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NEUROPROTECTIVE EFFECTS OF A STANDARDIZED FLAVONOID EXTRACT OF IPOMOEA AQUATICA FORSK AGAINST NEUROLOGICAL PROBLEMS

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Abstract

Parkinson's disease is a progressive neurodegenerative disorder characterized primarily by motor impairments, including tremors, rigidity, and bradykinesia. Additionally, cognitive deficits and neuropsychiatric symptoms often accompany the motor symptoms, contributing to the overall burden of the disease. The deposition of alpha-synuclein aggregates, known as Lewy bodies, in dopaminergic neurons of the substantia nigra is a pathological hallmark of Parkinson's disease. The present study aimed to investigate the potential therapeutic effects of hydroalcoholic extract of *Ipomoea aquatica* (HAEIA) in a mouse model of Parkinson's disease induced by neurotoxic agents such as 6-hydroxydopamine (6-OHDA) or MPTP. Animals received HAEIA treatment for a specified duration. Behavioral assessments, including rotarod test, pole test, and open field test, were conducted to evaluate motor function and exploratory behavior. Results from the study demonstrated that HAEIA treatment attenuated motor deficits induced by neurotoxic agents and exerted neuroprotective effects by modulating oxidative stress and neuroinflammation in the brain. These findings suggest that HAEIA may hold promise as a potential therapeutic agent for the management of Parkinson's disease.

Keywords: Neuroprotective effects, Flavonoid extract, *Ipomoea aquatica*, neurological problems.

Introduction

Neurological disorders, including Alzheimer's, Parkinson's, multiple sclerosis, epilepsy, and headache disorders, account for 3% of the global disease burden, with exponential growth expected in low and middle-income countries in the next decade [1]. PD, the most common neurodegenerative disorder among the elderly, is characterized by a progressive decline in muscular control, leading to premature mortality. It is more prevalent in the sixth decade of life, with men being more susceptible. Symptoms include tremors, rigidity, bradykinesia, and compromised balance. Non-motor disturbances include cognitive deficits, anxiety, stress, and other symptoms [2-4]. PD is a neurological disorder characterized by the gradual degeneration of dopaminergic neurons, with oxidative stress-induced disruption of dopamine metabolism contributing to the disease. Treatment strategies involve augmenting dopaminergic neuron activity or inhibiting cholinergic effects. PD is the second most prevalent neurological disorder globally, impacting 1.5 million Americans [5-10]. Cognitive impairment, a non-motor symptom of PD is a growing concern among PD patients. It can lead to feelings of distraction, difficulty planning, and difficulty focusing in group conversations. The global prevalence of PD is increasing, and studies have focused on motor symptoms, but less on non-motor symptoms like cognitive deficits. Cognitive impairment is a precursor to dementia, impacting life expectancy, daily functioning, and overall quality of life. Mild cognitive impairment (MCI) is an intermediate stage marked by variability, with some patients exhibiting stable cognition while others may revert to normal levels. Early screening and targeted management of cognitive deficits are crucial for delaying disease progression and improving patient prognosis [11-15].

The brain's significant oxygen consumption, accounting for 20% of the body's basal oxygen utilization, makes it vulnerable to oxidative stress. Oxidative DNA damage, impaired respiratory chain function, and mitochondrial DNA mutations are common in PD patients. Elevated dopamine metabolism contributes to toxic radical accumulation, while iron accumulation in neurons is significant in PD pathophysiology. The interplay between oxidative stress, dopamine metabolism, and iron accumulation is crucial in PD neuropathogenesis [16-39]. Natural products have been extensively studied for their potential protective effects against Parkinson's disease (PD) using animal models. Studies have shown that ceftriaxone, quercetin, Acacia catechu leaf extract, safflower flavonoid extract, isolecanoric acid, carnosic acid, ocimum sanctum leaf extract, hesperidin, and buddleja cordata methanolic extract have shown antioxidant and memory-enhancing activities. These studies highlight the therapeutic potential of natural products in mitigating neurodegenerative processes associated with PD [40-48].

Preparation of the plant extract

The fresh leaves of IA were harvested and thoroughly washed with running water to remove any impurities. Subsequently, they were shade-dried at ambient room temperature until completely dry. Upon drying, 1 kg of the prepared dried leaves was processed into coarse powder and sieved through a 60-mesh sieve to ensure uniformity of particle size. The air-dried powdered drug was then subjected to extraction using a mixture of ethanol and water (6:4) via the Soxhlet extraction method. This resulted in the production of HAEIA. The extract was then carefully filtered to

remove any insoluble impurities and concentrated using a rotary vacuum pump to obtain a solid mass. This methodical extraction process ensures the extraction of bioactive constituents from *Ipomoea aquatica*, facilitating further pharmacological evaluation and potential therapeutic applications.

In-Vivo Studies

Study Design

Animals

Swiss albino rats of either sex of two age groups, (i) neonatal pups (7 days old) and (ii) young adults (60 days old) were used for the study, obtained from the animal house of TAAB Bio Study services, Jadavpur, Kolkata-700032. Animals were kept in raised mesh bottom cages to prevent coprophagy. The animals were maintained in colony cages at 25±2°C, relative humidity of 50-55% maintained under a 12:12 h light and dark cycle. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by CPCSEA. The study was approved by the Institutional Animal Ethical Committee (Ref No: 1938/PO/Rc/S/17/CPCSEA; Dated - 01/01/23).

