RESEARCH ARTICLE

Pharmacological Evaluation of *Sedum lineare* Thunb Extract: Acute Toxicity and Anticonvulsant Effects in Swiss Albino Mice

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ABSTRACT

Aim: This study aimed to assess the acute toxicity and anticonvulsant effects of the hydroalcoholic extract of *Sedum lineare* Thunb in Swiss albino mice.

Materials and Methods: Acute toxicity was evaluated by administering a high dose (2000 mg/kg) of the hydroalcoholic extract and monitoring mortality, behavioral changes, body weight, and food/water consumption. Hematological parameters and organ histopathology were analyzed to assess systemic effects. The anticonvulsant activity was explored using pentylenetetrazole (PTZ) and picrotoxin-induced seizure models at various extract doses (100, 250, and 500 mg/kg).

Results and Discussion: The acute toxicity study revealed the safety of the hydroalcoholic extract, with no observed mortality, behavioral changes, or adverse effects on body weight and food/water consumption. While hematological changes were statistically significant, their clinical relevance requires further exploration. Histopathological examination confirmed the absence of organ toxicity. In the anticonvulsant assessment, the hydroalcoholic extract exhibited a protective effect against PTZ-induced seizures, significantly delaying jerk onset at the highest dose. Although not prevent clonic convulsions entirely, it dose-dependently reduces mortality rates. In the picrotoxin model, particularly at 250 mg/kg, the extract significantly increased clonic convulsion latency and suppressed mortality, indicating a potential modulatory role against seizures.

Conclusion: The hydroalcoholic extract of *S. lineare* Thunb demonstrated favorable acute toxicity and promising anticonvulsant effects in Swiss albino mice. The observed hematological changes warrant further investigation for clinical relevance, and additional studies, including long-term assessments and biochemical analyses, are recommended to comprehensively evaluate the extract's safety and therapeutic potential. These findings support further exploration of *S. lineare* Thunb as a potential candidate for neurological disorder interventions.

Keywords: *Sedum lineare* Thunb, Acute toxicity, Hematological analysis, Histopathological analysis, Anticonvulsant effects.

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INTRODUCTION

The native succulent *Sedum lineare* Thunb, which grows abundantly in the Maharashtra region of India's Pune district, is proof of how traditional medicine and nature may coexist perfectly. A staple of Punean folk medicine for many years, this resilient plant is deeply ingrained in the city's history and culture. *S. lineare* Thunb, with its thick, linear leaves and remarkable tolerance for dry environments, has lately caught the eye of scientists and is calling them to investigate its unexplored pharmacological possibilities.[1]

A multi-pronged investigation into the pharmacological profile of *S. lineare* Thunb has begun, including questions about the extracts' possible neuroprotective properties, physiological impacts, and safety. There may be a wealth of therapeutic potential within its botanical boundaries, given its slow growth and the multiplication of trailing stems by stolons, indicating the existence of bioactive chemicals.[2]

An integral part of Pune's traditional medical practices, *S. lineare* Thunb has added to the local pharmacopeia with its wide array of medicinal uses. A wide range of medical issues, from simple skin problems to gastrointestinal disorders, have traditionally been treated by the ingestion of plant extracts or infusions. Not only does this background support the plant's effectiveness, but it also gives a cultural basis for the modern scientific study of its pharmacological properties.[3]

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Previous research has recognized *S. lineare* Thunb's antioxidant and anti-inflammatory capabilities, but a crucial aspect the possible link between the phenolic or flavonoid content and anticonvulsant effects remains unexplored. Our work centers on this fascinating intersection of ancient knowledge and current scientific inquiry, inspired by our mentor's perceptive advice.[4]

We want to harmoniously combine conventional wisdom with cutting-edge scientific methods in our quest for understanding and health. A comprehensive evaluation of the physiological effects and safety profile of *S. lineare* Thunb extracts is one of our primary research aims. But our effort to deduce how the anticonvulsant effects of the plant's phenolic or flavonoid components relate to one another is where the true novelty resides.[5]

The importance of our work is becoming clearer as we set out on this scientific journey. Our research aims to do more than just record pharmacological effects; we want to help shape the future of treatment methods. Through the integration of traditional wisdom with scientific knowledge, we aim to create a comprehensive framework that honors and integrates ancient traditions into contemporary healthcare models.[5]

We are dedicated to discovering all the possible pharmacological uses of *S. lineare* Thunb, and it doesn't stop in the lab. Our goal is to provide more than simply facts; we want to tell a storey that honors and recognises this plant's historical and cultural background. We aim to bridge the gap between traditional wisdom and modern scientific rigour by doing thorough research to provide insights that cut across disciplines.

