B-5443
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RESEARCH ARTICLE Indian Journal of Animal Research

Neuroprotective Effect of Naturally Isolated Scopoletin (ISCN) from 3 Selected *Ipomoea* Species on Learning and Memory Impairment in Amnestic Mice Model

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10.18805/IJAR.B-5443

ABSTRACT

Background: The Convolvulceae family, which includes the several climbing herbs and weeds in the genus Ipomoea, is widely used in Ayurvedic, Unani and Siddha medicine. These plants are found across India. The current study to explore the Neuroprotective effect of naturally isolated scopoletin (ISCN) compound from *Ipomoea reniformis* (Choisy), *Ipomoea cairica* (L.) and *Ipomoea triloba* (L.) on learning and memory impairment in amnestic Swiss albino mice model.

Methods: There was no mortality observed at intraperitoneal maximum dosages up to 100 mg/kg body weight; the ISCN showed a significant safety margin. The Neuroprotective Effect of ISCN was evaluated by taking various doses (3, 6, 12 mg/kg i.p), standard piracetam (150 mg/kg; i.p) and scopolamine (1 mg/kg; i.p.)to produce amnesia in mice by using Interoceptive includes estimation of acetyl cholinesterase (AchE) activity and extroceptive animal models include the Morris-water maze (MWM), object recognition test (ORT), passive avoidance test (PAT).

Result: The memory score significantly improved after dosing of ISCN and standard piracetam in comparison with scopolamine-producing amnesia in mice. Dose-dependent ISCN shows significant (*P<0.01*)improvement in Escape Latency in MWM, improvement in step-down latency in PAT, mice spent more time exploring the novel object in ORT and dose-dependent ISCN shows significant inhibition of AchE in mice brain. This finding indicates that ISCN may exert a neuroprotective effect by inhibiting the AchE enzyme. ISCN has the potential to be therapeutically beneficial for reducing some of the memory impairments linked to Alzheimer's.

Key words: Albino mice, *Ipomoea cairica* (L.), *Ipomoea reniformis* (Choisy), *Ipomoea triloba* (L.), Neuroprotective, Scopoletin.

INTRODUCTION

A mental illness known as dementia is a significant loss of intellectual function that makes it difficult to perform social or professional tasks (Silvia *et al.,* 2017). Alzheimer's disease (AD) is a neurological condition that the most frequent cause of dementia (Makrania *et al.,* 2014 and Alatawi *et al.,* 2024). Globally, there are already 12 million instances of dementia and by 2040, there are predicted to be 25 million cases (Kawas *et al.,* 2000).

According to estimates, neurological illnesses will surpass cancer to become the second biggest cause of death worldwide by the middle of the 20th century and the eighth most common cause of death in developed nations by 2023 (Natthawut *et al.,* 2016).

In India, Approximately 65% of the population relies on Ethnomedicine for their primary medical needs (Rajasekhran *et al.,* 1996). Based on experimental and clinical findings, Acetylcholinesterase (AChE) inhibitors increase the concentration of Acetylcholine (ACh) (Blokland 1995), a primary neurotransmitter in the brain involved in the regulation of cognitive functions and neuroprotective action (Mirjana 2013 and Balamurugan 2018). Scopoletin (SCT) having IUPAC name 7-hydroxy-6-methoxycoumarin is a coumarin glycoside that enhances cholinergic neurotransmission by inhibition of AchE; it would be helpful for neurodegenerative diseases such as Dementia, Alzheimer's dementia (AD) (Hornick 2011 and Marilena 2012).

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How to cite this article: Khan, P.H.A. and Siddiqui, A.U.R. (2024). Neuroprotective Effect of Naturally Isolated Scopoletin (ISCN) from 3 Selected *Ipomoea* Species on Learning and Memory Impairment in Amnestic Mice Model. Indian Journal of Animal Research.1-7. doi: 10.18805/IJAR.B-5443.