Treatment Schedule

Seven-day-old neonatal rat pups and 60-day-old young adult rats were further subdivided into two groups.

Group A (Neonatal group) Consists of Seven-day-old neonatal rat pups, further subdivided into Group I (control group) received 10ml/kg normal saline, p.o. Group II and Group III (Treatment groups) animals received MEIA 200 and 400 mg/kg, p.o. for 30 days (from the 8th day to the 37th day in the neonatal group).

Group B (Adult group) Consists of 60-day-old young adult rats, further subdivided into Group I (control group) received 10ml/kg normal saline, p.o. Group II and Group III (Treatment groups) animals received MEIA 200 and 400 mg/kg, p.o. for 30 days (from 61st day to 90th day in young adult rats).

Intra cerebro ventricular injection of Aβ peptide

The administration of Aβ 25-35 was conducted by identifying the bregma as a reference point on the skull. Each mouse was injected at the bregma using a 50 µL Hamilton microsyringe fitted with a 26-gauge needle, which was inserted to a depth of 2.4 mm. Specifically, the needle was unilaterally inserted 1 mm to the right of the midpoint equidistant from each eye, angled slightly towards 45 degrees and perpendicular to the skull plane. Rats exhibited normal behavior within 1 minute after injection. The animals were then divided into six groups:

Group I: Animals injected with phosphate-buffered saline (10 µL)

Group II: Animals injected with Aβ peptide (10 µL) via intra-cerebroventricular (ICV) route

Group III: Animals injected with Aβ peptide (10 µL) via ICV route and treated with HAEIA at 200 mg/kg (per os)

Group IV: Animals injected with Aβ peptide (10 µL) via ICV route and treated with HAEIA at 400 mg/kg (per os)

Amnesia was induced by ICV injection of Aβ peptide (25-35) preparation to groups II, III, and IV on the 21st day after pretreatment with HAEIA, and this treatment continued for 7 days. Control animals were injected with phosphate-buffered saline only. This experimental setup enables the investigation of the effects of HAEIA on Aβ-induced amnesia in the tested animal model, providing valuable insights into its potential pharmacological efficacy.

Step down inhibitory avoidance

The experimental apparatus consisted of a 50×25×25 cm acrylic box, featuring a floor comprising parallel grids spaced 1.0 cm apart. Positioned at the center of the floor was a platform measuring 7.0 cm in width, 2.5 cm in height, and 25.0 cm in length. During the training session, upon stepping down and placing their four paws on the grid, the animals were subjected to a 0.4 mA, 2.0 sec scrambled foot shock. In the subsequent test session, no foot shock was administered, and the step-down latency served as a measure of retention, with a ceiling limit set at 300 seconds. The one-trial step-down inhibitory avoidance test in rats involves the activation of two distinct memory systems: a Short-Term Memory (STM) system and a Long-Term Memory (LTM) system. Therefore, retention tests were conducted 90 minutes after training to assess STM and 7 days after training to evaluate LTM. This experimental design enables the investigation of both short-term and long-term memory processes, providing valuable insights into the effects of the experimental variables on memory retention in the tested animals.

Water Maze Task

The experimental setup comprised a circular water tank with a diameter of 100 cm and a height of 35 cm. The tank was filled with water maintained at a temperature of 28°C; reaching a depth of 15 cm. Titanium dioxide was added to the water to render it opaque. Within the tank, a platform with a diameter of 4.5 cm and a height of 14.5 cm was submerged 1 cm below the water surface, positioned at the midpoint of one quadrant. Following several trial sessions, the actual test was conducted after the injection of β-amyloid peptide. During each training trial, the time taken by the subjects to escape onto the platform was meticulously recorded. This experimental design facilitates the assessment of spatial memory and learning abilities in the tested subjects, providing insights into the potential effects of the experimental variables on cognitive function.

Exploratory behavior (hole board)

The hole board apparatus serves as a tool to assess the locomotor activity of animals. Rats were subjected to exploration sessions twice, with a 24-hour interval, within a 40×40×60 cm open field. The floor of the field was covered with brown linoleum and divided into 16 equal squares by white lines. Following the administration of the test drug, each animal was placed in the rear left square and allowed to explore freely for 5 minutes. During this period, the number of line crossings, head dippings, and rearings were meticulously recorded. This method enables the evaluation of the locomotor and exploratory behaviors of the animals, providing valuable insights into the potential effects of the test drug on these parameters.

Y-maze task

The Y-maze task serves as a method to evaluate spatial working memory through the assessment of spontaneous alternation behavior. The maze itself is constructed from black painted wood,

with each arm measuring 40 cm in length, 3.5 cm in height, and 10 cm in width, converging at equal angles. During the training session, each mouse is positioned at the end of one arm and allowed to freely navigate through the maze for 15 minutes. Rats typically explore the maze systematically, sequentially entering each arm. The ability to alternate between arms indicates the rat's awareness of which arms they have previously visited. Observations are conducted for 5 minutes, during which the series of arm entries, including potential returns to the same arm, are visually recorded. Alternation is defined as the number of successive entries into the three arms, considering overlapping triplet sets. The percentage of alternation is then calculated as the ratio of actual alternations (total arm entries minus two), multiplied by 100. This method provides a quantitative measure of spatial working memory performance, offering insights into the cognitive effects of experimental variables or interventions.

