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LEARNING AND MEMORY ACTIVITY OF *CUCURBITA PEPO* AGAINST DIAZEPAM INDUCED AMNESIA AND ETHANOL INDUCED COGNITIVE IMPAIRMENT IN RODENTS BY USING MORRIS WATER MAZE (MWM)

Kakunuri Lakshmi *, Adapa Sowmya, Bathina Vara Lakshmi, Tejavathu Vijaya Lakshmi, Bhukya Ratna Madhuri, Peddarapu Sandhyarani, Sandipamu Savitri, Somavarapu Pravallika, Guda Swathi

Sri Siddhartha Pharmacy College, Nuzvid, Krishna District-521201

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Abstract:

The Cucurbitaceae family, also referred as cucurbits form a very large group with approximately 130 genera and 800 species and can be cultivated in warmer region of worldwide and make popular food crop plants some of these species include squashes, pumpkins, melons and gourds. Pumpkin belongs to the genus Cucurbita and the family Cucurbitaceae. The objective of this study was to identify the potential of Cucurbita pepo as protective and therapeutic agent against Alzheimer's disease. The learning and memory enhancing activity of Cucurbita pepo seed extract were investigated in rats by using the ethanol induced cognitive impairment and diazepam induced amnesia and its effects on learning and memory were examined by using Morris water maze (MWM) test. For Morris water maze test all groups showed significantly (P value is < 0.01 & < 0.05) decreases transfer latency time at all periods as compared to ethanol and diazepam inducing group Therefore seed extracts of Cucurbita pepo exhibited significantly learning and memory activity in Alzheimer's disease.

KEY WORDS: *Cucurbita pepo, learning and memory activity, Morris water maze and Alzheimer's disease.*

Corresponding author:

Kakunuri Lakshmi,

Sri Siddhartha Pharmacy College,
Nuzvid, Krishna District-521201

QR code

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INTRODUCTION:

Alois Alzheimer and Augusto D

The German psychiatrist and neuropathologist Dr. Alois Alzheimer is credited with describing for the first time a dementing condition which later became known as AD. In his landmark 1906 conference lecture and a subsequent 1907 article, Alzheimer described the case of Auguste D, a 51-year-old woman with a 'peculiar disease of the cerebral cortex,' who had presented with progressive memory and language impairment, disorientation, behavioural symptoms (hallucinations, delusions, paranoia), and psychosocial impairment.. Remarkably, many of the clinical observations and pathological findings that Alzheimer described more than a century ago continue to remain central to our understanding of AD today [1]. Alzheimer's disease is a form of brain degeneration in which abnormal particles called neurofibrillary tangles and neuritic plaques form in the brain and destroy healthy neurons (brain cells). These abnormalities tend to settle in brain areas that control the ability to learn a new fact and remember it 30 minutes, or a day later, a skill we refer to as "memory"[2].

Cucurbita pepo is one of the oldest known cultivated species, with Mexican archaeological evidence from 7000 BC. Thus, it was widely cultivated by indigenous peoples throughout Mexico, Central, and North America before the arrival of Europeans. This plant is native of Norther Mexico and south western and eastern USA. This family have medicinal and nutritional benefits. The immature fruits are consumed as a vegetable. The mature fruit is sweet and used to make confectionery, beverages are roasted, or cooked and can be incorporated into baked goods. The seeds, rich in oil, also are used in Mexico, with honey to prepare desserts known as palanquetas. Flower buds and flowers are also edible in Mexico to prepare quesadillas. Some fruit varieties are used with decorative purposes in Halloweenrty [3].

Figure -1: *Cucurbita pepo* seeds

MATERIALS AND METHODS:

Collection of plant material:

The seeds of *Cucurbita pepo* was identified and purchased from local market of Nuzvid.

Preparation of extract:

The *Cucurbita pepo seed* are powdered in a mechanical grinder. The collected powder was successively, extracted with water & ethanol by using Soxhlet apparatus. The extraction was carried out for 72 hrs at a temp not exceeding the boiling point of the solvent. Excess solvent was removed by the solvent evaporation to obtained the dry weight of the plant extracts.

