IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 5, Issue 2, May 2025
Impact Factor: 7.67

Evaluation of the Neuroprotective Effect of Galinsoga parviflora Leaves Extract in Memory-Impaired Rats

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Abstract: *This study investigated the potential of methanolic extract of Galinsoga parviflora leaves (MEGPL) to enhance learning and memory in a rat model of scopolamine-induced memory impairment. Spatial learning and memory were assessed using the Elevated Plus Maze (EPM) and Morris Water Maze (MWM) apparatus over a period of 21 days. Rats were divided into a normal control group, a negative control group (scopolamine-treated), and treatment groups receiving MEGPL at two different dosages (200 mg/kg and 400 mg/kg) and donepezil (5 mg/kg) as a standard drug, following scopolamine induction. In the EPM, transfer latency was significantly increased in the scopolamine group compared to the normal control. However, treatment with both doses of MEGPL and donepezil significantly reduced transfer latency. Similarly, in the MWM, the scopolamine group exhibited significantly increased escape latency and decreased retention time compared to the normal control. Treatment with both doses of MEGPL and donepezil significantly decreased escape latency and increased retention time. These findings suggest that the methanolic extract of Galinsoga parviflora leaves possesses learning and memory enhancing activity, likely due to the presence of flavonoids as major constituents.*

Keywords: Galinsoga parviflora, Memory Enhancement, Learning, Scopolamine, Elevated Plus Maze, Morris Water Maze, Flavonoids, Alzheimer's Disease

I. INTRODUCTION

Age-related memory loss, often categorized under cognitive impairment, represents a decline in cognitive function that falls short of dementia but can be a precursor to neurodegenerative conditions like Alzheimer's disease (AD) (Ju Yeon Ban et al., 2020). Dementia, characterized by a deterioration in cognitive abilities due to functional brain abnormalities, poses a significant global health challenge. Alzheimer's disease, the most prevalent form of dementia, is projected to affect an increasing number of individuals worldwide, leading to substantial social and financial burdens (Ju Yeon Ban et al., 2020). This necessitates extensive research into the mechanisms underlying dementia and the development of effective treatment strategies.

Various pharmacological agents, including scopolamine, streptozotocin, alcohol, and heavy metal dysregulation, are employed to induce AD-like symptoms in experimental animal models (Onesimus Mahdi et al., 2019). The scopolamine induction method in rodents is a widely used model to study cognitive deficits. Scopolamine, a muscarinic cholinergic receptor antagonist, induces cholinergic dysfunction and cognitive impairment in rats upon intraperitoneal administration (Samira Malekzadeh et al., 2017). Studies have also indicated that scopolamine-induced memory impairment is associated with alterations in brain oxidative stress (Samira Malekzadeh et al., 2017).

Despite significant advancements in pharmacotherapy for AD, including acetylcholinesterase inhibitors (AChEIs) like donepezil, rivastigmine, and galantamine, and the glutamate antagonist memantine, supportive care remains crucial (Thaiane Coelho Santos et al., 2018). The search for novel therapeutic agents, particularly from natural sources, has gained considerable attention. Numerous medicinal plants, including Ginkgo biloba, Centella asiatica, and Curcuma longa, have been investigated for their potential in treating AD (Gunter Peter Eckert 2010).

DOI: 10.48175/IJARSCT-26231

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Phytochemicals, especially flavonoids, present in many medicinal plants, are known for their potential to reduce the risk of chronic diseases and exhibit significant medicinal properties (Haroon Khan et al., 2020). Flavonoids, classified into various subclasses, have demonstrated potential in preventing neurodegenerative diseases by targeting multiple pathways. Their anti-inflammatory and antioxidant properties are particularly relevant to the pathophysiology of AD, and some flavonoids have shown the ability to cross the blood-brain barrier (BBB), suggesting their potential for neuroprotection (Haroon Khan et al., 2020).

