EVALUATION OF *HELIANTHUS ANNUUS* SEED EXTRACT FOR NOOTROPIC ACTIVITY

Ms. Ramya D[1], Mrs. Zeena Fernandes[1*]

[1]NGSM Institute of Pharmaceutical Sciences, Department of Pharmacology, Deralakatte, Mangalore-574018

Abstract

Objective

The present study was undertaken to carry out preliminary qualitative phytochemical screening and evaluate effect of ethanolic seed extract of *Helianthus annuus* on learning and memory of experimental animals.

Method

The phytochemical constituents were extracted by the maceration process using ethanol as solvent. The nootropic activity was assessed by Elevated plus maze, Y-maze, and Estimation of brain AchE activity after subsequent oral administration of the test extract for 7 days.

Result and Discussion

Preliminary qualitative phytochemical screening revealed the presence of phenols, alkaloids, flavonoids, triterpenoids, steroids, carbohydrates, and proteins. On pre-treatment with the test extract exhibited significant improvement in learning and memory activity in scopolamine-induced amnesic animals in a dose-dependent manner.

Conclusion

From the results obtained it can be concluded that ethanolic extract of *Helianthus annuus* seed has nootropic activity in a dose-dependent manner. However, there is a need for further investigation on the mechanism of action and result of the test extract on biochemical changes in the brain.

Keywords: *Helianthus annuus*, Cognitive disorders, Nootropics

Corresponding author

Mrs. Zeena Fernandes

Introduction: Learning is a process of gaining knowledge and, memory is its retrieval. Cognitive deficits have been identified as a neurological disorder associated with many

psychiatric and neurodegenerative states [1,2,3]. Alzheimer's disease is a progressive neurodegenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes, and ultimately death. It is a long-term progressive disabling organic brain disorder that is characterized by disturbance of multiple cortical functions such as memory, judgment, comprehension, orientation, language, and learning capacity[4]. There are currently many experimental models available for assessing agents that affect learning and memory processes.

Helianthus annuus is a coarse, robust, and erect 1-3 meter high annual plant which grows widely. It produces greyish-green or black seeds with black and white stripes which are enclosed in black or grey colored shells. Seeds contain diterpenes, oleic acid, monoterpenes (alpha-pinene, sabinene), triacylglycerol, glycosides (cyanogenic and cardiac), saponins, alkaloids, flavonoids, 45 to 48% fixed oil, tannins, polyphenols. *Helianthus annuus* is a medication for cough, bronchitis, fever, flu, diarrhea, fractures, dysuria, snakebite, rheumatism, urogenital ailments, wounds, epistaxis, dysentery, menorrhagia[5]. Based on the review of literature studies the plant *Helianthus annuus* has reported extensive work related to CNS and antioxidant activity and has great food value potential. This plant contains active constituents which may improve memory function and learning ability but the scientific reports are not available. This plant is been used as the dietary source in Alzheimer's disease, [6] but the exact role of seed extract of *Helianthus annuus* as a nootropic has not been reported anywhere, hence the study was undertaken to evaluate its role in the improvement of memory in experimental animals.

Material and Methods:

Plant material: The seeds of *Helianthus annuus* were fetched from the local areas of Mangalore, Karnataka. The seeds were washed, dried, and powdered. It was extracted with ethanol by using a Maceration process.

Animals: Albino Wistar rat of either sex, 4-6 weeks, weighing 150-200g were obtained from NUCARE animal house, Deralakatte, Mangalore. The animals were housed in cages, grouped together, and held under normal laboratory conditions (temperature 25 ± 2^0C,12h/12h light/dark cycle). They had unrestricted access to a normal dry pellet diet as well as unlimited water. The experiment was carried out in accordance with the guidelines proposed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drug treatment: In the present study, the rats were separated into six groups (n=6) to test several memory models. For 7 days, rats in various groups were fed daily doses of *Helianthus annuus*

seed extract (100mg/kg and 400mg/kg). On the 7th day, 90 minutes after the last feed, these rats were subjected to a training session using an elevated plus-maze or a Y maze. After 24 hours, (8th day), the learnt task's retention was noted. Scopolamine (1mg/kg, i.p.) was used to cause amnesia in different groups of rats (interoceptive model) on the 7th day, 90 minutes after the last feed. After 45 minutes of scopolamine injection, the animals were subjected to the training (on the 7th day). After 24 hours, the retention (memory) was assessed (on the 8th day). Piracetam (400mg/kg, i.p.), a well-known nootropic agent, was injected into positive control group of animals for seven days. The control group's animals were fed a standard wheat flour diet (3g/animal/day) for 7 days.

