Evaluation of *Clematis hedysarifolia* DC. for potential anticonvulsant activity in Pentylenetetrazol and Strychnine induced convulsion in albino mice

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ABSTRACT

Juice of Clematis hedysarifolia DC, has been employed in the treatment of seizures and various neurological disorders. Research objective was to examine anticonvulsant effects of different solvent extracts of leaf and stem parts of Clematis hedysarifolia DC by employing several experimental models. Anticonvulsant activity of plant extracts at dosages of 200 and 400mg/kg (administered orally) was evaluated using pentylenetetrazole (PTZ) as well as strychnine (STN)-induced seizure models in mice. Acute toxicity study indicated that LD50 exceeded 2000mg/kg in mice. Pre-treatment with the aqueous extract of Clematis hedysarifolia DC resulted in a dose-related protective effect against PTZ-induced seizures along with mortality, offering complete protection at highest dose. Extract significantly delayed onset of myoclonic jerks and shortened duration of tonic seizures in a dose-dependent fashion. In STN-induced seizure model, although extract did not prevent seizure occurrence, similar to standard diazepam, it significantly ($p<0.05$) delayed onset of seizures and extended survival period prior to death in dose-dependent manner.

Keywords: Anticonvulsant, *Clematis*, pentylenetetrazol, strychnine

INTRODUCTION Epilepsy is prevalent neurological condition that impacts individuals globally, with over 80% of cases occurring in countries with limited resources or underdeveloped healthcare systems (Beghi, 2020). Global incidence of epilepsy is estimated at 61.4 per 100,000 individuals (Fiest *et al.*, 2017). It manifests through recurring, sudden episodes characterized by consistent behavioral changes, indicating disruptions in neural function (Fisher *et al.*, 2017). Although precise cause of epilepsy is still unclear, it is believed to result from an imbalance between excitatory as well as inhibitory neurotransmission (Fokoua *et al.*, 2021). This imbalance often involves complex interactions among GABAergic, glutamatergic, as well as cholinergic systems (da Guedes, 2022). While various antiepileptic medications are available, many are associated with adverse effects, some of which can be severe or fatal. Additionally, more than 25% of patients do not respond effectively to existing antiepileptic drugs.

Therefore, there is a pressing need for identifying new, safer therapeutic agents, especially those derived from natural sources. Md. Sah *et al.* (2024) noted that tribal communities in hilly areas of Bangladesh are mainly depending on herbal treatment for their illness and primary health care. In most nations, medical plants also known as herbs, herbal remedies, pharmacologically active plants, or phyto-medicinals remain the principal source of medication. In Kashmir Valley, medicinal plants are also a major source of revenue for thousands of families (Khawaja *et al*., 2023). *Clematis hedysarifolia* DC, a member of the Ranunculaceae family, is a climbing plant commonly found in the Sahyadri hills of Maharashtra. Traditionally, its juice has been used to manage seizures and epilepsy. Among tribal communities in Maharashtra, such as Warli, Thakar, and Kokna, this plant continues to be used for treating convulsions and epilepsy without scientific substantiation. Kamble *et al.* (2009 and 2014) also reported the use of ethano-medicinal plants by the tribes of Maharashtra. Our research objective is to investigate anticonvulsant properties of *Clematis hedysarifolia* DC extracts by employing established experimental anticonvulsant models in mice (Ugwah-Oguejiofor *et al*., 2013).

MATERIALS AND METHODS

Fresh leaves along with stems of *Clematis hedysarifolia* DC are collected from area of Trimbakeshwar, District Nashik in October 2019. Plant was identified by the botanist Priyanka A. Ingle and the authentication No.isBSI/WRC/IDEN.CER/2018/112/88 (B). The plant materials were dried by natural drying method. The leaves and stems were dried under the shade of sunlight. The plant materials were pulverized in grinder as fine powder that was sieved through numbers (# 60-80). The powders of plant materials were then used for further study. Powdered plant material (500 g) underwent Soxhlet extraction with solvents including petroleum ether, ethyl acetate, butanol, and ethanol. Resulting extracts were concentrated by evaporation over a water bath, and percentage yield for each was calculated.