Object recognition test

The experimental apparatus consists of a wooden box measuring 70×60×30 cm, featuring a grid floor designed for easy cleaning with hydrogen peroxide after each trial. Two objects, differing in shape - a pyramid with an 8 cm side and a cylinder with a height of 8 cm - are positioned at diagonally opposite corners of the box. On day 0, the animals are granted a 2-minute exploration period within the box devoid of objects. During the first trial (T1), two identical objects are introduced into the box's opposite corners, and the time taken by each mouse to complete 20 seconds of exploration is recorded. Exploration is defined as directing the nose within a distance less than 2 cm of an object and/or making contact with the nose. Subsequently, during the second trial (T2), conducted 90 minutes after T1, one of the objects from T1 is replaced with a new object, and the rats are left in the box for 5 minutes. The time spent exploring the New (N) and Familiar (F) objects are recorded separately. Measures are taken to prevent place preference and olfactory stimuli by randomly altering the role (F or N) and the positions of the objects during T2, in addition to meticulous cleaning procedures. This methodological approach ensures accurate assessment of recognition memory in the tested animals, minimizing potential confounding factors and enhancing the reliability of the experimental results.

Elevated plus maze

The apparatus utilized in this experiment comprises two open arms measuring 35×6 cm each, along with two enclosed arms measuring 35×6×15 cm. These arms are interconnected by a central square measuring 5×5 cm. The entire maze is elevated to a height of 100 cm and is situated within a light and sound-attenuated room to minimize external stimuli. Each rat is individually placed at the end of an open arm of the Elevated Plus Maze (EPM), facing away from the central platform. The time taken by the rat to move from the end of the open arm to either of the enclosed arms referred to as Transfer Latency (TL) is meticulously recorded. Transfer Latency (TL) is defined as the duration taken by the mouse to transition into one of the covered arms with all four legs, and a maximum TL of 90 seconds is assigned if the mouse fails to enter a covered arm within this timeframe. Subsequently, the mouse is allowed to explore the maze for 10 seconds before being returned to its home cage. Memory retention is assessed 24 hours after the initial trial. The inflection ratio, indicative of memory retention, is calculated

using a specific equation tailored to the experimental design. This methodology enables the comprehensive evaluation of memory-related behaviors in the tested animals, facilitating the identification of potential effects of experimental variables on cognitive function. The inflection ratio was calculated by the equation.

$$IR = \frac{L0 - L1}{L0}$$

Where,
L0 is the initial Transfer Latency (TL) in seconds on first time,
L1 is the Transfer Latency (TL) in seconds on 2nd time.

ACh estimation

Acetylcholine (ACh) levels were determined utilizing a fluorimetric method. Initially, the pooled hippocampi were carefully weighed and homogenized utilizing a Teflon-glass homogenizer in freshly prepared cold 10% trichloroacetic acid. Subsequently, the homogenates underwent centrifugation at 10,000 revolutions per minute for 10 minutes at a controlled temperature of 4-8°C. The resulting supernatant was promptly collected and processed for the immediate estimation of acetylcholine levels. This meticulous procedure ensures the accurate assessment of ACh concentrations, facilitating the precise evaluation of neurotransmitter dynamics within the hippocampal region.

Estimation of acetylcholinesterase enzyme

The activity of the acetylcholinesterase (AChE) enzyme was assessed using the Elman method. This established enzymatic assay provides a reliable means of quantifying the activity of AChE, an essential enzyme involved in the regulation of acetylcholine levels. By employing the Elman method, we were able to accurately measure AChE activity, thereby gaining valuable insights into cholinergic neurotransmission and its modulation. This robust enzymatic assay forms a cornerstone in elucidating the pharmacological effects of compounds targeting the cholinergic system, contributing significantly to our understanding of neurobiological processes and drug development strategies.

Reagents

To prepare the phosphate buffer solution (0.1 M), two separate solutions were formulated. In solution A, 5.22 g of K_2HPO_4 and 4.68 g of NaH_2PO_4 were dissolved in 150 mL of distilled water. Solution B was created by dissolving 6.2 g of NaOH in 150 mL of distilled water. Subsequently, solution B was gradually added to solution A to achieve the desired pH level (typically pH 8.0 or 7.0). Finally, the volume was adjusted to 300 mL with distilled water, ensuring the accurate preparation of the phosphate buffer solution. This meticulously crafted buffer system provides the necessary pH stability for biochemical assays and pharmacological experiments, facilitating reliable and reproducible results in phytopharmaceutical research.

The DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) reagent was meticulously prepared by dissolving 39.6 mg of DTNB along with 15 mg of NaHCO3 in 10 mL of 0.1 M phosphate buffer, ensuring a precise pH level of 7.0. This formulation guarantees the optimal conditions for subsequent biochemical assays and pharmacological evaluations. Additionally, the

acetylthiocholine (ATC) solution was prepared by dissolving 21.67 mg of acetylthiocholine in 1 mL of distilled water, ensuring the solubility and stability of the compound for experimental use. These carefully prepared solutions are essential components in biochemical assays aimed at assessing enzymatic activity and pharmacological effects, facilitating accurate and reliable research outcomes in the field of phytopharmaceuticals.

Procedure

The experimental procedure commenced with the decapitation of rats, followed by the swift removal of their brains, which were promptly immersed in ice-cold saline to preserve their integrity. Subsequently, the frontal cortex, hippocampus, and septum were meticulously dissected out on a Petri dish chilled with crushed ice. These brain regions were then precisely weighed and homogenized in 0.1 M phosphate buffer at a pH of 8, ensuring optimal conditions for subsequent biochemical analyses. Next, a 0.4 mL aliquot of the tissue homogenate was carefully transferred to a cuvette containing 2.6 mL of 0.1 M phosphate buffer at pH 8, along with 100 µL of DTNB. The contents of the cuvette were thoroughly mixed by gentle bubbling of air, and the absorbance was measured at 412 nm using a spectrophotometer. Once the absorbance reached a stable value, it was recorded as the basal reading. Following the establishment of the basal reading, 20 µL of the substrate acetylthiocholine was added to the cuvette, and the subsequent change in absorbance was continuously monitored. This enabled the determination of the rate of change in absorbance per minute, providing valuable insights into the enzymatic activity under investigation. Such meticulous experimental procedures are essential for elucidating the pharmacological effects of phytopharmaceutical compounds on biochemical pathways within the brain.

Statistical analysis

All data are presented as mean ± SEM (standard error of the mean). Statistical analysis was performed using non-parametric ANOVA, followed by Dunnett's multiple comparison tests, with evaluation conducted using Graph Pad PRISM software. A significance level of $p < 0.05$ was deemed statistically significant, indicating differences between experimental groups. This rigorous statistical approach ensures robust analysis and interpretation of the experimental results, enhancing the reliability and validity of the findings.

Results:

***In-Vivo* Studies**

Step down inhibitory avoidance

In the investigation, it was observed that the Step Down Latency (SDL) time of group II animals exhibited a notable reduction in comparison to group I animals. However, upon administration of HAEIA (200 and 400 mg/kg) to groups III and IV, there was a noteworthy and statistically significant increase in SDL. This augmentation in SDL indicates a potential enhancement in both Short Term Memory (STM) and Long Term Memory (LTM). Detailed findings are presented in Table 1 and visually represented in Fig. 1.

Table no. 1 - Step down inhibitory avoidance test

Groups	STM (sec)	LTM (sec)
Group I	178.80±2.6	173.0±1.5
Group II	86.60±2.1a *	101.8±1.7a *
Group III	110.00±2.7b *	124.3±1.7b *
Group IV	136.50±1.8b *	139.8±2.0b *

Values are expressed as Mean±SEM, n = 6, symbols represent statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test, STM: Short term memory, LTM: Long term memory.

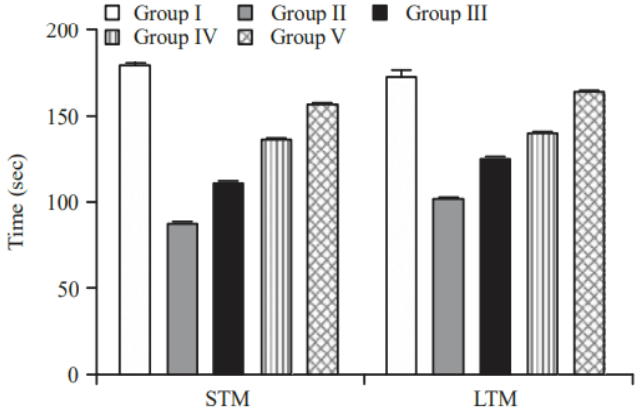


Fig no. 1 - Effect of HAEIA on step-down inhibitory avoidance test

Water maze task

In the study, it was observed that the escape latency of group II animals exhibited a notable increase compared to group I animals. However, upon treatment with HAEIA (200 and 400 mg/kg) in groups III and IV, a significant decrease in escape latency onto the hidden platform was noted in comparison to group II animals. This decrease in escape latency suggests an enhancement in memory retention. Comprehensive findings are summarized in Table 2 and visually depicted in Figure 2.

Table No. 2 - Water maze task

Groups	Escape latency time (sec)
Group I	**16.83 ± 0.7**
Group II	**56±1.1a ***
Group III	**43.5±1.4b ***

Group IV	**31.17±1.0b ***

Values are expressed as Mean±SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test