Submitted: 09-07-2024 **Accepted:** 03-10-2024 **Online:** 04-12-2024

Acetylcholinesterase (AChE) levels in the hippocampus are raised by scopolanide, a muscarinic acetylcholine receptor agonist that is useful for treating

experimental dementia or memory impairment (Barua *et al*., 2022).

With more than 700 species, *Ipomoea* is the biggest genus in the flowering plant family Convolvulceae (Santos *et al.,* 2020). There is another name for *Ipomoea reniformis* (Choisy) (I.R.). Burma f. Merremia emarginata A herb that grows horizontally and belongs to the Convolvulceae family. It is a much-branchedherb, growing several years on ground places (Chatterjee *et al*., 1997). I.R is Diuretic, purgative, in nature (Usnale *et al*., 2009), it is used to treat a variety of conditions including epilepsy, migraines, rheumatic conditions, neuralgia, headache, skin disease, cough, ulcers, abscesses and urinary and kidney related conditions (Jabeen *et al*., 2013). *Ipomoea reniformis* show in Fig 1.

Ipomoea cairica (L.) (I.C.)Sweet, also known as "Railway vine," is an evergreen perennial climbing plant with twining stems that are lignified at the base and exhibit roots at the nodes. It is found all over India in rainy seasons (Deepa *et al*., 2014). I.C. has antiviral, anti-inflammatory, antioxidant and strong anti-malarial, anti-inflammatory, anti-rheumatic properties (Ojha *et al*., 2016 and Ferreira *et al*., 2006), acts as a carminative agent and is beneficial for fever, jaundice, biliousness, bronchitis, liver problems and anti-cancer (Kour 2014 and Srivastava *et al*., 2015). *Ipomoea cairica* show in Fig 2.

Ipomoea triloba (I.T.) has anti-malarial, anti-tumor and wound-healing properties in addition to being utilized as an antioxidant, antimicrobial, antiviral, antibacterial, antifungal, hypotensive, analgesic and laxative (Essiet *et al*., 2014). *Ipomoea triloba* show in Fig 3.

Based on a literature survey these are 3 selected ethnomedicinal plants (IR, IC and IT) that grow all over India but little chemical and therapeutic exploration specially no studies has found regarding the effect on learning and memory has been done (Magesh *et al*., 2012 and Upadhyaya *et al*., 2010).

The current study to explore the neuroprotective effect of naturally isolated scopoletin (ISCN) from *Ipomoea reniformis* (Choisy), *Ipomoea cairica* (L), *Ipomoea triloba* (L.) on learning and memory impairment in amnestic mice model.

MATERIALS AND METHODS

Selection and authentification of the medicinal plant

The all 3 plants were having been collected from a nearby farm in the Aurangabad district between July and December 2020. The Department of Botany situated at Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra state, India has identified the authenticity of the plants as belonging to Accession Nos. Bot./2020/0720,021,026 for I.R., I.C. and I.T., respectively.

Preparation for extraction and isolation of scopoletin from 3 selected *Ipomoea* plant species

For extraction from powdered plant material, a Soxhlet extraction device was utilized, along with a 95% ethanol solvent at 60°C. 180 grams of coarse powder are included in each batch of material (Khandelwal 2008). Filter each time and remove the solvent from the combined extract under-dried by vacuum Rota evaporator to get % Yield of extract respectively. Suspend the residue in 1% v/v HCL (250 ml), Extract this suspension with chloroform (120 ml) (Omer 2017). Wash the combined chloroform layer with water till washing is neutral; Separate the organic and aqueous layers. A chloroform layer was used. Filter and remove the solvent under reduced pressure by rota evaporator to yield the crude residue and absorb the entire

Fig 1: *Ipomoea reniformis* (Choisy), Convolvulceae.

Fig 2: *Ipomoea cairica* (Linn), Convolvulceae.

Fig 3: *Ipomoea triloba* (Linn), Convolvulceae.

residue on a column-grade silica gel (Saxena *et al*., 2013). Place it on a column of silica gel (column grade, 80 gm). Performed the elusion using chloroform and combined the fraction. The eluted compound was a yellowish solid crystal. Shows blue fluorescence under UV light at 365 nm (Bhatt *et al*., 2011). Finally, get Crude and Natural SCN (Phytochemical Reference Standard 2010).