Literature indicates that the leaves of Galinsoga parviflora contain a variety of phytochemicals, including carbohydrates, phenols, flavonoids, steroids, alkaloids, anthraquinones, and amino acids (Ripanda A et al., 2010). Our current study confirmed the presence of alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, anthraquinones, and saponins in the methanolic extract of Galinsoga parviflora leaves (MEGPL). Given the rich phytochemical profile, particularly the presence of flavonoids, this study aimed to evaluate the learning and memory enhancing activity of MEGPL in scopolamine-induced memory-impaired rats using the Elevated Plus Maze (EPM) and Morris Water Maze (MWM) apparatus. These apparatus are widely used for screening learning and memory enhancing activities due to their economic viability, availability, accuracy, and specificity (Elevated plus maze apparatus, morris water maze apparatus, light and dark apparatus, elevated T maze, elevated zero maze, open field test and white lack box).

II. MATERIALS AND METHODS

2.1. Materials

2.1.1. Chemicals and Reagents

The list of chemicals used in this study are in Table No.1.

Table No.1: List of Chemicals Used in Study

Sr. No.	Chemicals	Company
1	Petroleum ether	Thermosil Fine Chem Industries
2	Methanol	Thermosil Fine Chem Industries
3	Mayer's reagent	Prayogina Laboratories India
4	α – napthol	Burgoyne Burbidges & Co.
5	Conc. H_2So_4	Thermosil Fine Chem Industries
6	Glacial acetic acid	Samar Chemicals (India)
7	Ferric chloride	Thermosil Fine Chem Industries
8	Ninhydrin solution	Thermosil Fine Chem Industries
9	Lead acetate	Thermosil Fine Chem Industries
10	Ammonia solution	Thermosil Fine Chem Industries
11	Scopolamine	Sovereign Pharma Pvt. Ltd.
12	Donepezil	Alkem Laboratories Ltd.

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13	Anesthetic ether	S D Fine Chem Ltd.
14	Acetylcholine	Thermosil Fine Chem Industries
15	DTNB	BLD Pharmatech (India) Pvt. Ltd.

2.1.2. Apparatus and Instruments

The list of apparatus and instruments used in this study is provided in Table No. 2.

Table No. 2: List of Instruments Used in Study

Sr. No.	Instruments	Company
1	Weighing balance	K- roy
2	Magnetic stirrer	Remi
3	Cooling centrifuge	Remi
4	Morris water maze apparatus	K- roy
5	Elevated plus maze apparatus	K- roy

2.2. Method

2.2.1. Collection & Authentication of Plant Materials

Galinsoga parviflora was collected from Nature India Nursery, Nashik, Maharashtra. The plant material was identified and authenticated by Mrs. A. M. Gaharwar, Assistant Professor of Vasantrao Naik College of Agricultural Biotechnology, Yavatmal (Ref No. VNCABT/Ytl/Hort/1881/2024).

2.2.2. Extraction of Galinsoga parviflora Leaves

Leaves of Galinsoga parviflora plant were collected, dried in shade, and coarsely powdered. The powdered leaves of Galinsoga parviflora were extracted in petroleum ether using a Soxhlet apparatus to remove fat content. The powder was then dried again and subsequently extracted in methanol using a Soxhlet apparatus.

2.2.3. Phytochemical Screening

The methanolic extract was subjected to phytochemical screening for the detection of various active constituents using the following tests:

- A. Test for Alkaloids: Mayer's test was performed, and the presence of a creamy/yellow precipitate indicated the presence of alkaloids (K. Sahira Banu et al., 2015).
- B. Test for Carbohydrates: Molish's test was performed, and the formation of a violet ring confirmed the presence of carbohydrates (Vandana Singh et al., 2017).
- C. Test for Cardiac Glycosides: Keller-killani test was performed, and the absence of a blue-colored solution in the acetic acid layer indicated the absence of cardiac glycosides (Rama Swamy Nanna et al., 2013).
- D. Test for Tannins: Braymer's test was performed, and the formation of a blue-green color confirmed the presence of tannins (Uma KS et al., 2017).
- E. Test for Proteins & Amino acids: Ninhydrin test was performed, and the absence of a purple-colored solution indicated the absence of proteins & amino acids (Gusthinnadura Oshadie De Silva et al., 2017).
- F. Test for Phenolic Compounds: Lead acetate test was performed, and the formation of a white precipitate confirmed the presence of phenolic compounds (Vishnu Balamurugan et al., 2019).
- G. Test for Flavonoids: Ammonia test was performed, and the formation of a yellow color confirmed the presence of flavonoids (R. Suman Kumar et al., 2013).
- H. Test for Anthraquinones: Borntrager's test was performed, and the formation of a pink, violet, or red colored solution confirmed the presence of anthraquinones (Mulugeta Alemu et al., 2024).

