Research Article

Screening of Anti-Alzheimer's Activity against Scopolamine-induced Amnesia in Mice Model

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Received: 13-06-2022; **Revised:** 22-08-2022; **Accepted:** 29-08-2022; **Published on:** 15-09-2022.

ABSTRACT

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. The main purpose of this research was to develop evidence-based medicine and achieve antioxidant and good neuroprotective activity of the fruit pulp of *Hylocereus undatus*, to assess the side effects and adverse drug reaction of the selected drug. To carry out biochemical estimations, the mice were sacrificed on the 21st day of the drug treatment. Brains were dissected carefully and kept in an ice-cold buffer; brains were subjected to homogenization with 10% NaCl in distilled water. All values were calculated as mean ± SEM (where, n=6). ****p≤0.0001, *p≤0.05, ***p≤0.001 as compared to scopolamine treated disease group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. ####p≤0.0001 as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)]. In this study, it was investigated that the animals (mice) showed an increase in levels of catalase, superoxide dismutase (SOD), glutathione, and malondialdehyde (MDA) with a decrease in levels of acetyl choline. Hence, through this study, it was found that results of *Hylocereus undatus* fruit pulp possess significant anti-Alzheimer's activity comparable to that of the standard drugs due to the presence of chemical constituents like tannins and phenolic compounds, flavonoids, terpenoids.

Keywords: Scopolamine, Alzheimers, Dementia, Acetylcholine, *Hylocerus udantu*.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v76i01.018

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v76i01.018

INTRODUCTION

Neurodegeneration is the progressive atrophy and loss of function of neurons. It primarily affects the neurons in the brain system.[1] Alzheimer's is a chronic neurodegenerative disease that progresses over time leading to worsening symptoms and can alternately cause death.[2] Alzheimer's disease is a progressive neurologic disease that results in the irreversible loss of neurons, particularly in the cortex and hippocampus.[3] It is the most common form of dementia (a continuous decline in thinking, behavioral, and social skills that disrupts a person's ability to function independently.[4] Currently, the only approved therapies for Alzheimer's may help identify some of its more advanced symptoms. However, there are ongoing efforts to develop treatments that when gets approved may delay, stop or prohibit the progression of AD.[5] In AD there are decreased and dysfunctional cholinergic neurons that show decreased choline uptake reduced ACH release/ decreased ChAT and decreased nicotine receptor.[6]

Today, there is still no cure for the disease or even to stop its progression. However, currently available therapeutic strategies are mainly symptomatic slowing the evolution of the disease.[7] The main treatments for the disease are acetylcholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists.[8] Unfortunately, these drugs have been connected to several adverse effects like nausea, vomiting, anorexia, and insomnia, due to nonselective action on a variety of organ tissues both centrally and peripherally.[9]

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.[10] *Hylocereus undatus*: Pitaya, often known as dragon fruit, is a tropical fruit that belongs to the cactus family, Cactaceae.[11] it is believed to be the native of Mexico and transplanted to Central America by Europeans.

It has been brought to Southeast Asian countries including Malaysia, Indonesia, Taiwan, Thailand, Sri Lanka, Bangladesh, and Vietnam.[12]

The fruit pulp is rich in chemical constituents like of Carbohydrate, Proteins and amino acids, Alkaloids, Terpenoids and Steroids, Glycoside and Flavonoids, Tannins and phenolic compounds, and Saponins.[13] The aim of the present study is to screen the possible antioxidant and neuroprotective effect of ethanolic extracts of *Hylocereus undatus* against scopolamine-induced memory impairment in mice. The goal of current study is to screen the possible antioxidant and neuroprotective effect of ethanolic extracts of *Hylocereus undatus* against scopolamine-induced memory impairment in mice.

The main purpose of this research was to develop evidence-based medicine and achieve antioxidant and good neuroprotective activity of the fruit pulp of *Hylocereus undatus.* To assess the side effects and adverse drug reaction of the selected drug. Natural products are gaining a renowned interest now a day for the prevention and treatment of various diseases because of their safety and better tolerance. Hence in the present study, we are using a natural product for the treatment of Alzheimer as they have least adverse effects.

