug phenytoin had exhibited significant anticonvulsant effect by abolishing the tonic extension phase and offered 100% animals protection.

Medium and high doses of ALETD but not lower dose (100 mg/kg) had delayed the onset of clonus, decreased the duration of tonic extensor phase and offered a good percentage protection of the animals (100 %) when compared to control group. (Table No. 2)

Effect of extract on PTZIC in mice: A standard drug diazepam treated group showed no convulsion after PTZ treatment it protects 100% of the animal. In all the doses of ALETD significantly increased latency, on set of tonic convulsion and protects up to 83%, 100% and 100% respectively of mice. (Table No.3)

Table 2: Effect of ALETD on MES induced convulsion in mice.

Groups No.	Treatment	Dose (Per kg)	Latency (on set of clonus) (sec/30 min) Mean ± SEM	Duration of tonic flexion (sec/ 30 min) Mean ± SEM	Duration of tonic extensor phase (sec/30min) Mean ± SEM	% Protection
Group I	Control (3% Tween 80)	10 ml p.o.	3 ± 0.57	3 ± 0.57	15 ± 0.73	33
Group II	Phenytoin	25 mg i.p.	10.16 ± 0.30**	---**	----**	100
Group III	ALETD	100 p.o.	3 ± 0.36ns	3.83 ± 0.65ns	12.16 ± 0.33**	100
Group IV	ALETD	200 p.o.	8.16 ± 0.30**	----**	3.16 ± 0.47**	100
Group V	ALETD	400 p.o.	8.16 ± 0.47**	0.33 ± 0.33**	5.33 ± 0.61**	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Table 3: Effect of ALETD on PTZ induced convulsion in mice.

Groups No.	Treatment	Dose (Per kg)	Latency (onset of clonus) (sec/30 min) Mean ± SEM	Onset of tonic convulsion (sec/ 30 min)Mean ± SEM	Status of animal(30min)(No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
Group I	Control (3% Tween 80)	10 ml p.o.	46±0.577	210.5±13.69	**3**	**2**	33
Group II	Diazepam	5 mg i.p.	792.66±46.37**	----**	ALL	ALL	100
Group III	ALETD	100 p.o.	267.16±14.96**	398.16±20.26**	ALL	**5**	83
Group IV	ALETD	200 p.o.	409.16±49.23**	457.5±23.85**	ALL	ALL	100
Group V	ALETD	400 p.o.	475.66±32.06**	651.5±34.00**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Table 4: Effect of ALETD on strychinine induced convulsion in mice.

Groups No.	Treatment	Dose (Per kg)	Latency (onset of clonus) (sec/30 min) Mean ± SEM	Onset of tonic convulsion (sec/ 30 min) Mean ± SEM	Status of animal (30min)(No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
Group I	Control (3% Tween 80)	10 ml p.o.	134.66 ±5.13	356.66±14.63	3	NIL	NIL
Group II	Diazepam	5 mg i.p.	----**	---**	ALL	ALL	100
Group III	ALETD	100 p.o.	147.33±5.95ns	365.33±8.74ns	ALL	4	66
Group IV	ALETD	200 p.o.	209.33±25.95**	464.83± 14.54**	ALL	ALL	100
Group V	ALETD	400 p.o.	237.5±6.11**	67.5±4.93**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Table 5: Effect of ALETD on picrotoxin induced convulsion in mice

Groups No.	Treatment	Dose (Per kg)	Latency (onset of clonus) (sec/30 min) Mean ± SEM	Onset of tonic convulsion (sec/ 30 min) Mean ± SEM	Status of animal (30min)(No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
Group I	Control (3% Tween 80)	10 ml p.o.	122.66±2.91	310.66±2.39	3	NIL	NIL
Group II	Diazepam	5 mg i.p.	----**	---**	ALL	ALL	100
Group III	ALETD	100 p.o.	136.83±13.12 ns	314.83±3.29 ns	5	4	66
Group IV	ALETD	200 p.o.	185.66±12.12**	441.66±14.76**	ALL	ALL	100
Group V	ALETD	400 p.o.	190.69±6.98**	513.5±12.42**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Effect of extract in SIC on mice: A standard diazepam treated group showed no convulsion after strychinine treatment it protects 100% of the animal. In all the doses of ALETD showed increased latency period, on set of tonic convulsion and protects up to 66%, 100% and 100% respectively of mice. (Table No.4)

