# NEUROPROTECTIVE EFFECT OF LINALOOL IN CHRONIC UNPREDICTABLE STRESS-INDUCED COGNITIVE IMPAIRMENT - *IN SILICO* AND *IN VIVO* STUDIES

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**KEYWORDS**
Chronic Unpredictable Stress**,** Linalool, Molecular Docking, Network Pharmacology

**ABSTRACT**
The growing prevalence of stress-induced cognitive impairment is becoming a significant concern, although the precise mechanism is still not fully understood. The chronic unpredictable stress (CUS) model is widely used to study stress-induced cognitive impairment, as it correlates with cognitive decline, behavioral changes, and abnormal cortisol levels in humans. Linalool, known for its traditional use in alleviating anxiety, acting as an antidepressant, sedative, and neuroprotective agent, was investigated in this study. The research explored the genes involved in stress-induced cognitive impairment through network pharmacology, molecular docking, and *in vivo* methods to assess linalool's neuroprotective effects. The study identified PTGS2, GSK3B, MAPK1, JAK2, PARP1, KDR, NR3C1, MDM2, PRKCA, and MAPK8 as key targets for therapeutic activity in CUS and a strong docking score with PTGS2. The network analysis revealed that neuroprotective effects of linalool have been associated with its modulation of neurodegeneration and sphingolipid signaling pathways, indicating that linalool may inhibit these pathways. The *in vivo* findings showed that Linalool ameliorated the chronic stress induced behavioral changes, reduced the acetylcholinesterase levels in the brain and corticosterone levels in blood. Both *in vitro* and *in vivo* studies indicate that linalool possesses neuroprotective properties and could be a potential target for treating cognitive impairment.

## INTRODUCTION

Cognitive impairment is an emerging problem which mainly affects memory, attention, decision-making, problem-solving, learning new things, and concentration in everyday life. The pathophysiology of cognitive impairment is multifactorial, one of the major contributors of cognitive impairment is stress. Stress is inevitable in life; studies have shown that chronic stress can result in neurodegeneration and metabolic disorders. Chronic stress leads to changes in hippocampal structure, such as the reduction of dendritic branching and the inhibition of neurogenesis, particularly in the dentate gyrus. Changes in neuronal pathways occur due to the release of inflammatory mediators that drive neurodegeneration. Stress-induced inflammation triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to the release of pro-inflammatory cytokines that can impair neuronal function and plasticity (Dantzer *et al.*, 2008). Activation of the HPA axis leads to the release of glucocorticoids (such as corticosterone

in animals). The increased levels of glucocorticoids and neuroinflammatory responses are thought to play a role in the cognitive deficits seen in chronic stress conditions.
Prolonged elevation of glucocorticoids can impair neurogenesis, synaptic plasticity, and neuronal survival, particularly in the hippocampus, a critical region for learning and memory (Meyers *et al.*,2012). Stress-induced activation of the immune system leads to the release of pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These cytokines can cross the blood-brain barrier and induce neuroinflammation, which negatively affects synaptic plasticity and neuronal health (Dantzer *et al.*, 2008).
BDNF is essential for neuronal growth, survival, and plasticity. Chronic stress reduces BDNF levels, which impairs synaptic function and contributes to cognitive deficits (Duman & Monteggia, 2006). Stress can alter the balance of neurotransmitters such as serotonin, dopamine, and glutamate. These changes can affect mood, cognition, and overall brain function. For instance, elevated levels of glutamate can lead to excitotoxicity, damaging neurons and impairing cognitive functions (Popoli *et al.*, 2011).

Since natural compounds have a long history and outstanding therapeutic benefits, they are indispensable in cures. Currently, the use of natural phyto-antioxidants as dietary supplements is commonly enhanced to address the changes in the body caused by stress. Curcumin, the yellow pigment derived from the rhizomes of *Curcuma longa*, has been widely researched for its therapeutic benefits, including its antioxidant properties (Nafisi *et al.*, 2009), as well as its anti-inflammatory and neuroprotective activities (Motterlini *et al.*, 2000). Pinene is a bicyclic monoterpene derived from pine, studies have shown protective effect against oxidative stress, inflammation and neuronal damage (Kim *et al.*, 2015).
Similarly, linalool is a monoterpene compound present in lavender, sweet basil, bergamot, which exhibits numerous beneficial properties, including anti-inflammatory, antioxidant, anxiolytic and sedative activities. Previous research has shown that linalool possesses significant neuroprotective properties (Gonçalves *et al.*, 2020). It has been particularly noted that linalool helps prevent cognitive decline in animal models of Alzheimer's disease, depression, and vascular dementia (Dos *et al.*, 2022). However, the protective effects of linalool on cognitive impairment induced by chronic unpredictable stress and its underlying molecular mechanisms remain unclear. Hence this study aimed to investigate the neuroprotective effects of linalool on CUS-induced cognitive impairment in rats and elucidate its molecular mechanisms through network pharmacology and docking studies.

## 2. Materials and Methods

### Network pharmacology analysis

#### Identification of potential targets of linalool

Using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), pertinent linalool files were retrieved and uploaded to the Swiss Target Prediction (http://www.swisstargetprediction.ch/) and the predicted targets were collected.