Finally, our investigation of *S. lineare* Thunb is emblematic of the coming together of ancient knowledge with cutting-edge scientific inquiry; it is more than just a scientific project. As we explore the pharmacological aspects of this hardy succulent, we hope that our discoveries will lead to a better knowledge of *S. lineare* Thunb and, more importantly, to new ways of thinking about medication that are based on traditional wisdom.

OBJECTIVES OF THE STUDY

Safety Assessment

First, an acute toxicity test is performed on Swiss albino mice in order to determine an appropriate dose range for *S. lineare* Thunb extract. This procedure is the first step in the research project. The OECD has created a set of guidelines, and this assessment complies with this set of criteria. It is very necessary to finish this first phase in order to conduct an assessment of the dangers that are connected to the use of extract.[6]

Physiological Impact

In order to explore the prospective physiological effects of the extract and its complete impacts, techniques from the disciplines of hematology and histopathology are used to conduct that investigation. This extensive analysis not only expands beyond the realm of acute toxicity but it also provides insights into the influence that the extract has on significant physiological markers.[7]

Neuroprotective Potential

Using confirmed seizure models is one of our study's most significant components, as it allows us to determine whether or not the extract has anticonvulsant characteristics. This inquiry aims to determine whether or not *S. lineare* Thunb has any neuroprotective properties. If successful, this investigation will add to a more thorough understanding of the function that botanical therapies play in the treatment of neurological disorders.[8]

Through these objectives, our study seeks to contribute to the scientific discourse surrounding *S. lineare* Thunb and to bridge traditional medicinal knowledge with contemporary scientific methodologies. This integrative approach holds promise for uncovering novel therapeutic interventions rooted in the rich biodiversity of botanical resources.[9]

MATERIALS AND METHODS

Plant Material

In December of 2022, the region of Pune in the state of Maharashtra, India, was the source of the succulent *Sedum linear*. Authentication of the reference voucher (B.U./Bot./PhD./2022/001) has been performed by Dr. Gaurav Nigam, who is a member of the Botany department at the Institute of Basic Science at Bundelkhand University. The voucher has been deposited for future reference.

Extraction

A hydroalcoholic solvent (ethanol: water, 70:30 volume/volume) was used to extract the marc of *S. lineare* Thunb after it had been air-dried, pulverized, and defatted. After that, the mixture was filtered using something called Whatman filter paper no. 1 before the solvent was evaporated in order to concentrate it. The crude extract was stored in glass vials at a temperature of 4°C, and its weight was recorded.[10]

Animals

Male and female Swiss albino mice evaluating 25 to 30 g were utilized in the experiment. Standard animal pellets (Amrut feeds, New Delhi) and municipal water were made available to them. As per CPCSEA rules (No.:- 716/GO/Re/S/02/CPCSEA), animal living conditions maintained a temperature of 22 ± 20°C with a 12-hour light/dark cycle. To lessen the animals' pain and shorten the duration of the experiments, we scrupulously adhered to the ethical rules for the study of seizures in conscious animals.[11]

Acute Toxicity Study

Principle of test

The OECD criteria for oral acute toxicity were adhered to throughout the investigation. Prior to the oral administration of the extract at a dosage of 2000 mg/kg, the animals were fasted for a full 24 hours. There was no evidence of death after close monitoring for a period of four hours after injection. On the basis of oral toxicity, further oral administration at one-fourth of the dosage was established. This resulted in the

establishment of the dose level for ethanol extracts of *S. lineare* Thunb at 2000 mg/kg.[12]

Description of Method

Selection of animal species

As a result of their sensitivity, young adult female albino mice that were healthy and between 8 and 12 weeks old were selected. The animals were acclimatized, tagged for identification, and placed in polypropylene cages according to the dosage they were given.[13]