Experimental animals:

SD rats of either sex (200-300g) were maintained for 7 days in the animal house of Chalapathi Institute of Pharmaceutical Sciences, Guntur under standard conditions temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. 5 rats/group were used in all sets of experiments.

Ethical approval:
All the protocols were approved by Institutional Animal Ethical Committee (IAEC) and conducted according to Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) registered no: 1048/PO/Re/S/07/CPCSEA at Department of Pharmacology, Chalapathi Institute of Pharmaceutical Sciences, and Guntur.

Drugs and chemicals:
For learning and memory activity in Alzheimer's disease:
Inducing agents -Ethanol - 60% (Ethanol was prepared as a 60% solution in distilled water and administered i.p at a dose of 2.5 mg/ kg) & Diazepam- was diluted in normal saline and administered i.p at a dose of (1 mg/kg).

Standard drug- Donepezil hydrochloride (2.5 mg/kg, p.o)

Investigation of learning and memory activity in Alzheimer's model by using Morris water maze (MWM):
Morris water maze (MWM): To assess hipocampal dependent spatial learning and memory, all rats were trained in a standard Morris water maze task (Morris et al., 1982; Stackman et al., 2002) [4]. Maze consisted of large circular pool (75cm &30cm) filled with water at a depth of 20cm. The pool was divided into four quadrants. A circular platform was placed at the centre of one quadrant. The rats performed four trials per day for four consecutive days. In the swimming trials, each individual rat was released gently into the water at a randomly chosen quadrant. The rats swim and learned how to find the hidden platform within 60 s. After reaching the platform rat was allowed to stay on the platform for 15 s and was then taken back into the cage. The rats were placed on the platform by hand for 15 s, if they could not escape to the platform within 60 s by themselves, and their escape latency was accepted as 60 s. During the inter-trial intervals, animals were kept in a dry home cage for 60 s. The time to reach the platform (latency) was recorded. 24h after the last day of training, subjects were tested on a probe trial, during which the escape platform was removed and the time spent in the correct quadrant was measured for a 60 s trial [5].

Table-1: The sequence of trials during the study period of MWM test [6]

1st day	2nd day	3rd day	4th day
Q1	Q2	Q3	Q4
Q2	Q3	Q4	Q1
Q3	Q4	Q1	Q2
Q4	Q1	Q2	Q3

Figure-2: MWM

Evaluation parameters:

- Transfer latency in sec

Ethanol- induced cognitive impairment: [6]
Ethanol is neurotoxin that able to alter behavioural and cognitive performance in experimental animals in addition to humans. It mainly impairs hippocampus-dependent learning and memory functions. The mechanism of ethanol-induced neurotoxicity is not well understood. Several studies show that free-radical mediated oxidative stress play an imperative role. The brain is extremely susceptible to oxidative stress due to high level of polyunsaturated fatty acids (PUFAs) and catecholamines, large amounts of oxygen (O_2) in relatively small mass and in conjunction with low antioxidant activities. Furthermore, certain regions of the central nervous system (CNS), especially hippocampus and cerebellum, may be more sensitive to oxidative stress because of their low endogenous antioxidant, in relation to other brain regions. Study showed that acetaldehyde dehydrogenase is responsible for the generation of reactive oxygen species (ROS) by converting cytotoxic acetaldehyde produced from oxidation of ethanol to acetate. It has been confirmed that ethanol induces the synthesis of CYP2E1 that lead to oxidative stress. It also increases the ratio of NADH/NAD, responsible for reduction of ferric ion (Fe3+) to ferrous ion (Fe2+)) which causes lipid peroxidation by generating hydroxyl radical.

Selection of dose and treatment period:
The learning and memory enhancing activity of the aqueous and ethanolic seed extracts of *Cucurbita pepo* was investigated using the ethanol- induced cognitive impairment [Ethanol (60%) is used to induce dementia like condition in the dose 2.5 mg/kg administered i.p for 15 days) [5]. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group- Ethanol (2.5 mg/kg was administered i.p for 15 days).

