Trop J Phytochem Pharm Sci, March 2025; 4(3): 102 – 110 **ISSN 2955-1226 (Print)**
ISSN 2955-123(Electronic)

Tropical Journal of Phytochemistry & Pharmaceutical Sciences

Available online at https://www.tjpps.org

Original Research Article

Evaluation of Memory Enhancing Potential of *Centella lujica* Supplement and Its Underlying Mechanisms of Action in mice model of Stress

Okporu N. Anthony[1], Anthony J. Anozie[2], Celestine O. Akpovwre[1]*, Ejiro P. Awhin[3], James O. Oni[4], Anthony T. Eduviere[1], Festus E. Avwotuhwaye[5]

[1]*Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Nigeria*
[2]*Department of Microbiology, Faculty of Science, Delta State University of Science and Technology, Ozoro, Nigeria*
[3]*Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Nigeria*
[4]*Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*
[5]*Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Edo State University, Uzairue, Nigeria*

ABSTRACT

Chronic stress is known to impair cognitive functions, particularly memory. This study investigated the neuroprotective and memory-enhancing effects of *Centella lujica* (*CL*) in a mouse model of chronic unpredictable mild stress (CUMS). Sixty adult male mice were divided into five groups: group 1 served as the control, groups 2 - 5 were exposed to CUMS, groups 3 and 4 were pretreated with *CL* at 25 mg/kg and 50 mg/kg orally, respectively, while group five was pretreated with donepezil (1 mg/kg, i.p.). Behavioral assessment using the novel object recognition test and biochemical analyses of the oxidative stress biomarkers, as well as the histology of the prefrontal cortex and hippocampus were done. Results showed that *Centella lujica* significantly enhanced memory performance (0.4120 ± 0.01715, 0.1920 ± 0.01281, 0.4240±0.02600, 0.4740±0.02293, and 0.3120±0.01985) and increased reduced glutathione levels (26.52±1.059, 17.8±1.499, 25.77±1.636, 29.7±1.535, and 24.82±1.631 in the prefrontal cortex; 18.8±0.6092, 13.21±0.6095, 18.38±0.773, 19.78±0.6057, and 17.31±0.5267 in the hippocampus), indicating the potential of *Centella lujica* in reducing oxidative stress. Histological evaluation confirmed improved structural integrity in the prefrontal cortex and hippocampus. These findings suggest that *Centella lujica* counteracts stress-induced oxidative damage and promotes neuroplasticity, offering potential therapeutic benefits for stress-related cognitive impairments. Further studies are needed to explore its clinical applications.

Keywords: Memory, Oxidative stress, Inflammation, *Centella lujica*

Received 12 February 2025
Revised 17 March 2025
Accepted 25 March 2025
Published online 01 April 2025

Copyright: © 2025 Okporu *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Stress is considered as any condition which results in perturbation of the body's physiological homeostasis.[1] It is perceived as an integral component of human life which requires some forms of adaptation for survival.[1] However, when stress persists over a long period of time and the body is unable to cope, it therefore induces a series of events which result in the generation of free radicals such as reactive oxygen and nitrogen species which are capable of driving the oxidative stress processes.[2] Almost all organs of the body including the immune system is susceptible to stress, of these organs the brain is more vulnerable to stressors, as it is the major organ of the body that regulates stress responses and determines the behavioral and physiological outcomes to aversive situations.[3,4] Oxidative stress has been reported as one of the major drivers of the aging process. Since the brain which regulates vital tasks such as cognition is said to be constantly susceptible to prolonged stress, thus stressors are capable of inducing a variety of degenerative disorders.[5]

*Corresponding author. Email: **akpovwrec@gmail.com**

Tel: +2348061143296

Citation: Ajani OS, Obiechefu CH, Ake AS. Therapeutic Effects of Cyperus esculentus on Monosodium Glutamate-Induced Haemo-Biochemical and Steroidogenic Anomalies in Male Wistar Rats. Trop J Phytochem Pharm. Sci. 2025; 4(3) 102 - 110 http://www.doi.org/10.26538/tjpps/v4i3.1

