IJPSR (2022), Volume 13, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 20 June 2021; received in revised form, 02 August 2021; accepted, 05 August 2021; published 01 April 2022

EVALUATION OF ANTI-ALZHEIMER'S ACTIVITY OF ALCOHOLIC EXTRACT OF *HYLOCEREUS UNDATUS* FRUITS IN WISTAR ALBINO RATS.

R. K. Shaikh * and V. G. Kanase

Department of Pharmacology, Oriental College of Pharmacy, Navi Mumbai - 400705, Maharashtra, India.

Keywords:

Alzheimer's disease, *Hylocereus undatus*, Scopolamine, Malonyl dialdehyde, Morris water maze

Correspondence to Author:
Dr. G. Vanita Kanase

Designation HOD,
Department of Pharmacology,
Oriental College of Pharmacy, Navi Mumbai - 400705, Maharashtra, India.

E-mail: vanita.kanse@ocp.edu.in

ABSTRACT: Alzheimer's is a degenerative brain disease associated with memory depletion and mild cognitive dysfunction, leading to dementia. Today's world attempts to treat various ailments have increased with medical advancements. There is still considerable research into medications capable of preventing or at least effectively altering the trajectory of AD. Medicinal plants play a major role in Alzheimer's and memory loss control. Ayurveda, homeopathy, Siddha & Unani medicine systems are the main conventional therapeutic approaches. In animal studies, AD caused by chemical compounds may be helpful in deeper understanding the mechanism of disease and AD treatment. The anticholinergic drug Scopolamine induces amnesia in rodents. In the present study, the efficacy of an Extract of *Hylocereus undatus* (HUFE) was evaluated against scopolamine-induced Alzheimer in the Wistar albino rat. Donepezil at a dose of 2.5 mg/kg, HUFE was given in rat at a dose of 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight. The anti-Alzheimer activity was assessed by Morris water maze, novel object recognition test, and Y-maze. And by a biochemical test like neurotransmitter Acetylcholinesterase activity, catalase activity, malonyl dialdehyde, and Nitric oxide assays. Results showed that extracts treatment prevents Alzheimer and increases the level of acetylcholine, catalase and phytochemical studies identified the presence of Phenolic compounds, flavonoids and alkaloids in the HUFE. According to the results, it was concluded that the fruits of *Hylocereus undatus* has significant anti-Alzheimer activity due to the presence of potent antioxidants such as phenolic compounds, flavonoids and alkaloids.

INTRODUCTION:

Neurodegenerative Diseases: Neurodegenerative disease is a prominent cause of illness and death in the elderly throughout the globe. Individual brain degenerative illnesses differ in their clinical manifestations and underlying physiology; however, they frequently share characteristics.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(4).1567-80

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(4).1567-80

Alzheimer's disease, frontotemporal dementia and its permutations, cortico-basal degeneration, Parkinson's disease, dementia with Lewy bodies, progressive supranuclear palsy, multiple system atrophy and Huntington's disease are the common neurodegenerative diseases [1].

Alzheimer's disease: Alzheimer's disease was first described and got its name after Alois Alzheimer, a German psychiatrist and neuropathologist in 1907. This disease begins slowly, increasing gradually to worsen in due course of time. AD is mainly characterized by the associated dementia, which is a decline of cognitive effects such as memory,

praxis and orientation [2]. Early disease shows a loss of short-term memory, inability to learn new things, mood swings and difficulty in finding words, forgetting names and losing items. Patients with Alzheimer's disease frequently express frustration, aggressiveness and irritation. In severe situations, patients become entirely incontinent, lose their memories and lose their sense of time and place. Patients become completely reliant on others and require full care at some point. Because the patient is completely reliant on others, he or she will need to be admitted to a nursing home and receive full-time nursing care. Thus, AD presents a considerable problem in patient management as well [3]. The development of neurofibrillary tangles & tau protein hyperphosphorylation and also the deposition of beta-amyloid plaques due to defective amyloid precursor protein (APP) metabolism, mark this illness. Excessive formation of reactive oxygen species causes oxidative stress that leads to abnormalities in brain cells, which is often accompanied by apoptosis, resulting in cognitive impairment & dementia. In this context, novel medicines for treating Alzheimer's disease are required. Antioxidants, for example, are attractive species for both prevention & treatment as they can break the radical chain reaction lowering the generation of ROS. These species have also been shown to raise the efficacies of conventional treatments when used in conjunction with them [4]. An antimuscarinic drug, Scopolamine enhances AChE activity in the cortex & hippocampus by competitively antagonizing the impact of Ach on muscarinic receptors by binding to the postsynaptic receptor with strong potential. Because of cholinergic hypofunction, Scopolamine reduces cerebral blood flow. Scopolamine also induces ROS, resulting in free radical damage, a rise in MDA levels, and a worsening in antioxidant capacity in the scopolamine-treated group.

Scopolamine causes neuroinflammation in the hippocampal region via increasing oxidative stress & pro-inflammatory cytokines. Scopolamine has been shown to raise APP & Tau levels. Chronic scopolamine administration resulted in significant histological alterations in the cerebral cortex, including neuronal loss. Scopolamine treatment has been employed to test efficacy of prospective new Alzheimer's disease treatment medicines in both healthy human subjects and laboratory animals of dementia [5].

Current Treatment of Alzheimer's disease in Medical Science:

TABLE 1: USFDA APPROVED DRUGS USED IN THE TREATMENT OF ALZHEIMER'S DISEASE

S. no.	Category	Drugs	Brand name
1	Cholinergic Activators	Tacrine Donepezil Rivastimine Galantamine Huperzine A	Cognex Aricept Exelon Razadyne Hup A
2	Glutamate (NMDA) Antagonists.	Memantine	Namenda
3	Miscellaneous cerebro-active drugs	Piracetam Pyritinol Dihydroergotoxin	Alcetam Renervol Hydergine
4	Combination Therapy	Memantine + Donepezil	Namzaric

Hylocereus undatus: Pitaya, often known as dragon fruit, is a tropical fruit that belongs to the cactus family, Cactaceae. Due to its white flesh, *H. undatus* is also referred to as white pitaya. *H. undatus* is native to the southern part of Mexico, but it is now spread throughout Asia, including Taiwan, India, Malaysia and Vietnam, as well as Northern Australia [6].

MATERIALS AND METHODS:

Plant Material: *Hylocereus undatus* fruits were obtained in November 2020 from a local vendor, Navi Mumbai and were authenticated by Mr. Mahesh Atale, M.Sc. Botany, Alarsin- Pioneers in Ayurvedic Research, Mumbai- 400 093 and specimen were submitted to Pharmacology department, Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400705. *Hylocereus undatus* fruits were peeled, and pulp was cut into thin slices and then kept for shade drying.