Elevated plus-maze: Elevated plus maze consists of two open arms and two closed arms with similar dimensions ,50cm (length) x10 cm (width) x40 cm(height) opposite to each other. From the floor, the entire maze is raised to a height of 50cm. Each animal will be placed at the edge of the open arm, facing against the central area, on the first day. The time it takes for the animal to transfer with all its four legs into one of the covered arms is Initial Transfer Latency (ITL). On the first day, ITL will be noted. The animal is pushed into one of the covered arms if it doesn't enter the covered arm within 90 seconds and the ITL will be considered as 90 seconds. The animal is given 10 seconds to explore the maze before being returned to its home cage. On the second day, 24 hours after the first day trial, memory retention will be assessed (retention transfer latency, RTL). The transfer latency evaluated on the starting 2 days of the trial will be used to measure acquisition (learning) and retention (memory) The inflexion ratio (IR) will be derived from these using the following formula:

IR = L0 – L1 / L1

IR = Inflexion Ratio, L0 = Initial transfer latency(sec) , L1 = Retention transfer latency (sec). A reduction in transfer latency on subsequent maze exposures was interpreted as a sign of good retention [7].

Y-maze: The spontaneous changes in the behavior of rats are observed in Y-maze. Temporary assessment and error scoring are the primary criteria for determining drug effects administered after training [8]. The Y-maze is a horizontal maze with symmetrically inclined arms (3 arms) at 120 angles from one another (40cm long, 3cm wide, 12cm high walls). Dark opaque polyvinyl plastic is used to build the walls and floor of the maze. The three arms, start arm (A), the reward arm (B), and the other arm (C) are all built at random. Each rat will be placed at the edge of arm A, free to move around, and the sequence and number of arm entries will be noted manually over

an 8-minute span. The maze appears to be discovered on a regular basis, with rats visiting each arm in turn. The ability to alternate needs the rats to know which arm they have already entered. To evaluate short-term memory, the percentage of triads with all three arms are represented, i.e., BCA, CAB or ABC, but not BCB, will be reported as a "alternation". Between tests, the arms are sprayed with water to eliminate residue and odors [9]. The following equation displays the percent alternation score for each species:

"% Alternation = [(Number of alternations) / (Total arm entries - 2)] X 100"

Locomotor activity was determined in terms of arm entries[9]

Collection of brain samples

The animals were anesthetized, soon after the experiment and the animals were sacrificed by cervical dislocation. The skull was cut open and the whole brain was removed carefully which was then weighed, placed on an ice water, washed with ice-cold saline, and homogenized. For homogenization, about 20mg of tissue per ml of phosphate buffer (pH 8.0, 0.1M) was used, which was then put in a Potter-Elvehjem homogenizer. The centrifugation of the homogenate was done for 10 minutes at 3000 rpm, and the supernatant liquid (cloudy) was used to determine the function of Acetylcholine esterase [10].

Estimation of Brain acetylcholinesterase activity

100µl of DTNB was transferred to a cuvette containing 0.4ml of rat brain homogenate and 2.6ml of phosphate buffer (0.1M, pH 8.0). By bubbling air, the contents of the cuvette were thoroughly mixed, and absorbance was recorded at 412nm. A shift in absorbance per minute was observed after 20µl of substrate, acetylthiocholine iodide, was added. The reaction rate was calculated by the equation below[11].

$$R=5.74\times10^{-4} \times A/C_0$$

Statistical analysis

GraphPad Prism software version 8.4 was used for the statistical study, and the findings were compared using one-way ANOVA and Tukey's Comparison Test. Statistical significance was described as a p value less than 0.05 and 0.001.

Results

Effect on Transfer latency (TL) using Elevated plus maze

The result showed that those groups of animals in which amnesia was caused by scopolamine (1mg/kg b.w. i.p.) after pre-treatment with seed extract of *Helianthus annuus* (100mg/kg, 400mg/kg) for seven days showed significant ($p<0.001$) reduction in ITL, when compared to the normal group.

The group treated with scopolamine has shown a significant ($p<0.001$) increase in ITL when compared to the normal group which shows the ability of the drug to disturb the cognitive functions.

The animals which were pre-treated with standard drug Piracetam and plant extract (100mg/kg and 400mg/kg) followed by Scopolamine exhibited significant ($p<0.001$) reduction in the RTL when compared to normal groups. This shows the ability of the extract to overcome memory deficiency produced due to scopolamine. Thus the results indicate that the *Helianthus annuus* seed extract possesses significant dose-dependent nootropic potential in both, normal and memory deficit animals.