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Oxidative stress is the condition arising from an imbalance between toxic reactive oxygen species (oxidants) and antioxidants defense system. Increased reactive oxygen species levels leads to deleterious effects including lipid peroxidation.[6] Also, chronic unpredictable mild stress (CUMS) has been reported to deplete antioxidant enzymes like glutathione (GSH), and therefore further damages brain cells through increased lipid peroxidation. Increased lipid peroxidation and decreased antioxidant defense system have been found in the brain of depressed patients, which further supports the role of oxidative stress in the pathophysiology of this disease.[6] Chronic stress can lead to structural alterations in synaptic terminals, a decrease in dendritic branches and neurons, and an increase in plasma cortisol, which can ultimately result in hippocampal neurodegeneration.[7] Glucocorticoids can cause these modifications by either altering the way neurons metabolize their cells or making hippocampus cells more sensitive to stimulatory amino acids.[8] These alterations can be brought about by glucocorticoids through their effects on neuronal cellular metabolism, augmentation of hippocampus cell sensitivity to stimulatory amino acids, and/or elevation of extracellular glutamate levels, all of which may contribute to neurodegeneration.[8]

The brain areas most susceptible to oxidative stress include the hippocampus, amygdala, prefrontal cortex, and cerebellar granular cells. Significant biochemical alterations in the hippocampal region occur during oxidative stress, which ultimately impact neuronal connections and function. The hippocampal cornu ammonis (CA3) neurons show considerable regeneration and remodeling capacity in addition to structural plasticity.[9]

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

The neurodegenerative disorder known as Alzheimer's disease (AD) is characterized by the accumulation of amyloid plaques, the formation of neurofibrillary tangles, oxidative stress, neuroinflammation, and the loss of cholinergic neurons in particular brain regions, including the prefrontal cortex and the hippocampus. AD is a multi-pathological disease; significant neuropathological characteristics associated with the illness include degeneration and functional impairment of the central cholinergic system.[10] One pathogenic feature of AD is the loss of neurons in the basal forebrain, specifically in the hippocampal circuits, which are important in learning and memory functions. The cholinergic theory of AD was subsequently proposed, indicating that a reduction in cholinergic transmission.[10]

Oxidative stress is characterized as an oxidative imbalance resulting from the inability of the body to detoxify its reactive products, which are created during cellular metabolism via the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[6] An increasing amount of research has highlighted the effect of oxidative stress on the brain, showing that the pathophysiology of neurodegenerative diseases, such as Alzheimer's, is closely linked to higher levels of reactive oxygen species (ROS).[11,12] The chronic unexpected mild stress (CUMS) procedure is a well-established technique.[13] It has been shown that mice subjected to the CUMS protocol display abnormalities in hippocampal structure and functions, including mental and cognitive deficiencies, because the hippocampus is vulnerable to CUMS. In the hippocampus of rats exposed to CUMS, preclinical investigations have revealed decreased expression of brain-derived neurotrophic factor (BDNF), changed synapse architecture, and impaired neurogenesis.[14] This paradigm is an ethologically relevant model to explore the effects of psychological stress because of the unpredictable period and sequence of exposure to them to prevent habituation.[15] Furthermore, the diversity of stressors inhibits the hypothalamic-pituitary-adrenal (HPA) axis from becoming accustomed to its function, resulting in a prolonged and heightened physiological stress response.[16] Previous studies have demonstrated that animals subjected to long-term mild stress or chronic restraint display cognitive impairment, cAMP-response element binding protein (CREB) and BDNF depletion, and dendritic atrophy of the hippocampal cornus ammonis-3 (CA3) sub-region. Research currently available indicates that CUMS leads to disruption of the HPA axis and results in an increase in the basal levels of stress hormones.[17] Moreover, CUMS exposure has been linked to decreased brain volume, dendritic atrophy, changes in synaptic plasticity indicators, and worsened methamphetamine-induced neurotoxicity.[13] These modifications are linked to changes in immune system, metabolic, and autonomic indicators.