METHODOLOGY

Procurement and Authentication of Sample

Hylocereus undatus (white pitaya) was collected in December 2019. It was procured locally from the vendors in Hyderabad. The plant was authenticated by Dr. Shaik Mohammed Aliuddin (Secretory, Hyderabad Unani Research Foundation, Hyderabad, Telangana)

Processing of Plant Sample

The fruit pulp of *Hylocereus undatus* was cut into pieces and then dried under shade. The pulp extract was stored in an airtight container and kept at room temperature.[14]

Preparation of Extract

Dried plant material (500 gm) was extracted with 1500ml of ethanol using the soxhlet extraction by placing a finely crude drug sample in the chamber of the soxhlet apparatus. The round bottom flask placed on the heating mantle was filled with ethanol and heated while its vapors condense inside the condenser. The solvent which is condensed drops onto the thimble with the crude powdered drugs and extracts on coming in contact with it. When the liquid in the chamber gets risen to the top, the liquid contents then fall into the flask containing the solvent.

This continuous cycle is performed until the siphon tube solvent drop leaves no more evaporated residue. The round bottom flask is filled with few boiling chips to avoid bumping during heating.

Phytochemical Analysis

EEHU was subjected to a preliminary phytochemical screening.

Toxicological Studies

After performing complete a survey on the acute toxicity test. It shows that EEHU does not cause any mortality or toxicity.

Based on the literature review done, a dose of 200mg/kg body weight was selected to screen the pharmacological activity of ethanolic extracts of *Hylocereus undatus* in scopolamine-induced Alzheimer mice model.

Thus, ethanolic extracts of *Hylocereus undatus* oral dose 200mg/kg and 400mg/kg were chosen.

Animals

Swiss albino mice have been used for this study. The mice were procured from Sai Nath agency, Hyderabad. The mice used were male(20-35gram). The mice were given pellets and water ad libtum. The animal experimental protocol has been approved by-

Institutional Animal Committee vide reference no: (IAEC/SUCP/2020/011).

Animal Groups

The animals were categorized into 5 groups containing 6 mice each.

Scopolamine was administered 30 minutes after the standard and test drugs were given.

Group I (Normal Control)

Animals of this group received normal diet and vehicle 0.5%w/v CMC by p.o. at a dose of 1ml/100gm body weight.

Group II (Negative Control

Animals of this group received Scopolamine 2mg/kg body weight in 0.5%w/v CMC by p.o.

Group III (Standard)

Animals of this group received Scopolamine 2mg/kg body weight +Donepezil HCL 5mg/kg body weight.

Group IV (Treatment group)

Animals of this group received Scopolamine 2mg/kg body weight + ethanolic extract of *Hylocereus undatus* 200mg/kg body weight in 0.5%w/v CMC by p.o.

Group V (Treatment group)

Animals of this group received Scopolamine 2mg/kg body weight +ethanolic extract of *Hylocereus undatus* 400mg/kg body weight in 0.5%w/v CMC by p.o

Scopolamine Induced Memory Impairment

Scopolamine (belladonna alkaloid) is an anticholinergic. Scopolamine acts as a competitive inhibitor at

postganglionic muscarinic receptor and on the smooth muscle that responds to acetylcholine.[15] Scopolamine acts by blocking the activity of muscarinic acetylcholine receptor and concomitant appearance of cognitive amnesia and electrophysiological changes.[16]

Screening Methods

Elevated Plus-Maze Model

Elevated plus-maze is used to estimate memory in mice. It is most widely used for measuring the anxiety-like behavior. The mice have access to all arms. The number of entries into both the arms is observed and the time spent in the respective arms is noted.

On day 7 of the 21days drug treatment, each mouse was put down at the end of the open arm facing away from the center area. Transfer latency was recorded for each mouse. The mice were then allowed to explore the maze for a specific amount of time and returned to their respective cages.

Locomotor Activity

Locomotor activity is measured by digital actophotometer. It also incorporates electric shock of up to 100 volts for activating the mice or rats.[17] Each animal is placed individually in the actophotometer. The animal is observed over 5 minutes following drug administration.