Experimental animals

Under typical weather circumstances, they were kept at 25 ±2°C and 45 to 55% relative humidity. Food and water were available to the animals without restriction (Achliya *et al*., 2004). Each trial ran for 9 to 18 hours. 7 animal groups each group containing 6 Swiss albino mice were randomly assigned to experimental and control groups and kept in sterile rice husk bedding in sterile polypropylene cages. The animals were habituated to the laboratory setting for 48 hours prior to the start of the experimental protocol (Swapnil *et al*., 2024).

Ethical considerations

Regarding animal experiments, the study was conducted between November 2021 to February 2022 after approval from the Institutional Animal Ethics Committee (IAEC) and the Indian government's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) accepted the experimental protocol (CPCSEA/IAEC/P'cology/72/2020-21/177). All the approved experiments were performed on daylight in the research center Y.B. Chavan College of Pharmacy's Animal House is located in Aurangabad, Maharashtra, India.

Safety studies of test drugs with various concentrations

The Organization of Economic Corporation Development (OECD)-423 criteria for category IV substances for the acute toxicity studies were followed in the safety studies. In this study, Swiss albino mice weighing 20-25 gm (n=3 from each group) of either sex were chosen at random for the study (Roselin, 2018). The animals had unrestricted access to water during their 4-hour fast. The maximal dosage of the ISCN was given 100 mg/kg *i.p.* of body weight (Tabana *et al*., 2016). After that, mice were checked by 1h, 2h, 4 h, 6 h and 24 h for 14 days of any indications of harm or toxicity against the dose (Jamuna *et al*., 2015). Any alterations to the body's weight, respiration rate, food and drink intake, skin, hair, eyes and mucous membranes, as well as alterations to the neurological, autonomic and behavioral profiles, were noted during the test period (Ding *et al*., 2005 and Porwal *et al*., 2017).

Study design

As per above data groups and treatment for experimental Swiss albino mice shown in Table 1.

Methods

Test of morris and water maze (MWM)

Animals are placed in the Morris water maze at the start of the path, given full reign to swim and use a secret platform to escape. A platform for escape was submerged one centimeter below the water's surface. The mice were divided into groups and placed around the perimeter of the pool (Tota *et al*., 2009). When the mice settled onto the hidden platform after 3 minutes, the experiment was deemed successful (Reddy *et al*., 2015)**.** Finding the hidden platform took longer than 3 minutes, which was noted as a mistake. Task performance was calculated using the percentage of successful mice. For 11 days, mice were given daily doses in addition to a daily training course (Habibur *et al*., 2010).

Test of passive shock avoidance test (PAT)

The step-down latency (SDL) was measured when the single mice was positioned in the shock-free zone (SFZ), which is an elevated wooden platform (Parle *et al*., 2004). SDL was the amount of time the mice needed to get off the wooden platform (Shinde *et al*., 2017). The 2nd session took place 09 days following the 1st test day. Retention was assessed 24 hours later in an identical fashion, with the exception that the grid floor was not shocked with electric shocks after the mice were taken out from the shock-free area if, after 60 seconds, they did not move down (Sharma *et al*., 1990).

Test of familiar and novel object recognition (ORT)

3 distinct phases make up the basic procedure: the acquisition, habituation and retention phases.

a) **Habituation phase:** To acquaint themselves with the equipment, on the 1st day, each mouse received a single 10-minute familiarization session during which they were brought into the empty arena (Katarzyna *et al*., 2023).

b) **Phase of acquisition:** The animals were given a single 10-minute session on the 2nd day with 2 floor-fixed items (X and Y) identical in terms of size, color and smells made of exactly the same material (Tursun *et al*., 2011).

Table 1: Groups and treatment (Every group comprises 6 animals in a cage).