Group II: Standard Group -Donepezil hydrochloride [6] (2.5 mg/kg was administered orally for 15days) + Ethanol.

Group III: Test-I -Aqueous seed extract of *Cucurbita pepo* [CPAE- 100mg/kg was administered orally for 15days) + Ethanol.

Group IV: Test -II -Ethanolic seed extract of *Cucurbita pepo* [CPEE- 100mg/kg was administered orally for 15 days) + Ethanol.

Diazepam induced amnesia:
Diazepam 1mg/kg, i.p was administered to rats and TL was noted after 45 min of injection on 8th day and after 24hrs. Extracts and standard Donepezil hydrochloride were administered for successive 8 days. After 60 min of administration of the last dose on 8th day, Diazepam 1mg/kg i.p was administered. TL was noted after 45 min administration of diazepam and after 24 hrs [7].

Selection of dose and treatment period:
The learning and memory enhancing activity of the aqueous and Ethanolic seed extracts of *Cucurbita pepo* was investigated using the [Diazepam is used to induce amnesia like condition in the dose 1 mg/kg administered i.p for 8 days) [7]. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group-Diazepam (1mg/kg was administered i.p for 8 days).

Group II: Standard Group-Donepezil hydrochloride [6] (2.5 mg/kg was administered orally for 8days).

Group III: Test-I- Aqueous seed extract of *Cucurbita pepo*[CPAE- 100mg/kg was administered orally for 8days).

Group IV: Test –II- Ethanolic seed extract of *Cucurbita pepo* [CPEE- 100mg/kg was administered orally for 8 days).

Statistical analysis:
The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

RESULTS AND DISCUSSION:

Effect of seed extracts of *Cucurbita pepo* on behavioural parameters i.e. MWM: Animals treated with ethanol [2.5 mg/kg] alone for15 days showed a increase in transfer latency in seconds on 1st, 7th& 15th days as well as diazepam [1mg/kg] alone for 8 days showed a increase in transfer latency in seconds on 8th day & after 24 hrs i.e. 9th day.

Table -2: Effect of seed extracts of *Cucurbita pepo* on transfer latency (ethanol- induced cognitive impairment)

S.No.	Group	Treatment	Transfer latency (In seconds)		
			1st DAY	7th DAY	15th DAY
1.	I	**Ethanol**	24.2±2.35	27.6±3.89	27.8±4.04
2.	II	**Standard+ethanol**	5.6±1.46	4.6±1.20	2.6±0.67
3.	III	**CPAE+ ethanol**	9.2±0.58	9.2±2.31	4.8±0.86
4.	IV	**CPEE+ ethanol**	5.8±1.62	4.4±1.54	2.6±0.75