Biochemical Estimations

To carry out biochemical estimations, the mice were sacrificed on the 21st day of the drug treatment. Brains were dissected carefully and kept in an ice-cold buffer; brains were subjected to homogenization with 10% NaCl in distilled water. This was then followed by centrifugation and centrifuged at 3000rpm.

The resultant supernatant which was obtained was further used for estimation of the following parameters Estimation of acetyl cholinesterase, Estimation of lipid peroxidation, Estimation of glutathione, Estimation of superoxide dismutase, and Estimation of catalase activity

Histopathological Evaluation

The brains of the animals were dissected on the 21st day of drug treatment and transferred into a container with 10% formalin solution. It was further used to carry out histopathological evaluation.

RESULTS

Estimation of behavioral parameters

Locomotor activity (Actophotometer)

The standard, EEHU (200mg/kg), and EEHU (400mg/kg) showed an improved locomotor activity in comparison to the disease control group.

Table 1: Effect of Treatment Groups on The Locomotor Activity of Rats

Groups	Locomotor activity (5 minutes)			
	Day 1	Day 7	Day 14	Day 21
Control	190.7 ± 4.73	190.7 ± 4.73	190.7 ± 4.73	190.7 ± 4.73
Disease control	115.8 ± 6.75	112.8 ± 5.31	101.7 ± 2.06	94.0 ± 2.92
Standard	166.7 ± 9.436	166.7 ± 9.43	166.7 ± 9.43	166.7 ± 9.436
EEHU (200mg/kg)	142.2 ± 4.98	137.3 ± 3.95	131.2 ± 3.81	113.2 ± 2.38

All values were expressed as mean ± SEM (where, n=6). On day 1 the values were ns as compared to Scopolamine treated disease control group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. Ns as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)].

On day 7, 14, and 21 the values were $^{****}p\leq0.0001$, $^{*}p\leq0.05$, $^{***}p\leq0.001$, as compared to Scopolamine treated disease control group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. $^{\#\#\#\#}p\leq0.0001$ as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)].

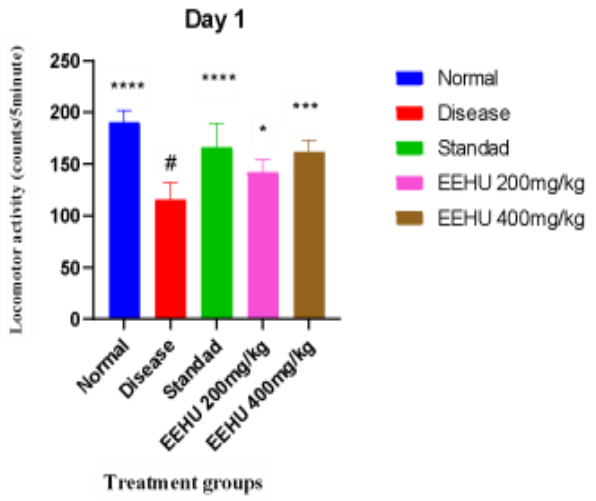

Figure 1: Effect of *Hylocereus undatus* on Locomotor Activity of Mice on Day 1

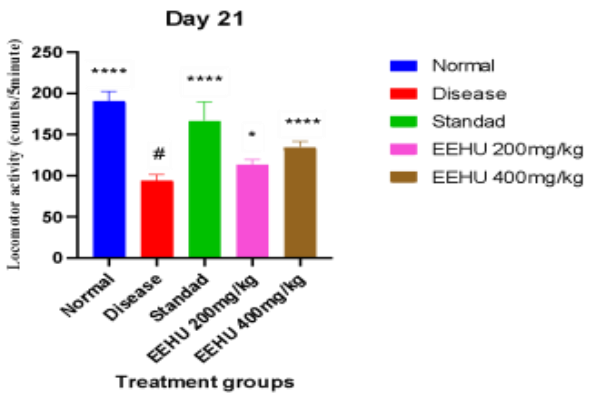

Figure 2: Effect of Treatment Group on Locomotor Activity of Mice on Day 21

Elevated Plus-Maze

The transfer latency was measured on the first day prior to 30 mins administration of the scopolamine. The transfer latency was similarly measured on the last day i.e., 21st day of the drug treatment. There was a decrease in transfer latency in the treatment group over 21 days.