Groups sr. no.	Treatments groups of swiss albino mice
1.	Normal control group (treated by Vehicle) (0.1 ml/100 gm; *i.p*)
2.	Negative control group (amnesia induced by Scopolamine) (1 mg/kg; *i.p.)* (Negative group)
3.	Positive control group Scopolamine (1 mg/kg; *i.p*) + STD (Piracetam) (100 mg/kg; *i.p*)
4.	Scopolamine (1 mg/kg; *i.p*) + ISCN (3 mg/kg *i.p*) (low dose)
5.	Scopolamine (1 mg/kg; *i.p*) + ISCN (6 mg/kg *i.p*) (medium dose)
6.	Scopolamine (1 mg/kg; *i.p*) + ISCN (12 mg/kg *i.p*) (High dose)
7.	Normal+ ISCN (12 mg/kg *i.p*) (High dose to Normal group)

c) **Retention phase:** On the 3rd day, mice were released into an open field with 2 new objects (Z) and a familiar object (X) that were different in shape but comparable in size and color (Takeshi *et al*., 2007).

Interoceptive models for evaluation of the inhibition of Acetyl cholinesterase (AchE) enzyme in mice brain

Cervical dislocation was the technique employed to sacrifice the animals and brain tissue was extracted with carefully. After Cervical dislocation brain tissue was extracted and underwent a variety of biochemical analyses. Brain tissue underwent a variety of biochemical analyses. Utilizing a Teflon homogenizer, the extracted brains were homogenized in phosphate buffer (pH 7.4, 10% w/v). To assess the enzyme activity of Acetylcholinesterase (AchE), the clear supernatant obtained after centrifugation at 3000 rpm for 25 min was tested (Tota *et al*., 2012). This was then mixed with the dithiobis nitro benzoic acid (DTNB) reagent and the substrate, acetyl Thiocholine iodide. AchE degraded acetylthiocholine iodide to produce Thiocholine and acetate (Joana *et al*., 2020). Using a spectrophotometer Acetylcholinesterase (AchE) level was check by Lowry technique was utilized to ascertain the protein composition of the brain sample homogenates At 412 nm, the sample's change in absorbance per minute was measured (Lowry *et al*., 1951).

Statistical analysis

All the below observation and evaluation data are shown as mean±SEM, n=6, with a one-way (Analysis of Variance) ANOVA and Dunnett's Test in between. **P<0.01, *P<0.05, consider as stastically significance.

RESULTS AND DISCUSSION

Outcomes from acute toxicity studies

The acceptable limit specified by OECD recommendations no. 423 was met by animals treated with ISCN, meaning they exhibited no toxicity and allowed for dosing given that gross behavioral alterations not observable occurred and no mortality has been identified to 100 mg/kg body weight

Table 2: Evaluation of effectiveness of ISCN on mice escape latency (EL) in MWM.

Groups sr. no.	Average weight of mice (gram)	Morris- water maze performance (Sec)	
		1st day treatments	11th day treatments
1.	21.63	73.50±0.33	58.25±0.61
2.	22.86	92.37±0.61	158.03±0.65*
3.	23.15	33.51±0.63	26.66±0.36**
4.	24.02	89.15±0.42	74.16±0.42**
5.	21.74	75.66±0.55	58.33±0.35**
6.	22.60	62.00±0.51	43.45±0.25**
7.	22.53	48.51±0.63	31.66±0.36**

Data are shown as mean ± SEM, n = 6, with a one-way (Analysis of Variance) ANOVA and Dunnett's Test in between. **P<0.01, *P<0.05, consider as stastically significance.