Centella lujica, often known as gotu kola, a perennial medicinal herb that is a member of the Apiaceae family. It is also known as Indian pennyworth in the United States of America (USA), Gotu Kola in Indonesia, and Pegaga in Malaysia, much like the well-known congener *Centella asiatica*, which is found in South-east Asia and India.[18] Growing amid moist places in tropical nations, *Centella lujica* is a slender, creeping plant that roots at the nodes.[19] *Centella lujica* is a perennial slightly scented creeper herb that grows up to 15 cm (6 inches) tall with glabrous stems. The plant thrives in loamy soil; its reniform leaves, which reach 1.5–5 cm in width and 2–6 cm in length, emerge from the stem nodes, and its blooms resemble fascicled umbels.[20] *Centella lujica* is rich in flavonoids which are naturally occurring bioactive phytochemical metabolites widely known to prevent and suppress several human diseases and are important sources of therapeutic compounds from plants.[21]

Centella lujica is considered a revitalizing herb and a neuro-tonic that improves memory and intelligence in Ayurvedic medicine. *Centella lujica* is utilized for its neuro-rejuvenating qualities as well as its psychotropic therapeutic qualities; in Ayurvedic medicine, for example, it is used to alleviate anxiety.[14] Hence this study seeks to evaluate the neuroprotective and memory-enhancing capabilities of *Centella lujica* supplement in unpredictable chronic mice stress model of depression in mice.

Materials and Methods

Laboratory animals

Sixty (60) adult albino Swiss mice weighing 25-28 g used in the study were obtained from the Central Animal House, Delta State University, Abraka and were housed in plastic cages at room temperature with 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet and water *ad libitum.* Mice were acclimatized for at least one week before commencement of experiments. All experimental procedures were performed in accordance with the Guide for Care and Use of Laboratory Animal (NIH guidelines), and approval was sort from the Faculty of Basic Medical Sciences ethical committee (RBC/FBMC/DELSU/25/653).

Equipment/Apparatus

Centrifuge (ATKE), water bath (Equitron), spectrophotometer (Inesa, 752N), pH meter (EDT instruments), weighing balance (Ohaus), test tubes, eppendorf tubes, test tube racks, dissection kits and boards.

Drug preparation

Centella lujica capsules were obtained from New Era Oluji Nig. Ltd©, Ondo State, Nigeria. One capsule (100 mg) was dissolved in 20 mL of distilled water to obtain a stock solution of 5 mg/mL, which was further diluted in distilled water to obtain the concentrations used in the study.[22]

Experimental design

Mice were divided into five groups of 6 mice per group: Control, CUMS + Distilled water (10 mL/kg), CUMS + *Centella lujica* (25 mg/kg), CUMS + *Centella lujica* (50 mg/kg), and CUMS + Donepezil (1 mg/kg). All mice except those in the control group were exposed to various mild stressors daily for a period of 14 days in an unpredictable pattern.[23] At the end of 14 days period of unpredictable chronic mild stress, one hour after the last treatment, the effect of *Centella lujica* on memory performance was assessed using novel object recognition test. Also, the animals were euthanized on the 15th day and their brain tissues were harvested and specific regions (prefrontal cortex and hippocampus) were kept aside for the investigation of the probable mechanisms of action of *Centella lujica* in chronic unpredictable mild stress utilizing spectrophotometric and ELISA techniques, immunohistochemical and histological evaluations**,** as well as electron microscopy where applicable.

