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Evaluation Pharmacological potential of *Tricholepis glaberrima* DC plant extracts for Management of Alzheimer's Disease

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ABSTRACT:

The aim was to assess the effect of anti-Alzheimer's activity of the alcoholic extract of *Tricholepis glaberrima DC* Leaves in Alzheimer induce Wistar albino rats.In the present study, the efficacy of an Extract of *Tricholepis glaberrima DC* was evaluated against scopolamine-induced Alzheimer in the Wistar albino rat. Donepezil at a dose of 2.5 mg/kg, ECPDD was given in rat at a dose of 100 mg/kg body weight, 150 mg/kg body weight, and 200 mg/kg body weight. Anti-Alzheimer activity was assessed by victimization novel object recognition test, elevated plus maze, and Y-maze. And by biochemical test like neurotransmitter esterase activity, catalase activity, malonyldialdehyd and glutathione assays. Results showed that extracts treatment prevents Alzheimer and increases the level of acetylcholine, catalase, glutathione peroxidase, and phytochemical studies identified the presence of flavonoid and alkaloid in the ECPDD. According to the results, it was concluded that the leave of *Tricholepis glaberrima DC* has significant anti-Alzheimer activity due to the presence of potent antioxidants such as flavonoid and anticholinesterase enzyme present in 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.

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. There is currently no cure for Alzheimer's disease. But currently some category of drugs like AChE inhibitors and NMDA antagonists were used along with some anti oxidants and some other supportive therapy. Therefore there is a lot of promising scope in the development of drug therapy for this serious and debilitating disorder.

Since ancient time the herbal medicines are effective in the treatment of various ailment, many plants have been used in the treatment of several dreadful disease but they are not scientifically exploited or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. The present study is to prove the memory enhancement and cognitive effect of *Tricholep is glaberrima* DC on Alzheimer's induced mice using various memory retention experiments such as Y maze, Morris water maze, Passive avoidance etc.

PREPARATION OF EXTRACTS:

Petroleum ether extract of whole plants of *Tricholep is glaberrima* DC

The shade dried coarsely powdered whole plants of *Tricholep is glaberrima* DC (500gm) were extracted with petroleum ether (60-80˚C), for 72 hrs. After completion of extraction, the defatted extracts were filtered while hot through What mann filter paper (No.10) to remove any impurities if present. The extract was concentrated by vacuum distillation to reduce the volume to 1/10. Then the concentrated extract was transferred to 100ml beaker and the remaining solvent was evaporated on a water bath. Dark greenish brown coloured extract was obtained. The concentrated extract was then kept in a desiccator to remove the excessive moisture. The dried extract was packed in air tight glass container for further studies.

EXPERIMENTAL ANIMALS:

The Swiss albino mice weighing 22-30gm were used for this study. The inbreed animals were procured from the animal house of C. L. Baid Metha college of pharmacy, Thoraipakkam, Chennai-97. They were housed six per kg under standard laboratory conditions at a temperature 22±2°C with 12:12 hrs light and dark circle. The animals were provided with standard animal feed, water and ad libitum. The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by Institutional Animal Ethical Committee (IAEC)

EXPERIMENTAL DESIGN:

On the first day of experiment the animals were divided randomly into five groups ofsix animals each. Amnesia is induced by i.c.v. of streptozotocin for the II, III, IV, V groups were performed on the 21st day of the pretreated animals and treatment was continued for 5days. Control animals were given 1%w/v of CMC orally by using intragastric catheter, where the last dose was given 60 min prior to behavioral testing and on 30th day scarification of animal was done for invitro studies.[68]

Experimental dementia of AD in mice was induced by i.c.v. STZ. Mice were anesthetized with anesthetic ether and i.c.v. injections were made with a hypodermic needle of 0.4 mm external diameter attached to a 10 µl Hamilton micro litre syringe. STZ was dissolved in freshly made ACSF (25mg/ml) solution. (ACSF (147m MNaCl;2.9mMKCl;1.6mM

ASSESSMENT OF HABITUATION BEHAVIOUR

Open field activity

Exploratory behavior was evaluated in an open field paradigm. The open field was made up of plywood and comprises of 40 x 50 x 60 cm dimension. The entire apparatus was painted black and divided into 16 squares with white lines on the floor.