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- I. Test for Saponins: Foam test was performed, and the persistence of foam for 10 minutes indicated the presence of saponins (Prashant Tiwari et al., 2011).
- J. Test for Terpenoids: Salkowski's test was performed, and the absence of a golden yellow layer at the bottom indicated the absence of terpenoids (Dipak Raj Pant et al., 2017).

2.3. Experimental Design

2.3.1. Experimental Animals

Eight weeks old healthy female Sprague-Dawley rats (weighing 150-250 gm) were used for this study. Rats were housed under controlled conditions (12h light/dark cycle, 25 ± 2 °C, 60 ± 5 % humidity) with free access to standard pellet diet and water. The experiments were performed during the day (8:00 – 16:00 hrs). The rats were housed and treated according to the rules and regulations of CCSEA and IAEC. The protocol for all animal studies was approved by the Institutional Animal Ethics Committee (IAEC) with research project number 650/Po/Re/S-2002/2025/CCSEA/04.

2.3.2. Animal Groups

For this study, rats were divided into the following groups (n = 6):

- Group 1 (Vehicle Control): Rats received only normal saline solution for 21 days.
- Group 2 (Negative Control): Memory & learning impairment was induced by scopolamine (2 mg/kg i.p.) for 21 days.
- Group 3 (MEGPL 200 mg/kg): Memory & learning impaired rats were treated with methanolic extract of Galinsoga parviflora leaves (200 mg/kg) orally for 21 days.
- Group 4 (MEGPL 400 mg/kg): Memory & learning impaired rats were treated with methanolic extract of Galinsoga parviflora leaves (400 mg/kg) orally for 21 days.
- Group 5 (Standard): Memory & learning impaired rats were treated with donepezil (5 mg/kg) orally for 21 days.

2.3.3. Learning & Memory Impairment Assessment

Learning and memory impairment state was checked in all animals using the Elevated Plus Maze (EPM) and Morris Water Maze (MWM) apparatus before (Day 0) and after (Day 21) the administration of scopolamine. Baseline readings were taken on Day 0 before scopolamine administration.

2.3.4. Induction of Memory Impairment

All groups, except the normal vehicle control group, were subjected to intraperitoneal administration of scopolamine (2 mg/kg i.p.) for 21 days to induce memory impairment (Fanta Sabine Adeline Yadang et al., 2020).

2.3.5. Drug Administration

Donepezil (5 mg/kg p.o.) was used as a standard drug. Methanolic extract of Galinsoga parviflora leaves was prepared in distilled water at two different concentrations (200 mg/kg and 400 mg/kg) and administered orally to the respective treatment groups for 21 days. All solutions were prepared fresh daily and administered via their respective routes.

2.3.6. Daily Dose of MEGPL

Daily oral doses of MEGPL (200 mg/kg and 400 mg/kg) were administered to the respective groups for 21 days.

2.3.7. Study of Learning and Memory Impairment Using Behavioral Models

Learning and memory impairment was assessed on Day 0 and after 21 days of treatment using the following models:

- 1. Elevated Plus Maze Apparatus: The EPM consisted of two open arms (50 x 10 cm) and two closed arms (50 x 10 x 40 cm) elevated 50 cm above the floor (Figure No. 6.1). Transfer latency (TL), the time taken for the rat

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to move from an open arm to a closed arm, was recorded on Day 0 and Day 21. A decrease in TL on Day 21 compared to the negative control group indicates improved learning and memory (Mani Vasudevan et al., 2007).

