

EVALUATION OF MEMORY-ENHANCING ACTIVITY FOR ERYTHRININE FROM LEAF EXTRACT OF *ERYTHRINA INDICA*KAMALRAJ R^{1*}, SUBHASHREE G R²¹PEH R and D, Pfizer Inc., Chennai, Tamil Nadu, India. ²Department of Physics, Dr. MGR Educational and Research Institute, Chennai, Tamil Nadu, India. Email: kamalrajkb@gmail.com

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ABSTRACT

Objective: The objective of the study was to investigate the memory-enhancing activity for erythrinine (ring-c-oxygenated Erythrina alkaloid) from leaf extract of *Erythrina indica* in mice.

Methods: The study protocol designed in the way that it was carried out for 21 successive days by administrating the 5 mg/kg, s.c doses of isolated erythrinine to mice and the profile was challenged against the mice feeds with normal saline as a positive control and the mice treated with 5 mg/kg, s.c corticosterone as a negative control for the amnesia using Morris water maze test method.

Results: Erythrinine-administered mice are showing remarkable retention of the memory (9.07±0.52) on the 21st day as such of the positive control normal saline (10.14±0.22) and against the negative control corticosterone-injected mice (70.86±0.54).

Conclusions: The dose of 5 mg/kg s.c of isolated erythrinine from the leaf of *E. indica* shows the remarkable memory enhancement when it was investigated with the memory weakening induced test by corticosterone in mice's using 5 mg/kg s.c. However, further studies required to elucidate the exact mechanism of action for developing its as potent memory-enhancing drug.

Keywords: Erythrinine, Memory enhancing, Amnesia.

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INTRODUCTION

Alzheimer's disease (AD) is a neurological brain disorder. AD is also known as dementia, a group of disorders that impair mental functioning. Memory loss is one of the earliest symptoms, along with a gradual decline of proper thinking abilities called cognitive function and change in personality [1-3].

On early stage of the person with Alzheimer disease, may have the symptoms like difficulty in remembering the daily planned activities and misperception (short term memory loss). On long term, the disease slowly goes worst such as start forgetting the words, language, loss of motivation, and faced behavioral issue in the society which may gradually isolated from the family and society and as a worst cause ultimately leading to death [4,5].

The exact causes of the AD were still under investigation, and most of the history of investigation is outcome reason as genetic or any inadvertent injuries and over the period of hypertension or depression [6].

Yoga, physical exercise, and creating the stress-free environment such as listening music, reading books, and maintain the stabilized blood pressure may help to decrease the threat of AD still there are no medications or supplements that used to decrease risk immediately [7].

Treating the people with antipsychotics drugs is recommended for the behavioral problems and psychosis due to dementia, but not usually recommended due to its adverse risks [8,9].

In early studies, different parts of Erythrina and Erythrina alkaloids are used as a traditional medicine in various diseases such as anti-inflammatory, antioxidant, sedative, antiasthmatic, and antiepileptic [10,11].

Even in our previous research, we proved the antihyperlipidemic, convulsion, and stress-induced alteration on lipid profile in rats using the leaf extract of *Erythrina indica* [12].

From the source of literature, we found the extract of Erythrina variegata already proved for the memory-enhancing activity and manages the dementia [13].

In continuation of our research, we attempt early erythrinine for its Insilco evaluation and QSAR studies. To explore further medicinal uses of the isolated erythrinine from the leaf extract of the *E. indica*, an attempt was made to carry out the memory-enhancing effect to confirm the specific activity from the individual Erythrina alkaloids (erythrinine).

MATERIALS AND METHODS**Material used**

The fresh leaf of *E. indica* was collected from the mature plant in and around the city of Chennai, Tamil Nadu, India, and dried under shade. The plant was authenticated by Dr. Sasikala, Department of Pharmacognosy, Captain Srinivasamoorthy Drug Research Institute of Ayurveda, Chennai [12].

Isolation of erythrinine

The powdered, air-dried leaf of *E. indica* was first extracted with hexane and then with methanol, and the alkaloids subsequently isolated by treating the crude leaf extracts of *E. indica* with 1% hydrochloric acid, followed by basification and extraction with chloroform. The alkaloidal fraction obtained from the methanol extract was fractionated by column chromatography on Sephadex LH 20 to yield a crystalline substance erythrinine with 85–90% purity (Fig. 1) [14-16].

