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EVALUATION OF NOOTROPIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CLERODENDRUM INFORTUNATUM* YOUNG STEMS IN MICE

Anamika Mistry[1]; Rajendra V Sundar[1]; Nanjappaiah H M[2]; Saifulla Khan M[1]; Kuntal Das[1]

[1]P.G. Department of Pharmacology, Krupanidhi College of Pharmacy, Bengaluru, India

[2]Department of Pharmacology, BLDEA's SSM College of Pharmacy and Research Centre, Vijayapur, India.

E-mail address: drrajendra1972@gmail.com

Abstract

Dementia is a loss of ability to create new memories after the events. The severe side effects of nootropic agent evoked us to search for new nootropic drugs from plant source. The aim of the present study was to evaluate the hydroalcoholic extract of *Clerodendrum infortunatum* young stems for nootropic activity in mice. Hydroalcoholic extract of *Clerodendrum infortunatum* young stems was administered orally at two different doses of 200 mg/kg and 400 mg/kg for successive 21 days and extent of improvement in memory was determined on 21st and 22nd day against diazepam (1 mg/kg) induced amnesia through behavioral models viz. Elevated plus maze, Y-maze, Morris water maze and Hole board model. The results were compared with standard drug Piracetam (200 mg/kg). The study reveals that *Clerodendrum infortunatum* extract (400mg/kg) has showed significant improvement in memory process when compared to control in all the models. Memory retention is more on 22nd day as compared with 21st day. It is clear from our study that lower dose (200 mg/kg) and higher dose (400 mg/kg) of *Clerodendrum infortunatum* young stems influence the nootropic activity in dose dependent manner.

Keywords: *Nootropic, elevated plus maze, Y-maze, diazepam.*

Introduction

According to the world health report (WHO 2001) approximately 450 million people suffer from a mental or behavioral disorder, yet only a small minority of them receives even the most basic treatment. About 12.3% of the global burden of this disease and will rise to 15% by 2020. Memory is ability of the brain to retain and recall previously experienced sensations, impressions, information, ideas and knowledge, which is experience is essential in the process of learning.

Impaired memory is a state in which a person is unable to remember or recall bits of information or behavioral skills. It can be acute or progressive and chronic. In acute there is sudden memory loss can occur many times, it is naturally reversed or progressive and chronic type of memory impairment causes permanent damage the brain and is usually difficult to be reversed. Memory loss is usually caused by brain trauma, stroke, or as a side effect of medications like statin drugs and chemotherapy, brain infections, brain surgery, or electroconvulsive therapy. Alzheimer's disease (AD), Parkinsonism, dementia complex cause extensive damage to the nervous system, including the impairment of learning and memory (1).

Nootropic agents are new class of psychotropic agents which facilitate the effect on integrative function on the central nervous system, particularly on intellectual performance, learning capacity and memory. Piracetam, pramiracetam, aniracetam and choline esterase inhibitors like donepezil are used to improve memory, mood and behavior, but their therapeutic effects is low and most of them have undesirable side effects so there is increasing tendency of people towards traditional medicine. Extensive research on different plants is underway worldwide. Plant extracts have more therapeutic benefits with fewer side effects. They may provide a source of new compounds.

Clerodendrum infortunatum (Lamiaceae) commonly known as Bhat is well known as a medicinal plant because of its wide therapeutic uses. In Ayurveda, the plant is used in postnatal care and to dress fresh wounds, in tumors, cirrhosis, jaundice, in scorpion-sting, and snake-bite, treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation, and epilepsy. *Clerodendrum infortunatum L.* was reported to possess analgesic, free radical scavenging, anticonvulsant and CNS activity, antihyperglycemic, antimicrobial, and repellant activities (2). *Clerodendrum infortunatum* contains flavonoids (3), saponinsand sterols like β-sitosterol present in leaf possess nootropic activity (4, 5). Leaves of plant have been exclusively used in cognitive impairment (6). Further, it is used in the Andaman and Nicobar Island as folk medicine for nootropic activity. However, there is no clear indication in the literature pertinent to nootropic activity of young stems of *Clerodendrum infortunatum*; hence the presence study was planned.