- 2. Morris Water Maze Apparatus: The MWM consisted of a circular pool (170 cm diameter, 45 cm height) filled with opaque water at 25°C (Figure No. 6.2 & 6.3). Acquisition trials (4 trials/day for 4 days, from day 6 to day 9 of drug administration) were conducted to measure escape latency (EL), the time taken to find the platform (Md. Sahab Uddin et al., 2016). A probe trial was conducted on day 10 (and the final reading on day 21) after removing the platform, and the retention time (RT), the time spent in the target quadrant, was recorded for 300 seconds. An increase in RT indicates improved spatial memory (Pankaj Phukan et al., 2019).

2.3.8. Statistical Analysis

All data were expressed as the mean ± standard deviation (SD). For statistical analysis, group means were compared using one-way analysis of variance (ANOVA) followed by Dunnett's test. A p-value of < 0.01 was considered statistically significant.

III. RESULTS

3.1. Phytochemical Screening

The phytochemical screening of the methanolic extract of Galinsoga parviflora leaves (MEGPL) revealed the presence of alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, anthraquinones, and saponins. Cardiac glycosides and terpenoids were found to be absent

Table No. 3: Phytochemicals Screening of MEGPL

Sr.No.	Phytochemicals	Test Performed	Extract Results
1	Alkaloids	Mayer's test	+
2	Carbohydrates	Molish's test	+
3	Cardiac glycosides	Keller-killani test	-
4	Tannins	Braymer's test	+
5	Proteins and Amino acids	Ninhydrin test	-
6	Phenolic compounds	Lead acetate test	+
7	Flavonoids	Ammonia test	+
8	Anthraquinones	Borntrager's test	+
9	Saponins	Foam test	+
10	Terpenoids	Salkowski's test	-

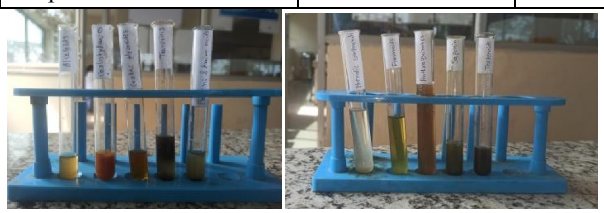

Figure No. 1: Phytochemicals screening of MEGPL

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3.2. Estimation of Behavioural Study

3.2.1. Elevated Plus Maze Apparatus

A. Transfer Latency

Table No. 4: Effect of MEGPL on transfer latency (TL) of rats in EPM apparatus

Sr.No.	Groups	Transfer latency in seconds on day 0	Transfer latency in seconds on day 21
1.	Normal Control	19.23 ± 0.75	20.54 ± 4.25
2.	Negative Control	21.67 ± 0.87^{ns}	34.43 ± 4.36$^{@}$
3.	MEGPL (200 mg/kg)	22.42 ± 0.78^{ns}	19.58 ± 4.36**
4.	MEGPL (400 mg/kg)	21.47 ± 0.66^{ns}	15.46 ± 4.73**
5.	Donepezil (5 mg/kg)	20.38 ± 0.86^{ns}	11.63 ± 4.86**

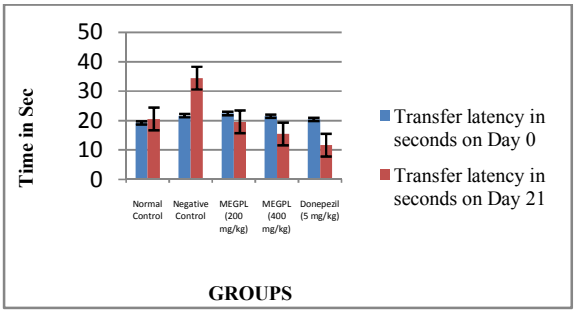

Figure No.2: Effect of MEGPL on transfer latency of rats in EPM apparatus

Table No. 4 and Figure No. 2 illustrate the effect of *Galinsoga parviflora* extract on transfer latency (TL) in the Elevated Plus Maze (EPM) in memory-impaired rats. On Day 0, there were no significant differences in TL among the groups. However, on Day 21, the negative control group exhibited a significantincrease in TLcompared to the normal control group, indicating memory impairment. Treatment with MEGPL at 200 mg/kg and 400 mg/kg significantly decreased the TL compared to the negative control group. Similarly,the donepezil-treated group showed a significan reduction in TL compared to the negative control group.

