

EVALUATION OF CRASSULA OVATA AGAINST EXPERIMENTAL INDUCED COGNITIVE IMPAIRMENT IN RATS

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ABSTRACT

Cognitive impairment is a major feature of neurodegenerative disorders such as Alzheimer's disease and is characterized by deficits in learning, memory, and executive function. Oxidative stress, neuroinflammation, and cholinergic dysfunction are considered major contributors to cognitive decline. *Crassula ovata* (Jade Plant), a succulent belonging to the family Crassulaceae, possesses flavonoids, phenolic compounds, triterpenoids, and other phytochemicals exhibiting antioxidant and anti-inflammatory activities. Preliminary phytochemical screening and acute oral toxicity studies were performed according to OECD Guideline 423. Cognitive impairment was induced using scopolamine, and animals were treated with different doses of *Crassula ovata* extract for 21 consecutive days. Cognitive performance was evaluated using the Morris Water Maze (MWM) and Novel Object Recognition (NOR) tests. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. The results demonstrated that *Crassula ovata* extract significantly improved spatial learning and memory, reduced escape latency, increased time spent in the target quadrant, and enhanced recognition memory compared to the disease control group. The neuroprotective activity may be attributed to its antioxidant phytoconstituents. The findings suggest that *Crassula ovata* possesses promising cognitive-enhancing potential and may serve as a therapeutic candidate for the management of neurodegenerative disorders.

Keywords:

Crassula ovata, Cognitive Impairment, Memory Deficit, Neuroprotection, Learning and Memory, Oxidative Stress, Nootropic Activity, Behavioral Studies, Neurodegenerative Disorders, Rat Model

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INTRODUCTION

Cognitive impairment is characterized by deficits in learning, memory, attention, and executive functions, and is a major feature of several neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and age-related dementia [1]. The increasing prevalence of cognitive disorders worldwide has created an urgent need for the development of effective and safer therapeutic agents. Although currently available drugs such as donepezil, rivastigmine, and memantine provide symptomatic relief, their long-term use is often associated with adverse effects and limited efficacy [2]. Oxidative stress, neuroinflammation, cholinergic dysfunction, and neuronal degeneration are recognized as key contributors to cognitive decline. Experimental models of cognitive impairment in rodents are widely used to investigate the pathophysiology of memory deficits and to evaluate the potential neuroprotective effects of natural products and novel therapeutic agents [3,4]. Medicinal plants have gained considerable attention due to their diverse bioactive constituents and lower incidence of side effects.

Crassula ovata (Mill.) Druce, commonly known as Jade Plant, belongs to the family Crassulaceae [5]. It is a succulent plant traditionally used in folk medicine for the treatment of inflammation, wounds, and various health disorders [6]. Phytochemical investigations have revealed the presence of flavonoids, phenolic compounds, triterpenoids, tannins, and other antioxidant constituents in *C. ovata*. These bioactive compounds are known to possess free radical scavenging, anti-inflammatory, and neuroprotective properties [7,8]. Recent studies suggest that plant-derived antioxidants may improve cognitive functions by reducing oxidative stress, enhancing cholinergic neurotransmission, and protecting neurons against degeneration. However, scientific evidence regarding the cognitive enhancing potential of *Crassula ovata* remains limited. Therefore, the present study was designed to evaluate the effect of *Crassula ovata* extract against experimentally induced cognitive impairment in rats using behavioral, biochemical, and neurochemical parameters. The findings of this study may provide

scientific validation for the traditional use of *Crassula ovata* and contribute to the development of novel plant-based therapeutic strategies for the management of cognitive disorders and neurodegenerative diseases.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh and healthy leaves of *Crassula ovata* were collected from a reliable source and authenticated by the Department of Plant Science, M.J.P. Rohilkhand University, Bareilly, Uttar Pradesh, India (Ref. No. RU/PS/PI/2025/318). The authenticated leaves were washed thoroughly with distilled water, shade-dried at room temperature, and pulverized into coarse powder for extraction.