Unpredictable chronic mild stress (CUMS) paradigm

The mice were adapted for one week and the CUMS procedure was performed for two weeks as previously described by Olayinka *et al*. (2023).[24] The control mice which were not subjected to CUMS was left in their cages except for normal handling and cleaning. The mice in the other groups were subjected to CUMS, with a minimum of two stressors per day for two consecutive weeks (Table 1). These stressors were randomly administered for the two weeks experimental period in an unpredictable fashion. In order to avoid prediction and adaptation, no two stressors were applied consecutively. Mice were allotted into five groups (n = 6) in a semi-randomized fashion, such that their mean body weights across the groups were comparable. One hour prior to exposure to CUMS, mice received orally *C. lujica* (25 and 50 mg/kg), Donepezil (1 mg/kg), or vehicle (10 mL/kg distilled water). The non-stress control group received the vehicle (10 mL/kg distilled water) but was not exposed to the stressors in the CUMS paradigm. Thereafter, behavioral tests for memory were carried out using validated behavioural procedures.

These stressors were randomly administered for the two weeks experimental period in an unpredictable fashion. In order to avoid prediction and adaptation, no two stressors were applied consecutively. Mice were allotted into five groups (n = 6) in a semi-randomized fashion, such that their mean body weights across the groups were comparable. One hour prior to exposure to CUMS, mice received orally *C. lujica* (25 and 50 mg/kg), Donepezil (1 mg/kg), or vehicle (10 mL/kg distilled water). The non-stress control group received the vehicle (10 mL/kg distilled water) but was not exposed to the stressors in the CUMS paradigm. Thereafter, behavioral tests for memory were carried out using validated behavioural procedures.

Table 1: Schedule of Stressors in Chronic unpredictable mild stress Paradigm in Mice

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Days		Stressors and duration
1	Forced swim (5 min)	food deprivation (12 h)
2	Damp sawdust (120 min)	hypoxia (15 min)
3	Tail pinch (5 min)	sawdust free cage (90 min)
4	Food deprivation (12 h)	change of cage mates (30 min)
5	45◦ cage tilting (120 min)	forced swim (5 min)
6	Food deprivation (12 h)	hypoxia (15 min)
7	Exposure to predator odor (30 min)	water deprivation (12 h)
8	45◦ cage tilting (180 min)	forced swim (6 min)
9	Sawdust free cage + 200 ml water (120 min)	tail pinch (5 min)
10	Hypoxia (15 min);	water deprivation (12 h)
11	Damp sawdust (120 min)	tail suspension (5 min)
12	Forced swim (5 min);	food deprivation (12 h)
13	Exposure to cat meowing (30 min)	sawdust free cage (120 min)
14	Social defeat (5 min),	food and water deprivation (12 h)

Behavioral test

Novel object recognition test

The effect of *C. lujica* on memory performance was assessed using the Novel Object Recognition Test (NORT) in an open-field chamber (60 cm × 50 cm × 40 cm) with discriminated objects (A, B and C) which were identically sized (4.5 cm diameter and 11.5 cm height) cylindrical bottles. Objects A and B were white, whereas object C had a black and white pattern. The NOR test consists of two phases: the trial phase and the test phase. Mice were randomly allotted into treatment groups (n = 6) and were administered *C. lujica* orally (25 and 50 mg/kg), donepezil (1 mg/kg) or vehicle (10 mL/kg) once daily for 7 days. Thirty minutes after the last treatment, the animals were acclimatized to the experimental set-up for a period of 5 min. The trial phase was carried out by placing each mouse in the middle of two identical objects (A and B) on opposite sides (at a distance of 8 cm from the walls and 34 cm from each other) of the open-field chamber for 5 min. Thereafter, the animals were returned to their home cages for a period of 4 h. In the test phase, object B was replaced with object C, which was novel to the mice and different from object A or B. Mice were then left to explore objects A and C for a period of 5 min. The apparatus was cleaned after each test and the duration of time spent (s) in exploring each of the objects were recorded in both phases. The discrimination index, which was used as a measure of non-spatial memory function, was calculated as the difference in time exploring the novel and familiar objects divided by the total amount of time spent with both objects.[6]

Sample collection

At the end of the experimental period, mice were decapitated after behavioural assessment. Blood samples were collected through the orbital vein. Brains were immediately removed, and specific brain regions (prefrontal cortex and hippocampus) were isolated on an ice trail, homogenized in phosphate buffered saline (PBS), centrifuged at 10000 rpm for 10 min at 4°C. The supernatants were collected, and stored at -20ºC until used for biochemical analysis.

