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ASSESSMENT OF NEUROBEHAVIOURAL AND NEUROCHEMICAL SCREENING OF SEDUM LINEARE IN ANIMAL MODEL

Arvind Patel, Prashant Gupta*, Vijay Nigam
Daksh Institute of Pharmaceutical Science, Chattarpur

***Correspondence Info:**
Prashant Gupta
Daksh Institute of Pharmaceutical Science, Chattarpur
Email:
researcharticle78@gmail.com

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ABSTRACT

Benzodiazepines, azapirones, SSRI, barbiturates drugs are available for the treatment of CNS disorders, but many patients suffer from significant adverse events; therefore, there is a need for new medications. Currently available antipsychotics are associated with variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. As a result there is high prevalence of usage of complementary and alternative medicines for treatment of psychiatric disorders. In the search for new therapeutic products for the treatment of neurological disorders. *Sedum lineare thunb. (SLT)*as per traditionally used in the treatment of CNS disorder including antihelminthic, antidysenteric and antiherpetic action From literature search, no scientific investigations have been conducted till date to verify the ethnobotanical use of *Sedum lineare thunb. (SLT)*in CNS disorders.

Keywords: Neurobehavioural, Neurochemical Screening, *Sedum lineare*, Phytoconstituents.

INTRODUCTION

India has an ancient heritage of traditional medicine. Materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural product. Indian traditional medicine is based on phytochemical, pharmacological & allied approaches including instrumental techniques like chromatography, microscopy and others. There is accumulating evidence suggesting medicinal plants are unlimited reservoirs of drugs. The amazing structural diversity among their active components makes them a useful source of novel therapeutic compounds. Researchers with interest in natural products have intensified their effort to towards scientific evaluation of traditional medicines (Prasathkumar *et al*., 2021).

Currently available antipsychotics are associated with variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. As a result there is high prevalence of usage of complementary and alternative medicines for treatment of psychiatric disorders. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models (Mazid *et al*., 2012).

Central nervous system (CNS)

The central nervous system (CNS) is the part of the nervous system that integrates the information that it receives from, and coordinates the activity of, all parts of the bodies of bilaterian animals-that is, all

multicellular animals except sponges and radially symmetric animals such as jellyfish. It contains the majority of the nervous system and consists of the brain and the spinal cord. Some classifications also include the retina and the cranial nerves in the CNS. Together with the peripheral nervous system, it has a fundamental role in the control of behavior. The CNS is contained within the dorsal cavity, with the brain in the cranial cavity and the spinal cord in the spinal cavity. In vertebrates, the brain is protected by the skull, while the spinal cord is protected by the vertebrae, and both are enclosed in the meninges.

Benzodiazepines, azapirones, SSRI, barbiturates drugs are available for the treatment of CNS disorders, but many patients suffer from significant adverse events; therefore, there is a need for new medications. Currently available antipsychotics are associated with variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects. As a result there is high prevalence of usage of complementary and alternative medicines for treatment of psychiatric disorders. In the search for new therapeutic products for the treatment of neurological disorders (Brodal, 2004; Nieuwenhuys, 2007).

*Sedum lineare thunb. (SLT)*as per traditionally used in the treatment of CNS disorder including antihelminthic, antidysenteric and antiherpetic action From literature search, no scientific investigations have been conducted till date to verify the ethnobotanical use of *Sedum lineare thunb. (SLT)*in CNS disorders (Wang *et al*., 2021).

Some plant show potent toxicity associated with the renal, cardiac, hematopoietic and reproductive systems. The development of controlled release delivery strategies would lead to significant advantages in the clinical use of these drugs to decreasing the toxicity. If the selected plant having toxicity and bad bioavailability, formulation of solid lipid nanoparticle associated with the isolate decreases the toxicity with increasing bioavailability.

MATERIALS & METHODS

Collection

Plants materials *sedum lineare* bark were collected from the local market of Bhopal, (M.P.) during the month of May –July, 2023.

Authentication

The specimens were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science & Education, Bhopal and their herbarium was deposited. Voucher specimen No.: 1757Bot/Safia/2023. These collected specimens were chosen for the extraction process and assessment of Neurobehavioural and Neurochemical screening activity.

Extraction

The plant materials so collected were cleaned properly and washed with distilled water to remove dust particles and dried in shade. The dried drugs were coarsely powdered and then exhaustively extracted with 90% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum.

The extract was subjected to qualitative phytochemical investigation to detect different phytoconstituents, TLC and Pharmacological studies.

Phytochemical profiling of extracts

Phytochemical investigation means to investigate the plant material in terms of its active constituents. It include following steps:

(A) Physical characteristics of extracts
(B) Qualitative chemical tests
(C) Chromatographic studies of extracts

Physical characteristics of extracts

Different physical parameters of extracts including their consistency, colour, odour and % yield are reported in Table.

