



Research Article

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NEUROPROTECTIVE ACTIVITY OF *TERMINALIA CHEBULA* RETZ AGAINST ETHANOL INDUCED COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS IN RATS: PROMISING FOR REGIMENTATION THE RISK OF ALZHEIMER'S DISEASE

Lakshmi K *, Karishma SK, Gopi Chand J, Maheswara Reddy M, Narendra Babu A, Bhargav Kumar N
Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur-522 034, Andhra Pradesh, India

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*Corresponding author

E-mail: lakshmikakunuri95@gmail.com

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ABSTRACT

Terminalia chebula Retz (*T.chebula*) which is a member of the Combretaceae family is frequently used medicinal herb in Ayurvedic, Unani, Siddha & Homeopathy system of medicine. *Terminalia chebula* is called the “King of Medicine” in Tibet and is always listed at the top of the list in Ayurvedic Materia Medica due to its extraordinary power of healing. To evaluate the neuroprotective activity of *Terminalia chebula* Retz against ethanol induced cognitive impairment and oxidative stress in rats brain. The learning and memory enhancing activity of *Terminalia chebula* Retz extract were investigated in Sprague Dawley rats for 21 days and its effects on learning and memory were examined by using 8-arm radial maze (or) radial arm maze (RAM) and histopathological studies. All the groups showed significantly (P value is <0.0001) decrease in the time taken to reach paired arm (sec) & number of entries in baited arms and non-baited arms as compared to ethanol inducing group and histopathological study of *Terminalia chebula* showed significant effect when compared to the standard drug for treating Alzheimer's disease. Therefore *Terminalia chebula* was proved to be effective agent for treating Alzheimer's. The present study suggests *Terminalia chebula* that modulate the oxidative stress and be involved in the protective effect against oxidative damage and neurodegenerative diseases in rat.

Key words: Ethanol, diazepam, Radial arm maze, learning and memory activity, Alzheimer's disease.

INTRODUCTION

Alois Alzheimer and Auguste 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, behavioral 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 today¹. 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.

Terminalia chebula Retz.(Family Combretaceae) commonly known as Haritaki and found all over the different parts of India, such as Assam, Gujarat, Mumbai, Konkan, Malbar, Chennai. The tree is great's significance to the Hindu and the dried twigs of tree are used in various vanjnas. *T. chebula* is commonly known as black myroblans in English and harad in Hindi. The *Terminalia* consists of 250 species and widely distributed in tropical areas of the world. This is one of the major components of wonder Ayurveda medicine known as Triphala Powder. Haritaki is known as “The King of Medicines”. In Ayurveda, it is described t as kind of mother because “At times even mother becomes angry but Haritaki never causes a harm to a person who takes it”.

T. chebula is a medium to large deciduous tree, attaining a height of up to 30 m with wide spreading branches and a broad-disk-shaped crown. The Sanskrit name “Haritaki” is rich with meaning, referring to the yellowish dye (haritak) that it contains, as well as indicating that it grows in the abode of god siva (Hari, that is the Himalayas) and that it cures (harayet) all diseases. Its other commonly used Sanskrit name, Abhaya, refer to the “fearlessness” it provides in the face of the disease³.

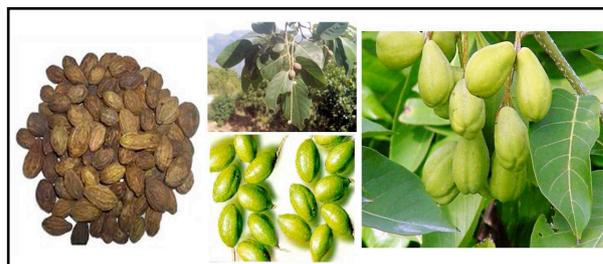


Figure 1: *Terminalia chebula* fruit leaf and tree⁴

MATERIALS AND METHODS

Collection & Authentication of Plant Material

The fruits of *Terminalia chebula* was identified and purchased from local market of Guntur and authenticated by P.Satyanarayana Raju (Plant taxonomist) of Department of Botany and Microbiology in Acharya Nagarjuna University, Guntur, India.

Preparation of Extract

The *Terminalia chebula* fruits 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 hours 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.

Experimental Animals

Sprague Dawley 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, Guntur.

Behavioral Study

Before starting the behavioral studies 1 week training was conducted. Only food and water was administered during this period⁸.

Radial Arm Maze (RAM)

The radial arm maze was developed by Olton and Samuelson (1976) and has become an essential tool for testing memory in rats⁵. The spatial memory was evaluated by the instrument. Open type radial arm maze 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 environment⁶. 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)⁷.



Figure 2: 8-Arm radial maze

Ethanol- Induced Cognitive Impairment

Ethanol is neurotoxin that able to alter behavioral 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 radical⁸.

Experimental Design

The learning and memory enhancing activity of the aqueous and ethanolic fruit extracts of *Terminalia chebula* 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)⁸. 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 hydrochloride⁸ (2.5 mg/kg was administered orally for 21 days) + Ethanol.

Group III: Test-I -Aqueous fruit extract of *Terminalia chebula* [TCAE- 100mg/kg was administered orally for 21 days) + Ethanol.

Group IV: Test -II -Ethanolic fruit extract of *Terminalia chebula* [TCEE- 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.

Histopathological Study

The rats were sacrificed on 22nd day by using the method decapitation and whole brain was collected. Routinely, brain tissue is fixed in 10% formalin.