Healthy adult Wistar albino mice (20-25g) and Wistar albino rats (175-200g) of either sex were obtained from National Center for Cell Sciences, Pune. Animals were housed in polypropylene cages under controlled environmental conditions, maintaining temperature of 22±2°C, 12-hour light/dark cycle, along with relative humidity of 55±5%. They were provided unrestricted access to a standard pellet diet (food) (Nutrivet Life Sciences, Pune) and water *ad libitum*".

All experimental procedures were reviewed and approved by Institutional Animal Ethical Committee (IAEC) of MGV's Pharmacy College, Panchavati, Nashik, Maharashtra, India (Registration No. 121/1999/CPCSEA), operating under guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)", Ministry of Environment and Forests, Government of India (GoI) (Certificate No. MGV/PC/CPCSEA/XXXV1/01/2019/19).

Diazepam (Valium®, Roche), "Pentylenetetrazol, Strychnine and Phenytoin (Sigma Chemical Co.), were standard chemicals" employed for the study. All extracts of *Clematis hedysarifolia* DC were analyzed for presence of saponins, alkaloids, glycosides, flavonoids, steroids, phenols, anthraquinones, as well as tannins using procedures outlined by (Trease *et al*., 2002). Quantitative phytochemical estimation was carried out following methodology reported by Narendra *et al*. (2013).

Oral acute toxicity examination was conducted for determining median lethal dose (LD50) among mice following Up and Down procedure using OECD guidelines No.425. In brief, in this method, six female albino mice were used (n=6). In the present study, Wistar albino mice were employed

and administered with different doses of two types of plant parts (leaves and stem) extracts (PE, EA, BE and EE) of *Clematis hedysarifolia* DC to test their lethality. None of the tested male Wistar albino mice died, no change in relative organ weight and there were no discernible behavioural alterations for administered extracts like PE, EA, BE and EE leaf and stem extracts in all three doses i.e. 175, 550, 2000mg/kg up to 2000mg/kg for 14 days. Data proved that the administration of PE, EA, BE and EE leaf and stem extracts from *Clematis hedysarifolia* up to the 2g/kg dose were secure to be used for our different anti-inflammatory and antiepileptic investigations throughout in vivo model, in a single administration (OECD guidelines 425). LD50>2000mg/kg was established.

Evaluation of anticonvulsant activity for all extracts of *Clematis hedysarifolia* was conducted using procedures outlined by Amabeoku (1999), Bum *et al.* (2001), Wannang *et al.* (2008), and Bum *et al.* (2011). Anticonvulsant assessment employed pentylenetetrazol (PTZ) as well as strychnine (STN) induced seizure models. For each test (PTZ and STN), a total of 50 mice were divided into 10 groups (Groups I–X) with 5 mice in each group (n=5). The sample size (n=5) was determined based on ethical guidelines to minimize animal use. Power analysis, considering the expected effect size and variability from prior studies, indicated that n=5 per group provides adequate statistical power to address the study objectives. Group I act as negative control and was given distilled water, whereas Group II functioned as positive control, receiving 5mg/kg diazepam for PTZ model or 25mg/kg for STN model. Groups III-X administered plant extracts at doses of 200mg/kg and 400mg/kg orally. Seizures were triggered by intra-peritoneal injection of 80mg/kg PTZ or 2.5mg/kg STN. Onset and duration of seizures were monitored for 30min. A mouse was considered protected if no seizures occurred during observation period. Mortality was recorded within 24hrs of administration.

Findings were indicated as "mean standard error (S.E.) of mean. Statistical analysis was conducted by employing one-way analysis of variance (ANOVA), and Dennett's post hoc test. The Kolmogorov–Smirnov test was employed to assess the normality of the data, as it is recommended for sample sizes of $n \geq 50$ and is generally preferred for medium to large datasets. Homogeneity of variances was evaluated using Bartlett's test. Data analysis was performed by employing Graph Pad Prism version-9.5.1 (Graph Pad®, San Diego, CA, USA)".

RESULTS AND DISCUSSION

Acute toxicity studies

The acute toxicity of the *Clematis hedysarifolia* extracts was evaluated in female mice at doses up to *2000 mg/kg body weight*, administered orally according to OECD guideline 425. No mortality or significant clinical signs of toxicity (such as tremors, convulsions, salivation, diarrhea, lethargy, or abnormal locomotor activity) were observed during the 14-day observation period. Body weight measurements taken on days 0, 7, and 14 showed normal weight gain compared to the control group, indicating no adverse effect on growth. Food and water intake remained within normal limits. Based on these findings, the oral LD_{50} of the extract was estimated to be greater than 2000 mg/kg, suggesting that the plant extract is relatively safe and non-toxic at the tested dose levels.