**Prediction of potential targets of cognitive impairment**

DisGeNET (https://www. disgenet. org/) and Genecards (https://www. genecards. org/) data bases were used to screen for potential Alzheimer's disease targets. Duplicate targets were eliminated, and related targets were retained.

**Construction and analysis of the protein-protein interaction (PPI) network**

The target genes were analyzed for overlap using Venn 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to identify common targets. These shared genes were then entered into the STRING database (version 11.0, https://www.string-db.org/) to construct a protein-protein interaction (PPI) network. To ensure the accuracy of the data, only interactions with a confidence score of ≥0.4 involving "Homo sapiens" were considered. The resulting PPI network was then visualized and analyzed using Cytoscape software (version 3.4.0, http://chianti.ucsd.edu/cytoscape-3.4.0/).

**Go and KEGG enrichment analysis**

Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted using Cluster Profiler (version 3.8.1, http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html).

**Molecular docking**

The molecular binding affinity of linalool with the core targets of dementia was assessed using AutoDock (http://autodock.scripps.edu/). The three-dimensional structure of linalool was obtained from GenBank (https://www.genecards.org/), while the crystal structures of the core target proteins were sourced from the RCSB Protein Data Bank (http://www.rcsb.org/). Structural modifications of these proteins, such as removing water molecules and heteroatoms, adding charges and hydrogen atoms, and converting them to PDBQT format for binding studies, were carried out using Chimera (https://www.cgl.ucsf.edu/chimera/.2.4) and AutoDock. A docking simulation was then conducted using AutoDock Vina, and the results were visualized with Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download).

***In vivo* experimental studies**

Linalool was obtained from Sigma Aldrich (Catalog No. L2602, USA), and all other chemicals used in the study were of standard analytical grades supplied by Himedia Pvt. Ltd., India.

**Experimental Animals**

Twenty-four adult male wistar rats, 12 weeks old weighing between 150 and 200 grams, were obtained from the Laboratory Animal Center at H/S Kedhar Biolabs, Mahabubnagar, Telangana. All the animals were maintained as per CCSEA guidelines with a temperature of 20 ± 2°C and a 12-hour light/dark cycle, and they had unrestricted access to food and water. The experimental protocol was approved by Institutional Animal Ethics Committee of Sri Padmavati Mahila Visvavidyalayam University, with approval no. CCSEA/1677/SPMVV/IAEC/IV-09.

**Chronic unpredictable stress (CUS) protocol**

The rats were exposed to each stressor daily and the same stressor was not scheduled for 3 consecutive days. The rats were subjected to different stressors include 15 min forced swim; 12 h cage tilting; reversal of the light/dark cycle; 12 h wet bedding; 1h crowded cage; 24 h food deprivation; 1h cage rotation; 24 h water deprivation; 1 min tail pinch; saw dust empty (Jagadeesan *et al.*, 2019).

**Drug administration and experimental group**

The dose of linalool was selected based on previous studies. Linalool was dissolved in 2% Tween. The animals were randomly divided into 4 groups(n=6). Group I vehicle (10ml/kg p.o);

Group II CUS and vehicle (10ml/kg p.o); Group III CUS and Linalool (25mg/kg p.o) and Group IV CUS and Linalool (100mg/kg p.o). The study protocol was represented in Fig. 1.

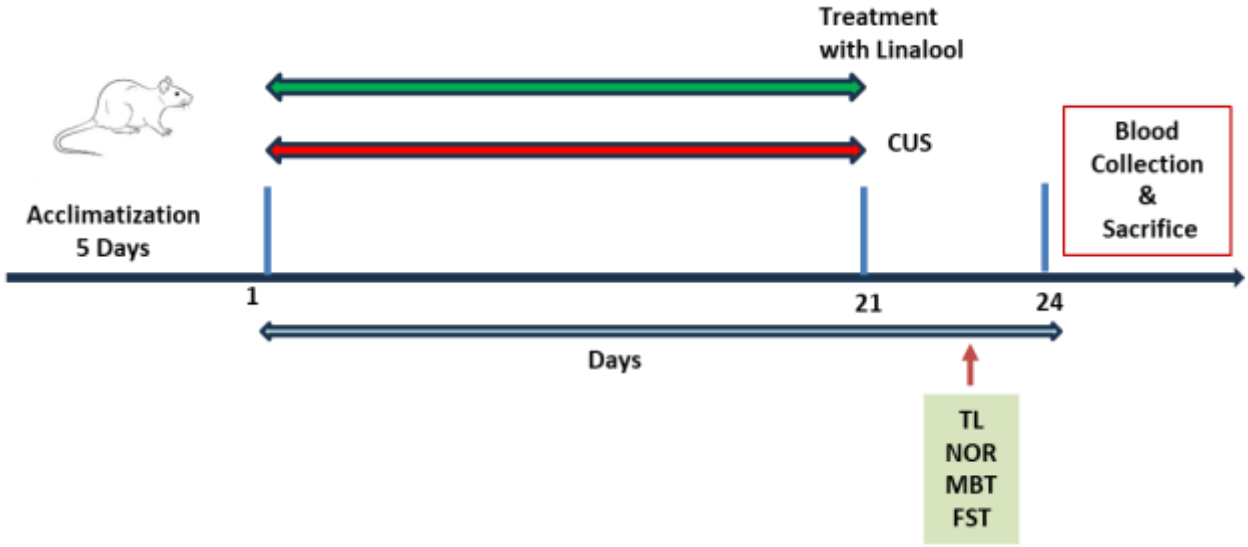



**Fig. 1:** Diagrammatic representation of the experimental procedure used in the study

**Behavioral parameters**

All the behavioral parameters were assessed between day 21 to day 24.