Material and methods

Plant material

Young stems of *Clerodendrum infortunatum* was collected from Andaman and Nicobar Island. The plant material was identified and authenticated by Mr. Lal Ji Singh, Deputy Director, Botanical Survey of India, Andaman & Nicobar regional center (Sl. No. BSI/ANRC/16/09/2017-18/Tech/609).

Preparation of extract

The plant stems were carefully washed with tap water, rinsed with distilled water, and immediately spread over tissue paper for air dried then Shade-dried, powdered, sieved (40 mesh size). Extraction of the plant was carried out by using Soxhlet extraction. 50g of powder was placed inside the Soxhlet extractor and 70% ethanol and 30% water (300 ml) was added to a round bottom flask, which is attached to a Soxhlet extractor and condenser (7). The round bottom flask was heated using heating mantle for 6 hr. at 60^{O}C (8). The extract was evaporated in a rotary evaporator at 50°C under vacuum and store at 2-4°C.

Experimental animals

Swiss albino mice of either sex weighing between 20 – 25 g were selected. The animals were procured from Krupanidhi College of pharmacy, Bangalore. All the animals were housed six per in polypropylene cage containing paddy husk for bedding and maintained under standard laboratory conditions. Prior Institutional Animal Ethical Committee approval bearing reference no. 2017/PCOL/01/KCP/IAEC was obtained for the procurement of animals.

Evaluation of In- vitro antioxidant activity of hydroalcoholic extract of Clerodendrum infortunatum

1. *DPPH Assay*

The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging antioxidant) and is reduced to the DPPHH (Diphenylpicrylhydrazine), results in de colorization (yellow colour) with respect to the number of electrons captured. More the de colorization more is the reducing ability (9).

0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml of different concentration (10, 20, 30, 40, 50 µg/ml). Here, only those extracts are used which are soluble in ethanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temp. for 30 min. then, absorbance was measured at 517 nm. by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid. Lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$

Where A_0= absorbance of control, A_1 = absorbance of test

2. *Reducing power assay*

The reducing power of hydroalcoholic extract of *Clerodendrum infortunatum* was determined by the slight modification of the method of Oyaizu (1986). Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex which has an absorption maximum at 700 nm (10).

Potassiumferricyanide → Potassium ferrocyanide + Ferric chloride + Ferrous chloride

Different concentrations of the hydroalcoholic extract of *Clerodendrum infortunatum* (10-50µg/ml) was added to 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using vortex shaker. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank with reference to standard using UV Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard. Percentage of increase in reducing power was calculated by using following equation:

Increase in reducing power (%) = (At – Ac)/Ac X 100

Where At= absorbance of test, Ac= absorbance of control

Evaluation of nootropic activity of hydroalcoholic extract of clerodendrum infortunatum young stems in mice

1. *Elevated plus maze model*

Swiss albino mice of either sex were divided into five groups. Each group containing six animals and treated as follows:

Group 1: Negative control, given normal diet.
Group 2: Positive control, Diazepam (Amnestic agent); 1mg/kg i.p, dissolved in double distilled water.
Group 3: Standard, Piracetam (200 mg/kg, i.p.) + Diazepam (1 mg/kg).
Group 4: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 200 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).
Group 5: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 400 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg)

Mice were administered with hydroalcoholic extracts of *Clerodendrum infortunatum* and standard drug (Piracetam) orally for 21days.After 60 min of administration of the last dose on the 21st day,

Diazepam (1 mg/kg, i.p.) was administered and positive control group (normal diet) was administered with Diazepam (1 mg/kg, i.p.) on the 21st day. Trials were conducted in the animal models after 1hr. and after 24 hrs. i.e. on the 22nd day. On the 21st day, each mouse was placed at the end of open arm, facing away from central platform and the time took to move from the end of open arm to either of closed arm with all its four legs (Transfer latency, TL) was recorded. If the animal did not enter into one of the enclosed arms within 90 sec, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 sec. TL was recorded on the 21st day for the each animal. The mouse was allowed to explore the maze for another 10 min. and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial (11).