Effect of *Clematis hedysarifolia* DC on Pentylenetetrazol-induced seizure

Leaf and stem extracts of *Clematis hedysarifolia* demonstrated a dose-dependent protective effect against PTZ-induced seizures, as presented in Table 1 and 2. Comparable to diazepam, petroleum ether extracts from both plant parts completely prevented onset of PTZ-induced seizures at dosage of 400mg/kg. The extracts

significantly increased seizure latency in animals that were not fully protected and significantly shortened seizure duration compared to control group. Specifically, the extracts led to 5.7-fold rise in latency and a 4-fold reduction in seizure duration at 200mg/kg and 400mg/kg doses. Moreover, complete survival was found in groups III, IV, VI, IX, and X of leaf extract-treated animals, along with groups III, IV, and VI of stem extract group within 24-hour period.

Treatments Vs Latency to Myoclonic spasm (sec) leaves extract

Group II-VI and IX-X demonstrated significant ($p<0.0001$) rise in latency in myoclonic spasm, compared with Group I, except Group VIII ($p<0.001$) showed significant increase in myoclonic spasm and group VII showed non-significant effect as shown in Figure 1. All values were presented as mean±S.E.M. Groups II- X were compared with groupI via "One-Way ANOVA followed by Dunnett's test *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Clonic spasm (sec) leaves extract

Groups II to X exhibited a significant increase ($p<0.0001$) in latency of clonic convulsions, compared with Group I, as illustrated in Figure 2. Data is represented as mean ± S.E.M. Statistical comparisons among Groups II-X and Group I were carried out by employing One-Way ANOVA followed by Dunnett's post hoc test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Clonic spasm (sec) stem extracts

Groups II-X exhibited a significant increase in latency of clonic convulsions ($p<0.0001$) when compared to Group I, as illustrated in Figure 4. All data are represented as mean±S.E.M. Statistical comparisons among Groups II-X and Group I were conducted by employing One-Way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to myoclonic spasm stem extract

Groups II-VI and IX-X indicated significant rise in latency of myoclonic spasms ($p<0.0001$), while Group VIII demonstrated statistically significant rise ($p<0.001$), compared with Group I. Group VII exhibited a non-significant increase in latency. These findings are depicted in Figure 3. All values are represented as mean±S.E.M. Statistical analysis was carried out by employing One-Way ANOVA followed by Dunnett's test, with comparisons made between Groups II-X and Group I. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Anticonvulsant activity by using – Strychnine (STR) induced seizure model:

Leaf and stem extracts of *Clematis hedysarifolia* demonstrated a dose-dependent protective effect against STR -induced seizures, as presented in Table 3 and 4. Extract demonstrated dose-dependent protection against STR-induced seizures. At both 200mg/kg and 400mg/kg p.o., animals treated with extract, similar to those given diazepam, exhibited no convulsions. Moreover, in animals that were not completely protected, extract significantly prolonged seizure latency ($p<0.0001$) and markedly decreased seizure duration ($p<0.0001$) when compared to control group. At lower dose of 200mg/kg, extract effectively enhanced latency and" reduced duration of seizures relative to control.

Treatments Vs Latency to myoclonic and clonic spasm Leaves extract

Group II-VI and IX-X demonstrated significant ($p<0.0001$) rise in latency in myoclonic and clonic spasm when compared to Group I, except Group VIII ($p<0.001$) showed significant increase in myoclonic spasm and group VII showed non-significant

effect as indicated in figure 5 and 6. All values "were presented as mean ± S.E.M. All Groups Viz II-X are compared with groupI by employing One-Way ANOVA and Dunnett's test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Myoclonic spasm stem extract

Groups II-VI and IX-X exhibited a significant increase in latency to myoclonic spasms (p<0.0001) compared to Group I. Group VIII also demonstrated statistically significant rise in latency (p<0.001). These findings are illustrated in Figure 7. All values are presented as mean± S.E.M and comparisons among Groups II-X and Group I were assessed by employing One-Way ANOVA and Dunnett's test.