2.2 Drugs and Chemicals

The chemicals and reagents used in the study were of analytical grade. Scopolamine hydrobromide was used for the induction of cognitive impairment. Donepezil hydrochloride (1 mg/kg) was used as the standard anti-amnesic drug. Distilled water Ethanol (analytical grade), Chloroform, Mayer's reagent, Benedict reagents, Potassium iodide, Diluted HCL, Sodium hydroxide (NaOH), Acetic anhydride, Conc. Sulfuric acid, Ferric chloride solution, Copper sulfate and Other analytical-grade chemicals and reagents procured from certified suppliers.

2.3 Experimental Animals

Adult Wistar albino rats of either sex weighing 150–200 g were used for the study. Animals were housed under standard laboratory conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity $55 \pm 5\%$, and 12 h light/dark cycle) with free access to standard pellet diet and water ad libitum. All experimental procedures were performed in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC). The treatment protocol was carried out for 21 consecutive days.

2.4 preparation of plant extract

The collected leaves of *Crassula ovata* were shade-dried at room temperature and coarsely powdered. Extracted the powdered crassula ovata leaves using ethanol in a Soxhlet apparatus until the plant material was fully exhausted. After filtering the solution, we concentrated it under reduced pressure and stored the resulting dry residue in an airtight container for further analysis. [9,10,11].

2.5 Physicochemical Studies

2.5.1. Determination of Loss on Drying (Moisture Content) [12].

- About 2 g of the air-dried powdered drug was accurately weighed, and take the sample and placed in a previously dried and weighed crucible.
- After that the crucible which is containing the sample was kept in a hot air oven at $105 \pm 2^\circ\text{C}$

for drying. Drying was continued until the sample reached a constant weight.

- Then the crucible was removed from the oven and allowed to cool in a desiccator, after cooling the sample was weighed again to determine its final weight, and the difference between the initial and final weights was used to determine the loss on drying.
- And the percentage of loss on drying was calculated using formula-
- Loss on Drying (%) =
$$\frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

2.5.2. Determination of Total Ash

- A silica crucible was washed, dried and heated in muffle furnace at 300°C for 15 minutes to remove any residual impurities.
- After heating, the placed crucible in desiccator for 10-15 minutes to cool and accurately weighed. The weight of the empty crucible was recorded.
- 3 g of powdered plant material (leaves) was accurately weighed and transfer into crucible.
- After that placed the crucible (containing powdered plant material) in a muffle furnace and sample was incinerated at $450-600^\circ\text{C}$ for almost 1 hour.
- Containing heat until the powdered plant material was completely burnt and a white or light grey ash was obtained, that indicating complete incineration.
- The crucible was carefully removed from the muffle furnace using holder and placed in a desiccator for 10-15 minutes to cool.
- After cooling the crucible (containing ash) accurately weighed and the weight was recorded
- The total Ash value (of powdered plant material) was calculated using formula and expressed as percentage (W/W)

$$\text{Total Ash Value (\%)} = \frac{\text{Weight of crucible with ash} - \text{weight of empty crucible}}{\text{weight of powder}} \times 100$$

2.5.3. Determination of Acid-Insoluble Ash

The total ash obtained was boiled with 25 mL of dilute hydrochloric acid for 5 minutes. The insoluble matter was filtered through ashless filter paper, ignited in a pre-weighed silica crucible. Acid-insoluble ash was calculated by subtracting the weight of the insoluble residue from total ash

2.5.4 Determination of Water-Soluble Ash

The total ash was boiled with 25ml of distilled water for 5 minutes. The insoluble matter was filtered

through ashless filter paper, and ignited in pre-weighed silica crucible to constant weight. The water-soluble ash was calculated by weight of the insoluble residue from the total ash.

2.6 Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was carried out to obtain the phytochemical of hydroalcoholic extract of *crassula ovata* leaves. Extract was spotted on silica gel G TLC plate and developed using ethyl acetate: formic acid: acetic acid: water (10:6:2:2) as the mobile phase. The plate was dried and observed under UV light, and the flavonoids spots and their RF values were recorded. [13,14]

$$R_f = \frac{\text{Distance traveled by the solute (compound)}}{\text{Distance traveled by the solvent front}}$$

2.7 Phytochemical analysis

The hydroalcoholic extract of *Crassula ovata* was subjected to preliminary qualitative phytochemical screening using standard phytochemical methods to detect the presence of various secondary metabolites. The procedures are described below.