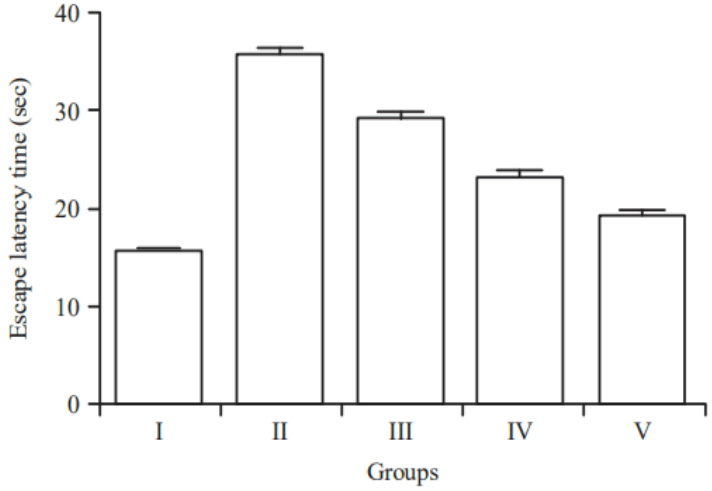


Fig no. 2 - Effect of HAEIA on escape latency in water maze

Open field exploratory behavior (Hole board)

In comparison to group I animals, group II animals displayed a notable decrease in head dipping, rearing, and line crossing. However, administration of HAEIA (200 and 400 mg/kg) to groups III and IV exhibited a statistically significant increase in open field habituation parameters such as head dipping, rearing, and line crossing when compared to group II animals. These findings are presented in detail in Table 3 and visually represented in Figure 3.

Table No. 3 - Open field exploratory behavior (Hole board)

Groups	Head dippings	Rearing	Line crossings
Group I	12.30 ± 0.6	27.17 ± 1.0	66.00 ± 1.5
Group II	4.00 ± 0.5 a **	13.17 ± 0.6 a *	22.40 ± 1.0 a **
Group III	5.6 ± 0.5 b *	16.3 ± 0.4 b *	28.0 ± 0.5 b
Group IV	7.8 ± 0.4 b *	19.5 ± 0.6 b *	38.3 ± 2.0 b *

Values are expressed as Mean ± SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test.

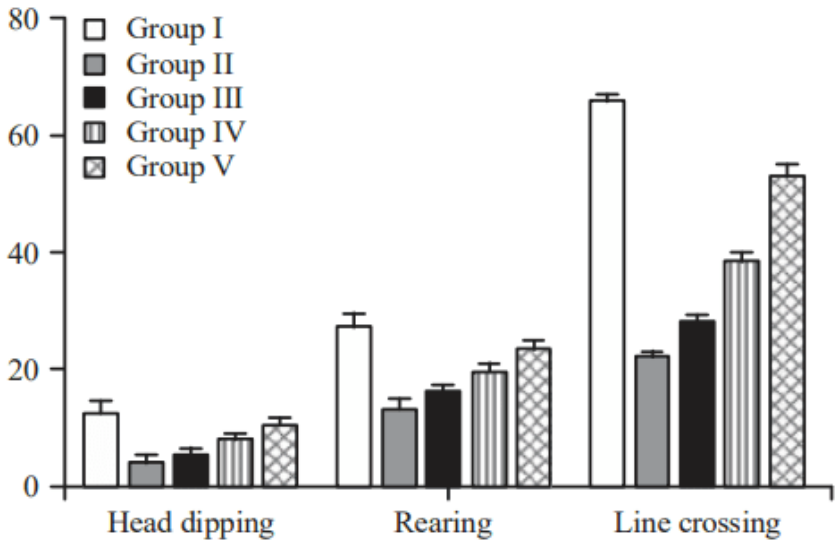


Fig no. 3 - Effect of HAEIA on open field exploratory behavior (Hole board)

Y-maze task

In comparison to group I animals, group II exhibited a significant reduction in the percentage of alteration. However, treatment with HAEIA (200 and 400 mg/kg) in groups III and IV demonstrated a noteworthy increase in alteration compared to group II animals. This increase in the percentage of alteration in the Y-maze suggests an enhancement of spatial working memory. Detailed results can be found in Table 4, with a graphical representation provided in Figure 4.

Table No. 4 - Y-maze task

Groups	Percentage alternation
Group I	**77.9 ± 2.1**
Group II	**33.2 ± 1.0 a ***
Group III	**52.6 ± 1.1*b**
Group IV	**63.0 ± 0.9 *b**

Values are expressed as Mean±SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test

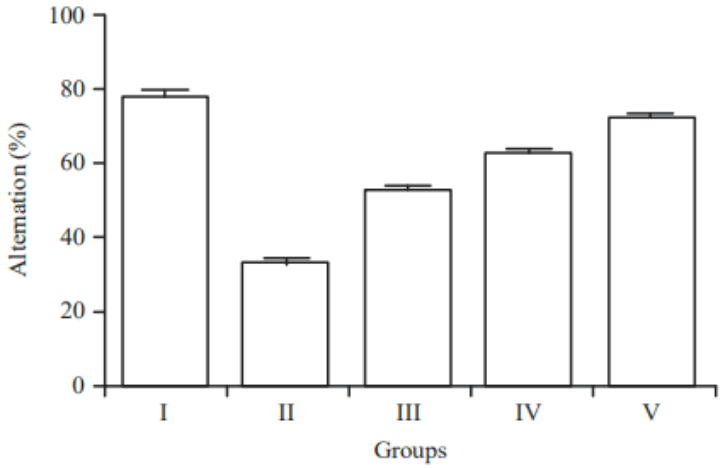


Fig no. 4 - Effect of HAEIA on Y-maze task

Open field exploratory behavior (hole board)

Exploration time towards newer objects was notably reduced in group II animals compared to group I animals. However, treatment with HAEIA (200 and 400 mg/kg) in groups III and IV resulted in a significant increase in exploration time compared to group II animals. Conversely, exploration time towards familiar objects showed a statistical increase in group II animals compared to group I animals. Yet, treatment with HAEIA (200 and 400 mg/kg) in groups III and IV led to a significant decrease in exploration time compared to group II animals. These findings are summarized in Table 5 and visually represented in Figure 5.