**Elevated Plus Maze Test**

Transfer latency (TL) was evaluated in rats using elevated plus maze which is a neutral behavioral model commonly used to assess rodent memory and anxiety like behavior (Mani *et al.,* 2022).

**Marble Burying Test (MBT)**

The marble burying method is used to assess obsessive-compulsive disorder (OCD) like behavior in rats. Marbles are placed in the cage with bedding and observed for number of marbles buried by each rat were calculated (Kedia *et al.,* 2014).

**Novel Object Recognition test (NOR)**

Novel object recognition test is commonly used to assess the cognitive ability of rodents, specifically their recognition memory, in various experimental CNS models. In rats, the exploration times of both familiar objects (FO1 and FO2), as well as the exploration times of the familiar object (FO1) and the novel object during the test session were recorded (Mani *et al.,* 2022).

**Forced swim test (FST)**

The forced swim test is the most used pharmacological model for assessing neurotoxicity and depressive behavior in animals. The onset of immobility, floating upright indicates the cessation of escape-directed behavior, often referred to as learned helplessness. After acclimatization, the duration of immobility in rats was recorded (Porsolt *et al.,* 1978).

**Biochemical studies**

At the end of the experiment, blood samples were obtained from the retroorbital plexus of animals and centrifuged at 2000 rpm for 10 minutes. The serum was then separated, and corticosterone levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit from Invitrogen (Thermo Fisher Scientific), following the manufacturer's guidelines. Subsequently, the brains were collected, homogenized in 0.1 M phosphate buffer (pH 8), and centrifuged at 10,000 rpm. The supernatant was then used to assess acetylcholinesterase activity, according to the procedure described by Ellman *et al.*, in 1961.

**Statistical analysis:**

The results were expressed as mean ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $p < 0.05$ was statistically significant.

**3. RESULTS**

**Network Pharmacological Analysis**

Through Swiss Target Database 100 potential targets for linalool were obtained, while 1,427 genes related to cognitive impairment were sourced from the DisGeNET and Gene Cards databases. The Venn diagram in Fig. 2: A illustrates 36 common targets derived by intersecting the linalool targets with the cognitive impairment-related genes.

Utilizing these 36 common targets a protein-protein interaction (PPI) network was constructed in the STRING database and a compound-target network using Cytoscape 3.0 (Fig.2: B and C).

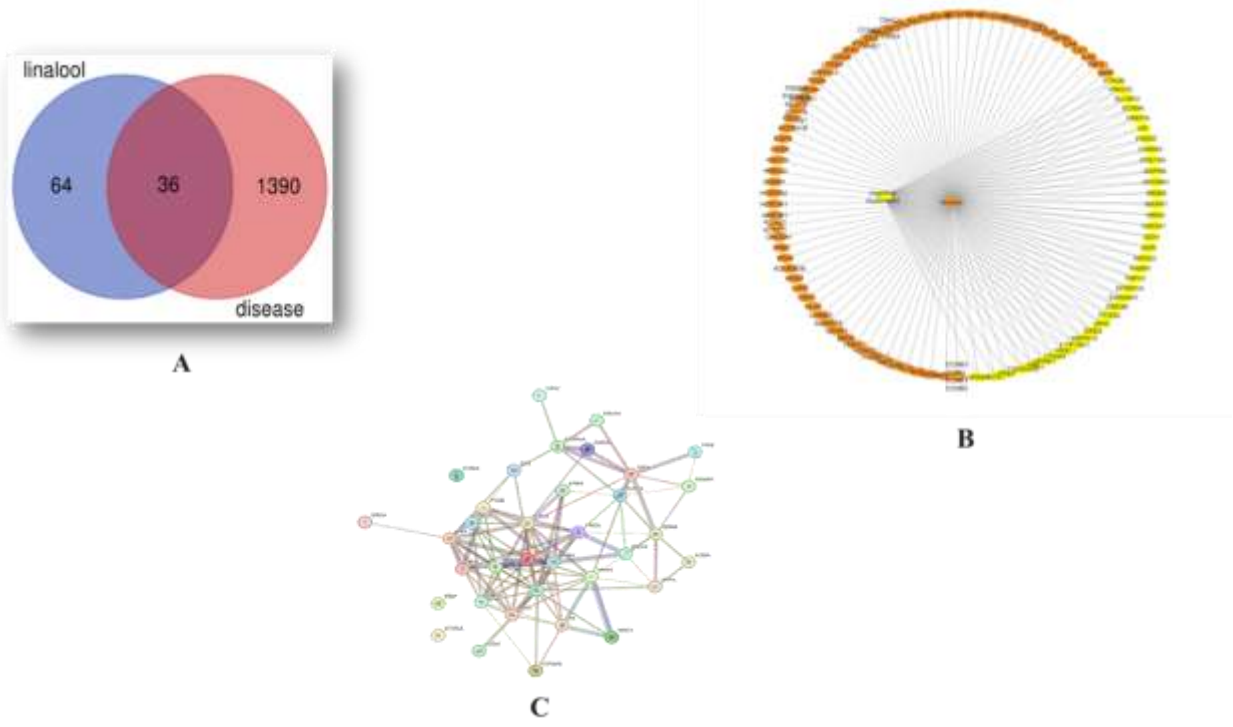



**Fig. 2:** A: Venn diagram of intersection of linalool targets and disease target genes; B: Construction of compound-target network- The network includes 100 Potential targets of linalool and 36 common targets of both disease and linalool C: PPI network for the 36 common targets between linalool compounds targets and cognitive impairment targets.

