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Antiepileptic activity of lobeline isolated from the leaf of *Lobelia nicotianaefolia* and its effect on brain GABA level in mice

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ABSTRACT

Objective: To investigate the anticonvulsant activity of the lobeline isolated from the *Lobelia nicotianaefolia* in chemoconvulsant−induced seizures and its biochemical mechanism by investigating relationship between seizure activities and altered gamma amino butyric acid (GABA) in brain of mice in Pentylenetetrazol (PTZ) seizure models. **Methods:** The anticonvulsant activity of the isolated lobeline (5, 10, 20 and 30 mg/kg, *i.p.*) was investigated in PTZ and strychnine induced seizures in mice and the effect of isolated lobeline on brain GABA level in seizures induced by PTZ. Diazepam was used as reference anticonvulsant drugs for comparison. **Results:** Isolated lobeline (10, 20 and 30 mg/kg, *i.p.*) significantly delayed and antagonized ($P < 0.050–0.001$) the onset of PTZ−induced seizures. It also antagonized strychnine induced seizures. The mortality was also prevented in the test group of animals. In biochemical evaluation, isolated lobeline (5, 10 and 20 mg/kg, *i.p.*) significantly increased the brain GABA level. And at dose of 30 mg/kg GABA level showed slight decrease in PTZ model. **Conclusions:** In our findings, isolated lobeline (20mg/kg) exhibited potent anticonvulsant activity against PTZ induced seizures. Also a biochemical evaluation suggested significant increase in barain GABA level at 20 mg/kg *i.p.* of isolated lobeline. Hence, we may propose that lobeline reduces epileptic seizures by enhancing the GABA release supporting the GABAergic mechanism.

1. Introduction

Epilepsy is the second most common neurological disorder in India. Epilepsy affects an estimated 7 million people in India, and 50 million worldwide. Approximately 40% of them are women. The prevalence of epilepsy is 0.7% in India, which is comparable to the USA and other developing nations. The estimated incidence rate range from 40 to 60 per 1 000 000 population per year. The WHO estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical treatment[1]. The introduction of each new antiepileptic drug into the market raises valid expectations in patients and physicians for more effective treatment of epilepsy. Although safety, tolerability, and interactions are important, better efficacy is a crucial feature for a new antiepileptic

drug[2].

In the Indian traditional system of medicine, various herbs have been used to treat epileptic seizures. *Lobelia* has been recognized and used in traditional systems of medicine for their antiepileptic activity in crude and their respective dosage forms. *Lobelia nicotianaefolia* (*L. nicotianaefolia*) is a rich source of alkaloids of the lobeline group and used as a substitute for *Lobelia inflata*[3,4]. The plant has been recorded to contain several alkaloids with the main alkaloid lobeline apparently observed, revealed higher concentrations. The total alkaloidal content ranges from 1.04−1.18%[5]. According to ethnobotanical survey, leaves and inflorescence are antispasmodic and used for the treatment of asthma, bronchitis and fever[6]. In Ayurveda, this plant is used for sciatia and back pain[7]. Roots are useful in the treatment of eye diseases. Decoction of flowers was given orally in asthma[8]. Leaves has been used for speedy healing of wounds[9]. The use of lobeline as a smoking deterrent was reported in 1936, but several later studies led to a dispute between positive and negative

reports[10]. In a recent patent, studies on a new formulation of drugs to deliver an effective amount of lobeline to sublingual mucosa were done. Sublingual formulation of lobeline sulfate does not appear to be an effective smoking cessation aid[11].

Lobeline was shown to attenuate self–administration of heroin and amphetamine[12,13], also used in the treatment of alcohol abuse[14–16]. Lobeline and its analogues could represent a novel class of therapeutic agents with a great potential for the treatment of psychostimulant abuse[17,18]. Lobelane, a molecule formed from deoxygenation of the lobeline molecule, has a shorter duration of action compared to lobeline[19]. The potassium large conductance calcium–activated channel KCNMA1 a protein might be targeted by Lobeline which was thought to be one of the reason in treating drug abuse[20]. Zheng et al. suggested that the noncompetitive inhibition of VMAT2 function by lobeline might be the mechanism in the treatment of drug abuse[21].

Lobeline is used as a pharmacotherapy for substance use disorder and attention deficit hyperactivity disorder[22]. The effect of lobeline in improving learning may be useful in the development of novel treatments for learning deficits[23].