Table No. 5 - Object recognition study

Groups	**Exploration time in sec (new object)**	**Exploration time in sec (familiar object)**
Group I	21.1 ± 0.7	5.3 ± 0.4
Group II	7.5 ± 0.5a *	14.5 ± 1.4a *
Group III	14.1 ± 0.6b *	8.8 ± 1.1b *
Group IV	16.5 ± 0.7b **	7.5 ± 1.5b *

Values are expressed as Mean ± SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test

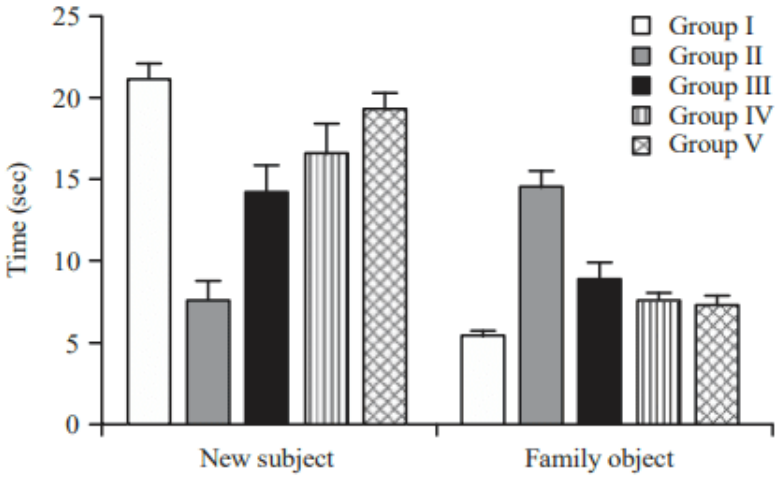


Fig no. 5 - Effect of HAEIA on object recognition study

Elevated plus maze

The Inflexion Ratio (IR) of group II animals exhibited a significant decrease compared to group I animals. However, treatment with HAEIA (200 and 400 mg/kg) in groups III and IV demonstrated a significant increase in IR compared to group II animals. These findings are detailed in Table 6 and visually depicted in Figure 6.

Table No. 6 - Elevated plus maze

Groups	Inflexion ratio (IR)
Group I	0.83 ± 0.02
Group II	0.24 ± 0.03 a *
Group III	0.42 ± 0.05 b *
Group IV	0.56 ± 0.02 b *

Values are expressed as Mean±SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test.

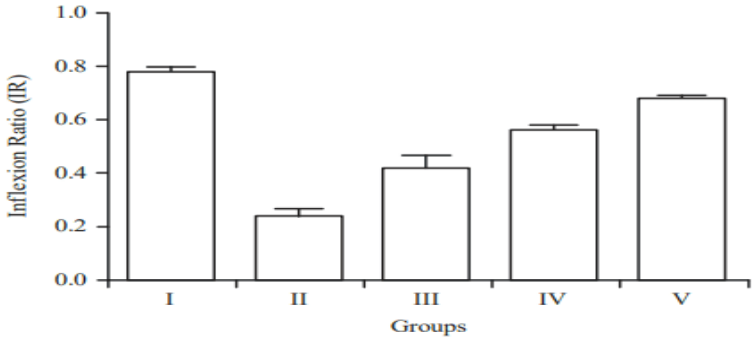


Fig no. 6 - Effect of HAEIA on the elevated plus maze

Estimation of acetylcholinesterase enzyme

Group II animals demonstrated a significant increase in the level of AchE compared to group I animals. However, treatment with HAEIA (200 and 400 mg/kg) in groups III and IV exhibited a significant reduction in the AchE enzyme level compared to group II animals. These results are summarized in Table 7 and depicted in Figure 7. Additionally, histopathological changes in the midbrain and cerebellum of rats stained with H&E at the original magnification (×200) are illustrated in Fig. 8 and Fig. 8, respectively.

Table No. 7 - Estimation of acetylcholinesterase enzyme

Groups	**Acetylcholinesterase (µM min^{-1} mg^{-1} protein)**
Group I	15.99 ± 0.60
Group II	35.79 ± 0.78 a *
Group III	29.02 ± 0.85 b *
Group IV	23.08 ± 0.74 b **

Values are expressed as Mean ± SEM, n = 6, symbols represent statistical significance: *p< 0.05, **p < 0.01, ***p < 0.001, one-way ANOVA followed by Dunnett's test.