Shiny GO was used to perform GO and KEGG enrichment studies to learn more about the biological processes and roles associated with these overlapping targets. According to the GO

functional analysis, the targets of linalool in cognitive impairment are mainly involved in cell and synaptic signaling processes, including responses to organic cyclic compounds, nitrogen compounds, and oxygen-containing compounds, as well as the regulation of transport, chemical synaptic transmission, and anterograde trans-synaptic signaling (Fig. 3: A, B & C).

The KEGG analysis identified the most significantly enriched pathways related to neurodegeneration, including the sphingolipid signaling pathway and dopaminergic synapse (Fig.3:D). Both GO and KEGG analyses suggest that chemical synaptic transmission, anterograde trans-synaptic signaling, and cell signaling are likely key mechanisms through which linalool may exert therapeutic effects in chronic stress-induced cognitive impairment.

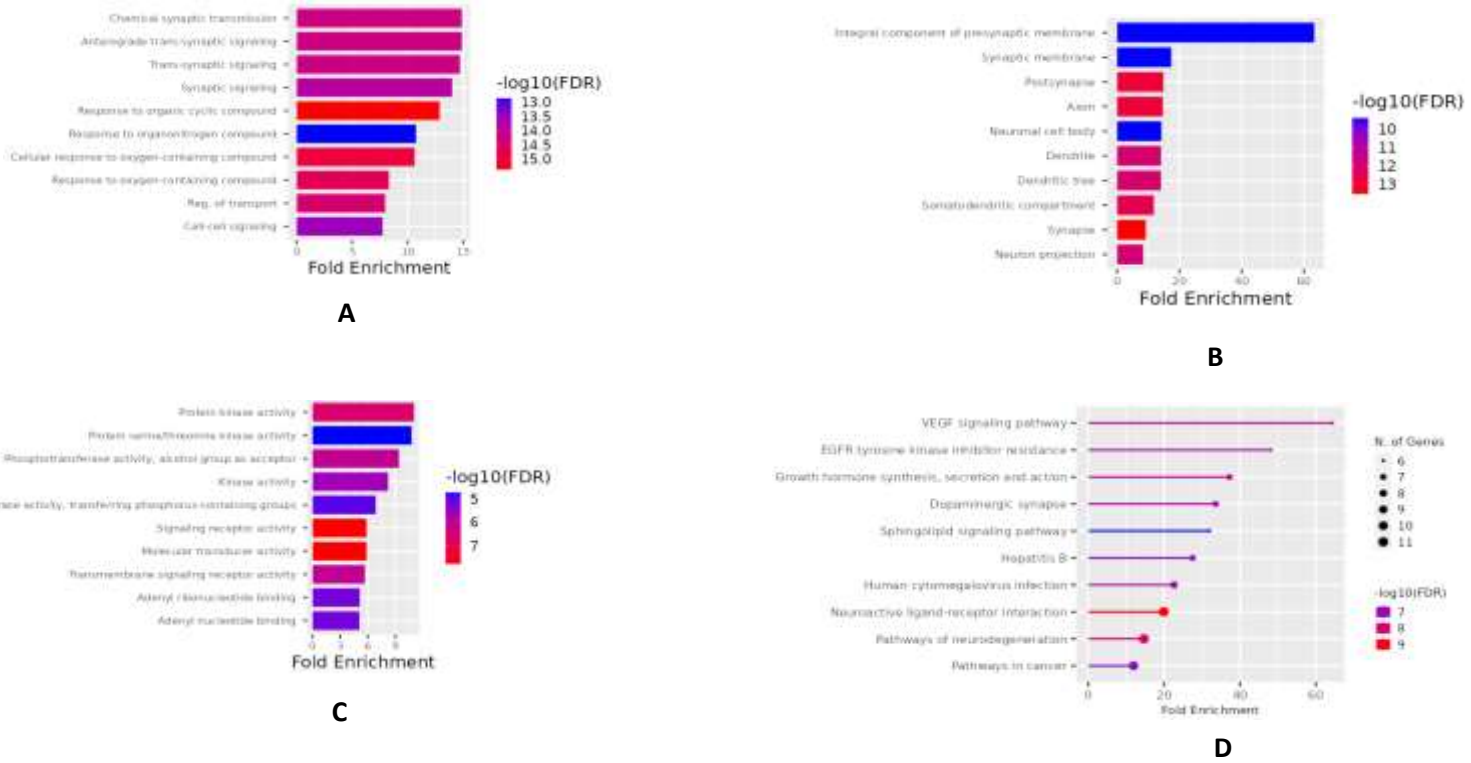



**Fig. 3:** GO and KEGG pathway analysis of the targets of Linalool against CI genes (A) GO-Biological processes plot (B) GO-Cellular component plot (C) GO-Molecular functions plot (D) KEGG enrichment pathway plot.