2.7.1. Test for Alkaloids

- **Mayer's Test**

Take a small amount of plant extract in a test tube, and add few drops of dilute hydrochloride acid (HCl). Then add Mayer's reagent. if alkaloids are present then a white or creamy participate appears, this indicates that the test is positive and alkaloids are present on the extract.[15]

2.7.2. Test for Flavonoids

- **Alkaline Reagent Test**

First, take a small amount of plant extract in a test and added a few drops of sodium hydroxide (NaOH), the solution turned yellow, which indicated the presence of flavonoids. Then, we added a few drops of dilute hydrochloride acid (HCl), and the yellow colour disappeared. Thus, the test confirmed the presence of flavonoids in the plant extract [16].

2.7.3. Test for Phenolic Compounds

- **Ferric Chloride Test**

We took a small amount of the plant extract in a test tube and added a few drops of ferric chloride solution (FeCl₃). after mixing it properly, the solution turned green. This colour change showed the presence of phenolic compounds in the plant extract.

2.7.4. Test for Tannins

- **Ferric Chloride Test**

First, we took a small amount of plant extract and added a few drops of ferric chloride solution after mixing it well then the solution turned into green, this showed that tannins present in plant extract [17].

2.7.5. Test for Saponins

- **Foam Test**

Take plant extract in a test tube and added some distilled water, shook the test tube well for a few

seconds. After shaking, foam appeared on the surface and remained for some time, the formation of foam showed the presence of saponins in the plant extract [18].

2.7.6. Test for Steroids

- **Salkowski Test**

Took a small amount of the plant extract in a test tube and added a little chloroform. Then, we carefully added concentrated sulfuric acid. A reddish-brown colour appeared where the two layers met, this showed that steroids were present in the plant extract [19].

2.7.7. Test for Terpenoids

- **Liebermann–Burchard Test**

First, we took a small amount of plant extract then added a little amount of chloroform. Then we added a few drops of acetic anhydride and carefully added concentrated sulfuric acid. After mixing it properly, a reddish-brown colour appeared in the solution, that showed the presence of terpenoids [19].

2.7.8 test for carbohydrates

- **Benedict's test**

Firstly, we took a small amount of plant extract in a test tube and then added some benedict's reagents. After that mix it well and heated the test tube in a water bath for approximately 5 minutes. After that the colour of solution changed into green color that indicates the presence of carbohydrates in plant extract.

2.7.9 test for proteins

- **Biuret test**

We took a little amount of plant extract in a test tube and added a few drops of sodium hydroxide solution. Then, we also added a some drops of copper sulfate (CuSO₄) the mix it well. No violet or pink colour appeared in the solution. That indicates the absences of proteins in the plant extract.

2.8 Toxicity study

2.8.1 Acute Oral Toxicity Study (OECD Guideline 423)

The acute oral toxicity study of the hydroalcoholic extract of *Crassula ovata* leaves was performed following the OECD guideline 423. To evaluate the safety profile of the extract and to determine a suitable dose for pharmacological studies. Healthy Wistar female rats (150-200g) was used for study. The animal was acclimatized to laboratory conditions for one week prior to the experiment. Before dosing, the animals were fasted overnight (approximately 12 hours), while water was provided [20]. The hydroalcoholic extract of *crassula ovata* was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally using an oral gavage.[21]

2.9 Experimental Design

. The hydroalcoholic extract of *Crassula ovata*, donepezil, and normal saline were administered orally (p.o.) using an oral gavage. Scopolamine hydrobromide (2 mg/kg, i.p.) was used to induce

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cognitive impairment and was administered 60 minutes before the behavioral assessment. The respective treatments were administered according to the following schedule [22].