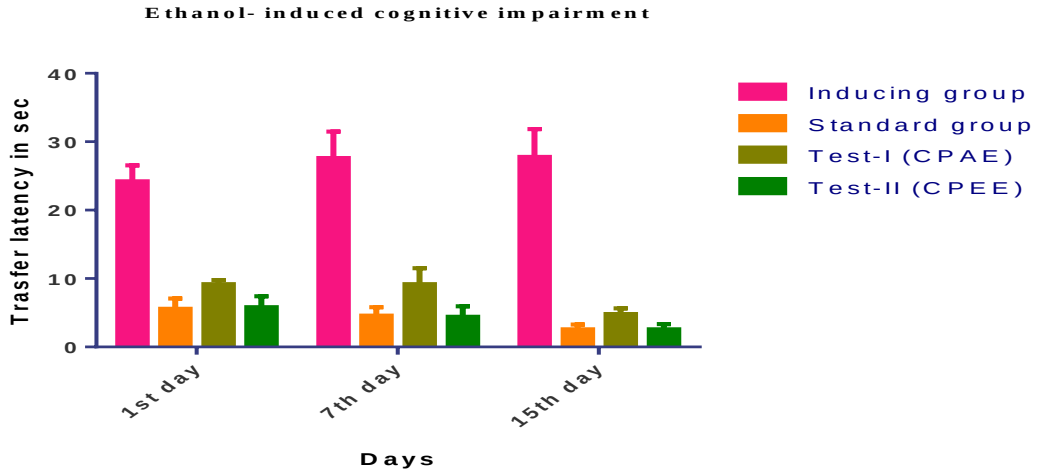

Figure-3: Effect of seed extracts of *Cucurbita pepo* on ethanol- induced cognitive impairment. Values are expressed as Mean ± SEM, P < 0.01 vs. control (n = 5 animals).

Table-3: Effect of seed extracts of *Cucurbita pepo* on transfer latency (diazepam induced amnesia)

S.No.	Group	Treatment	Transfer latency (In seconds)	
			8TH DAY	After 24 hours (i.e.9TH DAY)
1.	I	**Diazepam**	26.2±3.89	23±3.38
2.	II	**Standard+ diazepam**	4.4±0.81	2.0±0.32
3.	III	**CPAE+ diazepam**	9.6±2.40	6.6±1.12
4.	IV	**CPEE+ diazepam**	5.6±0.40	2.2±0.80

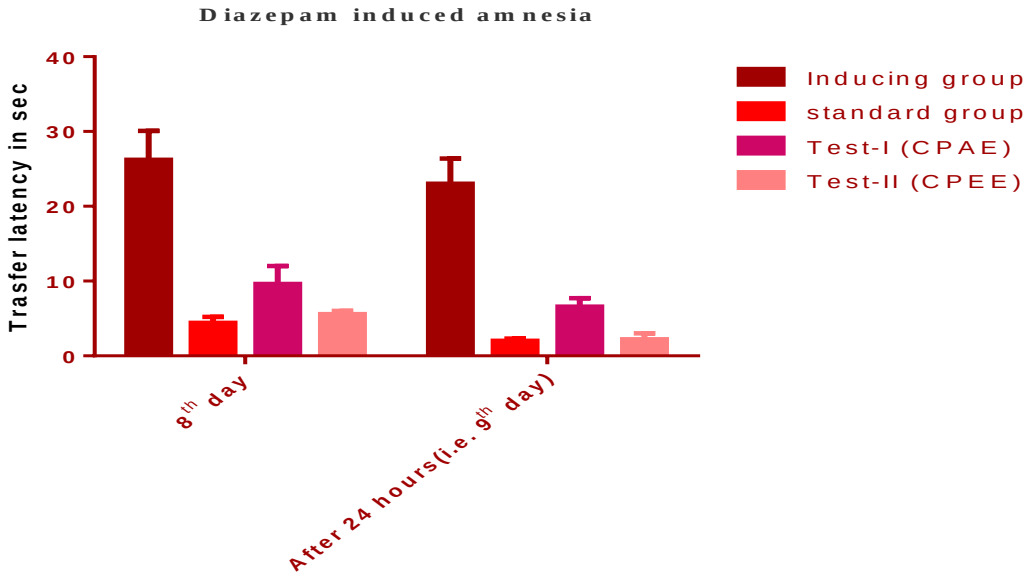

Figure-4: Effect of seed extracts of *Cucurbita pepo* on diazepam induced amnesia. Values are expressed as Mean ± SEM, P < 0.007 vs. control (n = 5 animals).

CONCLUSION:

Cucurbita pepo showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol- induced cognitive impairment & diazepam induced amnesia in rats. *Cucurbita pepo* ethanolic extract showed comparatively significant effect exerted to standard drug donepezil hydrochloride in the finding of transfer latency in sec (i.e. learning and memory activity). Transfer latency was recorded after administration of ethanol & diazepam at different days and graphs were ploted according to the results obtained. This effect is attributed to its ability to improve the levels of the acetylcholine that are decreased in the Alzheimer's disease.

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