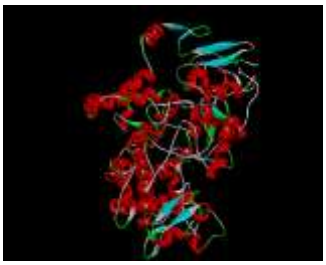
**Molecular Docking Analysis**

To understand the potential effects of linalool and disease targets, the top 10 components (target gene proteins) obtained from the network topology were subjected for molecular docking using Piracetam as standard. The molecular docking energies and optimal interaction between the target and the component were displayed in Table-1. The top three target genes that showed good binding affinity were in Fig.4: A, B & C. From the interpretation of results, linalool shows the highest binding affinity with the protein PTGS2.

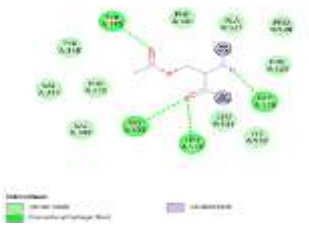
**Table 1:** Molecular docking energies of Linalool and targeted genes

| ID Gene | Molecular Docking Score | |
|---|---|---|
| | Linalool | Piracetam |
| PTGS2: 5F19 | **-6.01** | -4.31 |
| GSK3B: 1SGO | -3.39 | -3.28 |
| MAPK1: 5NHP | **-5.09** | -3.21 |
| JAK2: 6N77 | -2.43 | -3.73 |
| PARP1: 7KK2 | -4.72 | -4.57 |
| KDR: 6GQQ | -2.37 | -4.66 |
| NR3C1: 4UDD | -4.86 | -4.99 |
| MDM2: 4OGN | -4.17 | -3.58 |
| PRKCA: 6BJN | **-5.32** | -3.24 |
| MAPK8: 3ELJ | -4.42 | -2.22 |

**Table 1:** Molecular docking energies of Linalool and targeted genes. The targeted genes were PTGS2 - prostaglandin-endoperoxide synthase 2; GSK3B - glycogen synthase kinase 3 beta; MAPK1 - mitogen-activated protein kinase 1; JAK2 - Janus kinase 2; PARP1 – poly (ADP-ribose) polymerase 1; KDR - Kinase insert domain receptor; NR3C1 - nuclear receptor subfamily 3 group C member 1; MDM2 - murine double minute 2; PRKCA - protein kinase C alpha; MAPK8 - mitogen-activated protein kinase 8.

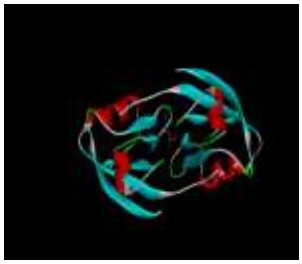


3D



2D

**A: PTGS2-Linalool**



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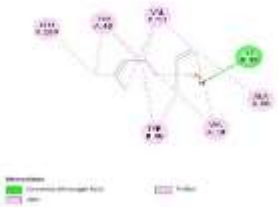
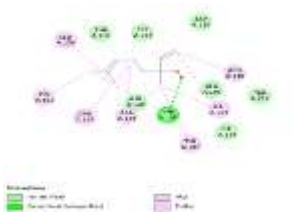


2D

**B: PRKCA -Linalool**

3D



2D

**C: MAPK1-Linalool**

**Fig. 4:** Molecular docking analysis of Linalool and top 3 target genes. (A) Molecular docking of Linalool to PTGS2 (PDBID: 5F19) (B) Molecular docking of Linalool to PRKCA (PDBID: 6BJN) (C) Molecular docking of Linalool to MAPK1 (PDBID: 5NHP).

**Treatment with Linalool reduced the transfer latency in CUS induced cognitive impairment**

After four weeks of chronic unpredictable stress, the untreated group showed a marked ($P < 0.05$) increase in the transfer latency compared to control group. The rats treated with linalool (25mg/kg p.o and 100mg/kg p.o) showed a significant reduction in the duration of transfer latency on day 2 compared to day 1 (Fig. 5)