However, no significant data supporting the efficacy of lobeline as an antiepileptic is available in official literature. Hence, the present study was designed to verify anti–epileptic effect of isolated lobeline and its effect on gamma amino butyric acid (GABA) level in mice brain.

2. Materials and methods

2.1. Collection and authentification of plant material

The plant *L. nicotianaefolia* Roth E. &S. was collected from the dense forest of Varandha Ghat, Bhor, cleaned and dried at room temperature in shade and kept away from direct sunlight and it was authenticated by Botanical Survey of India, Koregaon Park, Pune (Voucher specimen number ALLALON1BSI/WRC/Tech/2010/).

2.2. Extraction and isolation of lobeline

The powdered herb (800 g) was moistened with water (3.5 L), acidified with acetic acid (700 mL) and left macerated for three hours. The mass was then pressed and the process of moistening and pressing was repeated and the collected pressed solutions were filtered. The filtrate was alkalinized with 10% sodium bicarbonate solution and then extracted with petroleum ether successively (100 mL× 3) for purification. The ether extract was then shaken with water (100 mL×3) acidulated with sulphuric acid (pH 3). The acidic solution was rendered alkaline with 10% sodium bicarbonate solution and shaken with ether (50 mL×3). The ether fraction was evaporated to dryness, and the resulting yellow oily residue containing the total alkaloids was again

dissolved in water (100 mL) acidified with dilute hydrochloric acid (50 mL), then it was filtered and shaken successively with chloroform (50 mL×3); the chloroform extracted only lobeline hydrochloride leaving the salt of the other alkaloids in the aqueous layer. The chloroform was then evaporated under reduced pressure. The brownish oily residue was then stirred with twice its volume of hot water at about 60 ℃. The aqueous solution was then left sometimes under vacuum over sulphuric acid when lobeline hydrochloride crystallizes out. Isolated lobeline was recrystallized from alcohol to obtain off–white needle shaped lobeline[24,25].

2.3. Animals

Swiss albino male mice weighing around 22–25 g were obtained from the Yash Farm, Pune. The animals were housed in groups of 6–8 per cage and maintained at (24 ± 2) ℃, with relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard chew pellets and water. All the animals were fasted for 16 h, but still allowed free access to tap water before the commencement of our experiments. The Institutional Animal Ethics Committee of Allana College of Pharmacy, Pune approved the pharmacological and acute toxicity protocol (IAEC No: Ref/ACP/IAEC/10–11/18–09).

2.4. Chemicals

The PTZ was procured from Sigma chemicals, USA. Strychnine was procured from Indus Biotech Pvt Ltd., Pune. Diazepam was procured from Ranbaxy Lab, Mumbai.

2.5. Acute toxicity study

Acute toxicity study was performed in mice according to OECD guidelines. The isolated lobeline was administered intraperitoneally (*i.p*) at doses of 1.75, 5.5, 17.5, 55 mg/kg. They were then observed for signs of toxicity, continuously for 2 h, and for mortality up to 24 h, after injection[26].

2.6. Assessment of anti–convulsant activity

2.6.1. PTZ induced convulsions

The mice were divided into six groups of six animals each. The isolated lobeline was administered at doses 5, 10, 20, 30 mg/kg *i.p.* Group I was treated with vehicle (saline) and Group II with diazepam at a dose of 1 mg/kg *i.p.* Group III, IV, V and VI were administered with isolated lobeline in a dose of 5, 10, 20, 30 mg/kg respectively. All the drug treatment was given thirty minutes before the injection of PTZ (90 mg/kg *i.p*). Each animal was placed in individual plastic cage for observation lasting forty five minutes. The onset of general clonus was recorded and tonic clonic convulsions were studied. Protection of the animals from mortality was recorded[27].

2.6.2. Strychnine induced convulsions

For the evaluation of anti–convulsant activity against strychnine induced convulsions, the same procedure was adopted as mentioned in PTZ induced convulsions. Instead, strychnine (3 mg/kg *i.p*) was used for inducing convulsions[12].