B. Escape Latency

Table No. 5: Effect of MEGPL on escape latency (EL) of rats in MWM apparatus

Sr. No.	Groups	Escape latency In seconds on day 0	Escape latency in seconds on day 21
1.	Normal Control	29.45 ± 2.59	27.32 ± 8.61
2.	Negative Control	42.33 ± 2.51^{ns}	67.26 ± 8.38$^{@}$
3.	MEGPL (200 mg/kg)	34.57 ± 2.68^{ns}	32.46 ± 8.89**
4.	MEGPL (400 mg/kg)	36.53 ± 2.43^{ns}	32.48 ± 8.36**
5.	Donepezil (5 mg/kg)	39.24 ± 2.38^{ns}	30.52 ± 8.25**

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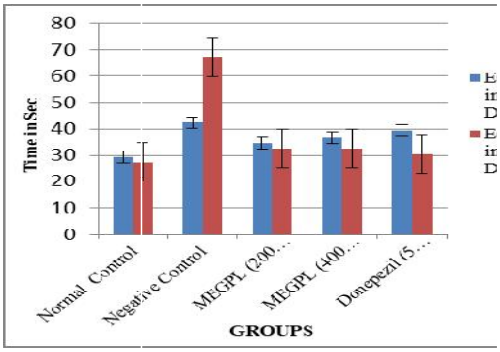

Figure No.3. Effect of MEGPL on escape latency of rats in EPM apparatus

Table No.5 and Figure No.3 depict the effect of Galinsoga parviflora extract on escape latency (EL) in the Morris Water Maze (MWM) in memory-impaired rats. On Day 0, there were no significant differences in EL among the groups. However, on Day 21, the negative control group exhibited a significant increase in EL compared to the normal control group, indicating impaired spatial learning. Treatment with MEGPL at 200 mg/kg and 400 mg/kg significantly reduced the EL compared to the negative control group. The donepezil-treated group also showed a significant decrease in EL compared to the negative control group.

C. Retention Time

Sr. No.	Groups	Retention time in seconds on Day 0	Retention time in seconds on Day 21
1.	Normal Control	38.44 ± 3.51	46.53 ± 7.41
2.	Negative Control	40.26 ± 3.78^{ns}	34.42 ± 7.73$^{@}$
3.	MEGPL(200 mg/kg)	46.37 ± 3.22^{ns}	58.29 ± 7.36**
4.	MEGPL`(400 mg/kg)	49.54 ± 3.36^{ns}	64.47 ± 7.36**
5.	Donepezil (5 mg/kg)	53.69 ± 3.89^{ns}	75.44 ± 7.86**

Table No. 6: Effect of MEGPL on RT of rats in MWM apparatus.

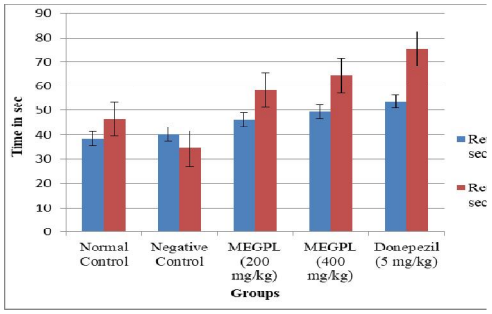

Figure No. 4: Effect of MEGPL on RT of rats in MWM apparatus.

Table No.6 and Figure No.4 demonstrate the effect of Galinsoga parviflora extract on retention time (RT) in the Morris Water Maze (MWM) probe trial in memory-impaired rats. On Day 0, there were no significant differences in RT among the groups. However, on Day 21, the negative control group showed a significant decrease in RT compared to the normal control group, indicating impaired spatial memory retrieval. Treatment with MEGPL at 200 mg/kg and 400 mg/kg significantly increased the RT compared to the negative control group. The donepezil-treated group also showed a significant increase in RT compared to the negative control group.