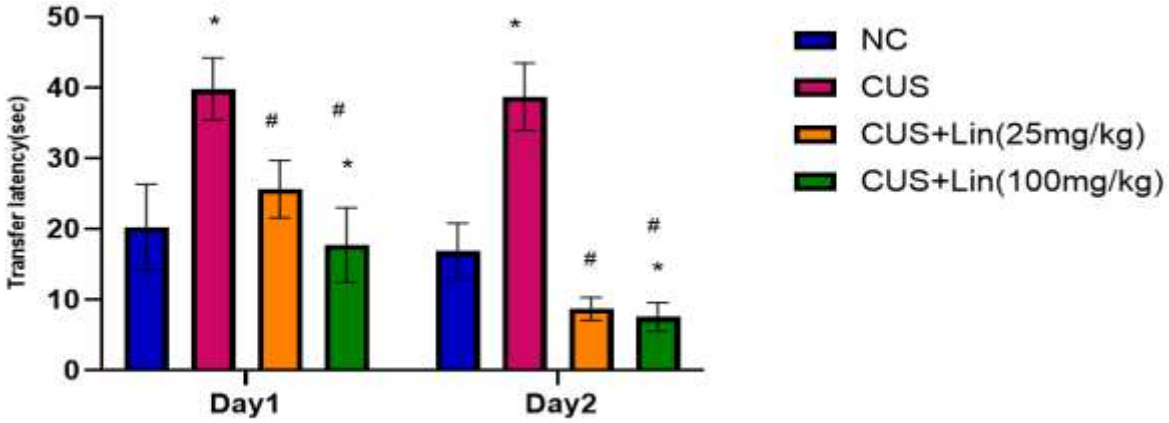
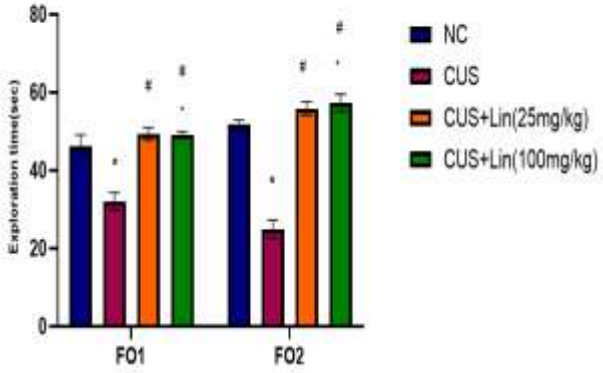



**Fig. 5:** Effect of Linalool (Lin) on transfer latency. The results were expressed as mean ± SEM(n=6); A one-way ANOVA was conducted followed by Tukey's multiple comparison test. *p<0.05 as compared to normal control group, # p<0.05 as compared to the CUS induced group.

**Effect of Linalool on stress induced cognitive impairment parameter using NOR test**

In the NOR test, CUS-induced animals spent less time exploring familiar objects (FO1 and FO2) and the novel object compared to normal control. However, rats treated with linalool (25mg/kg and 100mg/kg) spent more time on exploring both familiar objects and the novel object. These results reveal that linalool reversed cognitive deficits and helps to restore memory and recognition which are affected by CUS (Fig. 6 A & B).

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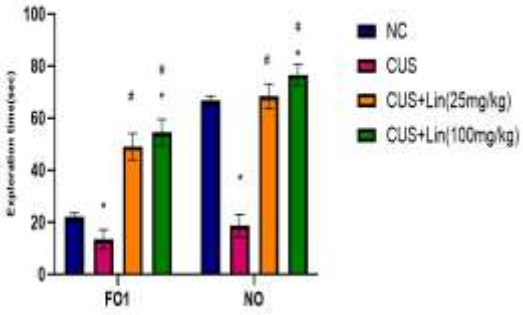
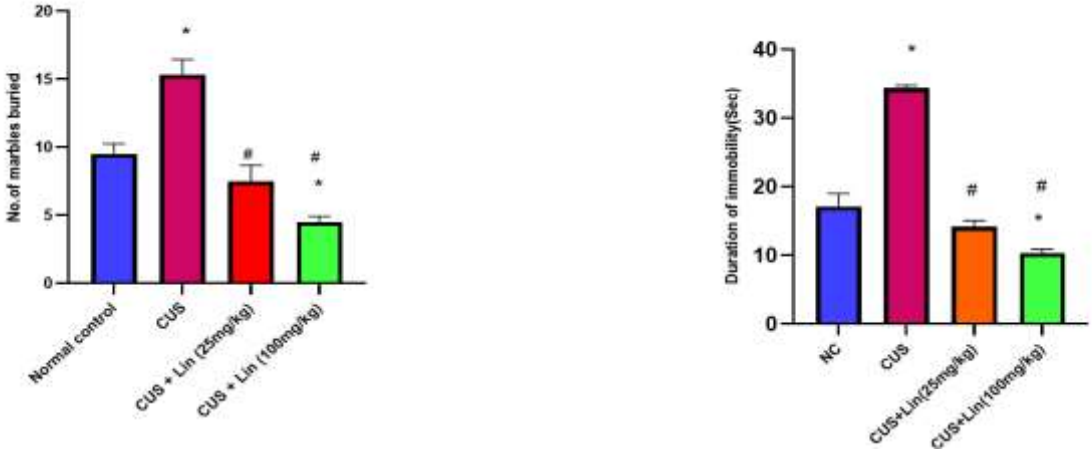



and anti-compulsive effects.

In forced swim test CUS-induced group exhibited a significant increase in immobility time compared to the control group. However, treatment with linalool (25 mg/kg and 100 mg/kg) significantly reduced the duration of immobility compared to the CUS group (Fig. 7B). This suggests that linalool effectively alleviates stress-induced depression and cognitive impairment.