- Normal Control: Received normal saline (1 ml/kg, p.o.) 30 minutes before behavioral testing.
- Scopolamine Control: Received scopolamine hydrobromide (2 mg/kg, i.p.) 60 minutes before behavioral testing.
- Standard Group: Received donepezil (1 mg/kg, p.o.) 30 minutes before the test, followed by scopolamine hydrobromide (2 mg/kg, i.p.) 60 minutes before the test.
- Low-Dose Treatment Group: Received *Crassula ovata* extract (100 mg/kg, p.o.) 30 minutes before the test along with scopolamine hydrobromide (2 mg/kg, i.p.) 60 minutes before the test.
- High-Dose Treatment Group: Received *Crassula ovata* extract (200 mg/kg, p.o.) 30 minutes before the test along with scopolamine hydrobromide (2 mg/kg, i.p.) 60 minutes before the test.[22,23]

Table 1: Treatment protocol of hydroalcoholic extract of *Crassula ovata* against induce cognitive impairment

Group	Treatment	Dose	Route
I	Normal Saline	10 mL/kg	Oral
II	Scopolamine Hydrobromide	2 mg/kg	Intraperitoneal (i.p.)
III	Donepezil	1 mg/kg	Oral
IV	<i>Crassula ovata</i> Extract	100mg/kg	Oral
V	<i>Crassula ovata</i> Extract	200 mg/kg	Oral

2.10. Behavioral Studies

Behavioral assessment was performed during the last week of treatment.

The following behavioral models were employed:

- Morris Water Maze Test
- Novel Object Recognition Test

2.10.1. Morris Water Maze Test

The Morris water maze is a commonly used behavioral test to study learning, memory and spatial navigation in rodents (rats). In this test, the rat is placed in a circular pool, filled with opaque water maintained at $24 \pm 1^\circ\text{C}$., with a small platform hidden just beneath 1 cm. before starting the experiment, allow rats to

acclimatize to the experimental environment. The experiment was carried out in the two phases, Phase I (training phase) and Phase II (probe trial phase). Each rat underwent four acquisition trials per day for four consecutive days. Parameters recorded included:

- Escape Latency Time (ELT): Lower escape latency: better learning and spatial memory Higher escape latency: poorer learning or memory
 - Time Spent in Target Quadrant: time spent in target quadrant means the amount of time the animal spends swimming in the quadrant where the hidden platform was previously placed during the prob trial, when the platform is removed.
 - Path length: Path length means the total distance travelled by the animal from the starting point until it reaches the hidden platform
- Reduced escape latency and increased target quadrant time were considered indicators of improved learning and memory [24].

2.10.2. Novel Object Recognition Test [25].

The Novel Object Recognition Test (NORT) apparatus consisted of an open-field arena. This test were performed to evaluate recognition memory in rodents. The test is based on the natural tendency of rats to spend more time exploring a new object than an object they have already encountered.

The procedure included:

- Habituation: Each rat was placed individually in the empty test arena. The animal was allowed to freely explore for 5–10 minutes. No object was placed in the arena during this phase. This helped animal become familiar with the test environment before the actual experiment. The arena was cleaned after each animal to minimize the effect of residual Oduors.
- Familiarization: After habituation phase, two identical objects were placed at fixed position inside the arena. Each rat was allowed to explore the two objects freely for 5 minutes. The object was kept identical so that the animal could become familiar with them. The time spent exploring the objects were recorded. After the training session, the arena and objects were cleaned before testing next animal.
- Test Session: After the selected retention interval, the rat was placing again in the arena.

During this phase, one familiar object was replaced with a novel object, while the other familiar object remained in the arena. The rat was allowed to explore both objects freely for 5minutes.

The time spent exploring the novel and familiar object was recorded. Greater exploration of the novel object indicated better recognition of the previously familiar object. Recognition memory was assessed by calculating the Discrimination Index (DI) using the following formula:

2.11. Statistical Analysis

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All experimental data were expressed as Mean $\pm$ SEM. Statistical analysis was performed using GraphPad Prism version 9.0. Differences among groups were analyzed using One-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. A value of $P < 0.05$ was considered statistically significant.

3.RESULT

3.1 Physicochemical Evaluation

Physicochemical Evaluation of *Crassula ovata* Leaf Powder given below in table 2.