Housing and feeding

The animals were kept in a controlled environment that was maintained at 22°C (+3°C), 30% humidity, and a 12-hour light/dark cycle. They were given conventional food pellets and water on an *ad libitum* basis.[14]

Observation

For a period of 14 days after administration, individual animals were monitored for toxicity reactions, changes in body weight, changes in food and water intake, appearance, activity, conditions affecting the furcoat and mucus, conditions affecting the body orifice and eyes, excretion, grooming, piloerection, gait posture, sedation, and mortality.[15]

Hematological analysis

A thorough haematological study was performed, which included blood collection via the tail both before and after administering the test material. This analysis included the collection of Hb, WBC, RBC, lymphocyte, monocyte, granulocyte, platelets, MCV, MCHC, PCV, and MCH.[16]

Histopathological analysis

Following the autopsy and isolation of organs (brain, kidney, liver, heart, lungs, and spleen), washing with normal saline, fixation in formalin at a concentration of 10%, and subsequent microscopy at magnifications of 20, 45, and 90, histopathology was performed upon the specimens.[17]

Methods

Pentylenetetrazole induced seizure

The *S. lineare* Thunb extract was administered in varying amounts to the animals, who were then separated into five groups. PTZ was used to induce seizures, and the characteristics were collected as they occurred.[18]

Picrotoxin induced seizure

Different dosages of the extract were administered to the groups, and the administration of picrotoxin brought on seizures. There was a record of the elimination of tonic extensor jerks and the percentage of protection.[19]

Table 1: Changes in body weight of mice treated with SLT for 14 days

S. No.	*Days*	*Group A (control mice)*	*Group B (treated mice)*
1.	Initial	24.31 ± 0.17	25.20 ± 0.41
2.	3rd day	23.33 ± 1.20	27 ± 0.57
3.	6th day	24.40 ± 0.30	26.4 ± 0.64*
4.	9th day	25.6 ± 0.92	26.07 ± 0.52
5.	12th day	25.4 ± 0.52	27.3 ± 0.51
6.	Final	26.4 ± 0.64	27 ± 0.57

Route of administration: Oral, Values are mean ± SEM, *p <0.05(S), compared to control, n = 5

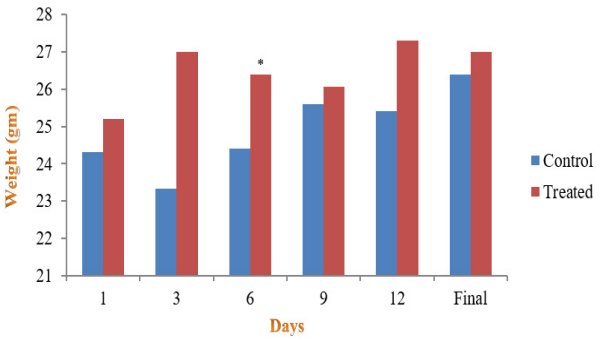

Figure 1: Mice body weight graphed over a period of 14 days, *p <0.05

Statistical Analysis

The values, expressed as Mean ± SEM for five mice, were subjected to analysis of variance (ANOVA), followed by Dunett's t-test.[20]

RESULT AND DISCUSSION

Acute Toxicity Study

Body weight observation

Hydroalcoholic extract of *S. lineare* Thunb up to 2000 mg/kg body weight caused a very significant (p <0.05) increase in body weight in the acute toxicity trial linked to the control group (Table 1). Notably, mice given oral doses of the extract up to 2000 mg/kg body weight showed no symptoms of death, behavioral abnormalities, or toxicity. The only observed effect was sedation on the 1st day after dose administration (Table 1 and Figure 1).

Food and water observation

The daily measurement of food and water consumption during the 14-day dosing period provided valuable insights into the potential effects of the hydroalcoholic extract of *S. lineare* Thunb on these essential parameters. There was a comparison between the initial food and water supply and the amount that remained after 24 hours, ensuring a comprehensive evaluation of the treatment groups in the assessment to the control (Table 2 and Figure 2, 3).