2. *Morris water maze model*

Swiss albino mice of either sex were divided into five groups. Each group containing six animals and treated as follows:

Group 1: Negative control, given normal diet.
Group 2: Positive control, Diazepam (Amnestic agent); 1mg/kg i.p, dissolved in double distilled water.
Group 3: Standard, Piracetam (200 mg/kg, i.p.) + Diazepam (1 mg/kg).
Group 4: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 200 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).
Group 5: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 400 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg)

Mice were administered with hydroalcoholic extracts of *Clerodendrum infortunatum* and standard drug (Piracetam) orally for 21days. After 60 min of administration of the last dose on the 21st day, Diazepam (1 mg/kg, i.p.) was administered and and positive control group (normal diet) was administered with Diazepam (1 mg/kg, i.p.) on the 21st day. Trials were conducted in the animal models after 1hr. and after 24 hrs. i.e. on the 22nd day. Every mouse was subjected to four continuous trials every day with a gap of 5 minutes, during which the mice were permitted to escape onto the hidden platform and to stay there for 20 seconds (12).

3. *Hole board model*

Swiss albino mice of either sex were divided into five groups. Each group containing six animals and treated as follows:

Group 1: Negative control, given normal diet.
Group 2: Positive control, Diazepam (Amnestic agent); 1mg/kg i.p, dissolved in double distilled water.
Group 3: Standard, Piracetam (200 mg/kg, i.p.) + Diazepam (1 mg/kg).
Group 4: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 200 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).
Group 5: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 400 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).

At the end of the treatment on the 21st, food-restricted mice received a single 15-min habituation session to acclimate to the apparatus. During this session, mice had to collect all the food pellets that were placed in every hole (one pellet per hole). The same four holes were baited with a 20-mg food pellet during each trial. The number of errors per trial an animal made each day was used as a measure of cognitive performance. Errors consisted of entering a hole that was never baited (reference-memory error), re-entering a hole (working-memory error). And also task completion time was recorded (13).

4. *Y Maze model*

Swiss albino mice of either sex were divided into five groups. Each group containing six animals and treated as follows:

Group 1: Negative control, given normal diet.
Group 2: Positive control, Diazepam (Amnestic agent); 1mg/kg i.p, dissolved in double distilled water.
Group 3: Standard, Piracetam (200 mg/kg, i.p.) + Diazepam (1 mg/kg).
Group 4: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 200 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).

Group 5: Hydroalcoholic extracts of *Clerodendrum infortunatum* (HECI) 400 mg/kg p.o., dissolved in double distilled water + Diazepam (1 mg/kg).

At the end of the treatment period each mouse was initially placed at the end of arm A, allowed to move freely and the sequence and number of arm entries were recorded manually over 8 min period. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate required that the mice knew which arm they had already visited. The percentage of triads in which all three arms were represented, i.e., ABC, CAB, or BCA but not BAB, was recorded as an 'alternation' to estimate short term memory (14). The % alternation score for each animal was defined as the ratio of the actual number of alternations to the possible number (defined as the total number of arm entries minus two) multiplied by 100 as shown by the following equation:

% alternation = [(number of alternations) / (total arm entries – 2)] x 100

Statistical analysis

All the data were presented as mean ± SEM values. The results of study were subjected to one way Analysis of Variance (ANOVA) followed by Dunnett's test. Values with P<0.05 were considered statistically significant.

Results

Antioxidant activity of HECI by DPPH assay

The young stems extract and the reference antioxidant (Ascorbic acid) promoted an inhibition response on the DPPH radical with increasing concentrations. HECI showed a significant effect in inhibiting DPPH, reaching up to 62.5% and 82.25% for the young stems extract and standard antioxidant (ascorbic acid) respectively at the highest concentration. IC_{50} of the HECI was (39.14µg/ml) while reference was (16.07µg/ml). The results of DPPH radical scavenging activity of *Clerodendrum infortunatum* and the reference antioxidant (Ascorbic acid) are presented in the table no 1.

Antioxidant activity of HECI by reducing power assay

The young stems extract and the reference antioxidant (Ascorbic acid) promoted % inhibition response on the reducing power assay with increasing concentrations. HECI showed a significant effect in % inhibition of reducing power assay, reaching up to 73.85% and 257.47% for the young stems extract and reference antioxidant (ascorbic acid) respectively at the highest concentration. IC_{50} of the HECI was (26.76µg/ml) while standard was (8.72µg/ml). The results of reducing power assay of *Clerodendrum infortunatum* and the reference antioxidant (Ascorbic acid) are presented in the table no 2.