Effect on Number of arm entries using Y maze

The groups in which the amnesia was induced by scopolamine (1mg/kg i.p) after pre-treatment with the extract (100mg/kg and 400mg/kg) for seven days successively showed a significant ($p<0.001$) decrease in the number of arm entries when compared to the normal group.

The scopolamine treated group exhibited a significant ($p<0.05$) rise in the number of arm entries when compared to the normal group, which shows the ability of the drug in disturbing the cognitive process. The test extracts showed almost comparable results as that of the Standard (Piracetam) treated group. The above results demonstrate the ability of the extract to improve cognitive dysfunction.

Effect of Percentage alteration using Y maze

The group of animals in which the amnesia was caused by giving scopolamine (1mg/kg i.p) on pre-treatment with extract (100mg/kg, 400mg/kg) successively for 7 days, showed significant ($p<0.001$), almost comparable results with normal groups. % alteration increased in all the extract and Piracetam treated group when compared to the normal group.

The scopolamine treated group resulted a significant ($p<0.05$) reduction in the % alterations when compared to the normal group, which shows the capability of the drug to produce

disturbance in cognition. The groups of animals treated with the test extracts showed almost comparable results as that of the Standard (Piracetam) treated group. The above results demonstrate the ability of the extract to improve the status of cognitive dysfunction.

Effect on whole-brain Acetylcholine esterase activity

The result of the above study suggested that there was a significant deviation of AchE in the whole brain upon treatment with scopolamine (1mg/Kg i.p). The amnesia-induced group exhibited a significant, ($p<0.05$) dose-dependent decline in AchE on a group of animals pre-treated with *Helianthus annus* seed extract (100mg/kg, 400mg/kg) successively for 7 days when compared with the scopolamine challenged group.

The scopolamine-treated group showed a significant increase in AchE levels when compared to the normal group. The study result reveals the acetylcholine esterase inhibitory activity of the extract *Helianthus annuus* seed at various dose levels.

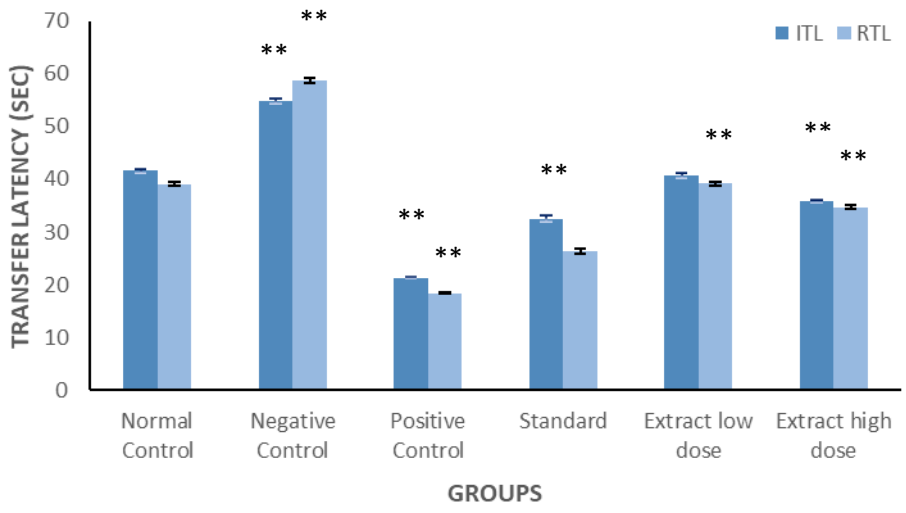

Fig 1. Effect of *Helianthus annuus* seed extract (100mg/kg and 400mg/kg) on transfer latency (s) using Elevated plus-maze.