Hematological analysis

At a 2000 mg/kg body weight dosage, the hydroalcoholic extract of *S. lineare* Thunb was administered to female mice before the hematological study was performed. The primary objective of the investigation was to evaluate critical parameters. Specific hematological indicators were found to

Table 2: Alterations in the free-feeding habits of female albino mice after 14 days of radiation therapy

Groups	*Food intake (in gm)*			*Water intake (in mL)*		
	Initial	*Middle*	*Final*	*Initial*	*Middle*	*Final*
Group A (control)	3.15	6.2	6.7	3.1	3.2	3.1
Group B (treated)	4.1	7.9	8.9	3.5	3.6	4.0

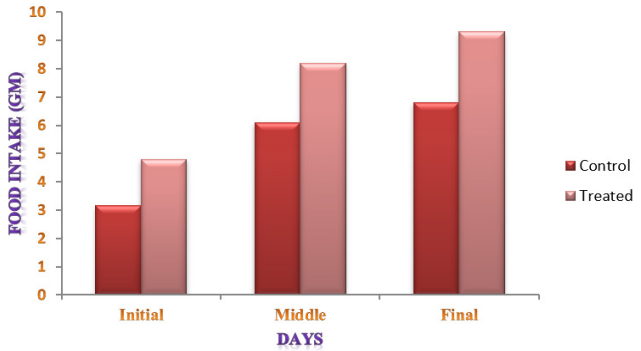

Figure 2: Female mice, both control and treated, with their food consumption shown graphically

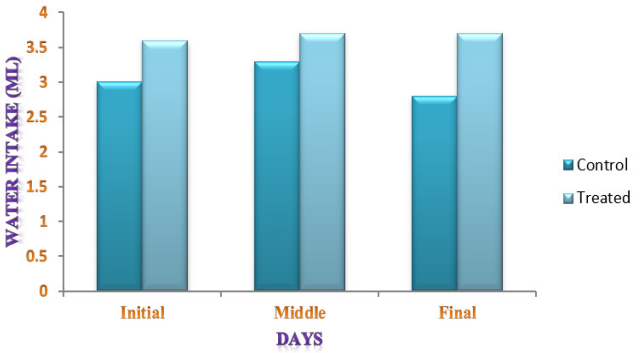

Figure 3: Visual depiction of fluid consumption in female mice (control and treatment)

have undergone significant shifts, as shown in (Table 3), which presents the findings.

Histopathological analysis

In the acute toxicity trial, female mice given 2000 mg/kg of the extract showed no toxicity symptoms over the course of 14 days. Necropsy was conducted to meticulously examine internal organ, including the kidneys, liver, brain, spleen, lungs, and heart. Macroscopic examination, presented in (Figures 4 and 5), along with organ weights relative to total body weight (Table 4), aimed to provide comprehensive insights into potential toxicity.

Figures 4 and 5 comparative histopathological study of untreated and treated mice in the SLT organs (Liver, Spleen, Kidney, Brain, Lungs, Heart).

Table 3: Hematological parameters after 14 days of treatment with SLT in female mice

S. No.	*Hematological parameter*	*Animals*	
		Control	*Treated*
1.	Hb (%)	14.53 ± 0.26	14.66 ± 0.35
2.	TLC (/mm)	12866.5 ± 32.36	12233.3 ± 669.95
3.	Granulocyte %	21.33 ± 0.87	28.33 ± 2.02*
4.	Lymphocytes	75.33 ± 1.45	77 ± 1
5.	Monocytes	2.6 ± 0.66	2.66 ± 0.66
6.	RBC (/mm^3)	5.7 ± 0.17	4.36 ± 0.13**
7.	Platelet count	4.2 ± 0.08	4.46 ± 0.28
8.	MCV (fl)	76.1 ± 0.81	79.5 ± 0.87
9.	MCH (pg)	25.1 ± 0.38	32.43 ± 0.29***
10.	MCHC (gm%)	33.03 ± 0.14	32.6 ± 0.30
11.	PCV (mL %)	43.33 ± 0.87	45.33 ± 0.32

Values are mean + SEM (Student t-test) n=5; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs* control group.

