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Evaluation of neuroprotective effects of *Vitex Leucoxylon* against Alzheimer's in rats

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ABSTRACT

Objective: The present work is aimed to evaluate the ethanolic extract of *Vitex leucoxylon* against aluminium chloride induced Alzheimer's in rats.

Methods: Male albino Wistar rats (150-200 g) were divided into five groups: normal control group animals received saline for 28 days, disease group animals received aluminium chloride 100mg/kg daily for 28 days through oral route, standard group animals received donepezil 10mg/kg and aluminium chloride 100mg/kg daily for 28 days through oral route, test group I and test group II animals received *Vitex leucoxylon* at doses 250mg/kg and 500mg/kg and aluminium chloride 100mg/kg through oral route for a period of 28 days. At the end of the study spontaneous alteration behaviour, spatial learning, reference memory (Y-maze, MWM, RAM), antioxidant parameters (SOD, GSH, MDA, CAT) , AchE and histopathology of the brain were performed.

Results: Disease group rats showed cognitive impairment, deposition of amyloid – beta plaques and neurofibrillary tangles and significant increase in oxidative stress markers (MDA) while significant reduction in antioxidant levels like (SOD, GSH, CAT). *Vitex leucoxylon* treated rats, particularly 500mg/kg showed improved cognition, and normalization of antioxidant parameters. Histopathological examination revealed that *Vitex leucoxylon* showed significant decrease in amyloid – beta plaques, preservation of neuronal structure compared to disease group animals. The findings suggest that *Vitex leucoxylon* exert neuroprotective effect against Alzheimer's disease through its antioxidant mechanisms, likely due to presence of polyphenolic and flavonoids.

1. INTRODUCTION:

Alzheimer's disease is a progressive neurological disorder that affects memory, thinking, and behaviour. It is the most common cause of dementia, gradually impairing cognitive functions and daily activities (1). Amyloid plaques and tau tangles build up in the brain, causing damage to nerve cells and atrophy of the brain. As time passes, the symptoms, which initially include minor memory loss and confusion, get worse and impact independence, language, and reasoning (2). The most common cause of dementia in older people

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(those over 65) is Alzheimer's disease, which is widespread throughout the world. Alzheimer's disease accounts for 60–70% of dementia cases, which affect about 55 million individuals worldwide. Alzheimer's disease is a major contributor to the almost 10 million new cases of dementia that occur each year. AD dementia is more common in women than in males, and its frequency rises with age. Age, heredity, high blood pressure, high blood sugar, obesity, smoking, excessive alcohol use, physical inactivity, social isolation, and depression are some of the factors that raise the risk of Alzheimer's disease.

Alzheimer's disease is currently the seventh largest cause of death worldwide and is one of the main causes of impairment and dependency among older persons (3)

Currently, there is no cure for AD, and existing treatments only provide symptomatic relief and slow down the progression of disease. The active molecules which are extracted from the natural sources like flavonoids, polyphenols and antioxidants decreases the amyloid formation and targeting the cholinergic deficits. There is no complete treatment for neurodegenerative disorders. In this study we evaluate the efficacy of ethanolic extract of *Vitex leucoxylon* in Alzheimer's in rats.

2. MATERIALS AND METHODS:

2. 1. Collection of plant:

Vitex Leucoxylon which belongs to family Verbenaceae were collected from a botanical garden at Tirupati, Andhra Pradesh which was confirmed by a taxonomist, Dr. Madhava Chetty, Sri Venkateswara University, Tirupathi with voucher number is 0889.

2. 2. Extraction:

Vitex leucoxylon was extracted through soxhlet apparatus. Shade dried *Vitex Leucoxylon* bark grinded into coarse powder using motor and pestle and passed the powder through the sieve to get fine powder. Plant powder was weighed and placed inside the thimble which is made of filter paper and inserted into soxhlet extractor. Filled the round – bottom flask with ethanol based on the amount of plant powder. Assembled the Soxhlet apparatus and placed it on heating mantle, heated the system at 60–70°C to allow ethanol to evaporate. The ethanol vapor condenses in the condenser and drips back into the thimble, extracting the bioactive compounds. This process continues for 4–8 hours or until the solvent in the siphon tube becomes colorless. Concentrated the filtrate using a rotary evaporator. The obtained extract is dried further to obtain a semi-solid extract. The final extract is stored in an airtight container for further analysis (4).

2. 3. Animals

Male albino Wistar rats (250 to 300g) were obtained from Vyas labs in Hyderabad, India. The rats were maintained according to CCSEA guidelines.

2. 4. Ethical Approval

The protocol was approved by Institutional Animal Ethics Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research (RIPER) as per CCSEA guidelines.

2. 5. Chemicals

5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), were purchased from Sigma Aldrich company. Trichloroacetic acid (TCA), Thio barbituric acid (TBA) were purchased from LOBA Chemie Pvt ltd, ketamine, xylazine, potassium chloride, ethanol, pyrogallol, hydrogen peroxide.

2. 6. Treatment Protocol

All the experimental animals were divided into five experimental groups. Each group consists of six animals.