A B



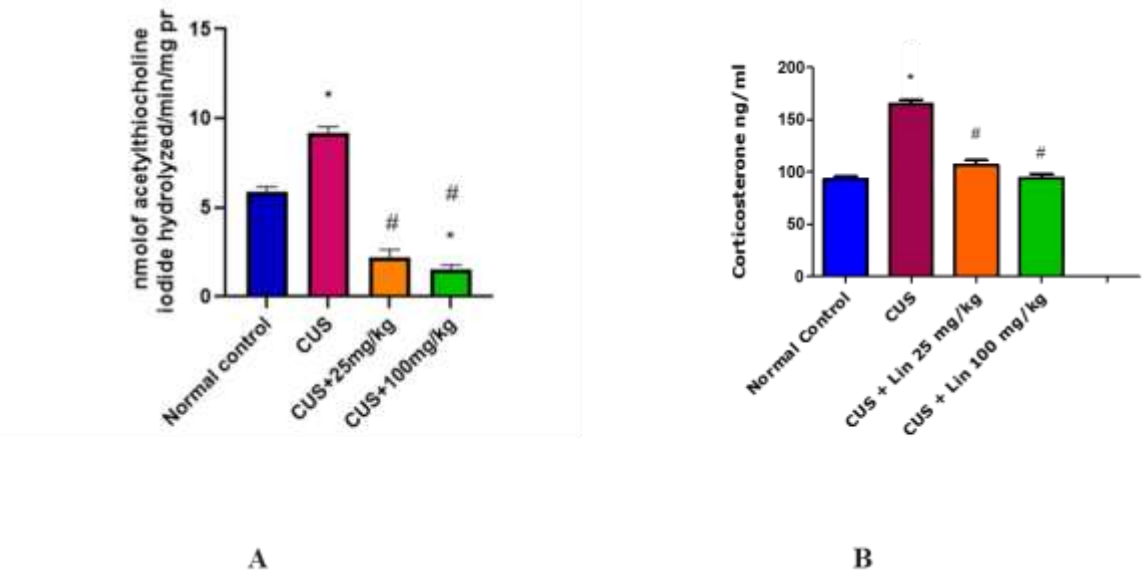

**A** **B**

**Fig. 7:** Effect of Linalool (Lin) on (A) marble burying test and (B) forced swim test. The results were expressed as mean ± SEM(n=6); A one-way ANOVA was conducted followed by Tukey's multiple comparison test. *p<0.05 as compared to normal control group, # p<0.05 as compared to the CUS induced group.

**Effect of Linalool on Acetylcholinesterase levels in brain and corticosterone levels in blood**

Chronic stress significantly elevated acetylcholinesterase (AChE) levels, enzyme associated with cognitive decline in the brains of rats. However, treatment with linalool (25 mg/kg and 100 mg/kg) notably reduced these elevated AChE levels, indicating a potential neuroprotective effect in learning and memory (Fig. 8 A). Corticosterone levels are significantly elevated in the CUS-

induced group, indicating that chronic stress increases corticosterone levels and disrupts the HPA axis. However, the groups treated with linalool showed a significant reduction in corticosterone levels in blood (Fig. 8B).




A B

**Fig.8:** A: Effect of Linalool (Lin) on Acetylcholinesterase levels. B: Effect of Linalool (Lin) on corticosterone levels. The results were expressed as mean ± SEM(n=6); A one-way ANOVA was conducted followed by Tukey's multiple comparison test. *p<0.05 as compared to normal control group, #p<0.05 as compared to the CUS induced group.

## 4. Discussion

The current study explored the neuroprotective effects of Linalool on chronic stress-induced cognitive impairment using network pharmacology, molecular docking, and *in vivo* animal models. The findings would thus provide comprehensive insight into the mechanisms and the therapeutic potential of Linalool in relation to its multi-targeting action through pathways that could affect neurodegeneration, oxidative stress, neuroinflammation, or synaptic plasticity.

Literature cites that chronic stress disrupts the HPA axis, increasing the levels of glucocorticoids and thus altering cognitive ability in regions including the hippocampus and prefrontal cortex (McEwen, 2005 and Sorrells *et al.*, 2009). Neurodegenerative alterations found in the present study resembled previous findings based on stress-related hormonal imbalance, oxidative stress, and neuroinflammation pathways in the deterioration of the hippocampus and disruption of cognitive activities.

Our network pharmacology analysis found that Linalool specifically targeting some crucial genes involved in the neurodegenerative pathways including PTGS2, GSK3B, MAPK1, and MAPK8, which are critical in neuroinflammation as well as response to oxidative stress, a basis of stress induced cognitive decline (Ajoolabady *et al.*, 2022). Another critical signalling pathway found as crucial to the neuronal integrity, synaptic plasticity are the levels of sphingolipids. Their pathophysiology and dysregulation have been commonly associated with the neurodegenerative diseases, and also to the cognitive deficiency. This is supporting the relevance of results exhibited by Linalool on sphingolipid pathway in the network analysis.

The dopaminergic synapse pathway, especially the mesocortical pathway connecting the ventral tegmental area to the prefrontal cortex, was also involved in CUS-induced cognitive impairments. Chronic stress disrupts dopamine neurotransmission, reducing receptor function and neuroplasticity, as shown in previous studies (Liu *et al.*, 2017; McEwen & Gianaros, 2011). Linalool's modulatory effects on this pathway suggest its potential in restoring cognitive functions impacted by stress.