Determination of acetylcholinesterase (AChE) activity in mice brain

Aliquots of supernatant of individual mouse brain (prefrontal cortex and hippocampus) of the various treatment groups were taken and used to measure AChE activity, a marker for cholinergic neurotransmission.[25] Briefly, AChE activity in the homogenate was measured by adding 2.6 mL of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) and 0.4 mL of the homogenate. Then, 0.1 mL of acetylthiocholine iodide was added to the reaction mixture. The absorbance was read using a spectrophotometer at a wavelength of 412 nm and change in absorbance for 10 min at 2 min interval was recorded. The rate of AChE activity was measured by following the increase in colour produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined and the rate of AChE activity was calculated and expressed as µmol/min/g tissue.

Determination of glutathione (GSH) concentration

Aliquots of brain (prefrontal cortex and hippocampus) supernatant of individual mouse in the respective treatment groups were taken and GSH concentration was determined using the method described by Moron *et al*. (1979).[26] Equal volume (0.4 mL) of brain homogenate and 20% trichloro acetic acid (TCA) (0.4 mL) were mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 mL) was then added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against a blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues were expressed as micromoles per gram tissue (µmol/g tissue).

Estimation of brain level of malondialdehyde

The brain (prefrontal cortex and hippocampus) level of malondialdehyde (MDA), a biomarker of lipid peroxidation, was estimated according to the method described by Ádám-Vizi and Seregi (1982).[27] An aliquot of 0.4 mL of the supernatant was mixed with 1.6 mL of Tris–KCl buffer to which 0.5 mL of 30% TCA was added. Then, 0.5 mL of 0.75% thiobarbituric acid (TBA) was added and placed on a water bath at 80°C for 45 min. The reaction mixture was allowed to cool in an ice bath, and then centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and the absorbance was measured at 532 nm against a blank of distilled water using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and values were expressed as µmoles of MDA per gram tissue.

Estimation of glutathione peroxidase (GPx)

The activity of glutathione peroxidase (GPx) in the brain (prefrontal cortex and hippocampus) was determined according to the method described by Mythri *et al*. (2011).[28] The following reagents; 1.49 mL phosphate buffer (0.1 M; pH 7.4), 0.1 mL EDTA (1 mM), 0.1 mL sodium oxide (1 mM), 0.05 mL glutathione reductase (1 IU/mL), 0.05 mL GSH (1 mM), 0.1 mL NADPH (0.2 mM), and 0.01 mL H_2O_2 (0.25 mM) were mixed with 0.1 mL of brain homogenate in a total volume of 2 mL. The disappearance of NADPH was determined at 25ºC using a spectrophotometer at 340 nm. The activity of the enzyme was calculated as nM NADPH oxidized per mg protein using molar extinction of 6.22×10^3 M^{-1} cm^{-1}.

Estimation of brain nitrite level

Brain (prefrontal cortex and hippocampus) nitrite concentration was estimated using Greiss reagent, which serves as an indicator of nitric oxide (NO) production. One hundred microliter of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 µL of the supernatant and absorbance was measured at 540 nm. The brain nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0 - 100 µM).