2.7. Biochemical evaluation by estimation of brain GABA

Animals were divided into seven groups consisting six mice in each group. Treatment schedule was as shown in Table 1. Forty–five min after vehicle or lobeline and 30 min after diazepam, mice were sacrificed. Group three was sacrificed as soon as onset of convulsions occurs or 65 sec after PTZ treatment. Brain was isolated immediately and transferred to homogenization tube containing 5 mL of 0.01 M hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 mL of ice cold absolute alcohol and kept for 1 h at 0 ℃. The content was centrifuged for 10 min at 16 000 rpm, supernatant was collected in petridish. Precipitate was washed with 5 mL of 75% alcohol for three times and washes were combined with supernatant. Contents in petridish were evaporated to dryness at 70 ℃ on water bath under stream of air. To the dry mass 1 mL water and 2 mL chloroform were added and centrifuged at 2 000 rpm. Upper phase containing GABA (2.0 mL) was separated and 10 μL of it was applied as spot on Whatman paper (No.41).

The mobile phase consisted of n–butanol (50 mL) acetic acid (12 mL) and water (60 mL). The chamber was saturated for half an hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% ninhydrin solution in 95% ethanol. The paper was dried for 1 h at 90 ℃. Blue color spot developed on paper was cut and heated with 2 mL ninhydrin solution on water bath for 5 min. Water (5.0 mL) was added to solution and kept for 1h. Supernatant (2.0 mL) was decanted and absorbance was measured at 570 nm[28].

Table 1

Table showing treatment schedule of biochemical evaluation by estimation of brain GABA.

Groups	Treatment	Dose
1	Vehicle	1 mL/kg *i.p.*
2	Lobeline	5 mg/kg *i.p.*
3	Lobeline	10 mg/kg *i.p.*
4	Lobeline	20 mg/kg *i.p.*
5	Lobeline	30 mg/kg *i.p.*
6	Pentylenetetrazole (PTZ)	80 mg/kg *i.p.*
7	Diazepam	5 (mg/kg *i.p.*

2.8. Statistical analysis

The statistical analysis of all the observations was

carried out using one–way ANOVA followed by Dunnett's test. Values were presented as Mean $\pm$ SEM using Graph Pad Prism – 5 Statistical Software Package. The level of significance was set as $P < 0.05$.

3. Results

3.1. Acute toxicity study

Intraperitoneal administrations of stepwise, escalated doses of isolated lobeline in mice gave an LD_{50} value as 40 mg/kg.

3.2. PTZ induced convulsions

The onset of seizure was significantly delayed in the animals treated with isolated lobeline at 10, 20 and 30 mg/kg dose (Figure 1). The onset was most significantly delayed in 20 mg/kg dose, when compared with the vehicle (saline) treated group. Also, lobeline in a dose of 20 mg/kg was found to be the most significant in duration of clonic and tonic seizures (Figure 2, 3). The mortality was also prevented in the test group of animals but not in a dose dependent manner (Table 2).

Table 2

Mortality of PTZ induced seizures.

Groups	Mortality	% Mortality
Control	6/6	100.00
Diazepam	0/6	0.00
Lobeline (5mg/kg)	4/6	66.66
Lobeline (10mg/kg)	2/6	33.33
Lobeline (20mg/kg)	1/6	16.66
Lobeline (30mg/kg)	2/6	33.33

Mortality = No. of animals dead after seizures / total No. of animals used;
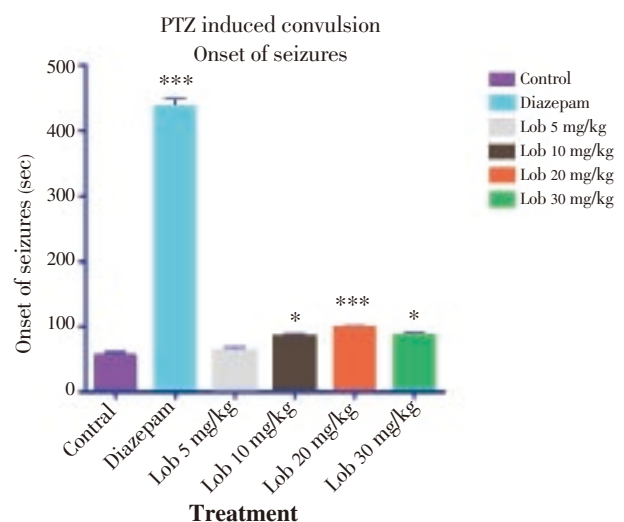% Mortality = (No. of animals dead after seizures / total No. of animals used) *100.