Closed field activity

The loco motor activity was measured by using an Acto photo meter. The acto photo meter consisted of a square arena (30 x 30 x 25 cm) with wire mesh bottom, in which the animal moves. Six lights and six photocells were placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam.

ASSESSMENT OF MEMORY AND RETENTION

Morris water maze test

The Morris water maze test is performed to evaluate spatial working and reference memory. In this model the animals are placed into a large circular pool of water and they can escape on to a hidden platform. The platform is hidden by its placement just below the water surface and by opaque water.

Passive Shock Avoidance Test:

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. The apparatus consisted of a box (27x27x27 cm) having three walls of wood and one wall of Plexi glass, featuring a grid floor (3mm stainless steel rods set 8mmapart) with a wooden platform (10x7x1.7cm) in the centre of the grid floor. Electric shock(20V,A/C) was delivered to the grid floor. During training session ,each mouse was gently placed on the wooden platform set in the centre of the grid floor, when the mouse stepped down and placed all its paw on the grid floor, shocks were delivered for 15 seconds and the Step Down Latency(SDL) was recorded. SDL was defined as time taken by the mouse to stepdown from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing, if they did not step down for a period of 60 seconds and subjected to retention test. On the 29th day, after the treatment of last dose training was given and memory retention was examined after 24 hours (i.e., on 30th day) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300seconds.

RESULT AND DISCUSSION:

PRELIMINARYPHY TO CHEMICAL ANALYSIS

The preliminary phytochemical analysis on HAECU revealed the presence of various phytoconstituents including alkaloids, carbohydrates, tannins, flavonoids, gums, mucilage, etc. which are given in Table 1.

Alzheimer's disease (AD) is now the most common cause of dementia. The incidence of AD increases with age. Impairment of short-term memory usually is the first clinical feature. When the condition progresses, additional cognitive abilities are impaired, as the ability to calculate, and use common objects and tools. Acetylcholine esterase inhibitors are the only agents approved by

Passive avoidance task, Y maze task, and Morris water maze test. It was found that treatment with HAECU protect cognitive deficits in STZ induced Alzheimer's disease.

Table 1: Phytochemical screening of *Tricho lepisglaberrima DC*

S.NO	CONSTITUENTS	REMARKS
1.	Alkaloids	Present
2.	Carbohydrates	Present
3.	Protein	Absent
4.	Steroids	Absent
5.	Phenols	Present
6.	Tannins	Present
7.	Flavonoids	Present
8.	GumsandMucilage	Absent

9.	Glycosides	Absent
10.	Saponins	Absent
11.	Terpenes	Present
12.	Sterols	Absent

Effect of HAECU on open field activity

The Group II animals showed significant decrease in head dipping and line crossing when compared with Group I animals ($p<0.001$). Treatment with HAECU (200 and 400mg/kg) showed increased head dipping and line crossing behaviour statistically ($p<0.01$and $p<0.001$ for Groups IV and V respectively) and Group III also showed increased head dippings and line crossings behaviour significantly ($p<0.001$) when compared with Group II.Results are given in Table 2.

Table 2: Effect of HAECU in Open field

S.no	Groups	No of head dipping	No of line crossing
1	Control	47.00±1.41	128.34±3.42
2	Negative control	$25.80\pm1.74^{a***}$	$89.60\pm3.07^{a***}$
3	Standard	$39.20\pm1.35^{b***}$	$122.25\pm4.49^{b***}$
4	HAECU200 mg/kg	$31.80\pm2.39^{b**}$	$102.80\pm2.85^{b**}$
5	HAECU400 mg/kg	$35.60\pm1.32^{b**}$	$110.22\pm1.11^{b***}$

Effect of HAECU on closed field activity

There was a significant ($p<0.001$) decrease in the activity scores produced by GroupIIanimalswhencomparedwithGroupIanimals.TreatmentwithHAECU(200and400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.001$ and $p<0.001$ forGroup III, IV and V respectively) increase in the activity scores when compared with Group II animals. Results are given Table 3.