Table 4: Organ Wgt. (g) of female mice in the acute toxicity study of SLT

S. No.	*Organs*	*Group A (control)*	*Group B (treated)*
1.	Brain	0.45 ± 0.005	0.46 ± 0.011
2.	Liver	1.06 ± 0.068	1.2 ± 0.066
3.	Kidney (right)	0.25 ± 0.008	0.27 ± 0.005
4.	Kidney (left)	0.21 ± 0.005	0.20 ± 0.003
5.	Heart	0.27 ± 0.005	0.28 ± 0.012
6.	Lungs	0.35 ± 0.005	0.37 ± 0.014
7.	Spleen	0.24 ± 0.005	0.25 ± 0.005
8.	Body weight	24 gm	29 gm

Anticonvulsant Activity

PTZ-induced seizures

PTZ-induced seizures were employed to assess the anticonvulsant activity of *S. lineare* Thunb (SLT) extract. PTZ (85 mg/kg, i.p.) induced generalized clonic tonic convulsions, resulting in a 100% mortality rate in the control group. At a dosage of 500 mg/kg, the extract was shown to delay the start of jerks statistically significantly ($p < 0.01$). However, the extract did not substantially ($p > 0.05$) raise the beginning of tonic-clonic with hind limb allowance phase at 100, 250, or 500 mg/kg. The latency to seizures revealed a dose-dependent increase. Clonic convulsions were not prevented; however, this extract greatly decreased mortality (Table 5 and Figure 6), displaying the full results.

Picrotoxin induced seizures

The administration of picrotoxin (PIC) delivered intraperitoneally (i.p.) at a dosage of 7.5 mg/kg resulted in