Preparation of Extract: *Hylocereus undatus dried pulp* (350 g) was extracted with700ml of ethanol using the maceration technique for 48 h. The extract was filtered through Whatman filter paper #1 and then concentrated in a vacuum at 40 ^{o}C using a rotary evaporator. The concentrated extract was stored in the dark at 40 ^{o}C till the time for their use. The alcoholic extract percentage yield was 9.9 percent w/w. The entire study was then done on the basis of alcoholic extract of *Hylocereus undatus*.

ALARSIN

(PIONEERS IN AYURVEDIC RESEARCH SINCE 1947
IN ● DENTAL ● MEDICAL ● VETERINARY FIELDS

ADMN. OFFICE : ALARSIN HOUSE, A/32, STREET NO.3, OPP. ESIS HOSPITAL, M.I.D.C., ANDHERI (E), MUMBAI - 400 093. (INDIA) P. BOX - 9444
TEL. : 2821 6836 ● FAX : 022 2836 4116
Website : www.alarsin.com ● e-mail id : alarsinmumbai@gmail.com feedback@alarsin.com

AUTHENTICATION CERTIFICATE

Drug Sample provided by **Ms. Rashda Khatoon Abdul Khalique,** of **Oriental College of Pharmacy, Navi Mumbai** are studied exomorphologically and microscopically.

We hereby Authenticate that the samples belongs to,

No.	Drug	Latin name	Family
1	**Dragon Fruit**	*Hylocereus undatus*	**Cactaceae**

This certificate is provided on demand of **Ms. Rashda Khatoon Abdul Khalique** for her research.

The above Fruit is authenticated.

Mahesh Atale
M.Sc. Botany
ALARSIN

FIG. 1: PLANT AUTHENTIFICATION CERTIFICATE

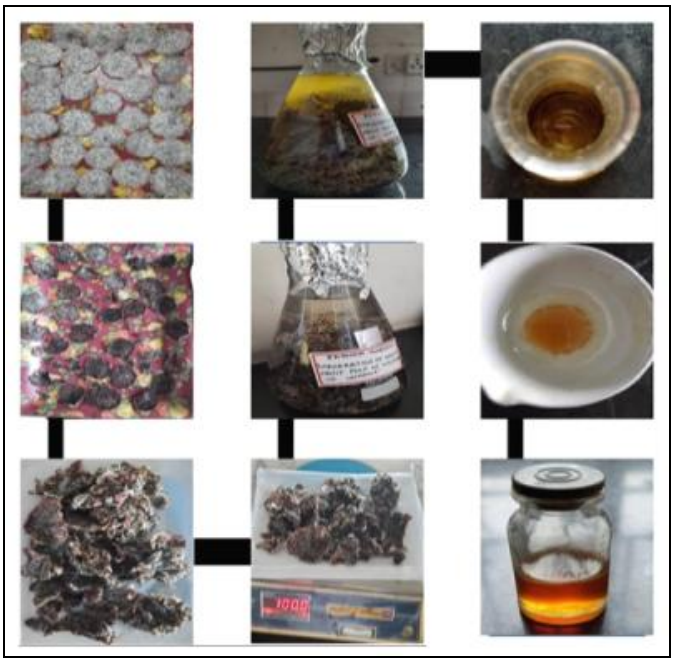

FIG. 2: PROCESS OF EXTRACT PREPARATION

Chemical Constituents: The chemical constituents present in *Hylocereus undatus* are Kaempferol, Quercetin, B-sitosterol, Isorhamnetin, Carbo-hydrate, Proteins, and amino acids, Alkaloids, Terpenoids and Steroids, Glycoside and Flavonoids, Tannins and phenolic compounds, Saponins, Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B6, Vitamin C and minerals like copper, iron, calcium, phosphate and magnesium [7].

Medicinal Properties: Antioxidant, Hepato-protective, Laxative, Anti Parkinson's, Antidiabetic, Antilipase, Antimicrobial, Cytotoxic, wound healing activity.

Toxicity: The alcoholic extract's LD_{50} was estimated to be 2000 mg/kg. This plant has also known to be edible, making it safe, and accordingly, $1/10^{th}$ and $1/20^{th}$ dose was selected for study, i.e., low dose 100 mg/kg, intermediate-dose 200 mg/kg, and high dose 300 mg/kg for the test groups with dose conversion [7].

Drug Treatment: The ethanolic extract was suspended in Normal saline in doses of 100, 200,300 mg/kg p.o. for the pharmacological tests. The doses were selected based on the previous studies on ethanolic extract. *Hylocereus undatus* fruits extract (HUFE) was administered to individual rats in groups 4,5,6,7. None of the rats was dead due to treatment till the end of the observation period.

The *Hylocereus undatus* fruits extract (HUFE) caused no abnormality till the duration of treatment.

Qualitative Phytochemical Screening: Preliminary chemical studies were performed on *Hylocereus undatus* alcoholic extract. The presence of following phytoconstituents were assessed: Molisch test, Resorcinol test, were done for carbohydrates, Lead acetate test, Ferric Chloride ($FeCl_3$) test, Conc. Sulphuric acid test for flavonoids, Modified Borntrager's test, Aqueous Sodium Hydroxide (NaOH) test for glycosides, Bromine water test and Braymer's a test for tannins, Dragendroff reagent test and Hager's reagent test for alkaloids, Iodine test, Ferric Chloride ($FeCl_3$) test, Lead Acetate test, Potassium dichromate test, and Ellagic acid test was done for phenolic compounds.

Equipment and Chemicals: Electronic balance, Morris water maze, Y-maze, novel object apparatus, syringes, and needles, ethanol, NS, and Scopolamine. Donepezil tablet, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine, thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2), Griess reagent.

Animals: The CPCSEA acknowledged the Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy; Sanpada approved the animals needed for the research work with

protocol no. OCP/ IAEC/2020-21/05 entitled "Evaluation of anti-Alzheimer's activity of ethanolic extract of *Hylocereusundatus* fruits in wistar albino rats". Animals procured were female Swiss Albino mice (15 to 20 g) for carrying out acute oral toxicity study & male Albino Wistar rats (100 to 120 g) were obtained from National Institute of Biosciences, Pune, Maharashtra 411051. The animals were kept in well ventilated, air-conditioned animal house at a constant temperature of 24 ± 2 °C, with 12:12 h dark: light cycle and with a relative humidity of 55-60%. The animals were placed on bedding material in spacious polypropylene cages with a paddy husk. The animals were held on a normal diet with pellets and filtered water.

Acute Oral Toxicity Study: Acute oral toxicity analysis was carried out for the alcoholic extract of dried fruits of *Hylocereus undatus* as recommended in OECD Guideline 423. The animals were noted keenly for the display of any toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for a period of 14 days. When this was conducted, any kind of toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg. Hence, it can be concluded that the LD_{50} of the alcoholic extract of dried fruits of *Hylocereus undatus* is greater than 2000 mg/kg.

Experimental Design: Grouping of Animals: Animals were divided into seven groups, each of six animals.