Animal's used

Male albino mice (25–40 g) were used for the proposed study. All the mice are well maintained in a ventilated room with suitable alternate source for light/dark cycle in polypropylene cages. The mice were free access to food and water. The standard pellet feed from Hindustan Lever

Limited, Bangalore, was used to feed for the mice to acclimatize for at least week before experimentation. Ethical committee clearance was obtained from the Institutional Animal Ethics Committee of CPCSEA and all cares were followed as per regulation.

Study plan

Experiments were designed as three groups, each group of six male albino mice with that the weight range of 25–40 g. The test drug, isolated erythrinine 5 mg/kg s.c doses were administered for 21 days in sequence as per study plan, 30 min before administration of corticosterone injection which is used as negative control. Corticosterone (5 mg/kg) was dissolved in absolute ethanol and subsequently diluted in water to the final concentration of 10% ethanol and injected subcutaneously in a volume of 1 ml/kg [17,18].

- Group-I: Positive control (Normal saline 2 ml/kg, p.o [0.9% w/v]).
- Group-II: Negative control (Corticosterone inj., 5 mg/kg s.c).
- Group-III: 5 mg/kg s.c doses of isolated erythrinine for 21 days, 30 min before corticosterone administration (5 mg/kg s.c).

Morris water maze test

Setting up the water maze

Water maze is set up as a round pool, about 6 feet in diameter and about 3 feet deep. Filled the water maze with tap water, which should be close to 26°C. Placed the escape platform in the center of the pool. During training, it must be exposed, 1 inch above the water. This teaches the mice are that there is a platform and that it is the way to get out of the water [13].

Then, escape platform was hidden and will not be visible color change due to milk. Pre-training for animals was given as per standard procedure [18].

Water maze testing

During execution of the experiments, the pool was added with milk for color difference with water and to differ from the training. The circular pool was filled up to 30 cm in height and maintained the temperature of 18°C. A white platform (6 cm in diameter and 29 cm in height) was centered in one of four quadrants of the pool (Southeast area) and submerged 1 cm below the water surface so that it was invisible at water level. In the water maze experiments, the 1st week of the experiment was dedicated to swimming training for 60 s for all group mice. Remaining

3 weeks of treatment were investigated with all the groups. In these days, the mice were given one session of two trails each day for 21 days. During each trial, the mouse's escape latency, measured with a stopwatch, was recorded. In each group, six mice were allowed for investigation and the parameter was averaged for each session of trials and for each mouse. If any one of the mice is located the correct platform earlier to 120s, will allow to remain the platform for 10s and note the readings. If the mice not identified the correct platform within 120 s, it was retested for 10 s on the platform for a break. The platforms are rearranged and bring back to testing condition and fixed position for next trail. The same testing was studied for more than each day up to 21 days study periods and all the parameters are noted. As a climax of the study protocol, on the 21 days, mice were given a probe trial which considered of removing the platform from the pool and allowing the mice to swim for 60 s in search of it and the outcome of the results and recorded. All the records calculated the swimming time of the mice in the pool quadrant, where the platform had previously been placed. Isolated erythrinine was administered subcutaneously 10 min before the consecutive training.

Statistical analysis

The statistical significance of the results of Morris water maze was analyzed using ANOVA, followed by Dunnett's test, $p < 0.05$ was considered as statistical significance.

RESULTS

The study protocol designed in the way it was carried out for 21 successive days by administrating the 5 mg/kg, s.c doses of isolated erythrinine to mice and the profile was challenged against the mice feeds with normal saline as a positive control and the mice treated with 5 mg/kg, s.c corticosterone as a negative control for the amnesia using Morris water maze test method. Erythrinine-administered mice are showing sowing remarkable retention of the memory (9.07 ± 0.52) on the 21st day as such of the positive control normal saline (10.14 ± 0.22) and against the negative control corticosterone-injected mice (70.86 ± 0.54).