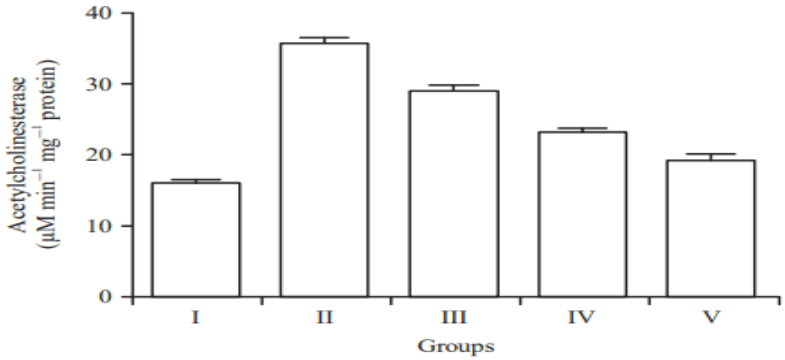


Fig no. 8 - Effect of HAEIA on acetylcholinesterase enzyme

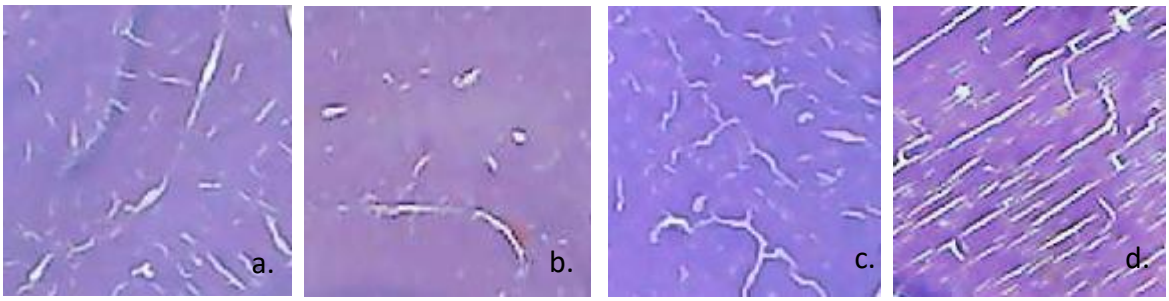
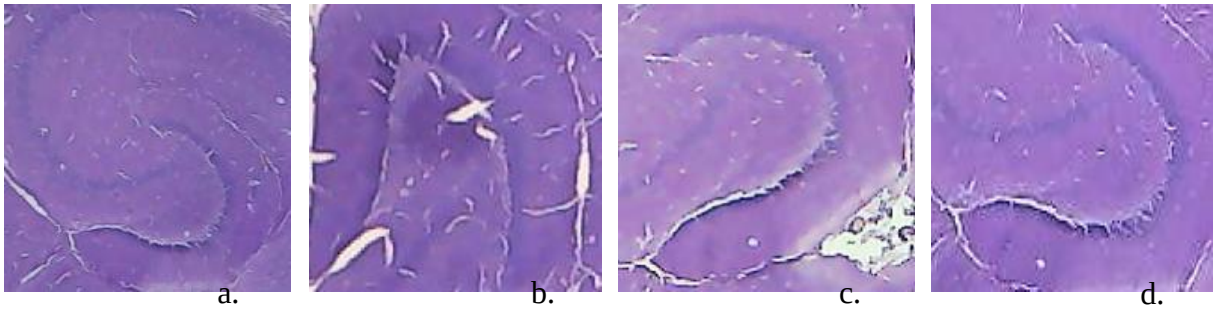


Fig no. 7 - Histopathological changes in the midbrain of rats: (a) Control section showing normal histoarchitecture. (b) Rats without treatment with prominent degeneration of neurons. (c) Rats treated with 200 mg/ kg HAIAE. (d) Rats treated with 200 mg/ kg HAIAE. Sections stained with hematoxylin and eosin × 400.



a. b. c. d.

Fig no. 8 - A section of the cerebellum stained with H&E is viewed at the original magnification (×200). (A) Rat cerebellar tissue showing the molecular layer (ML) of the Hippocampus (B) Rats without treatment with prominent degeneration showing a disturbance in the linear organization of the middle of the Hippocampus (C) Rats treated with 200 mg/ kg HAEIA showing a largely normal appearance. The Hippocampus retained a normal linear organization (D) Rats treated with 400 mg/ kg HAEIA showing a largely normal appearance. The Hippocampus retained a normal linear organization.

DISCUSSION

Cognitive impairment represents a significant challenge in PD, often leading to functional disability and skill loss among patients. A key pathological feature of PD involves the accumulation of amyloid-beta protein in the brain. Extensive behavioral research has underscored the central role of the cholinergic system in memory and learning. The Morris water maze is widely recognized as a prominent paradigm for assessing learning and memory. Given its established efficacy, we selected this maze for the present study. Our *in vivo* investigation revealed that administering HAEIA (200 and 400 mg/kg) to groups III and IV led to a noteworthy reduction in escape latency onto the hidden platform compared to group II animals. Our findings indicate that treatment with HAEIA effectively mitigated cognitive impairments in Aβ 25-35 injected mice. The step-down inhibitory avoidance task, renowned for its memory assessment capabilities coupled with an aversive element, was employed. Notably, HAEIA demonstrated significant enhancements in both short-term memory (STM) and long-term memory (LTM) at dosage levels of 200 and 400 mg/kg.