Table 3 :Effect of HAECU in Closed field

S.no	Groups	Activity scores
1	Control	407.2±7.31
2	Negative control	$309.6\pm3.89^{a***}$
3	Standard	$392.4\pm2.94^{\mathbf{b***}}$
4	HAECU200 mg/kg	$360.4\pm2.94^{b***}$
5	HAECU400 mg/kg	$378.2\pm1.56^{b***}$

Effect of HAECU on Step down Passive Shock Avoidance test

The Step Down Latency (SDL) of Group II animals were significantly decreased($p<0.001$)when compared with Group I animals. Treatment with HAECU (200 and 400 mg/kg) and standard drug ($p<0.001$, $p<0.01$ and $p<0.001$ for Group III, IV and V respectively) showed significant increase in step down latency when compared with Group II.The increase in SDL indicates increase in short term memory. Results are given Table 4.

Table4: Effect of HAECU in Passive avoidance

S.no	Groups	Latency(secs)
1	Control	43.83±1.24
2	Negativecontrol	$26.50\pm0.76^{a***}$
3	Standard	$39.50\pm0.53^{b***}$
4	HAECU200 mg/kg	$32.00\pm1.39^{b**}$
5	HAECU400 mg/kg	$35.33\pm1.30^{b***}$

Effect of HAECU on Ymazetask

Y maze task is one of the simplest versions of spontaneous alteration task which is used to measures partial working memory. The ability to alternate requires that the mice know which arm they have already visited. Normal mice are expected to exhibit an alteration percentage of 60-70.

Table 5: Effect of HAECU in Y maze

S.no	Groups	% Alterations
1	Control	48.17
2	Negative control	30.27^{a***}
3	Standard	44.40^{b***}
4	HAECU200 mg/kg	36.71^{b**}
5	HAECU400 mg/kg	39.65^{b***}

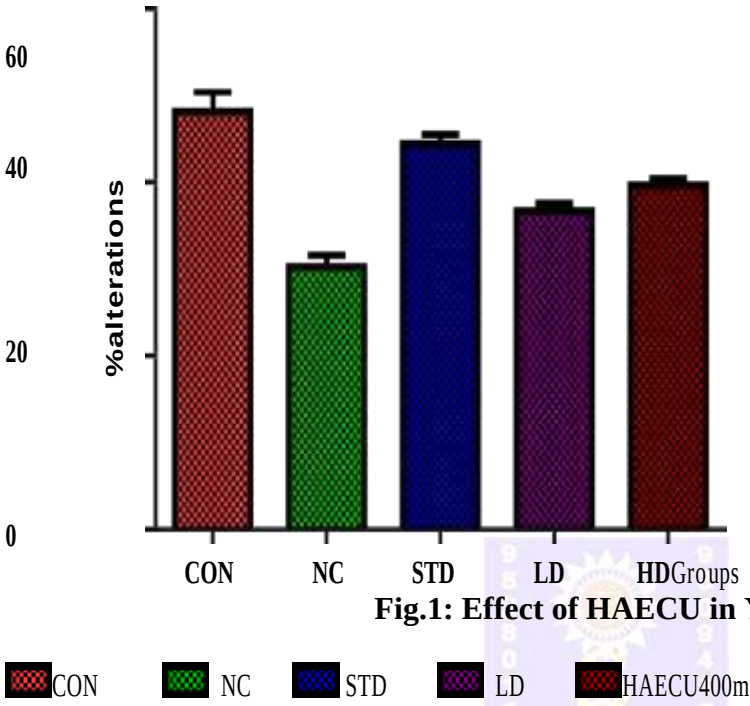

Fig.1: Effect of HAECU in Y maze

Conclusion: *Tricholepis glaberrima* DC have cholinesterase inhibitor property and useful anti-Alzheimer drug in delaying the onset and reducing the severity of AD when compared with that of reference drugs. The memory- improving activity is probably due to the presence of flavonoids. These memory-enhancing drugs showed that potential acting on cognitive functions by maintaining the acetylcholine level in the brain activity is of particular therapeutic importance. The ethanolic extract of *Tricholepis glaberrima* DC showed a significant increase in the onset of action and decrease in duration of action and recovery of time as compared to negative control thus justifying its anti-Alzheimer activity which may be due to the presence of alkaloid and flavonoid as a phytoconstituent present.

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