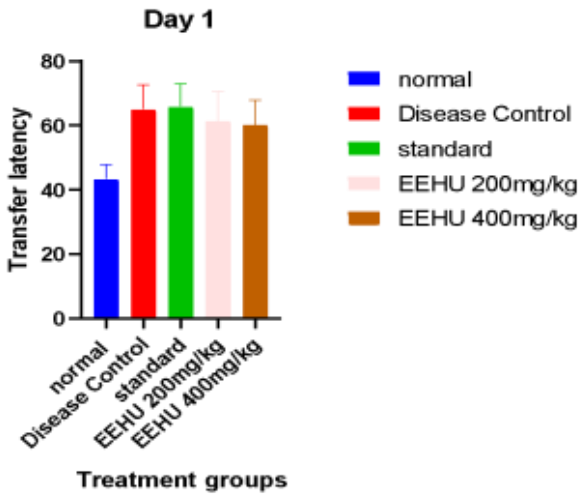

Figure 3: Transfer Latency on Day 1

Table 2: Transfer Latency of Mice

Groups	Locomotor activity (5 minutes)			
	Day 1	Day 7	Day 14	Day 21
Control	43.33 ± 4.41	28.17 ±2.18	26.0 ± 2.47	7.5 ± 0.76
Disease control	65.0 ± 7.63	65.0 ± 8.16	61.67 ± 6.66	25 ± 4.830
Standard	65.83 ± 7.12	32.50 ± 3.81	27.50 ± 3.819	8.83 ± 1.815
EEHU (200mg/kg)	61.33 ± 9.24	41.67 ± 4.41	35.83 ± 4.72	11.67 ± 2.17
EEHU (400mg/kg)	**60.17 ± 7.74**	**39.17 ± 4.72**	**37.50 ± 3.81**	**14.17 ± 3.26**

All values were estimated as Mean ± SEM (n=6). One-way Anova was carried out followed by Dunnett's multiple comparison test.

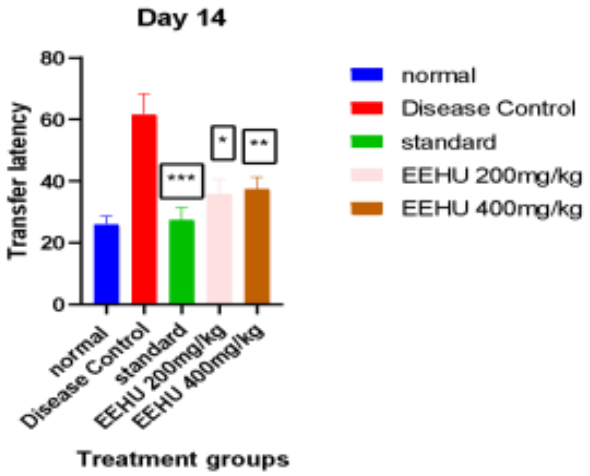

Figure 4: Transfer Latency on Day 14

Estimation of biochemical parameters

Estimation of catalase activity

An increase in the Catalase activity were observed in standard (1.78 ± 0.07), EEHU (200mg/kg) (1.60 ± 0.03), and EEHU (400mg/kg) (1.717 ± 0.09) when compared to disease control group (1.23 ± 0.07).

Table 3: Effect of Catalase Activity in the Rat Brain

Treatment groups	Catalase activity (MEAN ± SEM)
Control	2.00 ± 0.12
Disease	1.23 ± 0.07
Standard	1.783 ± 0.07
EEHU (200mg/kg)	1.60 ± 0.03
EEHU (400mg/kg)	1.717 ± 0.09

All values were calculated as MEAN ± SEM (where, n=6). ****p≤0.0001, *p≤0.05, ***p≤0.001 as compared to Scopolamine treated disease group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. ####p≤0.0001 as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)].

