

Original Research Article

Phytochemical Screening and Learning and Memory Activity of *Cucurbita pepo* against Ethanol Induced Cognitive Impairment in Rodents by Using 8-ARM Radial Maze [8-ARM]

Kakunuri Lakshmi^{1*}, Adapa Sowmya², Bathina Vara Lakshmi², Tejavathu Vijaya Lakshmi², Bhukya Ratna Madhuri², Peddarapu Sandhyarani², Puranam Supriya², Pilli Anusha², Shaik Sabeeha²

¹Assistant Professor in Department of Pharmacology, Sri Siddhartha Pharmacy College, Nuzvid, Krishna District-521201, Andhra Pradesh, India

²Sri Siddhartha Pharmacy College, Nuzvid, Krishna District-521201, Andhra Pradesh, India

***Corresponding Author:** Kakunuri Lakshmi

Assistant Professor in Department of Pharmacology, Sri Siddhartha Pharmacy College, Nuzvid, Krishna District-521201, Andhra Pradesh, India

Article History

Received: 28.03.2022

Accepted: 06.05.2022

Published: 09.05.2022

Abstract: The present study was undertaken to investigate the learning and memory enhancing activity of *Cucurbita pepo* seed extract in rats by using the ethanol induced cognitive impairment and its effects were examined by using 8-radial arm maze (8-RAM) test. For radial arm maze test all groups showed significantly (P value is < 0.0001 & < 0.001) decreased in the time taken to reach the paired arm and number of entries in baited arm and non-baited arm as compared to ethanol inducing group. Therefore seed extracts of *Cucurbita pepo* exhibited significantly learning and memory activity in Alzheimer's disease.

Keywords: *Cucurbita pepo*, learning and memory activity, radial arm maze and Alzheimer's disease.

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 Halloween [3].

Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

CITATION: Kakunuri Lakshmi *et al* (2022). Phytochemical Screening and Learning and Memory Activity of *Cucurbita Pepo* against Ethanol Induced Cognitive Impairment in Rodents by Using 8-ARM Radial Maze [8-ARM]. *South Asian Res J Pharm Sci*, 4(3): 48-53. 48



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 obtain the dry weight of the plant extracts.

Preliminary phytochemical screening [4-6]:

The preliminary phytochemical investigation was carried out with both aqueous & ethanolic extracts of *Cucurbita pepo seed* for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

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.

INVESTIGATION OF LEARNING AND MEMORY ACTIVITY IN ALZHEIMER'S MODEL BY USING 8-ARM RADIAL MAZE (8-ARM/8-RAM)

Behavioral study:

Before starting the behavioral studies 1 week training was conducted. Only food and water was administered during this period [7].

RADIAL ARM MAZE

The radial arm maze was developed by Olton and Samuelson (1976) and has become an essential tool for testing memory in rats [8]. The spatial memory was evaluated by the instrument. Open type radial arm maze' *Cucurbita pepo* was used in the study. It had a circular central arena and 8 equally sized arms (20 x 60 cm). Small dishes with animal food was kept at far end inside each arm was mounted. Initially animals were habituated to the environment [9]. In the present study, baited and unbaited arms were fixed throughout the tests. The 1st, 3rd, 5th, and 7th arms were baited while the 2nd, 4th, 6th, and 8th arms were unbaited. The rats was placed in the centre of the maze and allowed to freely explore the maze for 10 minutes on the first day. The rats were required to take the food pellets from each arm without making a re-entry into the arm already visited. The trail was terminated when the animal takes the food reward from all the eight

arms or after 10 minutes if all the eight arms were not visited. Correct score was give when the visits an arm and collects the food reward and a maximum score of '8' can be attained per trail. The first entry into the baited arm was recorded as a correct choice. An entry into an unbaited arm was considered a reference memory error (RME) .When a rat reenters an already visited arm it was taken as a working memory error (WME) [10].



Figure-2: 8-RAM

Evaluation parameters

- No. of entries in baited arms and non-baited arms.
- Time taken to reach the paired arm.

ETHANOL- INDUCED COGNITIVE IMPAIRMENT [7]:

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 (Fe^{3+}) to ferrous ion (Fe^{2+}) 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 (20%) is used to induce dementia like condition in the dose 4.5 mg/kg administered s.c for 21 days) [7]. The test animals were randomly chosen and divided into four groups having five rats in each as follows:

Group I: Inducing Group-Ethanol (4.5 g/kg was administered subcutaneously for 21 days).

Group II: Standard Group -Donepezil hydrochloride [7] (2.5 mg/kg was administered orally for 21 days) + Ethanol.

Group III: Test-I -Aqueous seed extract of *Cucurbita pepo* [CPAE- 100mg/kg was administered orally for 21 days) + Ethanol.