Positive control mice administered only with the normal saline (Group I), identified the same submerged platform as same as from the 1st day (13.14 ± 0.25) and 21st day (10.14 ± 0.22) with minimal change with remarkable transfer latency. When administering only corticosterone injection (5 mg/kg) group which act as a negative control, will show the memory impairment rapidly from the 0th day (9.76 ± 0.32) to 21st (70.86 ± 0.54) which will be higher rate when compare with the only saline treated mice's. Isolated erythrinine treated mice (5 mg/kg) shown good response in identifying the platform compare to Corticosterone injected mice's and also pointedly than the saline treated mice's from 9th (15.97 ± 0.27) day to 21st (9.07 ± 0.52) day which means the mice's start to response to the Isolated Erythrinine from the 9th day and when 21st day it shows the its show the equal response as such of saline only treated group (10.14 ± 0.22), Where has when it compared with the corticosterone only injected group shows impairment approx. 7 times (70.86 ± 0.54) (Table 1). The above results confirm that the isolated erythrinine-injected mice (9.07 ± 0.52) are having learning and memory-enhancing properties.

DISCUSSION AND CONCLUSIONS

The isolated erythrinine from the leaf of *E. indica* shows the remarkable memory enhancement when it was investigated with the memory

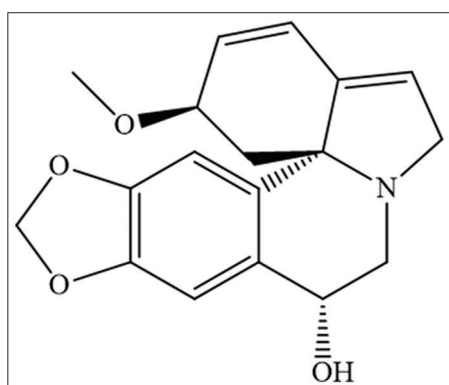


Fig. 1: Structure of erythrinine

Table 1: Effect of isolated erythrinine transfer latencies of mice on Morris water maze test

Group	Treatment	Transfer latency (s)							
		0	3	6	9	12	15	18	21
I	Normal saline 2 mL/kg, p.o	13.14±0.25	15.14±0.87	13.98±0.52*	12.14±0.59*	11.14±0.82*	11.64±0.52*	13.74±0.52*	10.14±0.22*
II	Corticosterone 5 mg/kg, s.c	9.76±0.32	14.00±0.52	25.04±0.22	36.04±0.52	49.25±0.47	56.87±0.52	67.34±0.92	70.86±0.54
III	Corticosterone+isolated erythrinine 5 mg/kg s.c	7.87±0.52	9.14±0.72	12.84±0.52	15.97±0.27	12.74±0.52*	11.76±0.98*	11.34±0.82*	9.07±0.52*

Values are expressed as mean±SEM, ANOVA followed by Dunnett's test, six male albino mice in each group. * $p < 0.001$, as compared to corticosterone-injected group

weakening induced test by corticosterone in mice using 5 mg/kg s.c. The dose of 5 mg/kg s.c of isolated erythrinine was challenged with the only saline group mice as positive control and corticosterone only administered mice as a negative control and established results proved that the dose of 5 mg/kg s.c having the memory-enhancing property and used in the treatment of dementia.

The probable mechanism of action, from literature is discussed in brief below. Corticosterone significantly impaired other forms of hippocampus-dependent memory such as object recognition and retrieval of the passive avoidance behavior. Corticosterone, the predominant glucocorticoid in rodents, chronic administration of corticosterone it damages hippocampal subregion CA3 that leads to impair spatial memory. Also chronically elevated levels of corticosterone injection administration in mice for 21 days can produce neuronal atrophy and cell death in the hippocampus while leaving other brain regions, the elevated levels of corticosterone changes in various neurotransmitters such as catecholamine's, serotonin and aminobutyric acid (GABA) in several brain structures. In the hippocampus, corticosterone impairs GABA-mediated inhibitory neurotransmission and causes neurodegeneration through diminished expression of GABA receptors. High amounts of corticosterone enhance action of norepinephrine through adrenal receptors and increased dopamine turnover in prefrontal cortex is accompanied by the decreased spatial memory performance [3,15].

Erythrinine is a ring-c-oxygenated Erythrina alkaloid which acts as inhibition of ache and increases the acetylcholine level in the brain through the involvement of GABA-benzodiazepine pathway virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function, thereby enhancing the memory activity. However, further studies required to elucidate the exact mechanism of action for developing its as potent memory-enhancing drug [3].

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AUTHORS' CONTRIBUTIONS

Both authors having equal contribution in overall studies.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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