Treatments Vs Latency to Clonic spasm stem extract

Groups II to VI and VIII to X indicated significant rise in latency to clonic spasms (p<0.001) when compared with Group I. Group VII also demonstrated significant rise in latency (p<0.05). These observations are presented in Figure 8. All "data are presented as mean±S.E.M. and comparisons of Groups II to X with Group I were carried out by employing One-Way ANOVA and Dunnett's test. One-Way ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$, ***$P < 0.001$, **** $P< 0.0001$. ns non-significant in comparison with disease control. Current investigation assessed the anticonvulsant potential of Petroleum ether, Ethyl acetate, Butanol, along with Ethanol extracts derived from leaves as well as stem of *Clematis hedysarifolia* DC in mice subjected to chemically induced seizures. Extracts displayed dose-dependent protective impacts in PTZ as well as STZ-induced seizure models. Among tested extracts, LPE exhibited most pronounced anticonvulsant activity compared to others. PTZ and STZ are recognized as convulsant agents commonly utilized for screening antiepileptic drugs (Brodie *et al.,* 2016). PTZ provokes seizures primarily through inhibition of Gamma-aminobutyric acid (GABA) pathway at GABAA-receptors (Salaudeen *et al.*, 2022) which play a key inhibitory role in central nervous system and are critical in epilepsy. Additionally, action of PTZ involves glutamatergic mechanisms (Chindo *et al*.,2014) particularly via activation of N-methyl-D-aspartate (NMDA) receptor, which contributes to initiation and propagation of PTZ-induced convulsions (Parmar *et al*., 2022). Therefore, agents that counteract PTZ-induced seizures are believed to enhance GAB Aergic transmission or inhibit NMDA receptor-mediated glutamatergic pathways (Ofokansi *et al.,* 2022). Substances that suppress PTZ-evoked seizures in mice are believed to block T-type calcium channels (Son *et al*., 2014) and such agents are effective against myoclonic and absence seizures in clinical practice (Ofokansi *et al*., 2022; Rahimi *et al.,* 2019). In present study, extracts provided full "protection against PTZ-induced convulsions, showing results similar to positive control, diazepam. Diazepam exerts its anticonvulsant effects by facilitating GABA-mediated neural inhibition" (Sarfo *et al*. 2022; Rang *et al*., 2000). Based on observed findings, it is plausible that the anticonvulsant effect of *Clematis hedysarifolia* extracts could be attributed to their role in enhancing GABAergic activity and/or suppressing NMDA receptor-mediated glutamatergic transmission. Consequently, these extracts may prove beneficial in managing clonic and myoclonic seizure types. However, further investigation is essential to clearly elucidate underlying mechanisms involved (Malami *et al*., 2016). CONCLUSIONS Findings of current study indicate that all extracts of *Clematis hedysarifolia* DC exhibit anticonvulsant activity in experimental seizure models. The observed anti-seizure effects may be attributed to the presence of secondary metabolites within the extracts. These outcomes offer scientific support for traditional use of *Clematis hedysarifolia* DC in managing epilepsy. The present study provides *in vivo* pharmacological evidences

for antiepileptic potential of secondary metabolites like saponine, steroids, volatile oils etc. present in Indian traditional plant *Clematis hedysarifolia DC* act as cheapest sources for multi-target agent with immense antiepileptic potentials.

List of Abbreviations: LPE: Leaf Pet ether extract; **LBU:** Leaf Butanol extract; **LEA**: Leaf Ethyl acetate **LET**: Leaf Ethanol Extract; **SPE:** Stem Pet ether extract; **SBU**: Stem Butanol extract; **SEA**: Stem Ethyl acetate extract; **SET**: Stem Ethanol extract; **PTZ**: Pentylenetetrazol; **STZ**: Strychnine; **NMDA**: N-methyl-D-aspartate receptors; **GABA**: Gamma- amino butyric acid; **Ca_2+:** Calcium; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethical Committee; **ANOVA**: Analysis of Varian.

CONFLICT OF INTEREST STATEMENT The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Anticonvulsant activity of *Clematis hedysarifolia* DC leaves extracts on PTZ induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm.