Table 2: Physicochemical Evaluation of *Crassula ovata* Leaf Powder (Sample weight: 3 g)

S. No.	Physicochemical Parameter	Result (%) w/w)
1	% of Total Ash	4.60 %
2	% of Acid Insoluble Ash	2.5 %
3	% of Acid Soluble Ash	3.60 %

The physicochemical evaluation of the powdered leaves of *Crassula ovata* revealed a total ash value of 4.60% w/w, indicating the total inorganic residue present in the crude drug. The acid-insoluble ash value was 2.5% w/w, suggesting a low level of siliceous impurities such as sand and soil. The acid-soluble ash value was 3.60% w/w, indicating that most of the ash consisted of physiologically occurring inorganic constituents. These values demonstrate the acceptable purity and quality of the plant material used in the present study.

3.2 Preliminary Phytochemical Screening

Preliminary Phytochemical Screening of Hydroalcoholic Extract of *Crassula ovata* Leaves given below in table 3.

Table 3: Preliminary Phytochemical Screening of Hydroalcoholic Extract of *Crassula ovata* Leaves

S. No.	Phytochemical Constituent	Test Performed	Observation	Result
1	Alkaloids	Mayer's Test	Green precipitate	Present
2	Flavonoids	Alkaline Reagent Test	Yellow colour	Present
3	Proteins	Biuret Test	No violet colour	Absent

4	Saponins	Foam Test	Stable persistent foam	Present
5	Carbohydrates	Benedict's Test	Green precipitate	Present
6	Tannins	Ferric Chloride Test	Dark green colour	Present
7	Phenolic Compounds	Ferric Chloride Test	Green colour	Present
8	Terpenoids	Liebermann-Burchard Test	Reddish-brown colour	Present
9	Steroids	Salkowski Test	Reddish-brown interface	Present

3.3. Thin Layer Chromatographic analysis

TLC Profile of Hydroalcoholic Extract of *Crassula ovata* given below in table 4.

Table 4: TLC Profile of Hydroalcoholic Extract of *Crassula ovata*

Spot No.	Distance Travelled by Spot (cm)	Solvent Front (cm)	Rf Value	Colour of Spot
1	1.68	8.0	0.21	Light Yellow
2	2.88	8.0	0.36	Green
3	4.24	8.0	0.53	Blue-Green
4	5.76	8.0	0.72	Brown

Thin Layer Chromatographic analysis of the hydroalcoholic extract of *Crassula ovata* revealed five distinct spots with Rf values of 0.21, 0.36, 0.53 and 0.72, indicating the presence of multiple phytochemical constituents in the extract. The chromatography suggests the presence of flavonoids, phenolic compounds, terpenoids, and other secondary

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metabolites, which may contribute to the neuroprotective and antioxidant activities of the plant.

3.4 Acute Oral Toxicity Study

Acute Oral Toxicity Study of Hydroalcoholic Extract of *Crassula ovata*, given below in table.

Table 5: Acute Oral Toxicity Study of Hydroalcoholic Extract of *Crassula ovata* (OECD Guideline 423)

Parameter	Observation
Test Guideline	OECD 423
Experimental Animals	Female Wistar rats (180–220 g)
Route of Administration	Oral (p.o.)
Dose Tested	300 mg/kg and 2000 mg/kg
Mortality	No mortality observed
Behavioral Changes	No abnormal behavior observed
Tremors/Convulsions	Absent
Salivation	Absent
Diarrhea	Absent
Food Intake	Normal
Water Intake	Normal
Body Weight	Normal increase throughout the study
Clinical Signs of Toxicity	Not observed
LD ₅₀	Greater than 2000 mg/kg

The hydroalcoholic extract of *Crassula ovata* was found to be safe up to an oral dose of 2000 mg/kg in female Wistar rats. No mortality, behavioral abnormalities, or signs of toxicity were observed during the 14-day observation period. Based on these findings, the LD₅₀ of the extract was considered to be greater than 2000 mg/kg.

3.5 Behavioral Studies

Behavioral assessment was performed during the last week of treatment.