Effect of HECI on plus maze in mice

It is observed that transfer latency (TL) of negative control was found to be 46.5 ± 2.61 and 47.5 ± 2.64 on 21st and 22nd day respectively. TL of positive control was found to be higher on both days when compared to negative control. TL has been decreased in a dose dependent manner in the treatment groups and it was found to decrease it more efficiently than those animals treated with standard group. The detail result are shown in table number 3.

Effect of HECI in water maze in mice

It is observed that escape latency (EL) of negative control was found to be 47.5± 2.71 and 46.5 ± 2.02 on 21st and 22nd day respectively. EL of positive control was found to be higher on both days where as in standard group and the treatment group, EL has been decreased in a dose dependent manner. The detail result are shown in table number 4.

Effect of HECI in hole board test

It is observed that reference memory error, working memory error and task completion time of positive control was found to be higher on both days where as in standard group and the treatment group, reference memory error, working memory error and task completion time has been decreased in a dose dependent manner. The detail result are shown in table number 5, 6 &7.

Effect of HECI in Y – Maze

It is observed that % alternation of negative control was found to be 56.72± 1.09 and 56.3 ± 1.44 on 21st and 22nd day respectively. The % alternation

in both the treatment groups was found to be enhanced when compared to Positive control in a dose dependent manner. The detail result are shown in table number 8.

Discussion

Learning is defined as acquisition of information and skill, and subsequent retention of that information is called memory. Learning and memory can be conceived as both a psychological process as well as a change in synaptic neural connectivity (15). To represent precisely the learning and memory process of human in experimental animals a number of neurobehavioral models are suggested in animals from pharmacological standpoint. A wide variety of physiological and neurotransmitter mechanisms are involved in learning and memory processes. Nootropic drugs belong to the category of psychotropic agents with selective facilitator, learning and memory (16). Nootropic drugs are the potential tools in the study of behavioral and neurobiological basis of learning and memory which may provide critical data for understanding and treating disorders of cognitive dysfunctions. A number of drugs including Piracetam and donepezil are being used for improving memory, mood and behavior but as with all other psychotropic drugs, the resulting side- effects arising from them (17).

The Indian traditional system of medicine offers a number of safe treatments for central nervous system-related disorders such as anxiety and memory loss. These nature-derived treatments are effective and devoid of any untoward effects. In the present study, hydroalcoholic extract of young stem of *Clerodendrum infortunatum* was studies for memory deficits and improving acquisition and memory retention in diazepam- induced amnesia in mice by elevated plus maze, morris water maze, hole board method and Y maze. It is well known that diazepam, a GABA mimetic, induces memory impairment and the inhibition of GABA-B receptor facilitates learning and memory.

The elevated plus maze is a widely accepted model to study nootropic activity. This observation has been strengthened by the finding the shortened in transfer latency on the elevated plus maze model indicating improvement in memory. In the Elevated plus maze test, mice show natural aversion to open and high spaces and therefore, spend more time in enclosed arms (18). The TL might be shortened if the animal had previous experience of entering the open arm and it could be related to memory. TL on day 21st and 22nd are taken as acquisition and retrieval, respectively (19). Diazepam (1mg/kg *i.p.*) prolong TL from the open arm to closed arm. The dose (400mg/kg *o.p.*) of HECI has produced significance in learning behavior and retention of memory and then 200mg/kg *o.p.* as seen in decreased TL on 21st and 22nd day as compare with standard and control.

The Morris water maze is behavioral procedure mostly used with rodents. It is widely used in behavioral neuroscience to study spatial learning memory (20). In this model, a reduction in escape latency (EL) indicated improvement of learning and memory. Diazepam (1 mg/kg, *i.p.*) caused significantly more in EL as compared with the control group. However, the nootropic agent, piracetam (200mg/kg, *i.p*) showed a significant reversal of diazepam induced deficits. HECI treated groups decrease in EL and increase the spatial memory in dose- dependent manner.

