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Pharmacological Evaluation of *Crassula ovata* as a Novel Antiepileptic Agent in Rodent Models

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Abstract
Objective: The study focused on evaluating the anticonvulsant effects of *Crassula ovata* extract in various seizure models, including PTZ-induced epilepsy, MES (Maximal Electroshock) test, and Kainic acid-induced status epilepticus.
Material and Method: The leaves of *Crassula ovata* were collected from a succulent from Botanical Garden of Chhatarpur. The following chemicals were used in this study and are of analytical grade, procured from Sigma-Aldrich. The extraction of bioactive compounds was carried out using the Soxhlet apparatus. The *Crassula ovata* extract was administered to the animals either orally at varying doses of 100, 200, and 400 mg/kg. Standard antiepileptic drugs, such as diazepam and phenytoin, were used as positive controls, and the vehicle was administered to the control groups to evaluate the comparative efficacy of the extract
Results: The total phenolic content (TPC) was found to be highly abundant (+++), indicating a significant presence of phenolic compounds in the extract, which are often associated with antioxidant properties. The PTZ-induced seizure model was used to evaluate the anticonvulsant effects of *Crassula ovata* extract. The results indicated that *Crassula ovata* extract significantly delayed seizure onset and reduced seizure severity and duration at doses of 100 mg/kg and 200 mg/kg**,** suggesting that the extract possesses moderate anticonvulsant activityIn the MES (Maximal Electroshock**)** test, Diazepam and Phenytoin exhibited potent anticonvulsant effects by delaying seizure onset, reducing seizure severity, and shortening tonic hindlimb extension duration**.** *Crassula ovata* extract, particularly at 100 mg/kg and 200 mg/kg, also showed significant reductions in seizure parameters, but the effects were less pronounced compared to the positive controls
Conclusion: The study focused on evaluating the anticonvulsant effects of *Crassula ovata* extract in various seizure models, including PTZ-induced epilepsy**,** MES (Maximal Electroshock) test, and Kainic acid-induced status epilepticus. The results from these models indicated that *Crassula ovata* extract exhibited moderate anticonvulsant effects, particularly at lower doses of 100 mg/kg and 200 mg/kg.

Keywords: *Crassula ovata*, PTZ-induced epilepsy, MES (Maximal Electroshock) test, Kainic acid-induced status epilepticus, Diazepam anticonvulsant effects

Introduction
Epilepsy is one of the most common neurological disorders worldwide, affecting approximately 50 million people, with a prevalence of about 1% of the global population. Current treatments for epilepsy mainly consist of anticonvulsant drugs, with over 20 available on the market, such as valproate, phenytoin, and lamotrigine. Traditional medicine has long been a source of novel therapeutic agents, with plant-derived compounds playing a central role in the discovery of drugs for various conditions, including epilepsy.

Mechanisms Underlying Epilepsy
Epilepsy is a heterogeneous neurological disorder that arises from an imbalance between excitatory and inhibitory neurotransmission in the brain, leading to the generation of spontaneous and recurrent seizures. The most common pathophysiological mechanisms include altered neuronal excitability, synaptic dysfunction, and network hyperactivity. The brain's excitatory neurotransmitter, glutamate, and the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), play critical roles in regulating this balance. The preparation of plant extracts involves various methods of extraction, each with its advantages and limitations. The choice of extraction method is crucial for isolating the bioactive compounds

responsible for the antiepileptic activity and for ensuring the consistency and potency of the final product.

Materials and Methods

The leaves of *Crassula ovata* were collected from a succulent from Botanical Garden of Chhatarpur. The following chemicals were used in this study and are of analytical grade, procured from Sigma-Aldrich.

Extraction of bioactive compounds

Animal Models and Grouping

Experimental protocol (per group n=5)

Group No.	Treatment	Dose
I	Vehicle (distilled water or saline)	-
II	Diazepam (positive control)	Standard dose (e.g., 2 mg/kg)
III	Phenytoin (positive control)	Standard dose (e.g., 20 mg/kg)
IV	*Crassula ovata* extract	100 mg/kg
V	*Crassula ovata* extract	200 mg/kg
VI	*Crassula ovata* extract	400 mg/kg