Qualitative chemical tests

(Shaikh and Patil, 2020)

Different methods of identification are enlisted here that are used to investigate phytoconstituents present in extracts of different plants.

Screening for Neurobehavioural and Neurochemical screening

Screening for Neurobehavioural and was carried out in wistar albino mice (25-35 g). Screening for Neurochemical screening was carried out in wistar albino rats of either sex weighing 100-200 g.

Assessment of Epileptic activity

Wistar albino mice (25-35 g) bred in Central Animal facility of the Institute, were used. They were housed under standard conditions, maintained on a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The mice were acclimatized to the laboratory environment 1 h before the experiments. All experiments were conducted during the light period (08.00-16.00 h). The usage of animals. During the experiments animals were free access to water only. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Determination of maximum tolerable dose was performed according to OECD (Organization for Economic Corporation and Development) guideline 423.

Pentylenetetrazol (PTZ)-induced convulsion test

Animal groups

Four groups of mice (n = 10) were used. Group I was administered the vehicle, i.e., normal saline (1 ml/100g body weight) and served as control, Group II received reference standard (diazepam, 2 mg/kg, i.p.) while Groups III and IV were administered extract 100 and 200 mg/kg, p.o., respectively, of the extracts. Two hours later, PTZ was administered (60 mg/kg, i.p.) to all four groups. The animals were observed for 30 min and the onset and duration of convulsion noted. (Dhir, 2012)

Estimation of Neurochemicals

Estimation of Adrenocorticotropin (ACTH), Corticosterone (CORT), and β Endorphin (β-EP)

The blood was collected and centrifuged at 4◦C; the serum was stored at −80◦C before assay. Serum levels of ACTH, CORT, and *β*-EP were determined using ELISA kit (obtained from R&D Systems). The sensitivity of the assay was 1.0 ng/mL. Intra-assay and interassay coefficients of variation were less than 4.85% and 6.08%, respectively. The test was performed according to the manufacturer's specification.

Wistar rats were divided into four groups of six rats each. Group I served as normal group. Animals of Group II serves as model group and III-V group were administered orally with respective test drugs, between 7:30 am–9:30

am daily during 14 days. These doses were calculated according to the conversion table of equivalent effective dose ratios from human to animals based on the body surface area. Food was withdrawn from the animals 2 h prior to drug administration but water was allowed freely. The pretreatment groups (Gr. III, IV, and V) received the same electric foot-shock one hour after drug administration (8:30 am–10:30 am).

I - Normal group (0.5% Sodium carboxyl methyl cellulose (CMC-Na) solution (20 mL/kg, b.w.)

II- Model group

III- Ethanolic extract of *Sedum lineare* (2.53 g/kg)

IV Ethanolic extract of *Sedum lineare* (5.06 g/kg g/kg)

V Ethanolic extract of *Sedum lineare* (10.12 g/kg)

Measurements of Monoamine Neurotransmitters by HPLC-FCD

Norepinephrine (NE), Dopamine (DA), Serotonin or 5-hydroxytryptamine (5-HT)

Levels of monoamine neurotransmitters (NE,DA, 5-HT, and) in serum and brain were measured by HPLC coupled with a fluorescence detector (FCD). Mice were sacrificed immediately after exposure to the stress. Blood was sampled into EDTA-containing tubes at 10:00 am, and separated in a refrigerated centrifuge at 10,000×g for 10 min at 4◦C. The serum was stored at −80◦C until assayed. After blood collection, the brains were quickly removed, frozen in liquid nitrogen, and stored at −80◦C until assayed. To determine serum monoamine neurotransmitter levels, an equal volume of 0.1M HCl was added to the serum samples containing 200 μg/mL of DHBA as an internal standard. The samples were then shaken and mixed for 1.5min in ice water. One drop of concentrated HCl was then added to the solution and mixed in ice water for another 1.5 min and then centrifuged at 3000 rpm, 4◦C for 10 min. The samples of brain tissue were homogenized in ice water solution of 0.1M HCl. Then, 0.1M HCl solution was added to the samples (1 μL/1mg tissue) containing 200 μg/mL of DHBA as an internal standard and centrifuged at 18000 rpm, 4◦C for 10min. The samples were filtered through 0.45 μm microfilters (MFS Inc., USA). Aliquots (10 μL) of supernatant were injected into a reverse phase HPLC column (condition: Agilent 110180 high-voltage pump coupled to a fluorescence detector, chromatographic column ZORBAX ODB C18 4.6mm × 150mm × 5 mm, voltage 121V, and wavelength 360 nm). All the brain samples were weighed on an electronic scale prior to HPLC analysis, and the results were expressed as ng of monoamine/mg of wet weight tissue (Amat *et al.*, 2012).