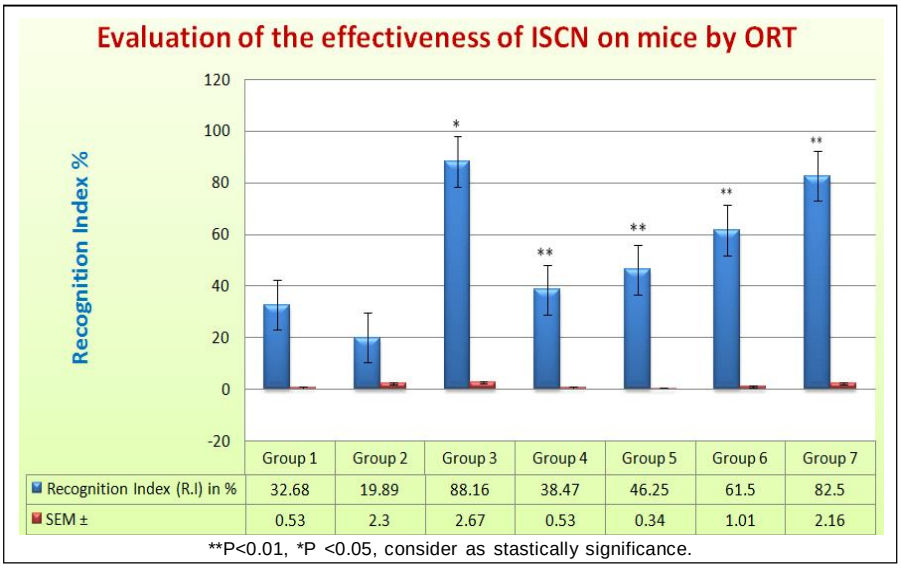


Fig 4: Evaluation of the effectiveness of ISCN on mice Recognition Index by using ORT.

intraperitoneal doses occurred for up to 48 hours, the ISCN demonstrated a large safety margin.

Outcomes from the morris-water maze model (MWM)

Table 2 provides the all observations evaluated by MWM. On the 11th day in the Morris-Water Maze, ISCN dramatically increases memory retention and decreases escape latency in mice treated with scopolamine. This demonstrates unequivocally that mice treated with ISCN greatly improved their learning and memory abilities.

Outcomes from object recognition test (ORT)

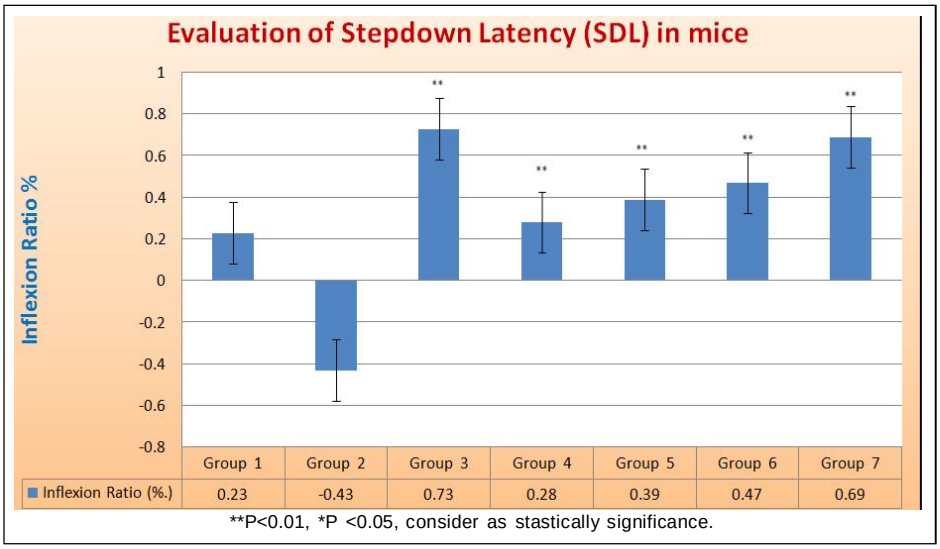
The findings are displayed in Fig 4. Following an acquisition trial, mice administered with ISCN spent more time investigating the novel object than mice given with scopolamine. This increased recognition index and counteracted the effects of scopolamine in mice with amnesia because ISCN enhanced their memory.

Outcomes from passive avoidance test (PAT)

Inflexion Ration (I.R.) was used to evaluate the Stepdown Latency (SDL).Every observation is listed in Fig 5. When compared to the control group on the 9th day, ISCN at different doses significantly increased both the step-down latency and I.R. Passive avoidance acquisition and memory retrieval both demonstrate improvement with ISCN. The ISCN showed decreases in the preferred shock zone and a greater occupancy rate in the shock-free zone (SFZ) of the paradigm. In the absence of cognitive impairment, the increase of step-down latency by ISCN suggested memory improvement.