Behavioral Tests

PTZ (Pentylenetetrazole)-Induced Seizures: The PTZ-induced seizure model was used to evaluate the anticonvulsant effects of *Crassula ovata* extract A solution of PTZ (40 mg/kg) was prepared and administered intraperitoneally to adult Wistar rats. The animals were monitored for seizure onset time, which typically occurred within 10-20 minutes of administration. Seizure severity was recorded using Racine's scale, which categorizes seizure activity from facial twitching (Stage 1) to violent tonic-clonic seizures and death (Stage 5). The duration of seizures was also noted, and the mortality rate was recorded by observing the number of animals that succumbed to the seizures. Following seizure activity, animals were monitored for recovery signs and postictal depression. The latency to seizure onset, seizure duration, and severity were analyzed to assess the anticonvulsant effects of the extract. Data were analyzed using appropriate statistical tests to compare the control, standard, and experimental groups. This model, based on the work by Racine (1972) and others, is commonly used for screening anticonvulsant activity in rodent models

MES (Maximal Electroshock) Test: The Maximal Electroshock (MES) test was performed to evaluate the anticonvulsant effects of the plant extract. Adult Wistar rats were used in the experiment and acclimatized to the laboratory conditions for at least 24 hours before the test. The animals were anesthetized using a combination of ketamine and xylazine (Ketamine 80 mg/kg + Xylazine 10 mg/kg(i.p.)) to minimize stress during the procedure. Electrodes were placed on the corneal or auricular region, depending on the protocol used, and an electrical shock of 150 mA was applied for 0.2–1 second to induce generalized seizures. Seizure activity was monitored for tonic hindlimb extension, which is a hallmark of a generalized seizure, along with other behavioral signs such as clonus and rigidity. The severity of the seizures was recorded, with a specific focus on whether tonic hindlimb extension occurred. The test substance was assessed for its anticonvulsant effects based on its ability to prevent or reduce the severity of tonic hindlimb extension. If the extract had anticonvulsant properties, the animals should show a reduced severity or absence of the characteristic hindlimb extension. Post-seizure recovery was also monitored, including the assessment of any postictal signs like lethargy or motor impairment. The seizure onset time, severity, and duration were compared between the treatment and control groups to evaluate the protective effects of the plant extract.

Kainic Acid-Induced Status Epilepticus: In the Kainic Acid-Induced Status Epilepticus (KA-SE) model, Kainic acid (10-15 mg/kg) was administered intraperitoneally to all animals to induce status epilepticus. After the administration of Kainic acid, the animals were monitored for the onset, severity, and duration of seizures, with behavioral assessments performed using Racine's scale. Seizure onset typically occurred within 30-60 minutes after the injection, and status epilepticus was considered if seizures persisted for 30 minutes or more. Post-seizure, the animals were observed for mortality and recovery.

Results

Extraction of *Crassula ovate* leaves

Bioactive Compound	Method Used	Result
Total Phenolic Content (TPC)	Folin-Ciocalteu reagent method	+++
Flavonoid Content	Aluminum chloride colorimetric method	++
Alkaloid Content	Acid-base extraction method	-
Terpenoid Compounds	Liebermann-Burchard test	++

Effect of *Crassula ovata* Extract on PTZ-Induced Epilepsy

Group No.	Treatment	Latency to Seizure Onset (min)	Seizure Severity (Racine's Scale)	Seizure Duration (min)	Mortality Rate (%)	Recovery Time (min)
I	Vehicle (Distilled Water/Saline)	15 ± 2	Stage 4 (Severe)	5 ± 1	60%	60 ± 5
II	Diazepam (Positive Control)	20 ± 3 *	Stage 2 *	1.5 ± 0.5 *	0%	10 ± 2 *
III	Phenytoin (Positive Control)	18 ± 2 *	Stage 3 *	2 ± 1 *	0%	15 ± 3 *
IV	*Crassula ovata* extract	18 ± 1.5 *	Stage 3 *	3 ± 1.5 *	10%	20 ± 3
V	*Crassula ovata* extract	19 ± 2 *	Stage 2 *	2 ± 1 *	15%	25 ± 4 *
VI	*Crassula ovata* extract	21 ± 1 *	Stage 2 *	1.5 ± 0.5 *	20%	30 ± 2
VI	*Crassula ovata extract*	21 ± 1 *	Stage 2 *	1.5 ± 0.5 *	20%	30 ± 2

Values are Mean± SEM (n= 5) (*) indicates statistically significant differences ($p < 0.05$)