Histology and estimation of neuronal density

Representative brain tissue sections of each treatment group were stained with Hematoxylin and Eosin to demonstrate general histology of the prefrontal cortex and cornu ammonis-3 (CA3) of the hippocampal region according to the method described by Amin *et al*. (2013).[29] Images were subsequently captured using an Optronics Digital Camera linked to a computer interface (MagnaFire) and an Olympus BX-51 Binocular research microscope. The morphology of the pyramidal, peri-glomerular, and granule cells was assessed through inter-reader variability. Viable neuronal cells were quantified using ImageJ at magnifications of X400 or X250 across different microscopic fields for all groups. Cells were considered viable if they exhibited a round shape,

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

an intact cytoplasmic membrane, and no signs of nuclear condensation or structural distortion. Neuronal density was determined by calculating the ratio of viable neuronal cells to the square area of the circular field in each section.

Statistical analysis
All data were presented as Mean ± Standard Error of Mean (SEM). The results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine the differences between mean values. The level of significance for all tests was set at $p<0.05$. Graph Pad InStat® Biostatistics software was used for the statistical analysis.

Results and Discussion

Effect of Centella lujica (CL) on memory performance in chronic unpredictable mild stress (CUMS)
The CUMS paradigm is generally believed to be the most suitable model for evaluating the deleterious effects of stress in animals, as it mimics the pathological changes in humans exposed to post-traumatic stress, or life-time events on daily basis.[6,30] Animals exposed to chronic stress present with behavioral changes; associated with memory loss, depression and anxiety.[31]
In the present study, mice subjected to CUMS were used as a model to induce memory deficits and the depletion of endogenous antioxidant defense molecules, and also to compare the effects of donepezil (an established anti-amnesic) with those of *CL*, which has not been previously tested in a CUMS model of memory deficits. Major findings from this study have shown that CUMS resulted in learning and memory impairments in mice.
The effects of *CL* on learning and memory abilities of the mice were evaluated using the novel object recognition test (NORT) for nonspatial recognition memory as shown in Figure 1. There were statistically significant differences in discrimination index in the treatment groups. Tukey's post hoc revealed significant decreases in discrimination index in the CUMS-induced mice when compared to the control group. Specifically, *CL* at 25 mg/kg showed the most significant ($p < 0.05$) improvement in discrimination index compared to the CUMS group. *CL* treatment also improved performance in the NORT in mice (Figure 1). While the control group did not display a preference for the novel object at either 2 h or 24 h post-training, *CL* treated mice spent significantly more time exploring the novel object than the familiar object at both time points, the results (Figures 1) revealed that the duration of new object recognition test decreased significantly in the CUMS group compared to the treatment group ($P < 0.05$).

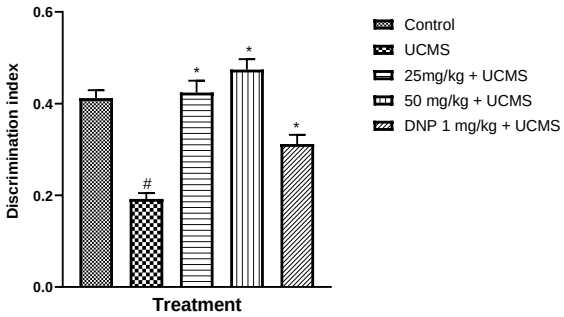

Figure 1: Effect of *Centella lujica* (*CL*) on memory performance in chronic unpredictable mild stress (CUMS) model using the novel object recognition test (NORT).
Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