Statistical analysis

Statistical evaluation of the data was done by Student's *t* test. (Graph PAD Instat software, Kyplot). A value of $p<0.05$ was considered to be significant

RESULTS AND DISCUSSION

The present project is done to explore the potential of herbal drugs for the treatment of CNS disorders with a view to perform phytochemical investigation and assess Neurobehavioural and Neurochemical screening. The various observation and results obtained from evaluations are discussed in this chapter.

Phytochemical profiling of crude extracts

Physical characteristics of extracts

Extraction of plant materials was done with 90 % ethanol. % yields of extracts as well as their characteristics are reported.

Chromatographic profile- Thin Layer chromatography (TLC)

The solvent system was developed on the basis of good resolution of ethanolic extracts.

Table 1: % Yield of extracts of different parts of plants

S. No.	Solvent	Part of Plant	Colour of extract	Yield (in gms)	% Yield (w/w)
1.	90% ethanol	bark	Dark Greenish Brown	92.3	13.14

Table 2: TLC profile in selected solvent system

S. No.	Solvent system	No. of spots	Separation pattern
I	Pet. ether: chloroform: methanol: glacial acetic acid (10:2:1:1) for *Sedum lineare*	4	Good Separation

Absorbent -Silica Gel G precoated plates

Detecting source- UV Chamber (254 and 366 nm)

The TLC study of all the four extracts was done with selected solvent systems.

Table 3: TLC profile of *Sedum lineare* ethanoilc extract

Spot No.	Colour of spots (at 254 nm)	Colour of spots (at 366 nm)	R_f value
I	Pale-green	Purple	0.72
II	Bluish-purple	Purplish-white	0.61
III	Dark green	Fluorescent-white	0.53
IV	Green	Red	0.45

Pharmacological studies

(Effect of ethanolic extracts of *Sedum lineare*) Pentylenetetrazol (PTZ)-induced convulsion test

Table 4: Effect of *Sedum lineare* on pentylenetetrazol (PTZ)-induced seizures in mice (n = 10)

Groups	Onset of convulsion (min)	Duration of convulsion (min)
Vehicle(control)	1.01 ± 0.11	28.2 ± 0.2
Standard (Diazepam)	0 ± 0†	0 ± 0 †
Etoh (100mg/kg)	4.94 ± 0.17#	6.9 ± 0.1#
Etoh (200mg/kg)	1.59 ± 0.13*	16.1 ± 0.1#

†P < 0.001; #p < 0.01; *p < 0.05

Assessment of anxiolytic study

Elevated plus – Maze test:

Table 5: Effect of *Sedum lineare* on Elevated plus Maze experiment

Mean ± SD (counts/5minutes)

Animal Group	Open arm Duration	Frequency of open arm	Closed arm Duration	Frequency of Closed arm
Vehicle(control)	2.6	0.8	257.8±11.19398	8.4±1.709
Standard (Diazepam)	2.4	0.2	261.8±12.49398	7.6±2.619
Etoh (200mg/kg)	16	3.2	207.6±10.29398	13.2±1.716
Etoh (400mg/kg)	8.2	2.6	203.2±11.39398	14.4±3.819

Assessment of nootropic study

Object recognition test:

Table 6: Effect of *Sedum lineare* on object recognition test

Animal Group (Dose in mg/kg)	Trial-1	Trial-2 (sec)		Recognition Index
	Time spent (sec)	Time spent familiar object	Time spent new object	
Control	20.00±3.05	10.10±0.47	11.10±1.27	51.58±1.84
Scopolamine(0.3)	31.11±3.84	31.98±20.98**	14.45±2.48	30.60±3.24*
Piracetam (100)	36.80±4.04	15.80±2.60	42.80±2.27**	73.89±2.65**
Ethanolic (10)	22.46±5.09	20.45±2.96 24.30±4.58		53.70±3.69

Assessment of antidepressant study

Forced Swimming Test (FST)

Table 7: Effect of *Sedum lineare* on Forced Swimming Test (FST)

Animal Group (Dose in mg/kg)	Immobility time (s) at first day	Change (%)	Immobility time (s) at third day	Change (%)
Control(0)	376.0 ± 31.5	-	385.0 ± 52.8	-
Haloperidol(1)	105.7 ± 4.5***	-71.84	74.8 ± 8.2***	-80.62
Fluoxetine(10)	201.7 ± 6.1***	-46.25	200.0 ± 7.0***	-48.06
ET(50)	380.3 ± 21.7ns	0.79	235.3 ± 41.8*	-38.17

Table 8: Effect of *Sedum lineare* on Tail suspension test (TST)