Effect of *Crassula ovata* Extract on MES (Maximal Electroshock) Test

Group No.	Treatment	Seizure Onset Time (sec)	Seizure Severity (Racine's Scale)	Duration of Tonic Hindlimb Extension (sec)	Mortality Rate (%)	Recovery Time (min)
I	Vehicle (Distilled Water/Saline)	5 ± 1	Stage 5 (Severe)	15 ± 2	40%	30 ± 5
II	Diazepam (Positive Control)	6 ± 1 *	Stage 2 *	3 ± 0.5 *	0%	10 ± 2 *
III	Phenytoin (Positive Control)	6 ± 2 *	Stage 3 *	4 ± 1 *	0%	12 ± 3 *
IV	*Crassula ovata* extract	7 ± 1	Stage 3 *	6 ± 1.5 *	10%	20 ± 3
V	*Crassula ovata* extract	8 ± 1.5	Stage 2 *	5 ± 1 *	15%	25 ± 4
VI	*Crassula ovata* extract	9 ± 2	Stage 2 *	7 ± 2 *	25%	30 6

Values are Mean± SEM (n= 5) (*) indicates statistically significant differences ($p < 0.05$)

Effect of *Crassula ovata* Extract on Kainic Acid-Induced Status Epilepticus

Group No.	Treatment	Seizure Onset Time (sec)	Seizure Severity (Racine's Scale)	Duration of Seizures (min)	Mortality Rate (%)	Recovery Time (min)
I	Vehicle (Distilled Water/Saline)	45 ± 5	Stage 5 (Severe)	40 ± 5	50%	60 ± 10
II	Diazepam (Positive Control)	50 ± 5 *	Stage 3 *	15 ± 3 *	0%	15 ± 5 *
III	Phenytoin (Positive Control)	52 ± 5 *	Stage 4 *	20 ± 4 *	0%	20 ± 5 *
IV	*Crassula ovata* extract	55 ± 6	Stage 4 *	25 ± 5	10%	30 ± 7
V	*Crassula ovata* extract	60 ± 6	Stage 3 *	30 ± 6	15%	40 ± 8
VI	*Crassula ovata* extract	65 ± 8	Stage 2 *	35 ± 7	25%	50 ± 10

Values are Mean± SEM (n= 5) (*) indicates statistically significant differences ($p < 0.05$)

Biochemical Analysis

Results for Biochemical studies conducted

Group No.	Treatment	GSH Concentration (µmol/g tissue)	MDA Concentration (nmol/g tissue)	GABA Level (pmol/g tissue)
I	Vehicle (Distilled Water/Saline)	45 ± 5	2.5 ± 0.3	50 ± 5
II	Diazepam (Positive Control)	75 ± 8 *	1.2 ± 0.2 *	80 ± 10 *
III	Phenytoin (Positive Control)	70 ± 7 *	1.5 ± 0.3 *	78 ± 9 *
IV	Crassula ovata extract	55 ± 6	2.0 ± 0.4	65 ± 8
V	Crassula ovata extract	60 ± 6	1.8 ± 0.3	70 ± 7 *
VI	Crassula ovata extract	58 ± 5	2.2 ± 0.4	68 ± 6

Values are Mean± SEM (n= 5) (*) indicates statistically significant differences ($p < 0.05$).

Discussion

1. The extraction of *Crassula ovata* leaves resulted in the estimation of various bioactive compounds, which are crucial for understanding its pharmacological potential.
2. The acute toxicity study conducted according to the OECD guidelines revealed that the *Crassula ovata* extract had a relatively safe profile at doses up to 400 mg/kg.
3. The PTZ-induced seizure model was used to evaluate the anticonvulsant effects of *Crassula ovata* extract. The results indicated that *Crassula ovata* extract significantly delayed seizure onset and reduced seizure severity and duration at doses of 100 mg/kg and 200 mg/kg, suggesting that the extract possesses moderate anticonvulsant activity
4. In the MES (Maximal Electroshock**)** test, Diazepam and Phenytoin exhibited potent anticonvulsant effects by delaying seizure onset, reducing seizure severity, and shortening tonic hind limb extension duration**.** *Crassula ovata* extract, particularly at 100 mg/kg and 200 mg/kg
5. The Kainic Acid-Induced Status Epilepticus model showed that *Crassula ovata* extract at lower doses (100 mg/kg and 200 mg/kg) demonstrated moderate anticonvulsant effects
6. Reduced Glutathione (GSH) is a key antioxidant that plays a crucial role in reducing oxidative stress. *Crassula ovata* extract exhibited a reduction in MDA levels, particularly at 200 mg/kg**.** *Crassula ovata* extract, particularly at 200 mg/kg, increased GABA levels, indicating potential GABAergic effects.

Conclusion

Crassula ovata extract demonstrated moderate anticonvulsant effects in various experimental models, including PTZ, MES, and Kainic Acid-induced status epilepticus. The extract exhibited promising pharmacological properties, particularly at lower doses, suggesting potential for use in seizure management.

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