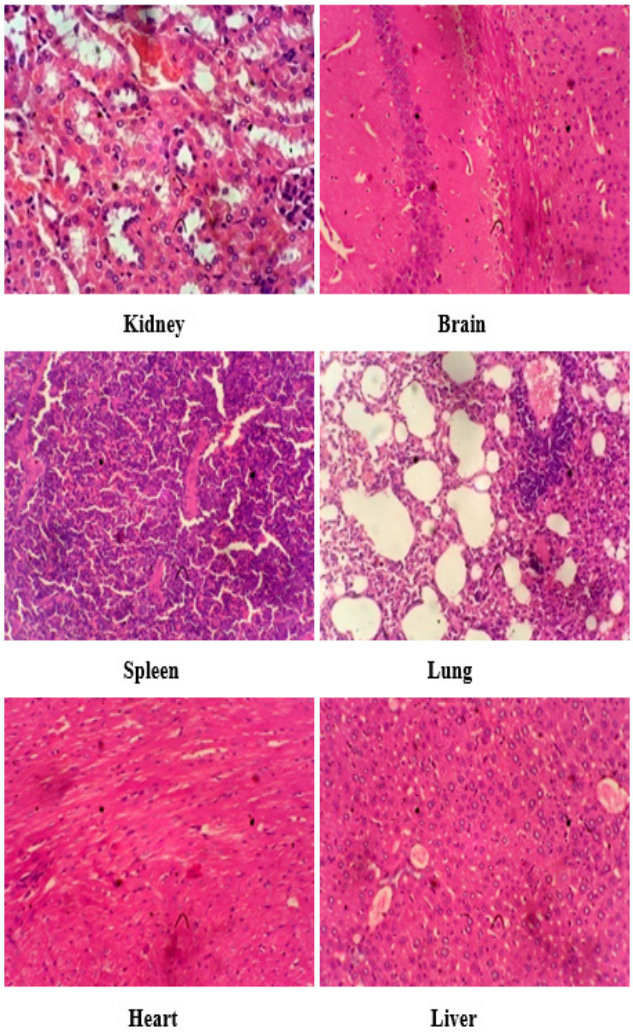

Figure 4: Control group

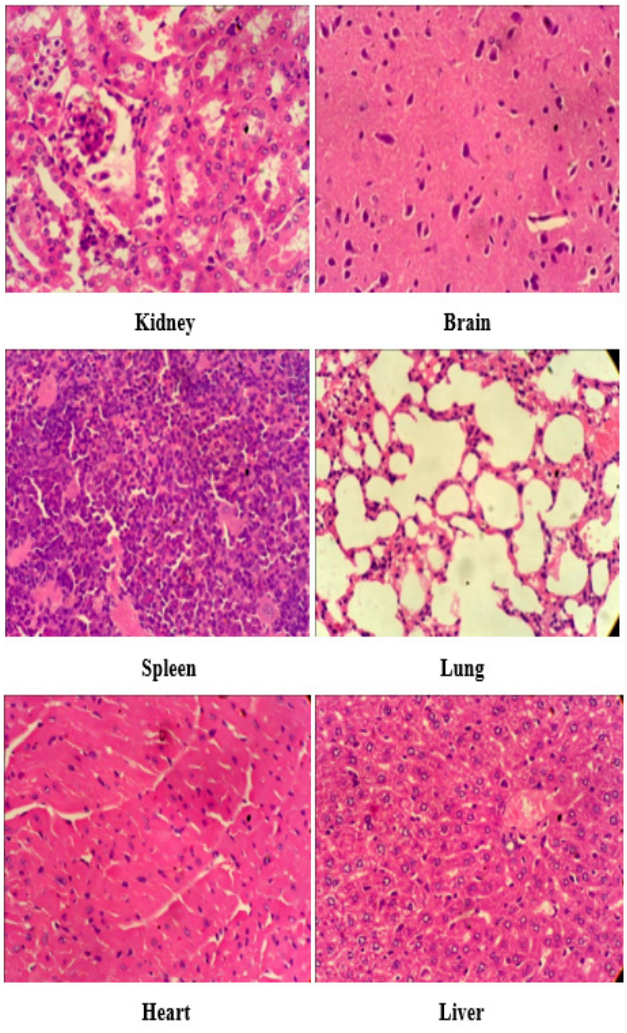

Figure 5: Treated group

the induction of widespread clonic tonic convulsions, which were followed by death in 50% of the mice. The effect of *S. lineare* Thunb (SLT) extract on seizures brought on by PIC was investigated, with a special emphasis placed on the latency of clonic convulsions and fatality rates associated with the condition. By administering the extract at a dosage of 250 mg per kg, it was shown that the latency of clonic convulsions was greatly enhanced ($p < 0.05$) and significantly reduced mortality (Table 6 and Figure 7) has a comprehensive presentation of the observations.

DISCUSSION

An acute toxicity investigation came to the conclusion that the hydroalcoholic extract of *S. lineare* Thunb is fully risk-free for intake. This conclusion followed the results of the study. The fact that a dosage of 2000 mg/kg did not result in any fatalities or noteworthy behavioral changes in the individuals who were given the medicine is evidence that this is the case. In addition, the fact that the extract promotes weight reduction without affecting the amount of food or drink consumed supports the claim that the extract is safe to consume.

Table 5: Effect of *S. lineare* Thunb extract on anticonvulsant activity in PTZ-induced seizures

Treatment	*Onset of jerk (sec)*	*Onset of clonic–tonic (sec)*
Dist. water (10 mL/kg, po)	80.24 ± 2.13	307.76 ± 2.7
Extract (100 mg/kg,po)	84.37 ± 1.33	310.05 ± 1.97 #
Extract (250 mg/kg,po)	132.53 ± 1.23	310.34 ± 0.86#
Extract (500 mg/kg,po)	110.82 ± 0.75*	119.86 ± 0.59#
Diazepam (4 mg/kg,ip)	_	_

Mice with PTZ-induced seizures responded differently to extract doses of 100, 250, and 500 mg/kg (po). Evaluations was conducted by PTZ (85 mg/kg,ip) after 1hr of administration of extract. Mean+SEM; *$p < 0.01$, #$p > 0.05$ against the distilled water treatment group; values are shown graphically

During the course of the hematological examination, it was seen that the characteristics of red blood cells (RBCs), as well as the percentage of granulocytes and the mean corpuscular hemoglobin (MCH), had undergone changes. These changes were observed. In spite of the fact that these adjustments