The Novel Object Recognition Test (NORT) is a widely used method to assess learning and memory in rodents, relying on their inherent tendency to explore new objects.[32-34] The NORT is also known to be less stressful and requires lesser time to run relative to other models.[6,34,35] The NORT behavioral tests have been widely used to assess animal learning and memory functions, the reliabilities of which have also been confirmed.[6,31] The data from the current study indicated that CUMS mice showed less interest in the novel object, as evidenced by a lower discrimination index, which suggests a decline in memory. In contrast, CL-treated mice displayed a stronger preference for exploring the unfamiliar object during the memory retention test, highlighting its potential to mitigate CUMS-induced cognitive impairment. Overall, the results from the NORT protocols demonstrated that CL significantly improved non-spatial memory recall in mice subjected to CUMS. Disruptions in the central cholinergic system have been linked to various cognitive deficits.[31,4]Previous research has demonstrated that exposing rodents to CUMS leads to long-term neuronal loss and dendritic atrophy in the hippocampus over several weeks, resulting in cognitive deficits.[36,37] However, there is increasing awareness in the use of medicinal plants with adaptogenic property to prevent stress-induced memory loss and related neuropsychiatric disorders.[3,38]

Effect of CL treatment on hippocampal and prefrontal cortex oxidative damage following CUMS exposure
The hippocampal tissue and prefrontal cortex tissue in CUMS-exposed mice demonstrated a disrupted oxidative status, characterized by increased MDA and NO production. These changes were accompanied by a significant decrease ($p < 0.05$) in the levels of endogenous antioxidant proteins, GSH, and its derived enzymes (GPx), compared with those in the control group. The administration of *CL* significantly ($p < 0.05$) inhibited the development of oxidative insults following exposure to CUMS by enhancing the levels of the examined antioxidant proteins and depleting the levels of pro-oxidants in hippocampal tissue and prefrontal cortex tissue. Similarly, donepezil (DNP) treatment significantly prevented ($p < 0.05$) oxidative damage associated with CUMS (Figures 2 and 3). The probable antioxidant capacity of *CL* in CUMS mice was evaluated by measuring the levels of MDA and NO in their brains. There were significant differences in the levels of MDA among the various groups of mice ($p < 0.05$) (Figure 2). CUMS-exposure resulted in significant increases in MDA and NO levels when compared to the control group ($p < 0.05$). However, pretreatment with DNP (1 mg/kg), *CL* (25 mg/kg), and *CL* (50 mg/kg) significantly decreased the levels of MDA and NO compared to the untreated CUMS group ($p < 0.05$).

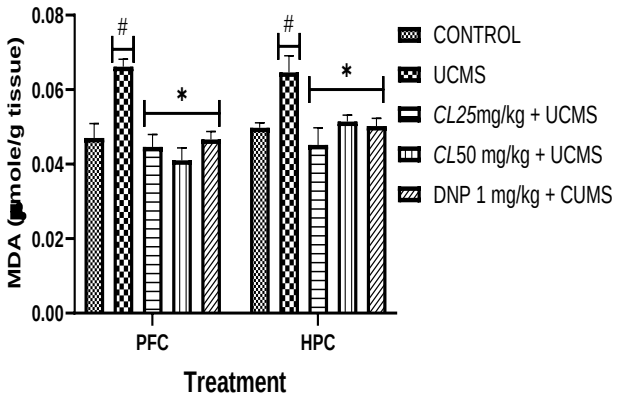

Figure 2: Effect of *Centella lujica* (*CL*) on malondialdehyde (MDA) levels in chronic unpredictable mild stress (CUMS) model. Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

The probable antioxidant capacity of *CL* in CUMS mice was evaluated by measuring the levels of GSH in their brains. There were significant differences in the levels of GSH and GPx among the various groups of

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

mice ($p < 0.05$) (Figures 4 and 5). Significant decreases in GSH and GPx levels were observed in the CUMS mice model ($p < 0.05$) when compared to the control group of mice. However, significant improvement in the levels of GSH and GPx was observed in the CUMS mice administered with DNP, *CL* at 25 and 50 mg/kg when compared to CUMS group ($p < 0.05$). among the treatment groups, the group of mice treated with CL 50 mg/kg exhibited the highest levels of GSH and GPx.