In hole board method reference- memory errors, working – memory errors and task completion time was used as a parameter for finding memory enhancing properties. Therefore reduction in errors and task completion time indicating memory improvement in mice (21). Diazepam (1mg/kg *i.p.*) showed increased in reference- memory errors, working- memory errors and task completion time indicated impairment in memory. Whereas HECI (200 and 400 mg/kg *o.p.*) and piracetam (200mg/kg *i.p.*) showed improvement in memory by decreased in errors and task completion time. HECI treated groups showed dose dependent effect.

In Y- maze, increase in % alternation indicated improvement of learning and memory. Diazepam (1 mg/kg, i.p.) caused significantly decrease in % alternation as compared with the negative control group. However, the nootropic agent, piracetam (200mg/kg, *i.p.*) showed a significant reversal of diazepam induced deficits. The % alteration in both the treatment groups was found to be enhanced when compared to Positive control in a dose dependent manner.

Various experiments reported that memory formation involves two types of glutamate receptors: the N-methyl-D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-

isoxazole propionic acid receptor (AMPAR). These receptors embedded on the surface of postsynaptic neurons. AMPARs allow sodium to flow into the postsynaptic cell, resulting in depolarization. NMDARs are permeable to both ionic sodium and calcium. The subsequent influx of ionic calcium into the postsynaptic terminals through the NMDAR activates biochemical cascades that trigger the up-regulation of AMPARs to the membrane while increasing the AMPAR's sensitivity to glutamate and thus strengthen the synapses.

HECI showed radical scavenging activity as measured by DPPH, across concentrations ranging from 10 to 50 µg/mL. The value for maximum % inhibition was noted at a concentration of 50 µg/mL with 62.5% inhibition, whereas that of standard (ascorbic acid) was found to be 86.25%. The radical scavenging activity, using DPPH exhibited the highest radical scavenging activity reveals that HECI contains powerful inhibitor compounds, which act as potential antioxidants and thus scavenge the DPPH radicals to form stable reduced DPPH molecules (22). Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was calculated. Therefore a lower IC_{50} value indicates a higher antioxidant activity (23). IC_{50} value of HECI was found to be 39.14µg/ml and ascorbic acid was 16.07µg/ml.

In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe3+ to Fe2+ by donating an electron. The amount of Fe2+ complex can then be monitored by measuring at 700 nm. Increasing absorbance indicates an increase in reductive ability (24). Since reducing power of a compound serves as a significant indicator of its antioxidant activity (25), HECI and the standard (ascorbic acid) was assayed for the reducing power activity. The absorbance at 700 nm increased with increase in concentration from 10 to 50µg/ml. The value for maximum % increase in reducing power was noted at concentration 50µg/ml with 73.85%, whereas the standard (ascorbic acid) was found to be 257.47%. IC_{50} value of HECI was found to be 39.14µg/ml and ascorbic acid was 16.07µg/ml.

Flavonoids, tannins are one of the important phytoconstituents that neutralize free radicals and toxic metabolites which are involved in loss of memory, concentration and in the pathogenesis of Alzheimer's disease (26).

In the present study hydroalcoholic extract of *Clerodendrum infortunatum* young stems showed nootropic activity which may be due to its antioxidant property and may be due to activation of glutamate receptors N-methyl-D-aspartate receptor (NMDAR) and the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR). Thus, the results suggest that extract of young stems of *Clerodendrum infortunatum* administration produced nootropic activity.

Conclusion

The present study was designed to evaluate the nootropic activity of hydroalcoholic extract of *Clerodendrum infortunatum* of young stems in swiss albino mice using several animal models. Piracetam was taken as standard reference drug. From the results it was concluded that low (200mg/kg) and high (400mg/kg) doses of the extract of *Clerodendrum infortunatum* of young stems showed a significant activity in dose dependent manner.