IV. DISCUSSION

The findings of this study demonstrate that the methanolic extract of Galinsoga parviflora leaves (MEGPL) exhibits significant learning and memory enhancing activity in scopolamine-induced memory-impaired rats. The results from

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both the Elevated Plus Maze and the Morris Water Maze tests consistently showed that treatment with MEGPL, particularly at doses of 200 mg/kg and 400 mg/kg, effectively reversed the memory deficits induced by scopolamine.

In the EPM, the scopolamine-treated group showed a marked increase in transfer latency on the retention test day, indicating impaired learning and memory consolidation. However, both doses of MEGPL significantly reduced this latency, suggesting an improvement in memory retention. Similarly, in the MWM, scopolamine administration led to a significant increase in escape latency during the acquisition trials, reflecting impaired spatial learning, and a decrease in retention time during the probe trial, indicating impaired spatial memory retrieval. Treatment with MEGPL at both doses significantly attenuated these scopolamine-induced deficits, as evidenced by the reduced escape latency and increased retention time. The efficacy of MEGPL was comparable to that of donepezil, a known acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease.

The observed learning and memory enhancing activity of MEGPL could be attributed to the presence of various bioactive compounds, particularly flavonoids, which were identified as major constituents in the extract. Flavonoids are known to possess antioxidant and anti-inflammatory properties, which are crucial in mitigating neurodegeneration (Haroon Khan et al., 2020). Furthermore, some flavonoids have the ability to cross the blood-brain barrier and interact with neuronal pathways involved in learning and memory.

The mechanism by which MEGPL exerts its memory-enhancing effects likely involves multiple pathways. Scopolamine induces memory impairment by blocking muscarinic cholinergic receptors, leading to a decrease in acetylcholine levels in the brain (Samira Malekzadeh et al., 2017). While this study did not directly measure acetylcholine levels or acetylcholinesterase activity, the comparable efficacy of MEGPL to donepezil suggests a potential involvement in the cholinergic system. Flavonoids present in MEGPL might exert a mild inhibitory effect on acetylcholinesterase, thereby increasing acetylcholine availability in the synaptic cleft.

Furthermore, scopolamine-induced memory impairment is also associated with increased oxidative stress in the brain (Samira Malekzadeh et al., 2017). The antioxidant properties of flavonoids present in MEGPL could contribute to its neuroprotective effects by scavenging free radicals and reducing oxidative damage to neuronal cells. This could indirectly improve neuronal function and enhance cognitive performance.

The results of this study align with previous research highlighting the neuroprotective potential of various medicinal plants and their phytoconstituents (Gunter Peter Eckert 2010). The presence of carbohydrates, phenols, tannins, anthraquinones, and saponins in MEGPL, in addition to flavonoids and alkaloids, might also contribute synergistically to the observed effects.

The dose-dependent effect observed in this study, with the higher dose of MEGPL (400 mg/kg) showing more pronounced memory-enhancing activity, suggests a potential for optimizing the therapeutic dosage. The comparable efficacy of MEGPL to donepezil warrants further investigation into its potential as a natural therapeutic agent for cognitive impairment.

V. CONCLUSION

The findings of this study demonstrate that the methanolic extract of Galinsoga parviflora leaves exhibits significant learning and memory enhancing activity in scopolamine-induced memory-impaired rats, as evidenced by the improved performance in the Elevated Plus Maze and Morris Water Maze tests. This activity is likely attributed to the presence of flavonoids and other bioactive compounds in the extract. The results suggest that Galinsoga parviflora leaves extract holds promise as a potential therapeutic agent for mitigating cognitive deficits associated with neurodegenerative conditions like Alzheimer's disease.

VI. FUTURE SCOPE

- To determine the specific Galinsoga parviflora leaves extract constituents responsible for producing anti-Alzheimer's activity.
- To elucidate the underlying mechanisms for the anti-Alzheimer's activity of Galinsoga parviflora leaves extract, including its effects on cholinergic neurotransmission, oxidative stress, and neuroinflammation.
- To formulate and develop Galinsoga parviflora leaves extract-based treatment regimens and to carry out.

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