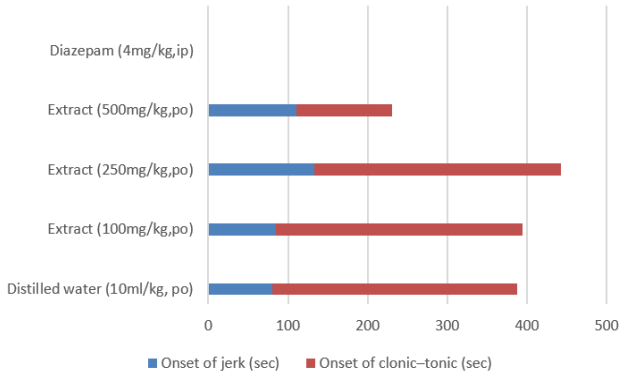

Figure 6: Effect of *S. lineare* Thunb extract on anticonvulsant activity in PTZ-induced seizures

Table 6: Effect of *S. lineare* Thunb extract on picrotoxin-induced seizures in mice (n = 5)

Treatment	*Onset of clonic-tonic seizures (min)*	*Mortality D/N*	*%Mortality*
Distilled water (10 mL/kg, po)	10.37 ± 0.79	3/5	60
Extract (100 mg/kg,po)	12.87 ± 0.68	5/5	100
Extract (250 mg/kg,po)	14.11 ± 0.63*	3/5	60
Extract (500 mg/kg,po)	11.76 ± 0.39	4/5	80
Phenytoin (25 mg/kg,ip)	19.16 ± 0.38	0/5	0

Increasing doses of extract (100, 250, and 500 mg/kg,po) reduced PIC-induced seizures in mice. After 1 hour, the effectiveness of the extract was measured using picrotoxin (7.5 mg/kg,ip). Mean standard error of the mean (SEM) values are shown. *$p < 0.05$ against the distilled water treatment group.

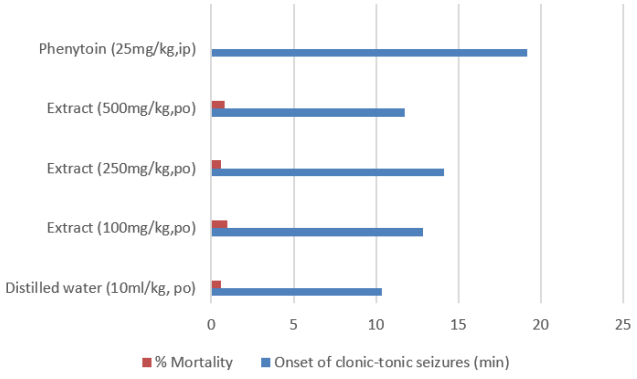

Figure 7: Effect of *S. lineare* Thunb extract on picrotoxin-induced seizures in mice (n = 5)

are statistically significant, there is a need for more studies to be carried out to ascertain the clinical relevance of these variations and the possible impact they might have on the health of the general population. Because the weights of the organs are within the normal range and histological studies did not uncover any obvious abnormalities, there is evidence that the extract is generally safe to use. This is demonstrated by the fact that the weights of the organs are within the natural range.

In terms of the anticonvulsant impact, our research results suggest positive consequences due to a paradigm that involves the development of seizures via the use of PTZ. It is quite likely that the maximum dosage of 500 mg/kg had a preventative effect on convulsions. This is due to the fact that the beginning of jerks was delayed. The jerks were delayed, which is the reason for this. Although the extract did not totally prevent clonic convulsions, it did have an effect that varied according to the amount of ingested extract. This was the case even if the extract did not completely prevent seizure activity.

When applied to a model of seizures brought on by picrotoxin, the extract demonstrated a considerable decrease in mortality as well as a delay in the onset of clonic convulsions. Specifically, this was seen when the extract was administered at a dosage of 250 mg per kg of body weight. The outcomes of this study lend credence to the need to research the possible processes by which the extract of *S. lineare* Thunb may serve to regulate seizures.

We took the choice to investigate whether or not the anticonvulsant effects of the plant were connected with the phenolic or flavonoid content of the plant. This decision was made in response to a suggestion made by our advisor. Nevertheless, our results align with prior research on the neuroprotective characteristics of flavonoids and phenolics. This is the case even though the precise bioactive components responsible for these benefits have not yet been discovered. The anticonvulsant properties of *S. lineare* Thunb ought to be the primary focus of future study. This research should concentrate on identifying the quantities of these components and exploring how they potentially contribute to the effects being studied.