Group I: Control group oral administered by distilled water (20 ml/kg).

Group II: Intraperitoneal injection by scopolamine hydrochloride (0.5 mg/kg) (Disease control).

Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with Scopolamine (Standard).

Group IV: Animals orally administered by extract which is dissolved in NS (100 mg/kg) and Alzheimer's induced with Scopolamine (Low dose).

Group V: Animals oral administered by extract which is dissolved in NS (200 mg/kg) and Alzheimer's induced with Scopolamine (Intermediate dose).

Group VI: Animals oral administered by extract which is dissolved in NS (300 mg/kg) and Alzheimer's induced with Scopolamine (High dose)

Group VII: Animals orally administered by extract which is dissolved in NS (300 mg/kg) (Extract only).

Anti- Alzheimer's Study:

Morris Water Maze Test: The apparatus consisted of a circular pool (45 cm in height and 100 cm in diameter) with a featureless inner surface. The pool was filled with opaque water (maintained at 22 ± 2 °C) to a height of 30 cm and was divided into four quadrants of equal area which were marked as I, II, III and IV. There was a platform (29 cm × 6 cm) placed one centimeter below the level of water at the center of one of the four quadrants (the target quadrant).

The position of the platform was unaltered throughout the experiment. The test was commenced on day 10^{th} of the treatment period when the rats were allowed to swim for 120 s without the platform to acclimatize.

During the next four consecutive days, each animal received four learning trials of 120 s with an intertrial interval of 60 s. For each learning trial, the rat was placed in the water facing the pool wall diagonally opposite to the quadrant in which the platform was kept. The time taken by the animal to locate the submerged platform was recorded as the escape latency time for each trial.

If any of the animals were unable to locate the platform within 120 s, they were directed to the platform and allowed to rest there for 60 s, and in this case, the escape latency time was recorded as 120 s. These sessions were recorded as the hidden platform trials or acquisition tests.

On day 15 (24 h after the final learning trial), the platform was removed from the pool, and they were subjected to a probe trial session to assess memory retention.

Each rat was placed into the water diagonally opposite the target quadrant, and for 60s it was allowed to swim and find the quadrant in which the platform was previously placed. The time spent by the animal in the target quadrant was recorded [8].

FIG. 3: MAZES: (A) MORRIS WATER MAZE, (B) NOVEL OBJECT RECOGNITION, (C) Y-MAZE

Novel Object Recognition Test: Object recognition was performedinasimple box, without a transparent wall. The procedure consisted of three different phases: a habituation phase, an acquisition phase and a retention phase. On the first day (habituation Phase- day 13 of treatment), rats were individually subjected to a single familiarization session of 10 min, during which they were introduced in the empty area, in order to become familiar with the apparatus. On the 2nd day (acquisition phase- day 14 of treatment) animals were subjected to a single 10-min session, during which floor- fixed two objects (A and B) were placed in a symmetric position in the central line of the area. 10cm from each and 8 cm from the nearest wall (each object occupies approximately 5 cm space by its size). The two objects, made of the same material with a similar color and smell, were different in shape but identical in size. Rats were allowed to explore the objects in the open field. The exploration time on each object was shown (as seconds) to indicate the exploring activity of rats. On the 3rd day day (retention phase- day 15 of treatment), rats were allowed to explore the open field in the presence of two objects: the familiar object A and a novel object C in different shapes but in similar color and size (A and C)**.** A recognition index (for retention session), calculated for each rat, was expressed as the ratio Recognition index (RI) = time exploring novel object/ (Time exploring novel object + time exploring familiar object) ×100%. [9]

Y- Maze Test: Y-maze task is used to measure working memory performance which was assessed by recording spontaneous alternation behavior in a single session in a Y-maze made up of black-walled cardboard. Each arm was 40 cm long, 12 cm height, 3 cm wide at the bottom and 10 cm wide at the top and converged in an equilateral triangular central area. Each rat was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. The ability to alternate requires that the rat know which arm they have already visited. The series of arm entries, including possible returns into the same arm, were recorded visually. Immediate working memory performance was assessed by recording spontaneous alternation behavior. Entry was considered to be completed when the hind paws of the animal had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B, and C) on overlapping triplet sets. The percentage of trials 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. On the 15th day of treatment, 90 min after the treatment of the last dose, arm entries were recorded and percentage alteration was calculated. [10]

Biochemical Test:

Preparation of Brain Sample: After assessing the learning and memory paradigms in Scopolamine-induced amnesia, rats from each group were euthanized by using a carbon dioxide chamber; brains were removed quickly and placed in ice-cold saline. The frontal cortex, hippocampus, and septum (and any other regions of interest) were quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). The samples of rat brain homogenates were collected in different test tubes to analyze Acetylcholinesterase, Catalase, MDA, nitric oxide. The supernatant was used for enzymatic assays [9].

Estimation Acetyl Cholinesterase Enzyme Levels in the Brain: 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in a spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20µl of substrate *i.e.,* acetylthiocholine, was then added, and change in absorbance was recorded. Change in the absorbance per minute was thus determined [11].

Determination of Catalase (CAT) Principle: Catalase activity was measured by the method of Aebi H. 0.1 ml of supernatant was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of catalase was expressed as units/mg protein. The reaction occurs immediately after the addition of H_2O_2. Solutions were mixed well, and the first absorbance (A1) was read after 15 seconds (t1) and the second absorbance (A2) after 30 seconds (t2). The absorbance was read at wavelength 240 nm [12].

Determination of Malonyl Dialdehyde (MDA): MDA was measured according to the method of Ohkawa *et al*. 1 ml. of suspension medium were taken from the tissue homogenate in a tube. 0.5 ml. of Trichloroacetic acid (TCA) was added to it, followed by 0.5 ml. of 8% Thiobarbituric acid (TBA) reagent. The tubes were covered with aluminum foil and kept in the water bath for 30 min. at 80 ºC. After 30 min. the tubes were taken out and placed in the cold water for 30 min. These tubes were centrifuged for 15 min at 3000 rpm. The absorbance of the supernatant was taken at 540 nm, at room temperature against appropriate blank solution (1 ml distilled water, 0.5 ml of 30% TCA and 0.5 ml of 0.8% TBA.). MDA value was expressed as n moles MDA/mg of protein. [13]

Determination of Nitric Oxide (NO): The production of nitric oxide was estimated by the accumulation of nitrate in the supernatant, which was determined by a colorimetric assay with the Griess reagent (1:1 solution of 1% sulfanilamide in 5% H3PO4 and 1% naphthylamine diamine dihydrochloric acid in water). An equal volume of the supernatant and the Griess reagent were mixed, and this mixture was incubated for 10 min in the dark at room temperature. The absorbance was taken at 540 nm using a spectrophotometer. The concentration of nitrite in the supernatant was estimated from a sodium nitrite standard curve [13].