The impairment of memory induced by intra-cerebro ventricular injection of Aβ 25-35 was evaluated using passive avoidance and the object recognition test. The object recognition task is particularly effective for assessing animals' aversion to new stimuli, thereby reflecting on their learning and memory capabilities. Our observations revealed that animals treated with beta-amyloid (25-35) exhibited a statistically significant decrease in exploration time towards newer objects compared to group I animals. However, treatment with HAEIA (200 and 400 mg/kg) in

groups III and IV led to a significant increase in exploration time, suggesting a potential ameliorative effect on memory impairment.

The administration of HAEIA at doses of 200 and 400 mg/kg resulted in an increased percentage of alterations observed in the Y-maze task, mirroring similar outcomes in open-field exploratory behavior. Specifically, animals treated with HAEIA exhibited an increase in line crossing compared to group II animals. Moreover, in the elevated plus maze task, there was a notable decrease in the inflection ratio (IR) of group II animals compared to those injected with beta-amyloid. However, treatment with HAEIA, as well as the standard drug, demonstrated a significant increase in IR, indicative of potential memory enhancement effects.

Two types of cholinesterase, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) play pivotal roles in the nervous system. AChE is primarily located in blood and neural synapses, where it rapidly hydrolyzes acetylcholine (ACh), while BuChE hydrolyzes butyrylcholine (BuCh) at a faster rate. Elevated levels of AChE can exacerbate the processing and deposition of beta-amyloid in PD. The presence of amyloid proteins triggers microglial activation, promoting neuroinflammation, which in turn leads to the degeneration of cholinergic and adrenergic neurons. The loss of neurons and decreased neuronal counts observed in PD are associated with reduced levels of acetylcholine, serotonin, dopamine, and noradrenaline.

The Aβ 25-35 peptide has been identified as having the potential to induce oxidative stress in the brain, leading to cholinergic hypo-function and an elevation of acetylcholinesterase (AChE) levels. Additionally, studies have shown that this peptide can stimulate the production of hydrogen peroxide and lipid peroxide in the neurons of the hippocampus in rat brains. In our current investigation, a notable elevation in acetylcholinesterase (AChE) levels was observed in the brains of mice following a single administration of Aβ 25-35 peptide. It is well-documented that AChE activity tends to increase around Aβ plaques in the brains of individuals with PD. This surge in AChE activity induced by the Aβ 25-35 peptide is attributed to calcium influx and subsequent oxidative stress, which disrupts cell membrane integrity and exposes more active enzyme sites. The observed augmentation in AChE activity by Aβ peptide suggests the potential to mitigate cholinergic dysfunction by inhibiting the Aβ-induced surge in AChE activity. Comparatively, mice treated with Aβ 25-35 displayed elevated AChE activity in their brains compared to untreated counterparts. This disparity in AChE activity may lead to heightened amyloid protein secretion, exacerbating the progression of PD. The findings strongly indicate the neuroprotective potential of HAEIA, highlighting its ability to counteract the detrimental effects associated with Aβ-induced AChE elevation.

CONCLUSION:

In conclusion, our pharmacological evaluation of HAEIA against amyloid-induced cognitive dysfunction in mice has provided compelling evidence. Treatment with both low and high doses of the drug demonstrated effectiveness in enhancing both short-term and long-term memory, as well as exploratory and learning memory in the animals. This is underscored by the notable performance of animals in the treatment groups across various memory and learning-related tasks, including the water maze, object recognition study, step-down inhibitory avoidance, Y-

maze, and plus maze, as observed during the in vivo studies. These findings collectively support the potential of HAEIA as a therapeutic agent for mitigating cognitive deficits associated with amyloid-induced dysfunction.

Drawing insights from the histopathological examinations of the midbrain and hippocampus in rats induced with PD and subsequently treated with Ipomoea aquatica extract (HAIAE), several conclusions can be deduced. In the midbrain, the control specimens exhibited a typical histoarchitecture, indicative of a healthy condition. Conversely, untreated rats exhibited noticeable neuronal degeneration, reflecting the adverse impact of PD. Conversely, rats administered with 200 mg/kg of HAIAE displayed signs of histological improvement in the midbrain. This suggests a potential neuroprotective effect of the extract, which may help alleviate neurodegeneration associated with PD. Likewise, in the hippocampus, untreated rats displayed signs of degeneration and disruption in the linear arrangement of the middle hippocampal region. This provides additional evidence of PD-induced damage to this brain area. Conversely, rats treated with 200 mg/kg of HAIAE maintained a predominantly normal appearance in their hippocampus. This indicates that the extract might be capable of preserving the linear organization and structural integrity of this crucial region implicated in memory and cognitive functions. Moreover, rats administered with a higher dose of 400 mg/kg HAIAE similarly exhibited a predominantly normal appearance in the hippocampus. This suggests that the higher dosage did not induce any adverse effects on the histopathological structure of the brain.

Finally, the histopathological assessments of the midbrain and hippocampus in rats afflicted with PD and administered with Ipomoea aquatic extract indicate promising neuroprotective properties. Specifically, the extract exhibited potential in attenuating neurodegeneration and maintaining the structural integrity of both the midbrain and hippocampus.

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