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (%)
I	Control	PTZ 80 mg/kg +Water(10ml /kg)	30.06 ± 3.2	43.38 ± 4.03	0/5	0%
II	Diazepam + PTZ	Diazepam 1 and PTZ 80	290.83 ± 3.93	480.90 ± 2.08	5/5	100 %
III	SPE+PTZ	200	178.03 ± 1.08	220.70 ± 3.15	5/5	100 %
IV	SPE+PTZ	400	220.80 ± 3.19	333.30 ± 6.08	5/5	100 %
V	SEA+PTZ	200	75.17 ± 2.90	101.72 ± 1.02	2/5	60 %
VI	SEA+PTZ	400	117.30 ± 3.19	143.10 ± 2.87	5/5	100 %
VII	SBU+PTZ	200	35.17 ± 2.90	78.72 ± 1.02	3/5	40 %
VIII	SBU+PTZ	400	50.73 ± 6.18	97.43 ± 2.41	3/5	40 %
IX	SET+PTZ	200	65.18 ± 5.32	126.87 ± 2.71	1/5	80 %
X	SET+PTZ	400	105.03 ± 1.08	135.70 ± 3.15	5/5	100 %

Table 2: Anticonvulsant activity of *Clematis hedysarifolia* DC Stem extracts on PTZ induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (%)
I	Control	PTZ 80 mg/kg +Water(10ml/kg)	30.06 ± 3.2	43.38 ± 4.03	0/5	0 %
II	Diazepam + PTZ	Diazepam 1 and PTZ 80	290.83 ± 3.93	480.90 ± 2.08	5/5	100 %
III	LPE+PTZ	200	209.03 ± 1.08	270.70 ± 3.15	5/5	100 %
IV	LPE+PTZ	400	253.80 ± 3.19	396.30 ± 6.08	5/5	100 %
V	LEA+PTZ	200	75.17 ± 2.90	101.72 ± 1.02	4/5	80 %
VI	LEA+PTZ	400	117.30 ± 3.19	143.10 ± 2.87	5/5	100 %
VII	LBU+PTZ	200	35.17 ± 2.90	97.72 ± 1.02	2/5	40 %
VIII	LBU+PTZ	400	50.73 ± 6.18	78.43 ± 2.41	3/5	60 %
IX	LET+PTZ	200	123.18 ± 5.32	143.87 ± 2.71	5/5	100 %
X	LET+PTZ	400	150.03 ± 1.08	196.70 ± 3.15	5/5	100 %

Table 3: Anticonvulsant activity of *Clematis hedysarifolia* DC leaves extracts on STR induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (24hr) (%)
I	Control	STR 1mg/kg +Water(10ml/kg)	35.06 ± 3.1	48.28 ± 5.03	0/5	0%
II	Diazepam + STR	Diazepam 1 and STR 1mg/kg	460.83 ± 3.43	680.90 ± 4.08	5/5	100 %
II	LPE+STR	200	230.03 ± 2.08	310.70± 5.15	5/5	100 %
IV	LPE+STR	400	397.80 ± 3.50	520.30± 6.08	5/5	100 %
V	LEA+STR	200	85.17 ± 2.80	125.72± 5.02	5/5	100%
VI	LEA+STR	400	147.30 ± 3.19	183.10± 6.75	5/5	100 %
VII	LBU+STR	200	65.17 ± 2.85	112.72± 4.02	5/5	100 %
VIII	LBU+STR	400	102.73 ± 5.28	123.42 ±5.31	5/5	100 %
IX	LET+STR	200	165.18 ± 5.42	176.87± 4.61	5/5	100 %
X	LET+STR	400	264.03 ± 2.08	225.70± 5.20	5/5	100 %