Effect of extract on PIC in mice: A standard drug diazepam exhibited anticonvulsant effect by increased time for onset and tonic convulsion after picrotoxin and offered 100% protection. The medium and high doses of ALETD significantly increased latency period and on set of tonic convulsionboth doses protects up to 100% of mice but not with lower dose of ALETD it protects only 66% of mice. (Table No. 5).

Effect of extract on IIC in mice: No convulsion was recorded with diazepam and it protect up to 100% of the mice. The medium and high doses of ALETD showed anticonvulsant effect by increasing latency, onset of tonic convulsion and protects up to 100 % of the animals, not with low dose but that offered only 100 % protection of the animals. (Table No.6)

Table 6: Effect of ALETD on INH induced convulsion in mice.

Groups no.	Treatment	Dose (Per kg)	Latency (onset of clonus) (sec/30 min) Mean ± SEM	Onset of tonic convulsion (sec/ 30 min) Mean ± SEM	Status of animal (30min)(No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
Group I Group II	Control (3% Tween 80)	10 ml p.o.	78.16±3.84	178.33±13.79	ALL	3	50
Group III	Diazepam	5 mg i.p.	----**	---**	ALL	ALL	100
Group IV	ALETD	100 p.o.	127.33±7.74ns	507±36.19ns	ALL	ALL	100
Group V	ALETD	200 p.o.	160.33±5.78**	628±19.38**	ALL	ALL	100
	ALETD	400 p.o.	172.66±3.32**	678.5±21.30**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Effect of extract on 4-APIC in mice:

A standard drug diazepam treated group significantly protects from convulsion up to 100 % of the animals. The medium and high doses of ALETD significantly increased latency period and on set of tonic convulsion but low dose was only significantly increased on set of tonic convulsion and those doses protects the animals up to 83 %, 100 % and 100 % respectively. (Table No.7)

Table 7: Effect of ALETD on 4-AP induced convulsion in mice

Groups No.	Treatment	Dose (Per kg)	Latency (onset of clonus) (sec/30 min) Mean ± SEM	Onset of tonic convulsion (sec/ 30 min) Mean ± SEM	Status of animal (30min)(No of Animal alive)	Status of animal (30min) (No of Animal alive)	% Protection
Group I	Control (3% Tween 80)	10 ml p.o.	242±12.92	312.5±3.21	5	2	33
Group II	Diazepam	5 mg i.p.	----**	---**	ALL	ALL	100
Group III	ALETD	100 p.o.	259.83±13.97ns	458.83±16.19**	ALL	5	83
Group IV	ALETD	200 p.o.	377.83±12.54**	571.33±6.89**	ALL	ALL	100
Group V	ALETD	400 p.o.	425.83±27.81**	597.83±23.65**	ALL	ALL	100

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Table 8: Effect of ALETD in serum GABA levels of mice

Groups No.	Treatment	Dose (Per kg)	Optical density (509 nm) Mean ± SEM	GABA levels (pmol/ml) (O.D of Test/O.D of Std=pmol/ml)
Group I	Control (3% Tween 80)	10 ml p.o.	0.021±0.0006	152.17
Group II	Gabapentin	25 mg i.p.	0.084±0.001	608.69**
Group III	ALETD	100 p.o.	0.030±0.0017	215.39ns
Group IV	ALETD	200 p.o.	0.038±0.0019	275.36**
Group V	ALETD	400 p.o.	0.067±0.006	485.50**
Group VI	Standard GABA solution 0.1ug/ml		0.138±0.005	1000**

n=6. Significance at P<0.05*, <0.01** and ns-not significant

Effect of extract on mice serum GABA levels: A standard gabapentin significantly increased GABA levels in the serum. Medium and high doses of ALETD treated groups showed increased GABA levels in the mice serum but not with low dose of the extract. (Table No 8)

DISCUSSION

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. Furthermore, undesirable side effects from the drugs used clinically often render treatment difficult so that a demand for new types of anticonvulsants exists. One of the approaches to search for new antiepileptic drugs is the investigation of naturally occurring compounds, which may belong to new structural classes[2].