Statistical Analysis: The data were analyzed using BioStat Pro (version 5.9.8). For each category, the results are expressed as mean ± SEM. A one-way Analysis of variance (ANOVA) was used to analyze statistical differences, followed by the Tukey-Kramer test. Tests at $P \leq 0.05$were considered statistically significant.

*** indicated $p < 0.001$,

** indicates $p < 0.01$,

* indicates $p < 0.1$

*is used to denote the comparison of standard and test groups with the disease control group. Experimental data were obtained from all the animals in different groups, recorded and tabulated on a broadsheet using an MS Excel program to prepare the graphs.

RESULTS & DISCUSSIONS:

Qualitative Phytochemical Screening:

TABLE 2: QUALITATIVE ANALYSIS OF *HYLOCEREUS UNDATUS* FRUIT EXTRACT

S. no.	Phyto Chemicals	Tests Performed	Inference	Result
1	Carbohydrates	Molisch test	Violet ring at the junction	+
		Resorcinol test	Rose colour	+
2	Flavonoids	Lead acetate test	Yellow ppt	+
		Ferric chloride test	Green ppt	+
		Conc. H_2SO_4 test	Orange colour	+
3	Glycosides	Modified Borntrager's test	Pink to Red colour	+
4	Tannins	Bromine water test	Discoloration of bromine	+
		Braymer's test	Blue-Green colour	+
7	Alkaloids	Dragendroff reagent test	Reddish Brown ppt	+
		Hager's reagent test	Yellow ppt	+
8	Phenolic compounds	Iodine test	Transient Red colour	+
		Ferric chloride test	Bluish Black colour	+
		Lead acetate test	White ppt	+
		Potassium dichromate test	Dark colour	+
		Ellagic acid test	Solution turns muddy	+
9	Proteins	Biuret test	No Violet/ Pink colour	-

* Present (+) /Absent (-)

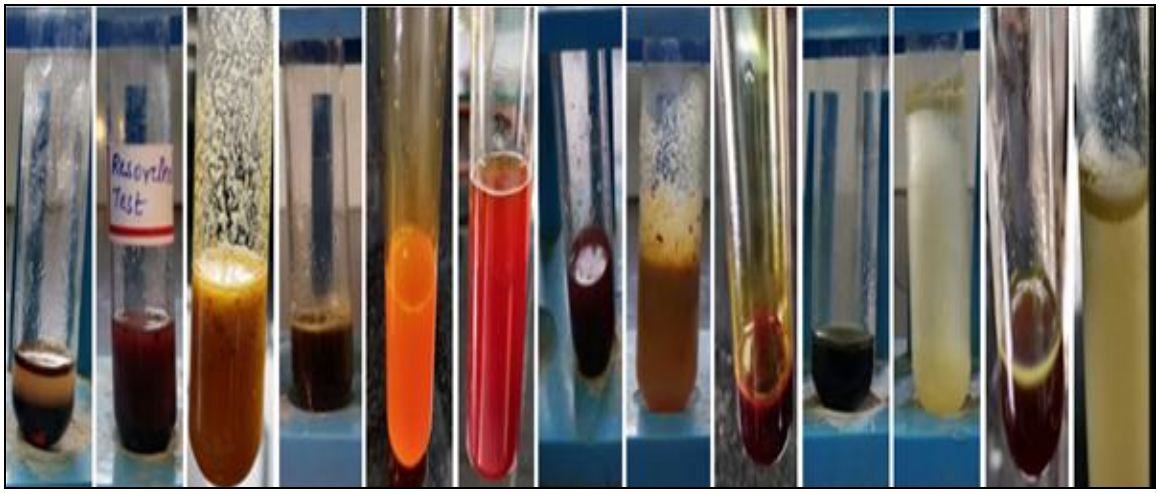

FIG. 4: RESULTS OF QUALITATIVE ANALYSIS

Acute Oral Toxicity Studies: Acute oral toxicity analysis was carried out for the alcoholic extract of dried fruits of *Hylocereus undatus* as recommended in OECD Guideline 423.

The animals were noted keenly for the display of any toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for a period of 14 days. When this was conducted, any kind of toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg.

Anti-Alzheimer's Study:

Morris Water Maze Test: During the acquisition sessions (days 11–14) scopolamine treatment resulted in significantly longer escape latencies when compared to the controls; this effect was mitigated by co-administration of the test formulation. Significant decreases in escape latencies were observed on days 2–4 (treatment days 12–14) when compared to day 1 (treatment day 11) of the acquisition sessions in the control group but not in the Scopolamine-treated rats. These differences were restored in rats co-administered with both Scopolamine and the test formulation. In the probe trial session on day 15, Scopolamine treated rats spent significantly less time swimming in the target quadrant when compared to the controls, whereas the time spent by the rats co-administered with the test formulation and Scopolamine in the target quadrant was more than the disease controls.

TABLE 3: EFFECT OF ALCOHOLIC EXTRACTS OF FRUITS OF *HYLOCEREUS UNDATUS* ON ESCAPE LATENCY TIME IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Day / Group	Escape Latency Time (Seconds)			
	Day 11	Day 12	Day 13	Day 14
Control	86.458 ± 4.04	77.2083 ± 0.62	56.625 ± 0.89	27.3333 ± 0.92
Disease Control	99.5 ± 2.69#	85.375 ± 0.98#	65.375 ± 0.82#	37.9583 ± 1.05 #
Standard	81.083 ± 3.23***	55 ± 0.75***	27.9167 ± 0.72***	15.2083 ± 1.23 ***
Low Dose (100 mg/kg)	94.875 ± 1.74**	77.0833 ± 1.04***	55.375 ± 0.79***	28.0417 ± 0.73***
Medium Dose (200 mg/kg)	85.125 ± 0.58**	69.7917 ± 1.79***	42.5 ± 0.47***	23.375 ± 0.53***
High Dose (300 mg/kg)	76.792 ± 0.72***	56.5833 ± 0.57***	38.4583 ± 1.53***	19.0417 ± 0.53***
Extract only (300 mg/kg)	78.5± 1.94***	55.5833 ± 1.17***	36.375 ± 1.54***	19.75 ± 0.67***

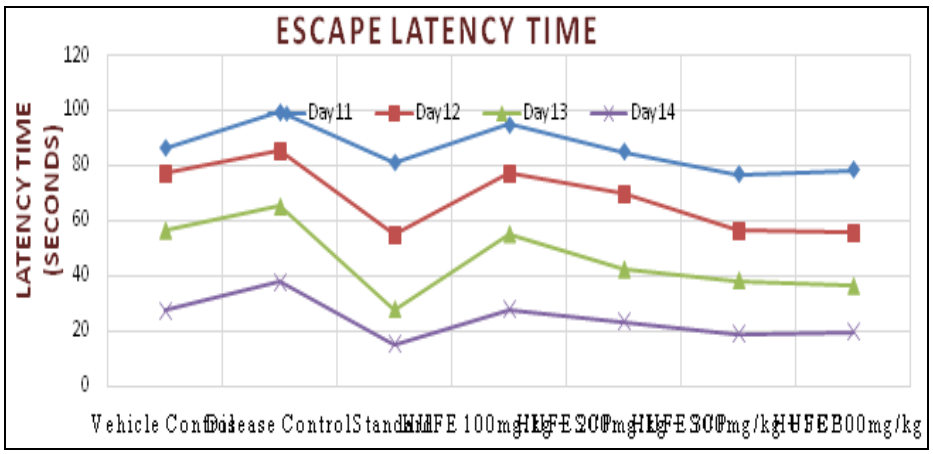

FIG. 5: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF *HYLOCEREUS UNDATUS* ON ESCAPE LATENCY TIME IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS

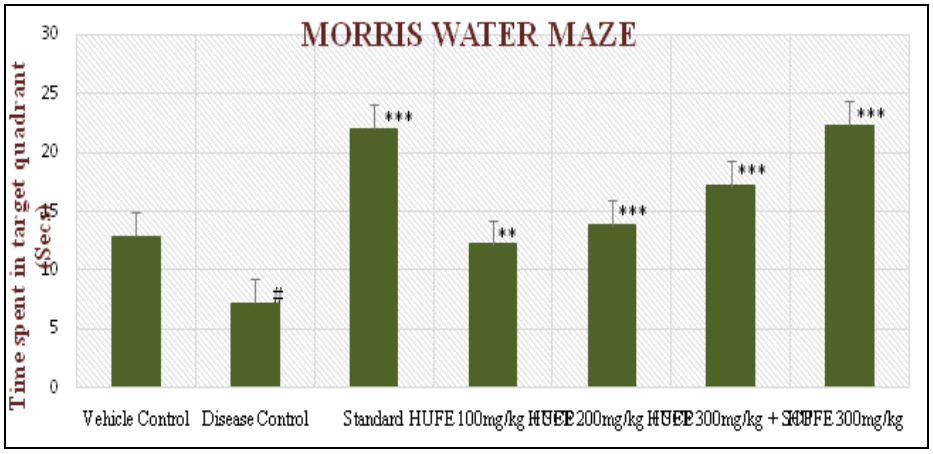

FIG. 6: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF *HYLOCEREUS UNDATUS* ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

TABLE 4: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF HYLOCEREUS UNDATUS ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Day / Group	Time spent in the target quadrant (seconds)
Control	12.833 ± 0.6
Disease Control	7.166 ± 0.74
Standard Control	22 ± 1.21
Low Dose (100 mg/kg)	12.166 ± 0.6
Medium Dose (200 mg/kg)	13.833 ± 0.74
High Dose (300 mg/kg)	17.166 ± 0.6
Extract Only (300 mg/kg)	22.333 ± 0.66

Novel Object Recognition Test: All the four groups treated with ethanolic extract of dried fruits of *Hylocereus undatus* showed dose-dependent increase in Recognition index when compared against disease control group (Scopolamine).

Y Maze Test: The scopolamine treatment was associated with a significant decrease in short-term memory performance, as indicated by a decreased spontaneous alternation percentage, compared with

the control group. All four doses of alcoholic extract of dried fruits of *Hylocereus undatus* pretreatment significantly improved the spontaneous alternation percentage, which Scopolamine decreased.

TABLE 5: EFFECT OF ALCOHOLIC EXTRACTS OF FRUITS OF *HYLOCEREUS UNDATUS* ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups	Recognition Index
Control	55.689 ± 1.06
Disease Control	42.168 ± 1.31
Standard Control	60.742 ± 1.27
Low Dose (100 mg/kg)	55.281 ± 0.92
Medium Dose (200 mg/kg)	56.429 ± 0.34
High Dose (300 mg/kg)	59.203 ± 0.97
Extract Only (300 mg/kg)	60.252 ± 1.24

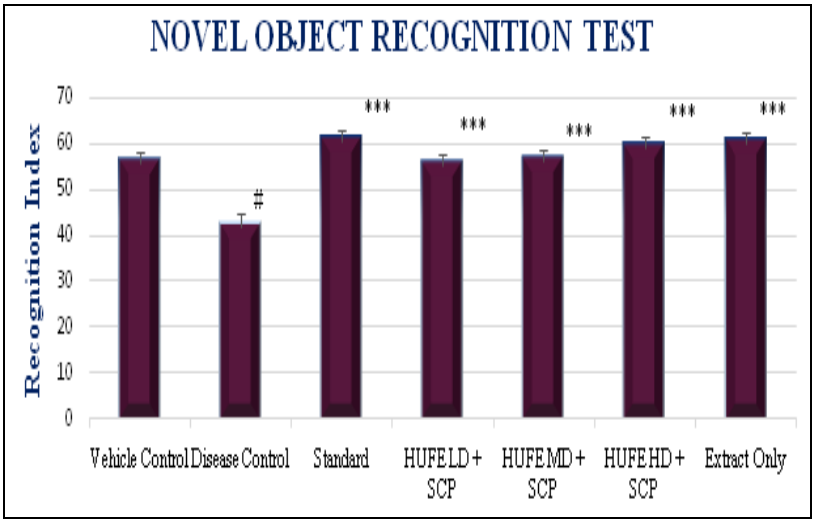

FIG. 7: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF *HYLOCEREUS UNDATUS* ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

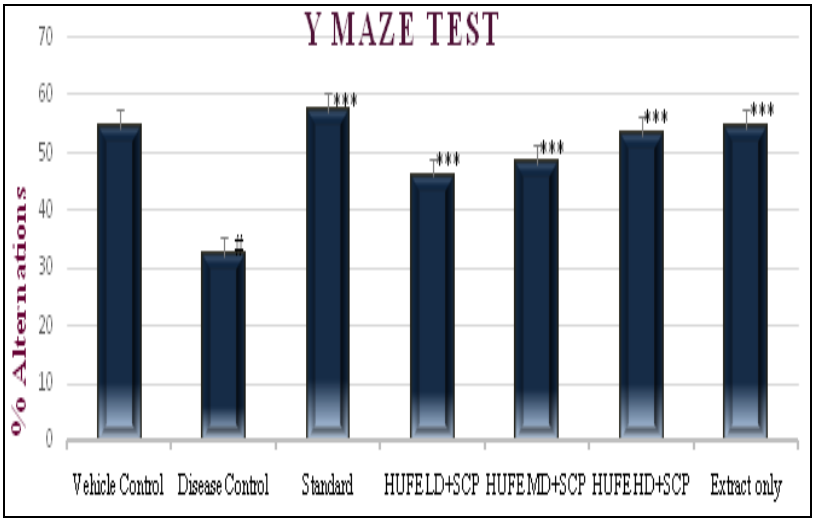

FIG. 8: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF *HYLOCEREUS UNDATUS* ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE-INDUCED ALZHEIMER IN WISTAR ALBINO RATS. Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by Tukey-Kramer test. ANOVA: Analysis of variance, SEM: Standard Error of Mean.