Estimation of Superoxide Dismutase Levels

The Scopolamine-induced group showed significantly reduced levels (0.215 ± 0.02) of SOD. Administration of Donepezil, EEHU (200mg/kg), and EEHU (400mg/kg) showed a significant increased level of SOD (0.75 ± 0.10), (0.505 ± 0.03), (0.638 ± 0.04) respectively as shown in figure 5.

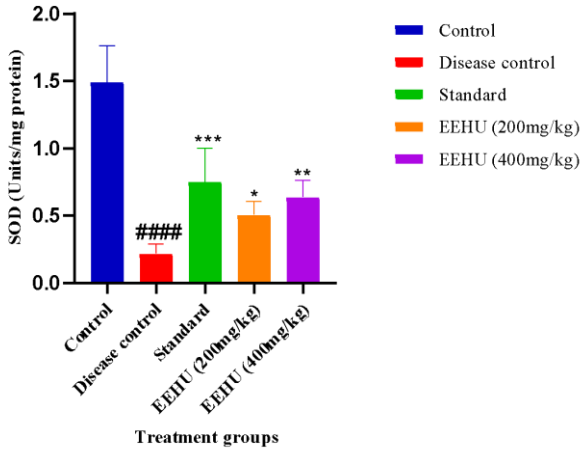

Figure 5: Estimation of Superoxide Dismutase Levels of Mice Brain

Table 4: Effect of Sod Activity in the Rat Brain

Treatment group	Superoxide dismutase activity (MEAN ± SEM)
Control	1.492 ± 0.10
Disease	0.215 ± 0.02
Standard	0.75 ± 0.10
EEHU (200mg/kg)	0.505 ± 0.03
EEHU (400mg/kg)	0.638 ± 0.04

All values were estimated as mean ± SEM (where, n=6). ****p≤0.0001, *p≤0.05, ***p≤0.001 as compared to Scopolamine treated disease group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg/kg) were compared with Group II (Disease control)]. ####p≤0.0001 as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)].

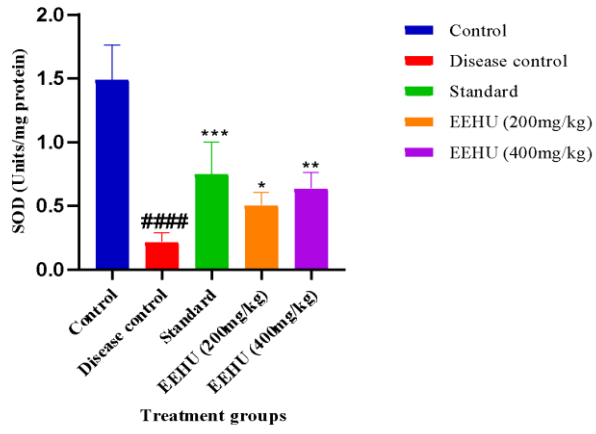

Figure 6: Estimation of Superoxide Dismutase Levels of Mice Brain

Estimation Of Brain GSH (Reduced Glutathione) Levels

An increase in the GSH levels were observed in standard (17.52 ± 0.75), EEHU (200mg/kg) (15.32 ± 0.76), and EEHU (400mg/kg) (20.33 ± 0.57) when compared to disease control group (16.35 ± 0.50) as shown in figure 7.

Table 5: Effect 9f GSH Levels in the Mice Brain

Treatment groups	Glutathione levels (MEAN ± SEM)
Control	31.23 ± 0.56
Disease	12.50 ± 1.00
Standard	17.52 ± 0.75
EEHU 200mg/kg	15.32 ± 0.76
EEHU 400mg/kg	16.35 ± 0.50

All values were expressed as mean ± SEM (where, n=6). ****p≤0.0001, *p≤0.05, ***p≤0.001 as compared to scopolamine treated disease group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. ####p≤0.0001 as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)].