The following behavioral models were employed:

1. Morris Water Maze Test
2. Novel Object Recognition Test

3.5.1 Morris Water Maze Test

In this experiment model was measure Escape Latency Time (ELT), Time Spent in Target Quadrant, Platform Crossings

3.5.1.1 Escape Latency Time (ELT)

Table 6: Statistical evaluation of Effect of *Crassula ovata* on Escape Latency Time

Group	Mean ± SEM
Normal control	9.633 ± 0.34
Standard	3.93 ± 0.1
Disease	51.1 ± 3.1
Low dose	5.71 ± 0.18
High dose	4.81 ± 0.13

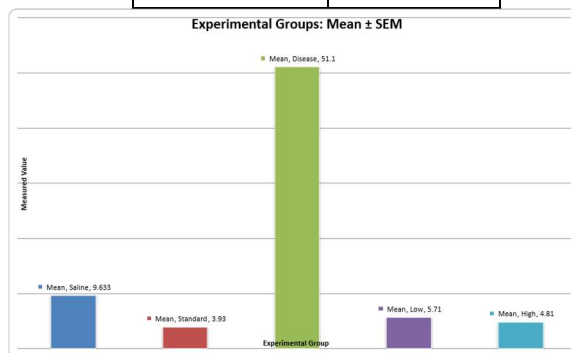


Figure 5: Graph represented of Effect of *Crassula ovata* on Escape Latency Time (ELT) in the Morris Water Maze Test

3.5.1.2 Path Length

Table 7: Statistical evaluation of Path Length

Group	Mean ± SEM (cm)
Normal Control	179.49 ± 6.48
Standard	72.54 ± 2.08*
Disease Control	896.41 ± 28.33####
Low Dose	103.41 ± 3.17*
High Dose	87.26 ± 2.37*

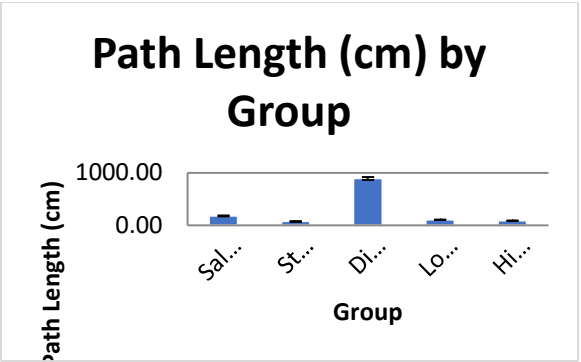


Figure 6: Graph represented of Effect of *Crassula ovata* on Path Length in the Morris Water Maze Test

3.5.1.3 Time Spent in Target Quadrant

Effect of *Crassula ovata* on Time Spent in Target Quadrant in the Morris Water Maze Test

Table 8: Statistical evaluation of Time Spent in Target Quadrant

Group	Mean $\pm$ SEM (s)
Normal Control	28.5 $\pm$ 1.2
Standard	25.8 $\pm$ 1.0*
Disease Control	13.4 $\pm$ 0.9####
Low Dose	20.7 $\pm$ 0.8
High Dose	23.9 $\pm$ 0.9*

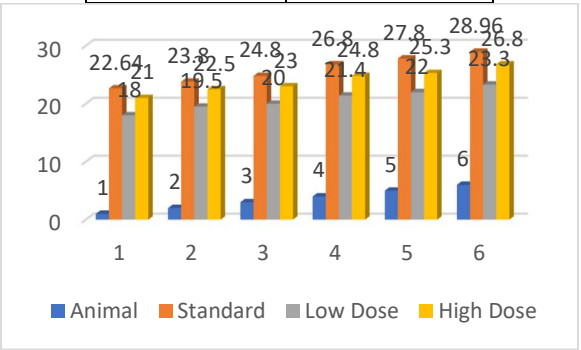


Figure 8: Graph represented of Effect of *Crassula ovata* on Time Spent in Target Quadrant in the Morris Water Maze Test

3.5.2 Novel Object Recognition Test

The Novel Object Recognition (NOR) test demonstrated that treatment with the hydroalcoholic extract of *Crassula ovata* significantly improved recognition memory in scopolamine-induced cognitively impaired rats. The high-dose extract (200 mg/kg) showed greater enhancement in novel object exploration, recognition index, and discrimination index, with effects comparable to the standard drug donepezil.