TABLE 6: EFFECT OF ALCOHOLIC EXTRACT OF FRUITS OF *HYLOCEREUS UNDATUS* ON SPONTANEOUS ALTERNATION PERCENTAGE IN SCOPOLAMINE INDUCED ALZHEIMER IN WISTAR ALBINO RATS

Groups	Percentage Alternation
Control	54.29 ± 0.80
Disease Control	32.281 ± 0.60
Standard Control	57.21 ± 0.54
Low Dose (100 mg/kg)	45.92 ± 0.43
Medium Dose (200 mg/kg)	48.313 ± 0.66
High Dose (300 mg/kg)	53.241 ± 1.30
Extract Only (300 mg/kg)	54.451 ± 0.67

Biochemical Studies:

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: All the four test doses of the ethanolic extract of dried fruits of *Hylocereus undatus* showed a dose-dependent decrease in acetylcholinesterase level when it was compared against the negative control group (Scopolamine).

TABLE 7: EFFECT OF ETHANOLIC EXTRACT OF DRIED FRUITS OF *HYLOCEREUS UNDATUSON* ACETYL-CHOLINE ESTERASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Enzyme Levels
Control	0.081 ± 0.0003
Disease Control	0.092 ± 0.0003
Standard Control	0.071 ± 0.0004
Low Dose (100 Mg/Kg)	0.080 ± 0.0004
Medium Dose (200 Mg/Kg)	0.078 ± 0.0004
High Dose (300 Mg/Kg)	0.075 ± 0.0003
Extract Only (300 Mg/Kg)	0.074 ± 0.0005

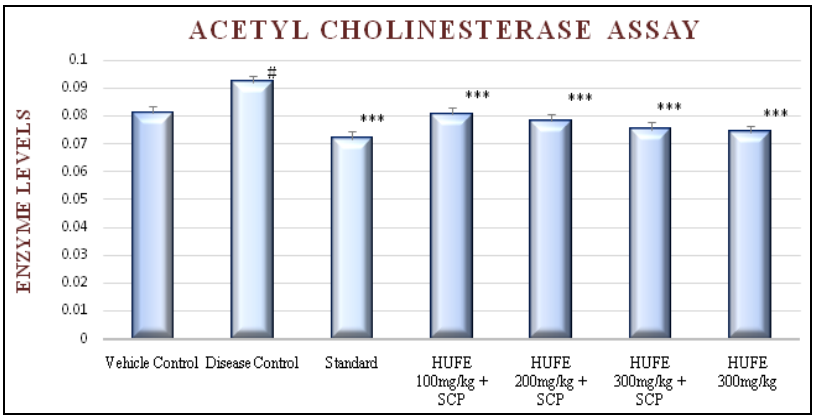

FIG. 9: EFFECT OF ETHANOLIC EXTRACTS OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON ACETYLCHOLINE ESTERASE LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with toxicant control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

Determination of Catalase (Cat) Principle: All the four test doses of the ethanolic extract of dried fruits of *Hylocereus undatus* showed dose-dependent increase in catalase activity when it was compared against the negative control group (Scopolamine).

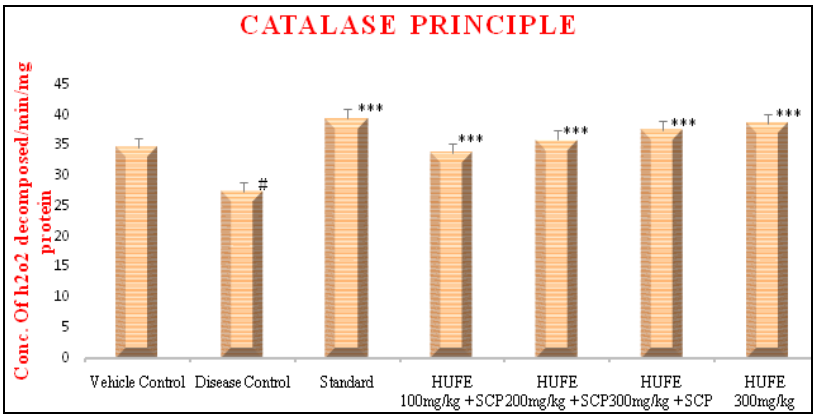

FIG 10: EFFECT OF ETHANOLIC EXTRACTS OF DRIED FRUITS OF *HYLOCEREUS UNDATUS*ON CATALASE LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with toxicant control, and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer multiple comparisons test.

TABLE 8: EFFECT OF ETHANOLIC EXTRACT OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON CATALASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Catalase Levels
Control	34.67 ± 0.12
Disease Control	27.388 ± 0.11
Standard Control	39.393 ± 0.15
Low Dose (100 mg/kg)	33.765 ± 0.08
Medium Dose (200 mg/kg)	35.846 ± 0.07
High Dose (300 mg/kg)	37.496 ± 0.15
Extract Only (300 mg/kg)	38.623 ± 0.13

Determination of Malonyldialdehyde (Mda): All the four test doses of the ethanolic extract of dried fruits of *Hylocereus undatus* showed dose-dependent decrease in Malonyl dialdehyde level when it was compared against the disease control group (Scopolamine).

TABLE 9: EFFECT OF ETHANOLIC EXTRACT OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON MALONYL DIALDEHYDE (MDA) LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Mda Levels
Control	0.025 ± 0.001
Disease Control	0.030 ± 0.0003
Standard Control	0.010 ± 0.0004
Low Dose (100 mg/kg)	0.022 ± 0.0004
Medium Dose (200 mg/kg)	0.020 ± 0.0003
High Dose (300 mg/kg)	0.018 ± 0.0004
Extract Only (300 mg/kg)	0.013 ± 0.0005

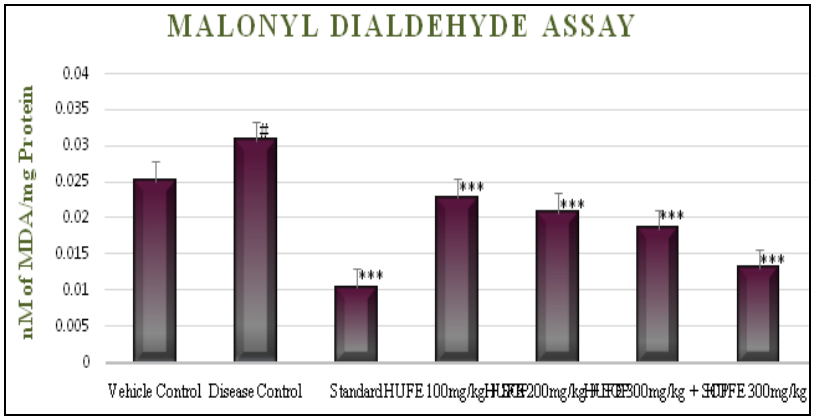

FIG. 11: EFFECT OF ETHANOLIC EXTRACT OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON MALONYL DIALDEHYDE (MDA) LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P <0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparisons test.