**Indicates $p<0.001$ compared to control group

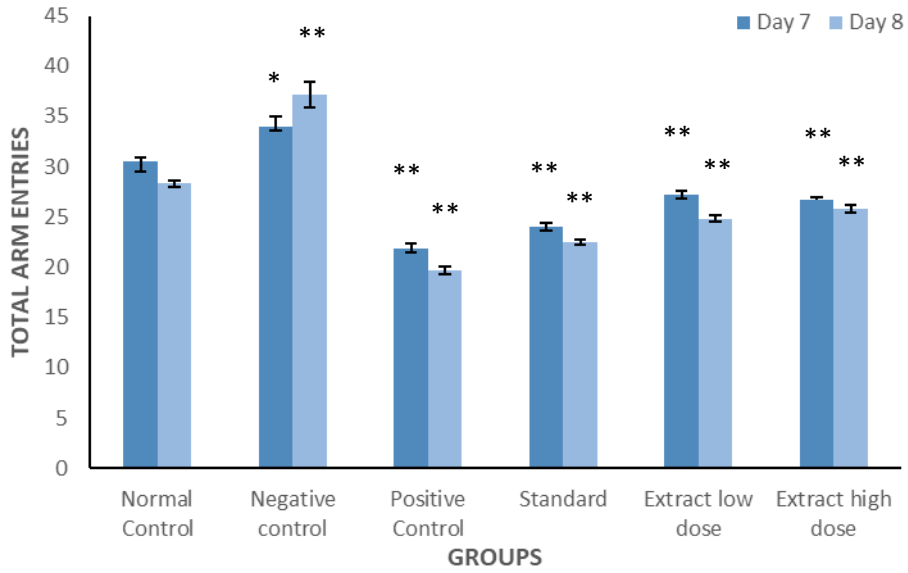

Fig 2. Effect of *Helianthus annuus* seed extract (100mg/kg and 400mg/kg) on no. of arm entries using Y-maze

*Indicates $p<0.05$ compared to control group

**Indicates $p<0.001$ compared to control group

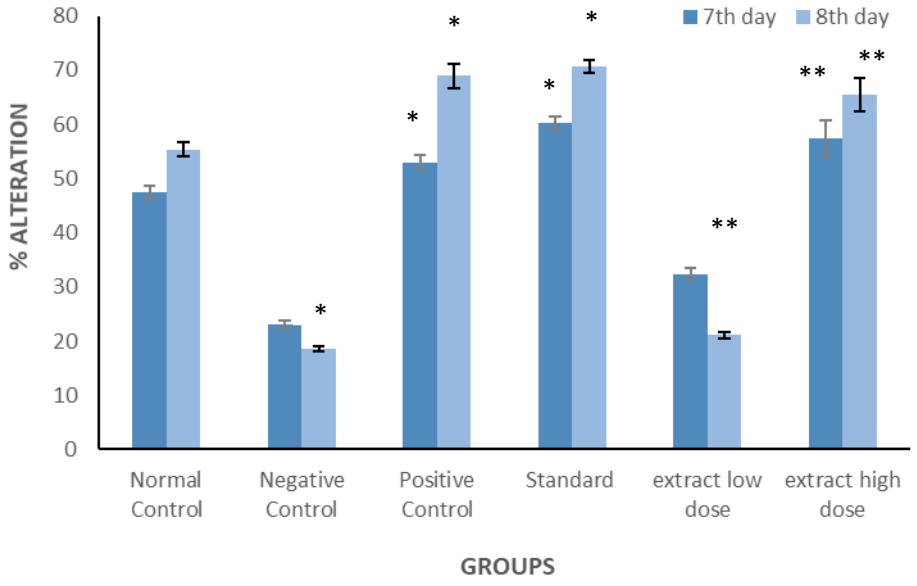

Fig 3. Effect of *Helianthus annuus* seed extract (100mg/kg and 400mg/kg) on % alteration using Y-maze