Table 1: Treatment protocol

Group	Treatment
Normal	Received normal saline only
Disease	$AlCl_3$ (100mg/kg) p.o
Standard	$AlCl_3$ (100mg/kg) p.o + Donepezil (10mg/kg) p.o
Vitex Leucoxylon (250mg/kg)	$AlCl_3$ (100mg/kg) p.o + *Vitex Leucoxylon* (250mg/kg)p.o
Vitex Leucoxylon (500mg/kg)	$AlCl_3$ (100mg/kg) p.o + *Vitex Leucoxylon*(500mg/kg)p.o

Statistical analysis

The data were presented as Mean ± SD for six animals in each group. All aggregated data were statistically assessed using GraphPad Prism V10. 4.2. Hypothesis testing procedures included one-way ANOVA and Tukey's multiple comparison test. P-values ≤0.05 were used to signify statistical significance.

3. RESULTS

Table 2: Effect of *Vitex Leucoxylon* in Y – maze test

Groups	Spontaneous alteration (%)
Control	123.3±2.71
$Alcl_3$ (100mg/kg)	47.33±1.28****
Vitex leucoxylon(250mg/kg) +$Alcl_3$ (100mg/kg)	104.0±2.92####
Vitex leucoxylon(500mg/kg) +$Alcl_3$ (100mg/kg)	119.2±4.59####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	84.83±3.005####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's

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multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 3: Effect of *Vitex Leucoxylon* in Morris water maze

Groups	Escape latency(sec)
Control	69.67 ± 2.765
$Alcl_3$(100mg/kg)	89.67 ± 2.906***
Vitex leucoxylon(250mg/kg) +$Alcl_3$ (100mg/kg)	63.17 ± 4.061####
Vitex leucoxylon(500mg/kg) +$Alcl_3$ (100mg/kg)	49.17 ± 2.301####
Donepezil (10mg/kg) + $Alcl_3$ (100mg/kg)	37.67 ± 2.565####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 4: Effect of *Vitex leucoxylon* on Reference Memory Error [RME] of RAM

Groups	RME Score
Control	2.500±0.22
$Alcl_3$(100mg/kg)	8.00±0.36****
Vitex leucoxylon(250mg/kg) +$Alcl_3$ (100mg/kg)	5.50±0.22####
Vitex leucoxylon(500mg/kg) +$Alcl_3$ (100mg/kg)	3.50±0.223####
Donepezil (10mg/kg) + $Alcl_3$ (100mg/kg)	1.83±0.30####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 5: Effect of *Vitex leucoxylon* on Correct Working Memory Error [CWME] of RAM

Groups	CWME Score
Control	1.500±0.22
$Alcl_3$(100mg/kg)	6.00±0.36****
Vitex leucoxylon(250mg/kg) +$Alcl_3$ (100mg/kg)	3.500±0.2236####
Vitex leucoxylon(500mg/kg) +$Alcl_3$ (100mg/kg)	2.33±0.2108####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	1.33±0.2108####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 6: Effect of *Vitex leucoxylon* on Incorrect Working Memory Error [IWME] of RAM

Groups	IWME Score
Control	2.500± 0.223
$Alcl_3$(100mg/kg)	8.500 ±0.42****
Vitex leucoxylon(250mg/kg) +$Alcl_3$ (100mg/kg)	6.667 ±0.33##
Vitex leucoxylon(500mg/kg) +$Alcl_3$ (100mg/kg)	4.500 ±0.223####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	2.500 ±0.2236####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001, ##P<0.01 compared to $Alcl_3$ group.

Table 7: Effect of *Vitex leucoxylon* on SOD levels

Group	SOD (U/g of tissue)
Control	1.31±0.01
$Alcl_3$ (100mg/kg)	0.96±0.02****
Vitex leucoxylon(250mg/kg) + $Alcl_3$ (100mg/kg)	1.32±0.0076####
Vitex leucoxylon(500mg/kg) + $Alcl_3$ (100mg/kg)	1.64±0.02####
Donepezil (10mg/kg) + $Alcl_3$ (100mg/kg)	1.45±0.0070####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 8: Effect of *Vitex leucoxylon* on CAT levels

Group	CAT (U/g of tissue)
Control	0.1105±0.0007
$Alcl_3$ (100mg/kg)	0.076±0.0009****
Vitex leucoxylon(250mg/kg) + $Alcl_3$ (100mg/kg)	0.107±0.0007####
Vitex leucoxylon(500mg/kg) + $Alcl_3$ (100mg/kg)	0.134±0.0008####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	0.127±0.0007####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and ####P<0.0001 compared to $Alcl_3$ group.

Table 9: Effect of *Vitex leucoxylon* on GSH levels

Group	GSH (U/gm of tissue)
Control	18.57±0.2826
$Alcl_3$ (100mg/kg)	14.46±0.3234****
Vitex leucoxylon(250mg/kg) + $Alcl_3$ (100mg/kg)	22.61±0.2806####
Vitex leucoxylon(500mg/kg) + $Alcl_3$ (100mg/kg)	31.56±0.3737####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	26.04±0.3435####

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The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and $^{\#\#\#\#}$P<0.0001 compared to $Alcl_3$ group.