Statistical Analysis

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. Comparisons were made between haloperidol group and test/standard groups. P-values <0.05 was considered statistically significant. The

statistical analysis was done by using Graph pad prism version no: 6.0.

RESULTS AND DISCUSSION

Effect of Fruit Extracts of Terminalia chebula Retz on Behavioral Parameters

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, 15th & 21st days.

Table 1: Effect of fruit extracts of Terminalia chebula Retz 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	147.4±0.75	138.4±0.93	120.4±2.73	100.4±1.72
2	II	Standard+ethanol	76.2±0.97	66.6±1.89	57.8±0.86	46.4±2.6
3	III	TCAE+ ethanol	127.8±0.86	121.6±0.93	102.2±0.86	76±1.76
4	IV	TCEE+ ethanol	103±0.84	96±0.71	89.2±1.43	75.2±1.46

Table 2: Effect of fruit extracts of Terminalia chebula Retz on number of entries in baited arms and non-baited arms

S.No.	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
1	I	Ethanol	104±0.3	1.2±0.2	1.2±0.2	1.6±0.3	1.2±0.2	1.6±0.3	0.8±0.2	0.8±0.2
2	II	Standard+ethanol	3.6±0.3	3.4±0.3	3.2±0.4	2.2±0.6	5.8±0.4	8.8±0.4	9.4±0.5	12.8±1.6
3	III	TCAE+ ethanol	2.2±0.4	2.4±0.4	2.6±0.2	4.4±0.5	5.4±0.5	6.8±0.8	5.8±1.0	7.2±1.6
4	IV	TCEE+ ethanol	6.4±0.5	7±1.3	7±1.3	11±0.9	10.4±0.9	9±1.0	8.8±1.0	10.2±1.2

Table 3: Effect of Terminalia chebula Retz fruit extracts on the different organs of rat by using ethanol cognitive impairment model

Organs	Weight in grams			
	Inducing group	Standard group	Test-I (TCAE)	Test-II (TCEE)
Brain	2.22±0.28	2.02±0.01	2.13±0.01	2.02±0.01
Heart	1.42±0.16	0.75±0.01	1.13±0.01	0.7±0.03
Lungs	2.01±0.02	1.62±0.01	1.55±0.01	1.54±0.02
Liver	8.18±0.02	5.43±0.01	6.28±0.03	5.37±0.02
Kidneys	2.37±0.21	1.38±0.03	1.35±0.02	1.05±0.01
Pancreas	1.18±0.03	0.51±0.02	0.65±0.02	0.51±0.01

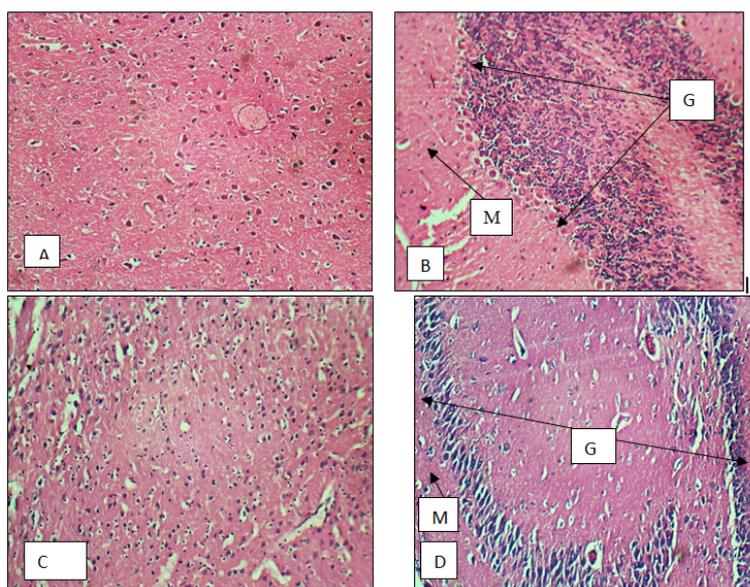


Figure 3 (A-D): Histopathological changes in rat brain treated with ethanol (i.e. inducing group), standard with ethanol (i.e. Standard group), TCAE (Terminalia chebula aqueous extract i.e. Test-I), TCEE (Terminalia chebula ethanolic extract i.e. Test-II) using light microscopy (Magnification 10 X). (A-Inducing group, B-Standard group, C- Test-I (TCAE), D-Test-II-(TCEE), M-Molecular layer, G-Granular layer)

Histological Findings

The results showed that ethanol significantly increased neuronal death, while the co treatment of *Terminalia chebula* with ethanol significantly inhibited the neuronal death compared to ethanol treated group, which suggest that *Terminalia chebula* an antioxidant, may effectively protects against the deleterious effects of ethanol-induced abnormalities by decreasing neuronal death in rat brain, furthermore the cell counting under light microscope also showed the same increased neurodegeneration upon ethanol treated group and decreased significantly when treated with ethanolic extract *Terminalia chebula* of as compare to alone ethanol treated group.

CONCLUSION

Terminalia chebula showed cholinesterase inhibitor mechanism at an effective dose of 100 mg/kg against ethanol- induced cognitive impairment. *Terminalia chebula* 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. The histopathological study showed that ethanol induced apoptosis neurodegeneration and the treatment of ethanolic extract of *Terminalia chebula* with ethanol decreased ethanol-induced apoptotic neurodegeneration in rat brain. This effect is attributed to its ability to improve the levels of the acetylcholine that are decreased in the Alzheimer's disease.

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