Determination of Nitric Oxide (No): All the four test doses of the ethanolic extract of dried fruits of *Hylocereus undatus* showed a dose-dependent decrease in Nitrite level when compared against the disease control group (Scopolamine).

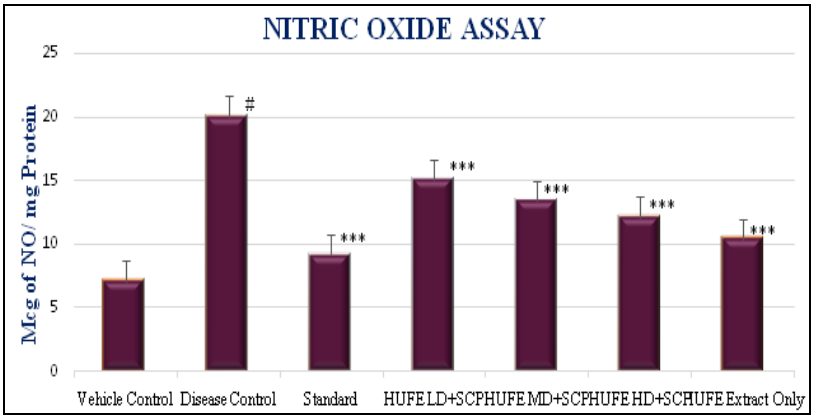

FIG. 12: EFFECT OF ETHANOLIC EXTRACTS OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON NITRITE LEVEL IN SCOPOLAMINE-INDUCED ALZHEIMER IN ALBINO WISTAR RATS. Note: Values are expressed as mean ± SEM (n=6). ***P<0.001 compared with disease control, **P<0.01 compared with toxicant control and #P<0.05 compared with vehicle control. Data was analyzed using one-way ANOVA followed by the Tukey-Kramer test. ANOVA: Analysis of variance, SEM: Standard Error of Mean

TABLE 10: EFFECT OF ETHANOLIC EXTRACT OF DRIED FRUITS OF *HYLOCEREUS UNDATUS* ON NITRITE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Nitrite Levels
Control	7.062 ± 0.02
Disease Control	20.047 ± 0.24
Standard Control	9.148 ± 0.10
Low Dose (100 mg/kg)	15.071 ± 0.10
Medium Dose (200 mg/kg)	13.383 ± 0.17
High Dose (300 mg/kg)	12.119 ± 0.14
Extract Only (300 mg/kg)	10.385 ± 0.08

DISCUSSION: Alzheimer's disease is a degenerative brain illness that causes loss of memory and mild cognitive impairment, eventually leading to dementia [14]. Congenital abnormalities, neurofibrillary tangle growth, poor processing of amyloid progenitors, mitochondrial deficiencies, neurotropic factor insufficiency, neurotoxicity of trace elements, energy metabolism defects, and oxidative damage are only a few of the reasons that can cause AD [15]. Short-term memory loss, inability to learn something new, mood swings, difficulty remembering terms, forgetting identities, and misplacing things are all symptoms of prior sickness [3]. Frustration, aggressiveness, and irritation are common emotional traits in Alzheimer's sufferers [16].

There are still numerous researches going on into treatments that can prevent or at least effectively change the course of Alzheimer's disease, also known as 'disease-modifying therapies [17]. The search for newer medications that are both safe and effective weapons in the fight against Alzheimer's disease has already required the collaboration of a huge number of researchers and thousands of consenting volunteers [18]. The fruit pulp of *Hylocereus undatus* tested for the anti-Alzheimer model in Morris Water Maze test. The MWM task has been widely used to test spatial learning and memory in rodents [19].

It showed a decrease in escape latency time and increased in time spent in the target quadrant in a dose dependent manner. The Y-maze task is an efficient spatial memory test for chronic stressed rats since behavioral evaluation can be initiated a day following the final stress session and completed within hours after its beginning. When the cumulative acts of stress are at their height, the evaluation of spatial recognition-memory from the output of the Y maze indicates the deterioration in the hippocampus [20]. Y maze test for AD rats pretreated with ethanolic extracts of dried fruit of *Hylocereus undatus* showed an increase in % alternation. The Novel Object Recognition experiment is employed in animal models of neurological disorders to assess memory, particularly memory recognition. This test is Reliedupon rodents' spontaneous propensity to invest more time investigating an unfamiliar object than a known one.

The decision of exploring the unfamiliar object represents the utilization of memory for learning and identification [9]. And Novel Object Recognition test for AD rats pretreated with ethanolic extracts of dried fruit of *Hylocereus undatus* showed an increase in Recognition Index. All of it indicates improve in spatial memory of rats. Acetyl-cholinesterase degrades acetylcholine, a neurotransmitter that promotes cholinergic transmission by activating nicotinic and metabotropic muscarinic receptors. As a result, Acetylcholinesterase inhibitors can facilitate cholinergic transmission by reducing ACh breakdown. The loss of ACh, which is caused by AChE's hydrolytic action, causes cognitive impairment [21].

Direct analysis of neurotransmitter content in the cerebral cortex of AD rats pretreated with *Hylocereus undatus* fruit extract showed a reduction in AchE level. CAT is a glycoprotein found in all cells' antioxidant defense mechanism that protects them from oxidative damage caused by reactive oxygen species. It protects the cell against extremely reactive hydroxyl radicals by catalyzing the breakdown of H_2O_2 to H2O and O2.[22] An increase in catalase activity has been observed in AD rats pretreated with *Hylocereus undatus* fruit extract.

MDA levels that are elevated are a sign of increased oxidative stress. It has been suggested that an abnormal rise in MDA is linked to memory impairment [23]. However, a decrease in the levels of the lipid peroxidation product Malonyl dialdehyde (MDA) has been observed in AD rats that were pretreated with *Hylocereus undatus* fruit extract. Oxidative & nitrosative stress have been described in AD brains before the accumulation of Aβ and phosphorylated tau due to increasing levels of

reactive oxygen and nitrogen species, respectively [24]. The AD rats pretreated with *Hylocereus undatus* showed a significant decrease in nitrite levels when compared against the negative control group. Reactive oxygen species are responsible for the age-related degradation of cognitive performance, which ultimately may cause Alzheimer's disease in elderly persons [25]. *Hylocereus undatus* has been reported to possess antioxidant properties as well. The neuroprotective effect of HUFE may be due to its antioxidant property; hence, the susceptible brain cells are exposed to lesser oxidative stress, thereby reducing brain damage. The symptoms of dementia are associated with the impaired neurotransmission in the affected brain regions.