Table 4: Anticonvulsant activity of *Clematis hedysarifolia* DC Stem extracts on STR induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	**Treatment**	**Dose mg/kg Orally)**	**Mean Duration of Myoclonic Spasm +/- SEM(s)**	**Mean Duration of Clonic Spasm +/- SEM(s)**	**No of Animals Recovered**	**Protection against Mortality (%)**
I	Control	STR 1mg/kg +Water(10ml/kg)	45.06 ± 4.5	83.38 ± 4.03	0/5	0%
II	Diazepam + STR	Diazepam 1 and STR 1mg/kg	463.83 ± 5.93	681.90 ± 5.08	5/5	100 %
III	SPE+STR	200	215.03 ± 5.08	298.70 ± 4.35	5/5	100 %
IV	SPE+STR	400	365.73 ± 5.29	502.25 ± 6.08	5/5	100 %
V	SEA+STR	200	81.17 ± 5.90	132.72 ± 5.22	5/5	100%
VI	SEA+STR	400	132.30 ± 4.19	162.10 ± 5.27	5/5	100 %
VII	SBU+STR	200	54.57 ± 5.20	104.62 ± 6.12	5/5	100 %
VIII	SBU+STR	400	98.73 ± 6.28	118.43 ± 4.41	5/5	100 %
IX	SET+STR	200	142.18 ± 5.32	156.87 ± 5.51	5/5	100 %
X	SET+STR	400	204.03 ± 4.08	258.70 ± 4.35	5/5	100 %

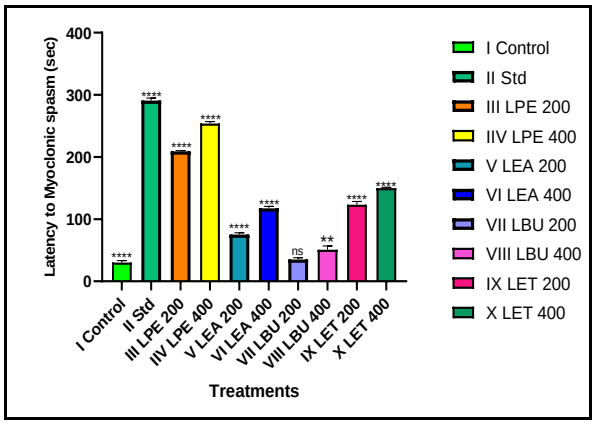

Figure 1: Effect of *Clematis hedysarifolia* leaf extracts on on PTZ induced Myoclonic spasm seizure model

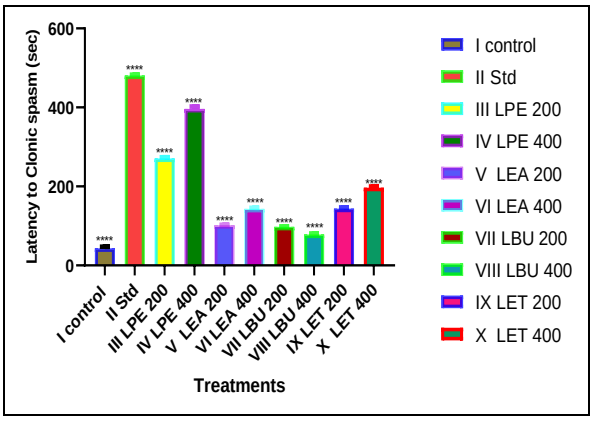

Figure 2: Effect of *Clematis hedysarifolia* leaf extracts on PTZ induced clonic spasm seizure model

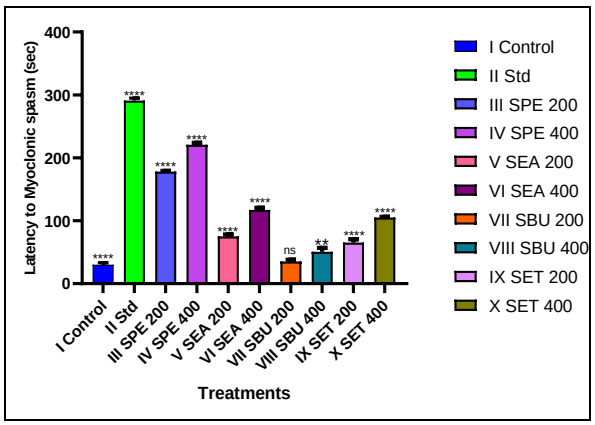

Figure 3: Effect of *Clematis hedysarifolia* stem extracts on on PTZ induced Myoclonic spasm seizure model