Table 10: Effect of *Vitex leucoxylon* on MDA levels

Group	MDA(µmol/g of tissue)
Control	1.44±0.055
$Alcl_3$ (100mg/kg)	5.66±0.063****
Vitex leucoxylon(250mg/kg) + $Alcl_3$ (100mg/kg)	3.95±0.069####
Vitex leucoxylon(500mg/kg) + $Alcl_3$ (100mg/kg)	2.100±0.060####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	2.74±0.060####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and $^{\#\#\#\#}$P<0.0001 compared to $Alcl_3$ group.

Table 11: Effect of *Vitex leucoxylon* on Acetylcholinesterase levels in brain

Group	AChE (U/gm of tissue)
Control	41.05±0.69
$Alcl_3$ (100mg/kg)	68.02±0.5653****
Vitex leucoxylon(250mg/kg) + $Alcl_3$ (100mg/kg)	48.77±0.6489####
Vitex leucoxylon(500mg/kg) + $Alcl_3$ (100mg/kg)	29.20±0.6851####
Donepezil(10mg/kg) + $Alcl_3$ (100mg/kg)	35.67±0.563####

The data were presented as Mean ± SEM for six animals per group. One-way ANOVA was used to assess statistical significance, followed by Tukey's multiple comparison test. ****P<0.0001 compared to control group and $^{\#\#\#\#}$P<0.0001 compared to $Alcl_3$ group.

4. DISCUSSION:

Alzheimer's Disease (AD) is a gradual, irreversible neurological condition that usually affects the elderly, causing cognitive decline, memory impairment, and functional disability. It is the most frequent cause of dementia, accounting for 60-80% of all cases globally (5). The disease is distinguished by the accumulation of two aberrant protein structures in the brain: amyloid-beta plaques, which form outside neurons, and neurofibrillary tangles made up of hyperphosphorylated tau protein, which form within neurons (6). These pathogenic changes impair neural connection, promote inflammation, and ultimately result in cell death. Alzheimer's disease begins with minor memory loss and confusion, which progresses to more severe cognitive deficits such as disorientation, language difficulty, impaired judgment, and behavioral or personality abnormalities. Individuals in advanced stages may lose the capacity to perform daily tasks and become completely dependent on caregivers.

While the specific etiology of Alzheimer's disease is unknown, a mix of genetic, environmental, and lifestyle factors are thought to contribute to its start and progression. The APOE ε4 gene variation is a known genetic risk factor (7). Other factors include age, cardiovascular health, head trauma, and a lack of mental or physical activity. Alzheimer's illness is now incurable. Available therapies, such as cholinesterase inhibitors and NMDA receptor antagonists, only provide symptomatic relief. Recent advances focus on early diagnosis through biomarkers and the development of disease-modifying therapies (8). As life expectancy increases globally, Alzheimer's represents a growing public health challenge requiring urgent attention in both medical research and policy (9).

Vitex leucoxylon which belongs to family Verbenaceae is a medicinal plant traditionally used in Ayurveda for its anti-inflammatory, antioxidant, and neuroprotective properties. Phytochemical studies have indicated the presence of flavonoids, glycosides, and essential oils, all of which may contribute to its medicinal potential. These bioactive chemicals may aid in the reduction of oxidative stress and neuronal damage in neurodegenerative illnesses such as Alzheimer's. Preliminary studies suggest that *Vitex leucoxylon* extracts could inhibit acetylcholinesterase activity, supporting cognitive function. As a result, this plant has potential as a natural source for creating complementary medicines that delay the progression of Alzheimer's disease or improve patient outcomes (10).

In the current study, we attempted to evaluate the preventive and therapeutic benefits of ethanolic extract of *Vitex Leucoxylon* on Alzheimer's disease. In the current study, Alcl3-induced rats demonstrated a significant decrease in cognitive impairment, neurochemical alterations such as decreased levels of acetylcholine, oxidative stress, which includes elevated MDA levels, decreased antioxidant enzymes such as (SOD), catalase, and (GSH), increased production of reactive oxygen species (ROS), and histopathological changes such as neuronal loss in the hippocampus and cortex, and the formation of amyloid-like plaques.
Treatment with ethanolic extract of *Vitex Leucoxylon* (250mg/kg and 500mg/kg) showed enhanced cognition, decreased acetylcholinesterase (activity, significant increase in antioxidant enzyme levels such as SOD, catalase, and GSH, reduction in lipid peroxidation (MDA), when compared to $Alcl_3$ induced rats

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which indicates neuroprotective effect of *Vitex Leucoxylon* against $Alcl_3$ induced Alzheimer's in rats.

5. CONCLUSION:

The findings suggest that *Vitex leucoxylon* exert neuroprotective effect against Alzheimer's disease through its antioxidant mechanisms, likely due to presence of polyphenolic and flavonoids.

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