CONCLUSION: The findings of the current study indicate that alcoholic extract of the fruits of *Hylocereus undatus* can be beneficial for the prevention or treatment of Alzheimer's by significantly reducing the Acetylcholinesterase levels in the brain. Preliminary phytochemical analysis of an alcoholic extract of *Hylocereus undatus* showed the presence of carbohydrates, flavonoids, glycosides, tannins, alkaloids, phenolic compounds. The present study concluded that ethanolic extract of *Hylocereus undatus* possesses anti-Alzheimer activity by improving spatial memory and also showed a significant increase in the onset of action and decreased in the duration of action and recovery of time as compared to the negative control, thus justifying its anti-Alzheimer activity which may be due to presence of Phenolic compounds, flavonoid, alkaloid, and glycoside, as a phytoconstituent present. Additional studies are suggested to classify the active phytochemicals and illustrate the mode of action.

ACKNOWLEDGEMENT: The author thanks her guide Dr. Mrs. Vanita G. Kanase and the department of pharmacology for constantly supporting and providing facilities during the work.

CONFLICTS OF INTEREST: The authors have no conflicts of interest regarding this investigation.

REFERENCES:

1. Erkkinen MG, Kim M and Geschwind MD: Major Neurodegenerative Diseases. Published Online 2018.
2. Pradeep S, Jain AS and Dharmashekara C: Alzheimer's disease and herbal combination therapy: a comprehensive review. J of Alzheimer's Disease Repo 2020; 4(1): 417-29.
3. Rao RV, Descamps O, John V and Bredesen DE: Ayurvedic medicinal plants for Alzheimer's disease: A review. Alzheimer's Res and Therapy 2012; 4(3): 1-9.
4. Teixeira JP, de Castro AA, Soares FV, da Cunha EFF and Ramalho TC: Future therapeutic perspectives into the Alzheimer's disease targeting the oxidative stress hypothesis. Molecules 2019; 24(23): 1-17.
5. Kaur R, Parveen S, Mehan S, Khanna D and Kalra S: Neuroprotective Effect of Ellagic Acid against Chronically Scopolamine Induced Alzheimer's Type Memory and Cognitive Dysfunctions: Possible Behavioural and Biochemical Evidences. International J of Preve Med Res 2015; 1(2): 45-64.
6. Sudha K and Baskaran DKSD: Evaluation of Functional Properties of *Hylocereus undatus* (White Dragon Fruit). International J of Agri Scien and Res 2017; 7(5): 451-56.
7. Kanchana P, Devi SKS V, Pushpa Latha P and Spurthi N: Phytochemical evaluation and pharmacological screening of Antiparkinson's and Laxative activities of *Hylocereus undatus* (White Pitaya) in rodents. IOSR Journal of Pharmacy www.iosrphr.org. 2018; 8(4): 78-92.
8. Upadhyay P, Sadhu A and Singh PK: Revalidation of the neuroprotective effects of a United States patented polyherbal formulation on Scopolamine induced learning and memory impairment in rats. Biomedicine and Pharmacotherapy 2018; 97: 1046-52.
9. Khooshbu P and Ansari I: Evaluation of Anti-Alzheimer Activity of Alcoholic Extract of Costus Pictus D. Don Leaves in Wistar Albino Rats. Asian Journal of Pharmaceutical and Clinical Research 2019; 13(2): 36-43.
10. Hughes RN: The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neuroscience and Biobehavioral Reviews 2004; 28(5): 497-05.
11. Ellman GL, Courtney KD, Andres V and Featherstone RM: A new and rapid colorimetric determination of acetyl cholinesterase activity. Biochem Pharma 1961; 7(2): 1-2.
12. Aebi H: Methods in Enzymology [13] catalase *in-vitro*. Published online 2021; 2.
13. Khare P, Chaudhary S, Singh L, Yadav G and Verma S: Evaluation of Nootropic activity of *Cressa cretica* in scopola-mine- induced memory impairment in mice. International Journal of Pharmacology and Toxicology 2014; 2(2). doi:10.14419/ijpt.v2i2.2004.
14. Kumar A, Aggarwal A, Singh A and Naidu PS: Animal Models in Drug Discovery of Alzheimer's Disease: A Mini Review. EC Pharm and Toxicology 2016; 21: 60-79.
15. Malekzadeh S: Galen Medical Journal, Vol 6, No 3 (2017) Drugs Induced Alzheimer's disease in Animal Model. 2020; 6(3): 1-12. doi:10.22086/gmj.v6i3.820.
16. Grossberg GT and Desai AK: Management of Alzheimer's Disease 2003; 58(4): 331-53.
17. Yiannopoulou KG and Papageorgiou SG: Current and future treatments for Alzheimer's disease. Therapeutic Advances in Neurological Disorders 2013; 6(1): 19-33.
18. Ellison JM: What's New in the Alzheimer's Treatment Pipeline ? Published online 2020: 1-7.
19. Jawaid T, Shakya AK, Siddiqui HH and Kamal M: Evaluation of Cucurbita maxima extract against scopolamine-induced amnesia in rats: Implication of Tumour necrosis factor alpha. Zeitschrift fur Naturforschung - Section C Journal of Biosciences 2014; 69(9-10): 407-17.
20. Acid G and Impairment SC: Methods Preparation of the Glycyrrhizic Acid Animals Y-maze Test 2020; (853): 1-8.
21. Balkrishna A, Pokhrel S and Tomer M: Anti-acetylcholinesterase activities of mono-herbal extract and

exhibited synergistic effects of the phytoconstituents: A biochemical and computational study. Molecules 2019; 24(22): 1-21.

22. Sahab Uddin MD: Evaluation of Nootropic Activity of <i>Persicaria flaccida</i> on Cognitive Performance, Brain Antioxidant Markers and Acetylcholinesterase Activity in Rats: Implication for the Management of Alzheimer's Disease. American Journal of Psychiatry and Neuroscience 2016; 4(2): 26.
23. Güven A, Yıldız Dalgınlı K, Çulhaoğlu H, Huseyinoglu N and İlhan Alp S: Investigation of the Levels of Blood MDA, GSH and Nitric Oxide Levels in Patients with Probable Alzheimer's Disease. Kafkas J of Medical Sciences 2020; 10(3): 188-94.
24. Asiimwe N, Yeo SG, Kim MS, Jung J and Jeong NY: Nitric oxide: Exploring the contextual link with Alzheimer's disease. Oxidative Medicine and Cellular Longevity 2016; 2016. doi:10.1155/2016/7205747.
25. Teixeira JP, de Castro AA, Soares FV, da Cunha EFF and Ramalho TC: Future therapeutic perspectives into the Alzheimer's disease targeting the oxidative stress hypothesis. Molecules 2019; 24(23): 1-17.

How to cite this article:
Shaikh RK and Kanase VG: Evaluation of anti-alzheimer's activity of alcoholic extract of *Hylocereus undatus* fruits in Wistar albino rats.. Int J Pharm Sci & Res 2022; 13(4): 1567-80. doi: 10.13040/IJPSR.0975-8232.13(4). 1567-80.