Figure1. The onset of seizures using PTZ. * *P<0.05*, ** *P<0.01*, *** *P<0.001*. All data compared with the control using one way ANOVA followed by Dunnet's post hoc test.

Figure 2. Duration of clonic seizures using PTZ. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with the control using one way ANOVA followed by Dunnet's post hoc test.

Figure 3. Duration of tonic seizures using PTZ. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with the control using one way ANOVA followed by Dunnet's post hoc test.
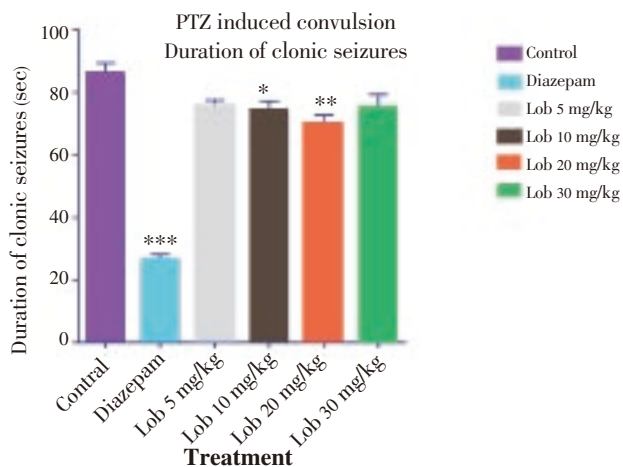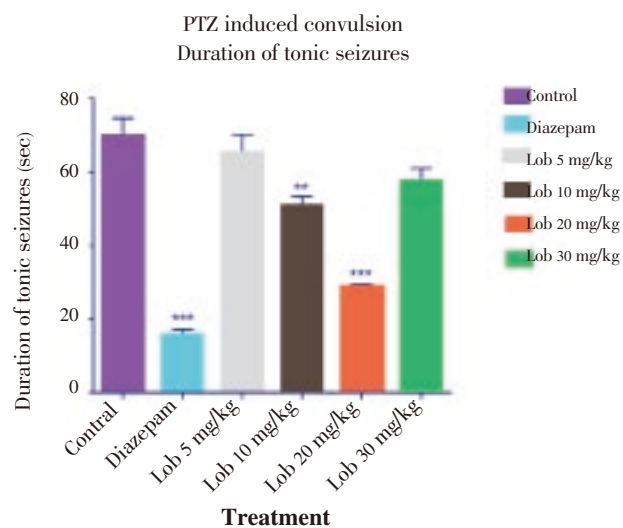
3.3. Strychnine induced convulsions

The onset of seizure was comparatively less significantly delayed in the animals treated with isolated lobeline at 10, 20 and 30 mg/kg dose with 20 mg/kg dose showing better results (Figure 4). Also with reference to Figure 5 and Figure 6, it was observed that lobeline in a dose of 20 mg/kg significantly reduced duration of clonic and tonic seizures however the results were not as impressive as that of PTZ induced seizure including percentage mortality (Table 3).

3.4. Biochemical evaluation by estimation of brain GABA

In biochemical evaluation, lobeline exerted a biphasic effect on GABA level *i.e.* GABA level was found to be increased up to dose of 20 mg/kg and it decreased at dose of 30 mg/kg (Figure 7). Hence, we may propose that lobeline reduces epileptic seizures by enhancing the GABA release thereby supporting GABAergic mechanism.

Table 3

Mortality of strychnine induced seizures.

Groups	Mortality	%Mortality
Control	6/6	100.00
Diazepam	0/6	0.00
Lobeline (5mg/kg)	4/6	66.66
Lobeline (10mg/kg)	3/6	50.00
Lobeline (20mg/kg.)	1/6	16.66
Lobeline (30mg/kg)	4/6	66.66