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Table 1: Antioxidant activity of HECI by DPPH assay

Concentration (µg/ml)	Absorbance of HECI (517nm)	% inhibition	Absorbance of ascorbic acid (517nm)	% inhibition
10	0.214	13.76	0.239	3.62
20	0.194	21. 75	0.182	26.67
30	0.167	32.66	0.141	43.14
40	0.128	48.36	0.076	69.35
50	0.093	62.5	0.034	86.25
IC_{50}	39.14µg/ml		16.07µg/ml	
Control	0.248			

Table 2: Antioxidant activity of HECI by reducing power assay

Concentration (µg/ml)	Absorbance of HECI(700nm)	% increase in reducing power	Absorbance of ascorbic acid (700nm)	% increase in reducing power
10	0.248	15.16	0.336	57.06
20	0.256	19.6	0.449	109.81
30	0.307	43.45	0.530	147.66
40	0.359	67.75	0.655	206.07
50	0.372	73.85	0.765	257.47
IC_{50}	26.76µg/ml		8.72µg/ml	
Control	0.214			

Table 3: Effect of HECI on plus maze in mice

Sl. No.	Experimental group	Transfer latency in second (MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	46.5±2.61	47.5± 2.64
2	Positive control; Diazepam1mg/kg *i.p*	101.3±6.19*	108.7±4.47*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam (1mg/kg *i.p.)*	32.5±3.08	28.5±2.89*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg *i.p.*)	32.83±0.79	26.6±2.48*
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg *i.p.*)	23.5±1.99*	18.17±1.01*

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant.

Table 4: Effect of HECI in water maze in mice

Sl. No.	Experimental group	Escape latency (sec) (MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	47.5±2.71	46.5±2.02
2	Positive control; Diazepam1mg/kg *i.p*	68±3.42*	73.83±3.97*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam (1mg/kg)	39±2.06	28.5±2.43*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg)	36.17±2.08	25.67±2.61*
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg)	21.83±2.62*	15.17±1.90*

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant.

Table 5: Effect of HECI in hole board test (Reference memory)

Sl. No.	Experimental group	Reference memory error (MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	31.83±2.79	32.17±1.04
2	Positive control; Diazepam1mg/kg *i.p*	44.83±1.30*	49.83±0.94*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam (1mg/kg)	24.67±2.44	19.5±0.88*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg)	20.33±1.28*	15.67±1.66*
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg)	10.67±0.84*	6.66±0.76*

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant.

Table 6: Effect of HECI in hole board test (Working memory)

Sl. No.	Experimental group	Working memory error (MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	26.83±0.90	25.67±1.05
2	Positive control; Diazepam1mg/kg *i.p*	38.5±1.99*	45.5±1.60*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam(1mg/kg)	19.33±0.76*	15.67±0.55*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg)	12.83±1.66*	9.66±2.18*
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg)	7.83±0.79*	3.83±0.60*

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant

Table 7:- Effect of task completion time of HECI in hole board test

Sl. No.	Experimental group	Task completion time (sec) (MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	262.7±14.17	258.8±13.5
2	Positive control; Diazepam1mg/kg *i.p*	343.5±3.32*	355.2±4.22*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam(1mg/kg)	215.5±17.05*	202.7±14.76*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg)	204.7±15.9*	196±16.55*
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg)	174.8±8.03*	166.5±6.84*

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant.

Table 8: Effect of HECI on % Alternations in mice by using Y – Maze

Sl. No.	Experimental group	% Alteration(MEAN ± SEM)	
		Day 21	Day 22
1	Negative control; Normal diet.	56.72±1.09	56.3±1.44
2	Positive control; Diazepam1mg/kg *i.p*	26.44±0.71*	22.31±0.63*
3	Standard; Piracetam (200 mg/kg, *i.p.*) + Diazepam (1mg/kg *i.p.*)	71.29±1.53*	74.91±2.64*
4	HECI; 200 mg/kg *p.o.*, dissolved in double distilled water + Diazepam (1mg/kg *i.p.*)	48.39±0.89*	51.87±1.12
5	HECI 400 mg/kg *p.o.*, dissolved in double Distilled water + Diazepam (1mg/kg *i.p.*)	55.94±1.21	60.19±0.75

Values are expressed as (Mean ± SEM), n= 6. Statistically analyzed by one- way analysis of variance (ANOVA) followed by Dunnett's test. All groups were compared with Negative control group. P<0.05 is considered as statistically significant