Table 9: Effect of *Crassula ovata* on Novel Object Recognition Test

Rat	TF	TN	TT	RI (%)	DI
1	13.26	30.18	43.41	69.45	0.389
2	14.16	29.02	43.18	67.21	0.344
3	12.10	31.05	43.15	71.96	0.439
4	13.05	30.26	43.31	69.87	0.397
5	12.29	32.14	44.43	72.34	0.447
6	11.30	29.11	40.41	72.04	0.441

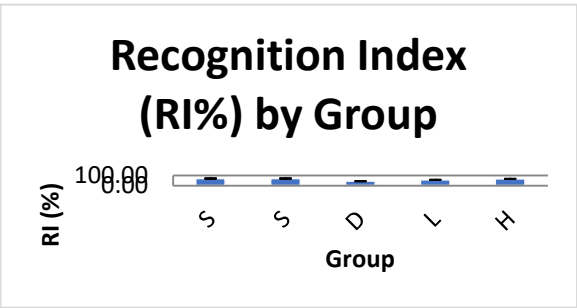


Figure 9: Graph represented of Effect of *Crassula ovata* on Recognition Index (RI%) Novel Object Recognition Test

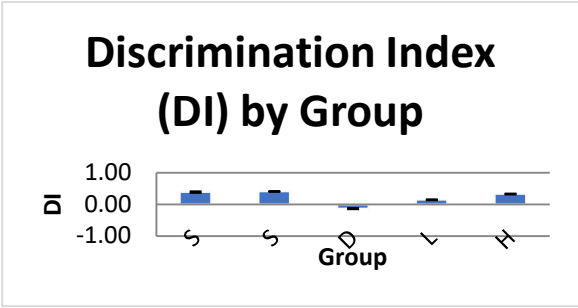


Figure 10: Graph represented of Effect of *Crassula ovata* on Discrimination Index (DI) Novel Object Recognition Test

Table 10: Statistical Analysis of Novel Object Recognition Test

Parameter	Mean $\pm$ SEM
Familiar Object Exploration Time (TF, s)	12.69 $\pm$ 0.41
Novel Object Exploration Time (TN, s)	30.29 $\pm$ 0.48

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Total Exploration Time (TT, s)	42.98 ± 0.55
Recognition Index (RI, %)	70.48 ± 0.82
Discrimination Index (DI)	0.410 ± 0.016

Values are expressed as Mean ± SEM (n = 6).

4. INTERPRETATION

All the results were expressed as mean ± SEM (standard error of the mean) for each group (n=6). data for the MWM (Morris water maze) and NOR (Novel Object Recognition) test were analyzed by using GraphPad Prism (Version 9.0 or later). Differences among the experimental groups were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's multiple comparison test for post hoc analysis. A value of $p < 0.05$ was considered statistically significant. The results were presented in the form of tables and graphs wherever appropriate.

5. CONCLUSION

The present study was undertaken to evaluate the neuroprotective and cognitive-enhancing potential of the hydroalcoholic extract of *Crassula ovata* against scopolamine-induced cognitive impairment in Wistar rats. Preliminary phytochemical screening revealed the presence of several bioactive secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, and terpenoids, which are known to possess antioxidant and neuroprotective properties. Physicochemical evaluation confirmed the quality and purity of the plant material, while the acute oral toxicity study (OECD Guideline 423) demonstrated that the extract was safe up to an oral dose of 2000 mg/kg, indicating a wide margin of safety. Behavioral assessment using the Morris Water Maze (MWM) and Novel Object Recognition (NOR) tests demonstrated that scopolamine significantly impaired learning and memory, as evidenced by increased escape latency time, reduced time spent in the target quadrant, and decreased recognition index. Treatment with the hydroalcoholic extract of *Crassula ovata* significantly improved spatial learning and recognition memory in a dose-dependent manner. The 200 mg/kg dose produced greater cognitive improvement than the 100 mg/kg dose and showed efficacy comparable to the standard drug donepezil. The observed neuroprotective activity may be attributed to the synergistic action of flavonoids, phenolic compounds, and other phytoconstituents present in the extract, which are capable of reducing oxidative stress and protecting neuronal function. In conclusion, the present study hydroalcoholic extract of *Crassula ovata*

possesses significant cognitive-enhancing and neuroprotective activity against experimentally induced cognitive impairment in rats. These results support the traditional medicinal importance of the plant and indicate that *Crassula ovata* may serve as a promising natural therapeutic of the management memory deficits and neurodegenerative disorders such as Alzheimer's disease.

6. REFERENCE

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