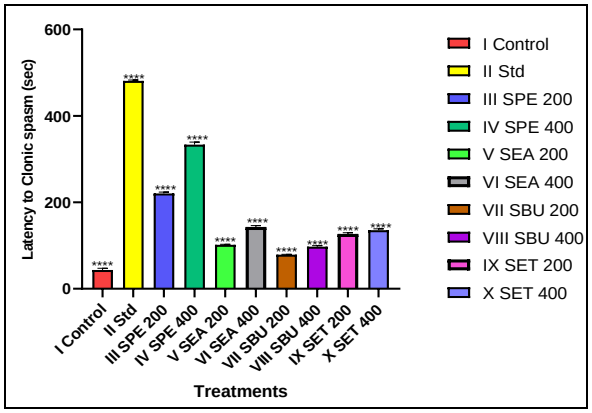

Figure 4: Effect of *Clematis hedysarifolia* stem extracts on PTZ induced clonic spasm seizure model

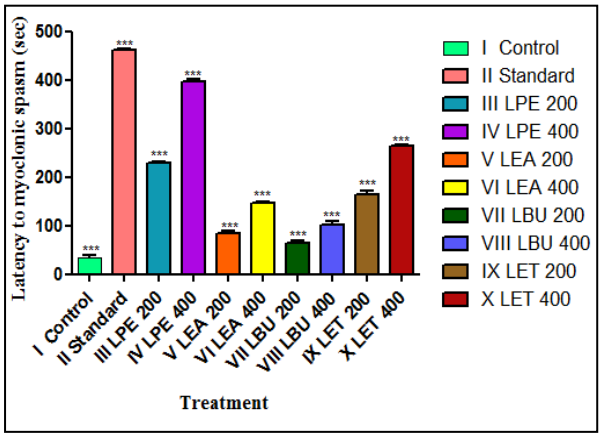

Figure 5: Effect of *Clematis hedysarifolia* leaf extracts on on STR induced Myoclonic spasm seizure model

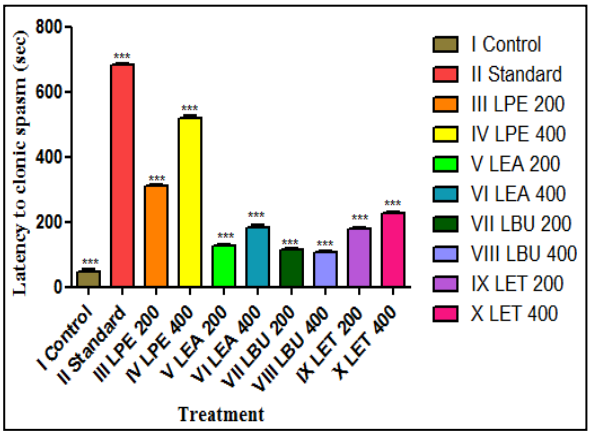

Figure 6: Effect of *Clematis hedysarifolia* leaf extracts STR induced clonic spasm seizure model

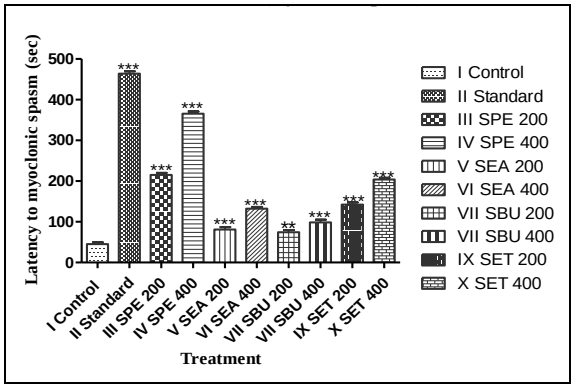

Figure 7: Effect of *Clematis hedysarifolia* stem extracts on on STR induced Myoclonic spasm seizure model

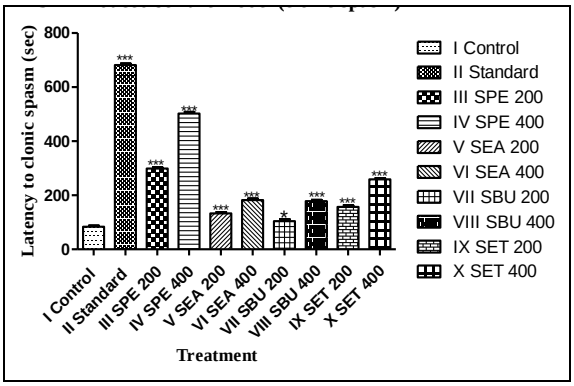

Figure 8: Effect of *Clematis hedysarifolia* stem extracts STR induced clonic spasm seizure model