*Indicates p<0.05 compared to control group

**Indicates p<0.01 compared to control group

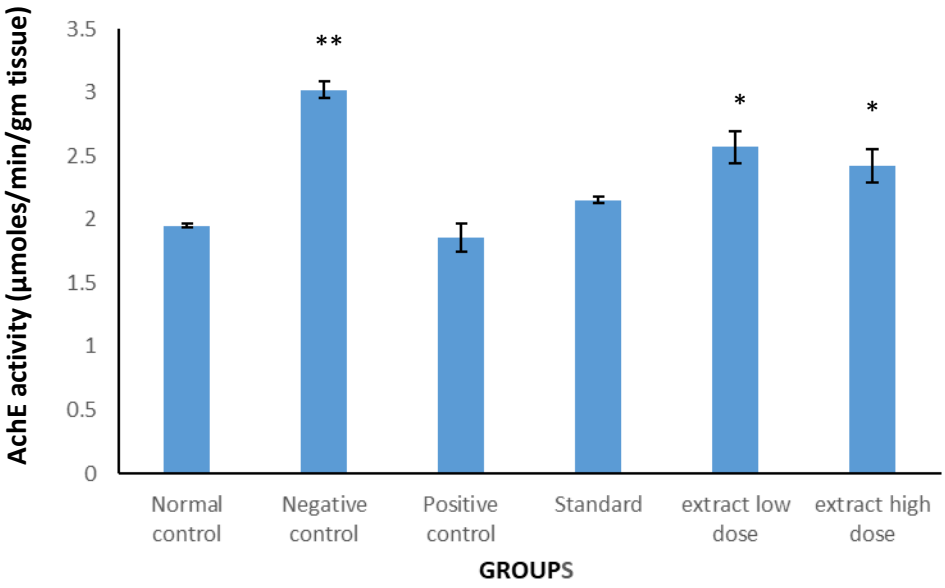

Fig 4. Effect of *Helianthus annus* seed extract (100mg/kg and 400mg/kg) on brain Acetylcholine esterase activity

*Indicates p<0.05 compared to control group

**Indicates p<0.01 compared to control group

Discussion: Cognition is about the processes behind human thinking and experiences. Cognition refers to "a process of identifying, selecting, interpreting, storing, and using the information to make sense of and interact with the physical and social world, to conduct one's everyday activities, and to plan and enact the course of one's occupational life".

Several plants have been reported to possess nootropic activity. There are many medicinal plants in Indian medicine that are said to help with learning, memory and intellect. Plants like *Withania somnifera ,Bacopa monniera, Azadirachta indica,* as well as *Ocimum sanctum,* have been investigated for their effect on cognitive functions [12].

In the present study, the nootropic potential of *Helianthus annuus* seed was evaluated using three different *in-vivo* models i.e. Elevated plus maze, Y-maze, and one *in-vitro* model to determine the AchE activity was carried out. Piracetam was used as a standard drug and scopolamine was used to induce amnesia in rats. The phytochemical screening revealed that the ethanolic seed extract of *Helianthus annuus* possesses flavonoids, triterpenoids, alkaloids, carbohydrates, tannins, steroids, and proteins.

The data obtained from the elevated plus-maze reveals the ability of the ethanolic seed extract of *Helianthus annuus* in enhancing the nootropic potential, which showed almost comparable results with that of the standard drug Piracetam. There was a significant reduction in ITL, which was 40.33 and 35.88 seconds in groups treated with 100 and 400mg/kg doses of extract respectively, when compared to that of the scopolamine treated group (54.75 sec). A similar reduction in RTL values was also observed (39.17and 34.71 seconds at 100 and 400mg/kg doses of extract) in scopolamine challenged groups. These findings justify the nootropic potential of *Helianthus annuus* seed extract.

The finding obtained from the Y maze model also revealed the increase in nootropic potency of the ethanolic seed extract of *Helianthus annuus* in a dose-dependent manner. The result of this study revealed a decrease in the number of arm entries (25.83 and 24.83 at doses of 100 and 400 mg/kg) on day 8 and an increase in % alterations (21.15% at 65.38% at 100 and 400 mg/kg of extract) on day 8 against animals challenged with scopolamine. This study also showed comparable results with that of Piracetam in a dose-dependent manner. These findings gave us promising scientific evidence to establish the nootropic potential of seed extract of *Helianthus annuus.*

The study of *Helianthus annuus* seed extract on AchE was carried out using Ellman's method which was analyzed with a UV spectrophotometer. The hypothesis behind this study was that reduced activity of AchE might reduce the breakdown, thus increasing the acetylcholine available in the brain. This might play a significant role in the enhancement of cholinergic-based cognition.

The results showed a significant decline in AchE levels in the scopolamine challenged group pre-treated with extract (2.57 and 2.42 μmoles/min/gm tissue at 100 and 400 mg/kg doses). The group treated with extract also showed comparable results with that of the Standard (2.15 μmoles/min/gm tissue). The ethanolic seed extract of *Helianthus annuus* exhibited AchE inhibitory activity in a dose-dependent manner.

Based on the results obtained from all the above studies, it can be concluded that the ethanolic seed extract of *Helianthus annuus* showed significant nootropic potential against animals induced by amnesia using scopolamine.

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Authors:

First Author- Ms. Ramya D,
Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Deralakatte, Mangalore-574018, Karnataka, India.

Second Author- Mrs. Zeena Fernandes
Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Deralakatte, Mangalore-574018, Karnataka, India.

Corresponding Author*
Mrs. Zeena Fernandes
Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences,
Nitte (Deemed to be University), Deralakatte,
Mangalore-574018, Karnataka, India.