Mortality = No. of animals dead after seizures / Total No. of animals used; % Mortality = No. (of animals dead after seizures / Total No. of animals used) *100.
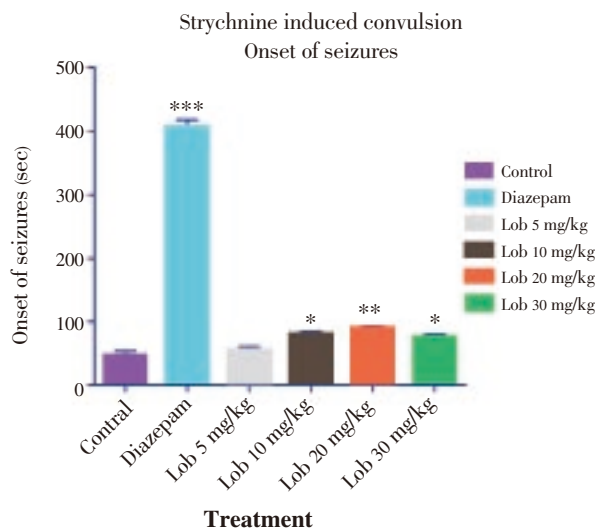

Figure 4. Onset of seizures using strychnine. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with control using one way ANOVA followed by Dunnet's post hoc test.
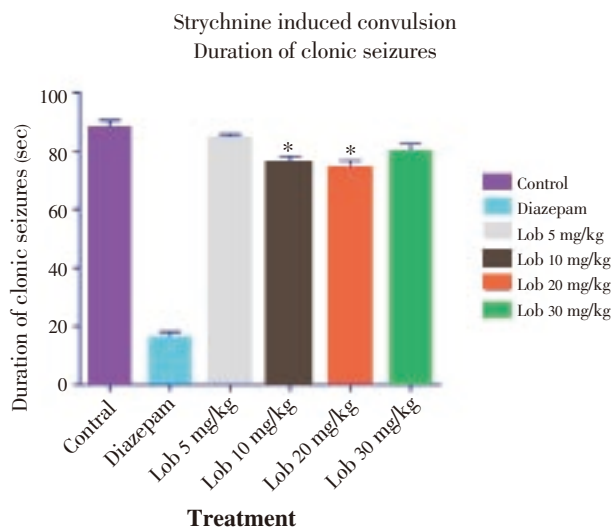

Figure 5. Duration of clonic seizures using strychnine. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with control using one way ANOVA followed by Dunnet's post hoc test.

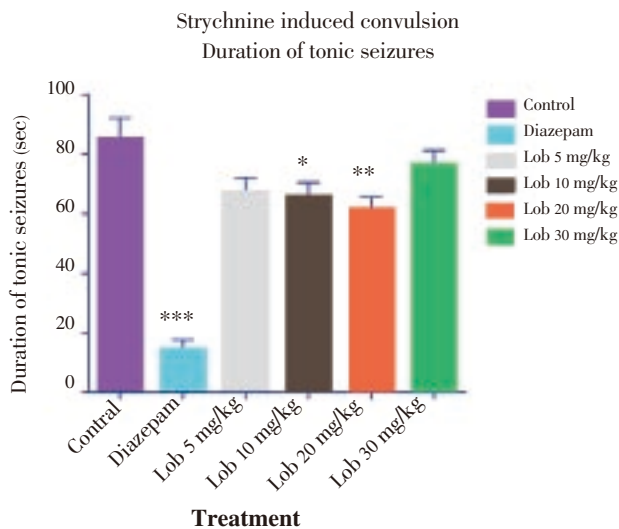## Strychnine induced convulsion
Duration of tonic seizures

Figure 6. Duration of tonic seizures using strychnine. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with control using one way ANOVA followed by Dunnet's post hoc test.

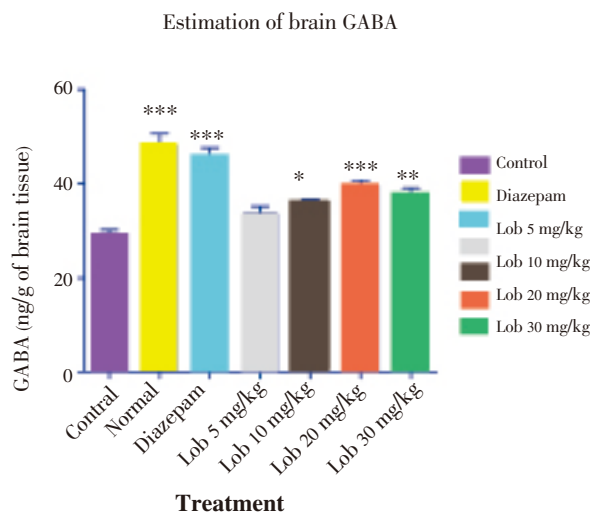### Estimation of brain GABA

Figure 7. Estimation of brain GABA (ng/g of brain tissue). * $P<0.05$, ** $P<0.01$, *** $P<0.001$. All data compared with control using one way ANOVA followed by Dunnet's post hoc test.