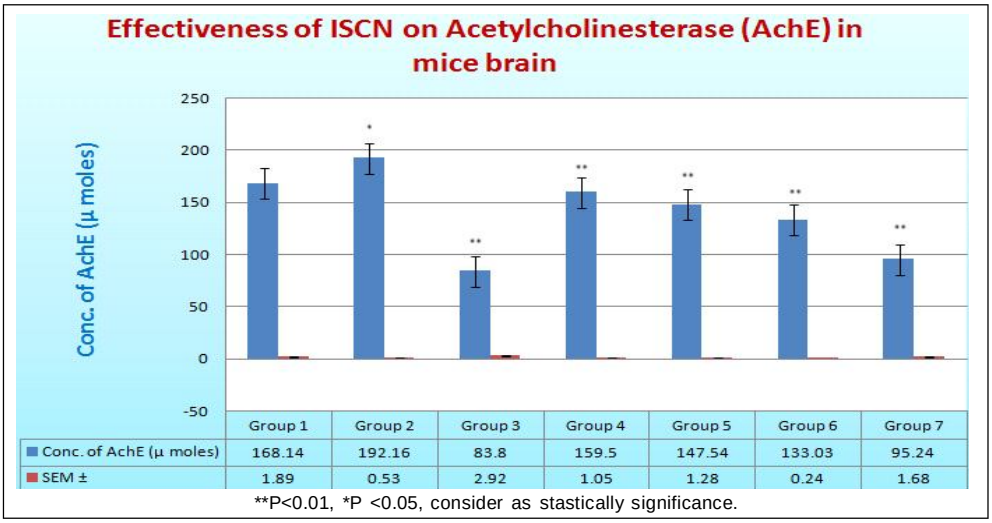
Estimation of Acetylcholinesterase (AchE)

The results shown in Fig 6. The most significant neurotransmitter thought to be involved in the control of cognitive processes is acetylcholine. There is a wealth of research that links improved memory with a reduction in



**P<0.01, *P <0.05, consider as stastically significance.

Fig 5: Evaluation of the stepdown latency (SDL) in mice.



**P<0.01, *P <0.05, consider as stastically significance.

Fig 6: Effectiveness of ISCN on acetylcholinesterase (AchE) in mice brain.

cholinesterase activity. It is discovered that treating ISCN at different doses inhibits the increase in AchE activity while promoting central cholinergic activity. This improves memory and protects mice's brains from scopolamine-induced amnesia.

CONCLUSION

According to the findings of the current results, ISCN protected mice's memory in all behavioral models against scopolamine-induced memory impairment, including the EPM, MWM, ORT and PAT tests. Additionally, the neuroprotective impact of ISCN was linked to an elevated AchE level and a decrease in AchE level in mice brains and ISCN has a potential anti-cholinesterase agent with its neuroprotective activity. This finding indicates that ISCN may exert a neuroprotective effect by inhibiting the AchE enzyme. It has the involvement of the cholinergic system to protect against scopolamine-induced amnesia in mice as well as enhance the recall of memories. ISCN has the potential to be therapeutically beneficial for reducing some of the memory impairments linked to Alzheimer's.

ACKNOWLEDGEMENT

The Authors express their sincere gratitude to the PhD research center at Y.B. Chavan College of Pharmacy in Chh. Sambhajinagar (Aurangabad) for their constant encouragement and for providing well maintained animal house and its facilities required for conduction of experimental work.

Ethical considerations

Regarding animal experiments, The Institutional Animal Ethics Committee (IAEC) and the Indian government's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) accepted the experimental protocol (CPCSEA/IAEC/P'cology/72/2020-21/177). All the approved experiments were performed on daylight in the research center Y.B. Chavan College of Pharmacy's Animal House is located in Aurangabad, Maharashtra, India.

Conflict of interest

All authors declared that there is no conflict of interest.

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