Group IV: Test -II -Ethanolic seed extract of *Cucurbita pepo* [CPEE- 100mg/kg was administered orally for 21 days) + Ethanol.

All the treatment group animals received respective control, standard and test treatment 30 minutes prior to the ethanol administration for 21 days of experimental period.

STATISTICAL ANALYSIS

The values are expressed as mean \pm 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

In this study, we found that aqueous & ethanolic seeds extract of *Cucurbita pepo* Possess the following chemical constituents.

Table-1: Phytochemical screening of CPAE & CPEE

Phytochemical constituents	Aqueous seed extract of <i>Cucurbita pepo</i>	Ethanolic seed extract of <i>Cucurbita pepo</i>
Alkaloids	-	-
Carbohydrates	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Terpenoids	-	-
Sterols	+	+
Tannins	+	+
Proteins	+	+
Amino acids	-	-
Glycosides	-	-
Fixed oils and fatty acids	-	-

+ indicate the compulsory present and – indicate the absent.

CPAE – *Cucurbita pepo* aqueous extract.

CPEE- *Cucurbita pepo* ethanol extract.

Effect of seed extracts of *Cucurbita pepo* on behavioural parameters i.e. 8-ram:

Animals treated with ethanol [4.5 mg/kg] alone for 21 days showed an increase in time taken to reach paired arm & number of entries in baited arms and non-baited arms in 1st, 7th, 14th & 21st days.

Table-2: Effect of seed extracts of *Cucurbita pepo* on time taken to reach paired arm (ethanol- induced cognitive impairment)

S. No	Group	Treatment	Time Taken to reach Paired arm (Sec)			
			1 st day	7 th day	14 th day	21 st day
1	I	Ethanol	144.4 \pm 3.66	134.2 \pm 3.69	120.2 \pm 2.65	103.2 \pm 2.98
2	II	Standard+ethanol	76.2 \pm 0.96	66.6 \pm 1.98	57.8 \pm 0.86	48.4 \pm 2.08
3	III	CPAE+ ethanol	121.2 \pm 5.58	112.4 \pm 5.97	98.2 \pm 2.47	92.0 \pm 3.33
4	IV	CPEE+ ethanol	99.0 \pm 1.30	90.0 \pm 3.70	83.2 \pm 4.54	69.2 \pm 2.95

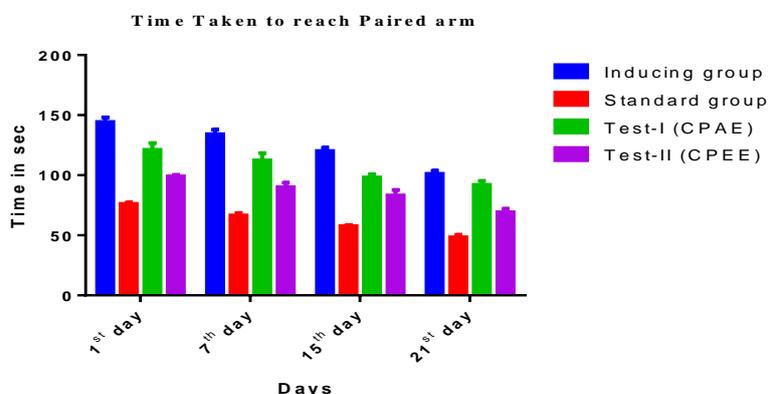
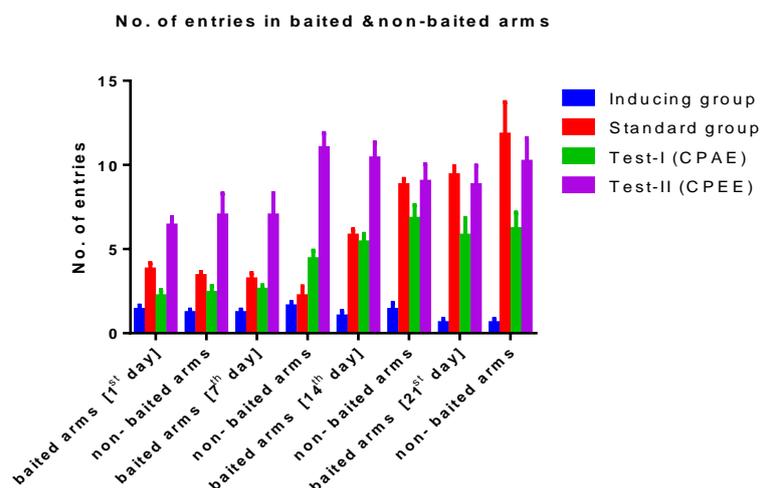