Similarly, molecular docking studies showed a strong binding affinity of Linalool to PTGS2, a key enzyme involved in neuroinflammation. This finding supports its anti-inflammatory effects, which likely contribute to the observed neuroprotective outcomes. The multi-target action of Linalool, as evidenced by its interaction with genes in various signaling pathways, underscores its potential as a holistic therapeutic agent for cognitive impairments.

The behavioral tests in animal model indicated that Linalool significantly attenuated anxiety-like behaviors, depressive symptoms, and compulsive behavior with improved cognitive performance in a chronic unpredictable mild stress model of rats. These observations were in accordance with its conventional use as an anxiolytic and neuroprotective drug (Efferth *et al.*, 2020). A significant decrease in corticosterone levels in the serum samples of rats also further supports its involvement in the normalization of the hyperactive HPA axis observed in CUS models (Herman *et al.*, 2005)

These multi-faceted effects of Linalool on stress-induced interlinked pathways regulating neuronal survival, synaptic plasticity, neurotransmission, and neuroinflammation point toward its potential role as a novel intervention in stress-induced cognitive impairments. Validation of these results in clinical settings requires further research. In order to translate these preclinical results into therapeutic applications, long-term safety and efficacy assessment along with detailed pharmacokinetic and pharmacodynamic profiling are needed.

The study provides strong evidence for the neuroprotective effects of Linalool in alleviating chronic stress-induced cognitive impairments. Targeting multiple pathways, Linalool has great potential to restore cognitive function and decrease stress-related behavioral abnormalities. Such findings open avenues for future research to investigate Linalool as a promising therapeutic agent for stress-related cognitive disorders.

**Author contributions**

Latha P: Methodology, supervision and original draft preparation; T. Sukeerthi: Experimental data analysis; U. Vasundhara: Executed the experiment work; D. Sujatha: Supervision, reviewing and manuscript editing. The authors read and approved the final manuscript.

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**Conflict of interest**

The authors declare that they have no conflicts of interest.

**References:**


- Ajoolabady A, Lindholm D, Ren J, Pratico D. ER stress and UPR in Alzheimer's disease: mechanisms, pathogenesis, treatments. Cell death & disease. 2022 Aug 15;13(8):706.
- Dantzer R, O'connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature reviews neuroscience. 2008 Jan;9(1):46-56.
- Dos Santos ÉR, Maia JG, Fontes-Júnior EA, Maia CD. Linalool as a therapeutic and medicinal tool in depression treatment: A review. Current Neuropharmacology. 2022 May 5;20(6):1073.
- Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. Biological psychiatry. 2006 Jun 15;59(12):1116-27.
- Efferth T, Greten HJ, Pandey A, Bishayee A. The dawn of traditional Asian and ethnomedicine in modern times. J Ethnopharmacol. 2020 Sep 15; 247:112255.
- Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of pharmacology. 1961 Jul 1;7(2):88-95.
- Gonçalves S, Mansinhos I, Romano A. Aromatic plants: a source of compounds with antioxidant and neuroprotective effects. InOxidative stress and dietary antioxidants in neurological diseases 2020 Jan 1 (pp. 155-173). Academic Press
- Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Progress in neuro-psychopharmacology and biological psychiatry. 2005 Dec 1;29(8):1201-13.
- Myers B, McKlveen JM, Herman JP. Neural regulation of the stress response: the many faces of feedback. Cellular and molecular neurobiology. 2012 Jul;32:683-94.
- Jagadeesan S, Chiroma SM, Baharuldin MT, Taib CN, Amom Z, Adenan MI, Moklas MA. *Centella asiatica* prevents chronic unpredictable mild stress-induced behavioral changes in rats. Biomedical Research and Therapy. 2019 Jun 29;6(6):3233-43.
- Kedia S, Chattarji S. Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice. Journal of neuroscience methods. 2014 Aug 15; 233:150-4.
- Kim DS, Lee HJ, Jeon YD, Kee JY, Kim HJ, Shin XJ, et al. Alpha-pinene exhibits anti-inflammatory activity through the suppression of MAPKs and the NF-κB pathway in mouse peritoneal macrophages. Am J Chinese Med. (2015) 43:731–42.
- Liu J, Wang C, Zhao Y Chronic social stress induces changes in dopamine neurotransmission and working memory impairment.2017;95(8):1550-1563.
- Mani V, Rabbani SI, Shariq A, Amirthalingam P, Arfeen M. Piracetam as a therapeutic agent for doxorubicin-induced cognitive deficits by enhancing cholinergic functions and reducing neuronal inflammation, apoptosis, and oxidative stress in rats. Pharmaceuticals. 2022 Dec 14;15(12):1563.
- McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. 2011;306(6):648-658
- Motterlini R, Foresti R, Bassi R, Green CJ. Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radical Biology and Medicine. 2000 Apr 15;28(8):1303-12.

- Nafisi S, Adelzadeh M, Norouzi Z, Sarbolouki MN. Curcumin binding to DNA and RNA. DNA and cell biology. 2009 Apr 1;28(4):201-8.
- Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nature Reviews Neuroscience. 2012 Jan;13(1):22-37.
- Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978 Feb 15;47(4):379-91.
- Sorrells, S.F., Caso, J.R., Munhoz, C.D., & Sapolsky, R.M. The stressed CNS: When glucocorticoids aggravate inflammation. Neuron. 2009 64(1), 33-39.