Animal Group (Dose in mg/kg)	Immobility time (s) at first day	Change (%)
Control(0)	325.3 ± 30.4	42.39
Haloperidol(1)	63.8 ± 15.1***	-80.39
Fluoxetine(10)	97.8 ± 11.5***	-69.97
ET(50)	187.0 ± 9.5***	-42.39

Assessment of Muscle relaxant study

Grip Strength Test

Table 9: Grip strength in seconds

Group	Treatment	Dose (mg/kg)	0 hr	30 min	1 hr	2 hr	4 hr	6 hr
1	Control	--	357.17 ± 11.620	369.33 ± 19.416	378.23 ± 12.456	380.50 ± 12.132	376.33 ± 11.071	360.17 ± 19.511
2	Diazepam	26	369.50 ± 18.936	* 201.17 ± 13.893	* 154.17 + 13.946	** 124.23 ± 11.923	** 100 ± 33 ± 4.310	** 102 ± 33 ± 4.310
3	Ethanolic extract	200	331.50 ± 12.233	* 308.17 ± 10.778	* 325.23 ± 9.127	ns 360.01 ± 11.923	358.50 ± 10.987	337.23 ± 6.940

Effects of *Sedum lineare* on the Serum Levels of ACTH, CORT, and β-EP in the Chronic Stress Mice

Table 10: Effects of *Sedum lineare* on the serum level of ACTH

GROUP	ACTH (pg/mL)
Gr. I	16.0 ± 3.12
Gr. II	32.10 ± 4.14*
Gr. III	22.25 ± 3.89**
Gr. IV	19.60 ± 4.21**
Gr. V	17.51 ± 2.84**

*Results are given as means ±SEM, when compared to the normal group (Gr. I), $P < 0.01$. **Results are given as means ±SEM, when compared to the model group (Gr. II), $P < 0.05$.

Table 11: Effects of *Sedum lineare* on the serum level of CORT

GROUP	CORT (pg/mL)
Gr. I	12.18 ± 4.9
Gr. II	29.18 ± 4.5*
Gr. III	19.90 ± 5.6**
Gr. IV	21.50 ± 3.34 **
Gr. V	21.40 ± 2.1 **

Table 12: Effects of *Sedum lineare* on the serum level of *β*-EP

GROUP	*β*-EP (pg/mL)
Gr. I	153.17 ± 27.19
Gr. II	255.21 ± 23.12*
Gr. III	200.32 ± 34.25**
Gr. IV	193.10 ± 19.65**
Gr. V	176.00 ± 20.56**

Table 13: Effects of *Sedum lineare* on the serum level of NE (ng/mL)

GROUP	NE (ng/mL)
Gr. I	422.22 ± 20.19
Gr. II	695.55 ± 23.10
Gr. III	625.32 ± 30.25**
Gr. IV	600.11 ± 18.65**
Gr. V	400.01 ± 20.56**

Table 14: Effects of *Sedum lineare* on the serum level of 5-HT (ng/mL)

GROUP	5-HT (ng/mL)
Gr. I	100.5 ± 14.19
Gr. II	40.50 ± 21.10*
Gr. III	103.5 ± 31.25**
Gr. IV	102.11 ± 18.65**
Gr. V	104.01 ± 21.56**

Table 15: Effects of *Sedum lineare* on the serum level of DA(ng/mg)

GROUP	DA (ng/mg)
Gr. I	2900.25 ± 21.19
Gr. II	2212.50 ± 18.10*
Gr. III	4450.5 ± 27.25**
Gr. IV	5250.11 ± 38.65**
Gr. V	5650.01 ± 27.56**

CONCLUSION

The central nervous system (CNS) is the part of the nervous system that integrates the information that it receives from, and coordinates the activity of, all parts of the bodies of bilaterian animals—that is, all multicellular animals except sponges and radially symmetric animals such as jellyfish. It contains the majority of the nervous system and consists of the brain and the spinal cord. Some classifications also include the retina and the cranial nerves in the CNS. Together with the peripheral nervous system, it has a fundamental role in the control of behavior. The CNS is contained within the dorsal cavity, with the brain in the cranial cavity and the spinal cord in the spinal cavity. In vertebrates, the brain is protected by the skull, while the spinal cord is protected by the vertebrae, and both are enclosed in the meninges

The present project is done to explore the potential of herbal drugs for the treatment of CNS disorders with a view to perform phytochemical investigation and assess Neurobehavioural and Neurochemical screening. The study also involves development of solid lipid nanoparticle formulation associated with the extracts followed by characterization and other evaluation parameters employing *Sedum lineare*. The plant materials were collected, dried, coarsely powdered and then exhaustively extracted with 90% ethanol in Soxhlet apparatus for 72 h. The ethanolic extracts so obtained were freed of solvent under vacuum. The % yield (w/w) of *Sedum lineare*. ethanolic extracts were found to be 13.14%.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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