4. Discussion

In the present investigation, it is evident that lobeline was able to ameliorate the epileptic seizures induced by PTZ and strychnine in laboratory animals. The various epileptogenic agents used in the investigation were PTZ and strychnine. It is understood from the literature that GABAergic neurotransmission is closely associated with the induction of epilepsy in the animals[29,30]. GABA is the major inhibitory neurotransmitter in the central nervous system and even slight deficiencies in GABAergic transmission may lead to hyperexcitability and pathological neuronal discharges leading to epilepsy. GABA is an endogenous agonist at GABA$_A$ receptor (ionotropic receptor) thereby opening the channels to Cl$^-$ ions in the neuronal membrane.

An enhancement of GABAergic inhibitory transmission is responsible for the antiepileptic effects of drugs that directly bind and activate GABA$_A$ receptors or influence GABA release, transport and metabolism. GABA mediated Cl$^-$ channel GABA–benzodiazepine receptor complex are closely associated with induction and onset of seizures[31]. The benzodiazepines do not substitute for GABA but appear to enhance GABA's effects allosterically without directly activating GABA$_A$ receptors or opening the associated chloride channels. Since PTZ is a noncompetitive antagonist it blocks GABA–mediated Cl$^-$ influx. Hence, this accentuates the excitatory neurons to release neurotransmitters such as The α–amino–3–hydroxy–5–methyl–4–isoxazolepropionic acid receptor (AMPA) and N–Methyl–D–aspartic acid (NMDA). This leads to neuronal excitotoxicity leading to seizures and convulsions characterized by clonic and tonic phases. PTZ–induced convulsion decreases the GABA content in brain when compared to the vehicle group[32].

Lobeline was able to inhibit the epileptic seizures at doses of 5, 10, 20, 30 mg/kg. However, an interesting phenomenon of ceiling effect of efficacy was seen. At doses of 5, 10 and 20 mg/kg lobeline exhibited dose dependant attenuation epileptic seizures. However at higher doses of 30mg/kg effect waned off. This shows that at higher doses lobeline was ineffective. There are numerous molecular mechanisms through which drugs can block seizure spread and/or elevate seizure threshold.

The paradoxical nature of lobeline is exhibited in the pharmacological activity at a dose of 20 mg/kg. Lobeline protects the animals from epilepsy at 20 mg/kg, whereas at 30 mg/kg it might be an epileptogenic agent.

Strychnine is an alkaloid which is an inhibitor of glycine (inhibitory neurotransmitter) synthesis. It is an antagonist to excitatory monotransmittors like AMPA and NMDA and it also opposes Ca^{2+} induced excitotoxicity in the neurons. These factors are pro epileptogenic and precipitate seizures. Hence, inhibition of glycine leads to clonic and tonic seizures in laboratory animals. Thus, it is used as a research tool to induce seizures in the animals.

In the present investigation, lobeline was able to halt the progression and precipitation of seizures. This leads to suppression of clonic–tonic seizures and delays the onset of seizures. In the estimation of GABA levels in the PTZ model, lobeline exerted a biphasic effect on GABA level *i.e.* GABA level was found to be increased up to dose of 20 mg/kg and it decreased at dose of 30 mg/kg. However, the induction of seizures via strychnine does not involve GABA mediated Cl$^-$ channel or benzodiazepine modulation. Hence, GABA levels were not determined in the study. Hence, we may propose that lobeline reduces epileptic seizures by enhancing the GABA release thereby GABAergic mechanism.

However, since lobeline exhibited antiepileptic profile, it could be postulated that lobeline exerts its pharmacological effects via multiple mechanisms through GABAergic transmission. Concrete proof to this hypothesis could be

derived by estimating the levels of glycine, AMPA, NMDA and monoamines such as noradrenalin, 5–hydroxytryptamine in the further study. And further studies are required to check any binding of lobeline with GABA receptor which can be achieved by radioligand binding assays.

Conflict of interest statement

We declare that we have no conflict of interest.

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