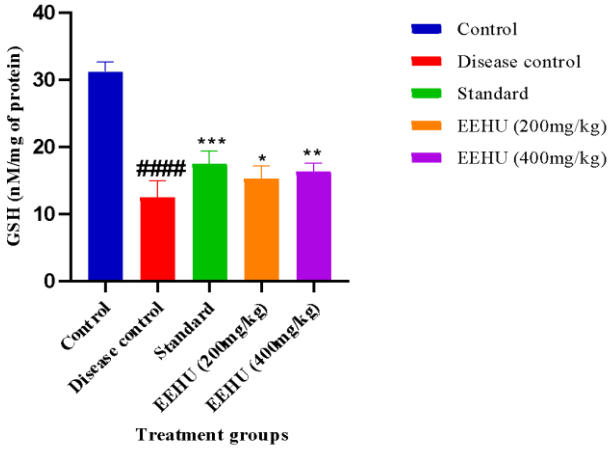

Figure 7: Estimation of GSH levels of mice brain

Estimation of malondialdehyde (MDA) levels

A rise in MDA levels in Scopolamine treated group (1.802 ± 0.12) was observed. Administration of Donepezil EEHU (200mg/kg), and EEHU (400mg/kg) resulted in decreased levels of MDA (0.88 ± 0.19), (1.22 ± 0.05), (0.966 ± 0.19) respectively as shown in figure 8.

Table 6: Effect of MDA Levels in the Rat Brain.

Treatment groups	Malondialdehyde levels (MEAN ± SEM)
Control	0.2417 ± 0.06
Disease	1.802 ± 0.12
Standard	0.88 ± 0.19
EEHU 200mg/kg	1.22 ± 0.05
EEHU 400mg/kg	0.966 ± 019

All values were calculated as mean ± SEM (where, n=6). ****p≤0.0001, *p≤0.05, ***p≤0.001 as compared to scopolamine treated disease group (Group II) [Group III (Standard), Group IV (EEHU 200mg/kg), Group V (EEHU 400mg.kg) were compared with Group II (Disease control)]. ####p≤0.0001 as compared to the vehicle-treated group (Group I) [Group II (Disease control) was compared with Group I (Control)]

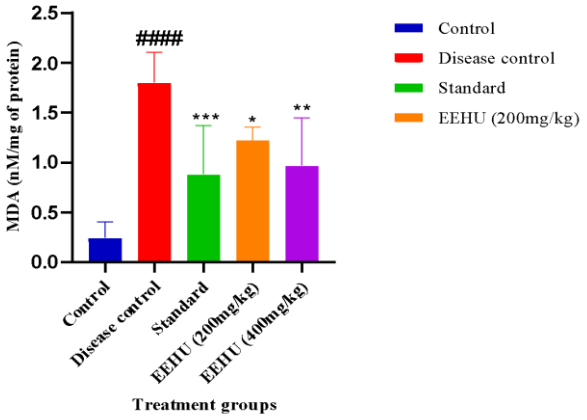

Figure 8: Estimation of MDA Levels of Mice Brain

Histopathology of mice brain

Disease control group

The mice of this group showed multifocal necrosis which was detected in the cortex region of the cerebral hemisphere. Meningeal hemorrhage was observed. Multiple foci of necrosis and apoptosis were observed in the neurons of the hippocampus

Treatment Group 1

The mice of this group showed necrosis with infiltration of the inflammatory cells in cortex region of the cerebral hemisphere. The hippocampus region appeared normal. Hemorrhages were observed in the meninges covering the brain.

Treatment Group 2

The mice of this group showed the presence of the normal hippocampus and cerebral cortex. Meninges appeared normal with mild a proliferation of neurons.

DISCUSSION

Alzheimer's disease is the most common neurodegenerative disease resulting in progressive damage to the brain. Alzheimer's disease is identified as a protein misfolding disease called proteopathy which is caused by plaque accumulation of amyloid and tau proteins in the brain. The plaques formation and tangles in the brain are the main cause of Alzheimer's disease. There is a destruction of function of neurons i.e., loss of connection between the nerve cells of the brain.[16] Thus, this results in progressive memory loss, impaired thinking, the deterioration, and changes in personality and mood. It includes deterioration of language, comprehension, memory, and thinking, and learning capability.[19]

Early diagnosis may help in the clinical study investigating new potential treatments.