It is of the utmost importance that the hematological alterations discovered in this study be taken into serious consideration by researchers conducting future investigations. This is because these alterations have the capability of providing insight into the influence that the extract has on the physiological system. Furthermore, this is the reason why this is the case. The purpose of this study is to provide light on the therapeutic potential and safety of the extract. It may be advantageous to undertake longitudinal studies and conduct a more in-depth investigation of biochemical markers in order to accomplish this goal. As a result, the data we have gathered serve as the basis for future research into the potential therapeutic use of *S. lineare* Thunb for treating neurological conditions. More specifically, we will explore whether or not there is a relationship between the anticonvulsant effects of this plant's phenolic or flavonoid content and the benefits of employing this plant as a treatment for neurological illnesses. This will allow us to be more specific.

CONCLUSION

The results of our in-depth research into the pharmacological properties of *S. lineare* Thunb have led to the discovery of

an intriguing new piece of information regarding the drug's tolerability, its physiological effects, and the therapeutic applications it has received. This information has been uncovered as a result of our investigation. An acute toxicity test was conducted on Swiss albino mice, and the results showed that the hydroalcoholic extract that was administered to the mice at a dose of 2,000 mg per kg showed an astonishing lack of toxicity. The findings of this test were published in the journal laboratory investigation. In addition, the fact that it does not adversely impact health outcomes and does not alter dietary or hydration routines adds credence to the idea that it is safe to use when taken as directed.

After an examination of the patient's blood, it was discovered that the granulocyte percentage, mean corpuscular hemoglobin (MCH), and many other hematological indicators exhibited variations. This was discovered after the examination of the patient's blood was finished. The fact that histological examinations did not reveal any abnormalities in the organs and that the weights of the organs were normal lends credibility to the notion that the extract is typically well tolerated. On the other hand, more study is necessary to ascertain whether or not these modifications have therapeutic importance. More investigation is necessary at this time.

Through the use of the PTZ-induced seizure paradigm, it was shown that the *S. lineare* Thunb extract has anticonvulsant action by demonstrating its presence. The amount of anticonvulsant effect that was exhibited by this particular paradigm was especially noteworthy. Following the administration of the medicine, there is a dose-dependent impact that includes a delayed onset of jerks and a reduction in the number of fatalities associated with the condition. The occurrence of this reaction is proof that the treatment was effective in achieving its intended purpose. In addition, the extract substantially delayed the initiation of clonic convulsions and lowered mortality in the picrotoxin-induced seizure model. This suggests that the extract has a modulatory function in seizures, which should be investigated further. Based on these data, it seems that the extract may have the capability of lowering death rates. On the basis of all that has been taken into account, it is evident that the extract has the potential to significantly reduce the rates of mortality.

If we consider the suggestion that our instructor made, we would like to suggest conducting additional research into the potential connection between the phenolic or flavonoid content of *S. lineare* Thunb and the anticonvulsant effects reported regarding this plant. As a consequence of the fact that our findings agree with the neuroprotective properties associated with these chemicals in the body of research that has been done in the past, we have discovered a potential path for doing research on the mechanism of action of the extract.

In addition to providing a solid foundation for further investigation, the findings of our analysis provide a solid basis for further exploration. It is essential that any researchers conducting research in the future pay particular attention to the discovered hematological irregularities. To achieve the goal of getting a comprehensive understanding of the extract's potential therapeutic effectiveness and safety, there is a need for more research into biochemical markers, in addition to clinical tests that are carried out over a more extended length of time.

S. lineare Thunb seems to be a potentially effective choice for the treatment of neurological illnesses, according to the results of our inquiry, which we have presented here. This is as a result of the fact that it strikes a balance between the most recent discoveries in the field of pharmacology and the knowledge that has been handed down from generation after generation. Because we now know that the anticonvulsant effects of this native succulent are associated with the concentration of phenolic or flavonoid chemicals that it contains, this is a significant step forward in unlocking this succulent's medicinal potential. This is because the concentration of these chemicals is associated with the properties of the succulent.

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