Figure-3: Effect of seed extracts of *Cucurbita pepo* on Time taken to reach Paired arm. Values are expressed as Mean \pm SEM, P< 0.0001 vs. control (n = 5 animals)

Table-3: Effect of seed extracts of *Cucurbita pepo* on number of entries in baited arms and non-baited arms (ethanol- induced cognitive impairment)

Group	Treatment	Number of entries in baited arms and non-baited arms							
		1 st day		7 th day		14 th day		21 st day	
		B.A	N.B.A	B.A	N.B.A	B.A	N.B.A	B.A	N.B.A
I	Ethanol	1.4±0.24	1.2±0.20	1.2±0.20	1.6±0.24	1.0±0.31	1.4±0.4	0.6±0.24	0.6±0.24
II	Standard+ethanol	3.8±0.37	3.4±0.24	3.2±0.37	2.2±0.58	5.8±0.37	8.8±0.37	9.4±0.50	11.8±1.93
III	CPAE+ ethanol	2.2±0.37	2.4±0.40	2.6±0.24	4.4±0.50	5.4±0.50	6.8±0.80	5.8±1.02	6.2±0.96
IV	CPEE+ ethanol	6.4±0.50	7.0±1.30	7.0±1.30	11.0±0.89	10.4±0.92	9.0±1.04	8.8±1.16	10.2±1.39

**Figure-4: Effect of seed extracts of *Cucurbita pepo* on number of entries in baited arms and non-baited arms. Values are expressed as Mean ± SEM, P < 0.0001 vs. control (n = 5 animals)**

CONCLUSION

In the present investigation *Cucurbita pepo* possesses the presence of carbohydrates, tannins, flavonoids, phenols, sterols and proteins. *Cucurbita pepo* showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol- induced cognitive impairment. *Cucurbita pepo* ethanolic extract showed comparatively significant effect exerted to standard drug donepezil hydrochloride in the finding of time taken to reach paired arm (sec) & number of entries in baited arms and non-baited arms (i.e. learning and memory activity). Time taken to reach paired arm (sec) & number of entries in baited arms and non-baited arms was recorded after administration of ethanol at different days and graphs were plotted 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. Therefore finally suggests ethanolic extract of *Cucurbita pepo* show same effects exerted to the standard drugs.

REFERENCES

- Korolev, I. O. (2014). Alzheimer's disease: a clinical and basic science review. *Medical Student Research Journal*, 4(1), 24-33.
- FTD, F. D., & PPA, P. P. A. (2009). Cognitive Neurology and Alzheimer's Disease Center (CNADC) Frontotemporal Dementia (FTD) & Primary Progressive Aphasia (PPA) Caregiver Information and Resources CNADC.
- Perez Gutierrez, R. M. (2016). Review of *Cucurbita pepo* (pumpkin) its phytochemistry and pharmacology. *Med. Chem*, 6(1), 12-21.
- Sharada, L. D. (2018) Pharmacognosy and phytochemistry: A Companion Handbook, PharmaMed Press, 2nd edition, 69-77.
- Beena, P., Rajesh, K. J., & Arul, B. (2016). Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 3(3), 155-157.
- Manohar, V. R., Chandrashekar, R., & Rao, S. N. (2012). Phytochemical Analysis of Ethanolic Extract of Fruits of *Terminalia chebula* (EEFTC). *Drug Invention Today*, 4(10), 491-492.
- Uddin, M. S., Asaduzzaman, M., Mamun, A. A., Iqbal, M. A., Wahid, F., & Rony, R. K. (2016). Neuroprotective activity of *Asparagus racemosus* Linn. against ethanol-induced cognitive impairment and oxidative stress in rats brain: Auspicious for controlling the risk of Alzheimer's disease. *J Alzheimers Dis Parkinsonism*, 6(4), 1-10.

8. Guitar, N. A. (2014). The Interaction Between Spatial Working and Reference Memory in Rats on a Radial Maze. Western University, Scholarship@Western, Undergraduate Honors Theses, Psychology, 1-35.
9. Jahanara, H., & Sibi, P. I. (2017). Biosynthesis, Characterization and Evaluation of Silver Nanoparticles of *Alternanthera sessilis* (linn.) and its Ethanolic Extract in High Fat Diet Induced Dementia Model. *International Journal of Pharmaceutical Sciences Review and Research*, 42(2), 160-165.
10. Sahak, M. K. A., Mohamed, A. M., Hashim, N. H., & Hasan Adli, D. S. (2013). Nigella sativa oil enhances the spatial working memory performance of rats on a radial arm maze. *Evidence-Based Complementary and Alternative Medicine*, 2013, 1-5.