In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage activated Na^+ channels or enhance GABA mediated synaptic inhibition[1].

SMA is considered as an index of alertness and decrease in it is considered in it is indicative of sedative activity**.** The study on SMA showed that ALETD (100, 200 & 400 mg/kg) decreased the frequency and the amplitude of movements. The reduction of the locomotor activity could be attributed to the sedative effect of the extract that may be due to increase in concentration of the GABA in GABAergic system[31]**.**

In MES-induced convulsion animals are represent grandmal type of epilepsy. It has often been suggested stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread. Moreover, MES-induced tonic extension phase can be prevented either by drugs that inhibit voltage-dependent Na^+ channels, such as phenytoin, valaproate, feblamate and lamotrigine or by drugs that block glutaminergic excitation mediated by the N-methyl-D-Aspartate (NMDA) receptor such as feblamate. The ALETD showed anticonvulsant activity against MES-induced convulsion, it was abolished tonic extension phase due to it might be either inhibit voltage-dependent Na^+ channels or act as a NMDA antagonist.

PTZ is a most frequently used substance as well as an acute experimental model in the preliminary screening to test potential anticonvulsant drugs and it is produced petitmal type epilepsy. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the $GABA_A$ receptor complex. Several biochemical hypotheses have been advanced involving the inhibitory GABAergic system and the system of the excitatory amino acid glutamate and aspartate[32]. The ALETD exhibited anticonvulsant activity as a result of increased time taken for onset of convulsion and tonic convulsion induced by PTZ. The anticonvulsant effect of extract against PTZIC might be it acts as a $GABA_A$ agonist.

In the strychnine-induced seizure model, it is known that strychnine a potent spinal cord convulsant, blocks glycine receptor selectively to induce excitatory response in the CNS[25]. The ALETD offered a significant inhibition against SIC; it might be interfere with glycine transmission. The suppression of seizure by diazepam might be indirectly enhancing glycine inhibitory mechanisms[35].

Picrotoxin is a noncompetitive antagonist at $GABA_A$ receptors and it blocks the GABA-activated chloride ionophore[22]. However, the extract was counteracting the action of picrotoxin, that effect might be the extract modified the function of $GABA_A$ receptor mediated chloride channel.

INH is used widely for the treatment and chemoprophylaxis of tuberculosis, but can have serious effects on the CNS causing seizures and comas[33]. The INH is thought to be inhibition of GABA synthesis in the CNS. So ALETD treated groups were showed up to 100% of protection of the animals. It indicated the extract enhanced GABA synthesis either by stimulation of L-glutamate or prevention of GABA degradation by GABA transaminase.

4-AP is a K^+ channel blocker and Ca^{2+} channel stimulator, both voltage dependent gated[34,35] which shows convulsant action when administered systemically to a variety of species. Furthermore, the convulsant effects of 4-AP is due to the release of excitatory neurotransmitters[36], where the glutamate is release results in over activation of excitatory amino-acid receptors, mainly the NMDA-type. Indeed, an enhancement in the glutamatergic neurotransmission has been linked to the 4-AP convulsant action[37], since the administration of NMDA receptor antagonists protectes against 4-AP induced seizures[38]. The ALETD exhibited anticonvulsant effect might be due to it inhibited glutamate signal pathway.

Significantly increased GABA concentration in the serum of the animals were observed with medium and high doses of the extract and gabapentin[39] treated groups it indicated the extract enhances synthesis of GABA in GABAergic neurons.

In conclusion, our result showed that ALETD possesses anticonvulsant activity probably due to the modulation of GABAergic system and reduction of neuronal excitability mainly through the voltage-dependent Na^+ channels. Isolation of the effective components of *T.divaricata* and clarification of their pharmacological mechanisms is needed to confirm the findings in this study.

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