There are only a few drugs available for the treatment of Alzheimer's disease, which give only symptomatic relief to the patient and have no permanent cure. The current treatment options are limited to 2 main approaches that are the use of anticholinesterases to maintain the Ach levels and use of NMDA receptors to prevent toxicities that are responsible for damage of neurons in the brain.[20]

There is significant oxidative damage in the brain of AD which is associated with abnormal marked accumulation of amyloid-beta and deposition of neurofibrillary tangles. Aβ not only induces oxidative stress but its generation is also increased as a result of oxidative stress.[21]

Thus, in the current study ethanolic extract of *Hylocereus undatus* was used.

The phytochemical test of EEHU confirmed the presence of flavonoids, alkaloids, terpenoids, phenols, etc. showing the potential antioxidant activity. In this study, Swiss albino mice were used. Mice were treated with EEHU 200mg/kg and 400mg/kg and were subjected to actophotometer, there was a rise in the locomotor activity similar to Donepezil treated group when compared with scopolamine treated group. The EEHU decreased the transfer latency same as Donepezil treated group on the last day of drug treatment.

Acetylcholinesterase undergoes catalyzation of choline and choline esters that functions as neurotransmitters. AchE levels were decreased in the treatment group of EEHU 200mg/kg and 400mg/kg when compared to the scopolamine treatment group.

The EEHU shows favorable antioxidant activity. Levels of SOD, catalase, GSH were significantly increased. And there was marked decrease in levels of malondialdehyde in mice treated with EEHU.

Catalase enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen.[22] Catalase levels were increased in mice treated with EEHU 200mg/kg and 400mg/kg respectively in comparison to the disease control group.

Superoxide dismutase undergoes catalyzation to form hydrogen peroxide and molecular oxygen. SOD levels increase in mice treated with 200mg/kg and 400mg/kg respectively in comparison with the disease control group. [23]

Reduced GSH levels impair the clearance of H_2O_2 and formation of hydroxyl radicals which results in the formation of an oxidative environment. Levels of GSH were increased in mice treated with EEHU 200mg/kg and 400mg/kg respectively in comparison to the disease control group. [24]

One of the important markers of oxidative stress is lipid peroxidation which is estimated by malondialdehyde levels.[25] The lipid peroxide radicals are produced due to the assault of the free radicals on the double bond of unsaturated fatty acid and arachidonic acid. Malondialdehyde levels showed a decrease in mice treated with EEHU 200mg/kg and 400mg/kg respective in comparison to the disease control group. [26]

Therefore, EEHU (200mg/kg and 400mg/kg) showed increase levels of catalase, superoxide dismutase, and GSH. Whereas acetylcholine levels were decreased.

EEHU (400mg/kg) showed similar effects to that of donepezil (5mg/kg) disclosing its antioxidant property.

CONCLUSION

In this study, it was investigated that the animals (mice) showed an increase in levels of catalase, superoxide dismutase (SOD), glutathione, and malondialdehyde (MDA) with a decrease in levels of acety choline

The behavioral studies of mice showed an increase in locomotor activity in comparison to disease control i.e scopolamine treated group. Elevated plus-maze showed significant improvement in the retention of the mice treated with EEHU against the scopolamine-induced group.

Hence, through this study, it was found that results of *Hylocereus undatus* fruit pulp possess significant anti-Alzheimer's activity comparable to that of the standard drugs due to the presence of chemical constituents like tannins and phenolic compounds, flavonoids, terpenoids. Based on some literature review it confirms that these chemical constituents can be used in treatment of Alzheimer's disease.

Thus it can be concluded that ethanolic extracts of *Hylocereus undatus* can be regarded as safe, economic, beneficia, l and as a natural source for Anti-Alzheimer's activity. Because of its easy availability, White pitaya (*Hylocereus undatus*) can be used as a cost-effective alternative for Alzheimer's disease.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.