Endogenous antioxidant enzymes, for example, GPx, CAT, SOD, and GST, make up the main constituents of the antioxidant defense of the organism.[39] A significant decline in these enzyme activities suggests that the brain is either unable to detoxify hydrogen peroxide or that chronic stress has led to ROS-induced enzyme depletion. As a result, the neuroprotective effect of CL against CUMS-induced neurological deficits may be linked to its ability to enhance the activity of these antioxidant enzymes.

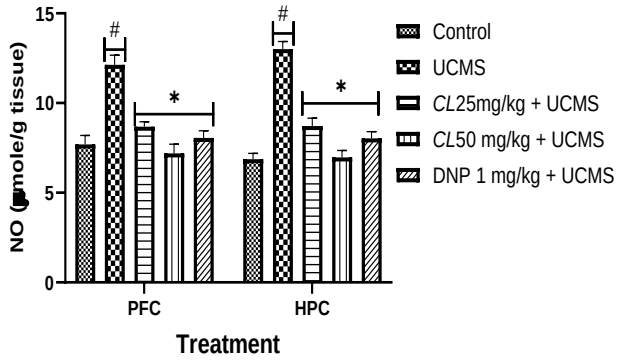

Figure 3: Effect of *Centella lujica* (*CL*) on Nitic oxide (NO) levels in chronic unpredictable mild stress (CUMS) model
Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

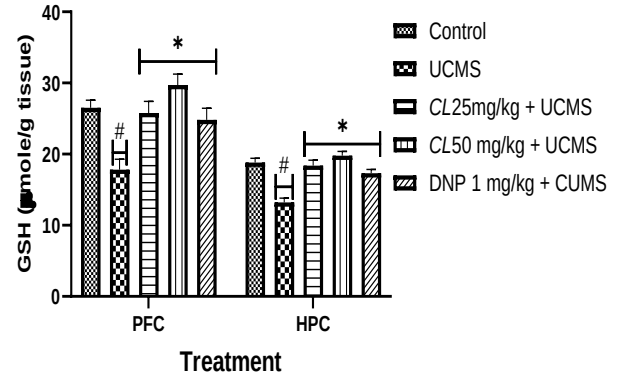

Figure 4: Effect of *Centella lujica* (*CL*) on glutathione (GSH) levels in chronic unpredictable mild stress (CUMS) model
Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

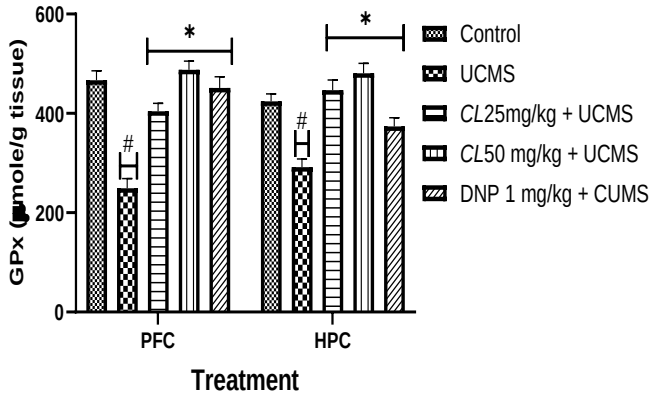

Figure 5: Effect of *Centella lujica* (*CL*) on glutathione peroxidase (GPx) levels in chronic unpredictable mild stress (CUMS) model. Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

Furthermore, the quantification of non-enzymatic oxidative stress markers has shown that CUMS elevates NO level while concurrently reducing brain levels of total sulfhydryl (***TSH***) groups, non-protein-bound sulfhydryl (***NPSH***) content. NO exhibits concentration-dependent neuronal activity, offering neuroprotection at lower levels but triggering neurotoxic effects through immune and inflammatory responses at higher concentrations.[40] At levels exceeding physiological concentrations, nitric oxide (NO) interacts with various free radicals, including superoxides, leading to the formation of reactive nitrogen oxide species like peroxynitrite, which can damage macromolecules.[41] The findings from the present study indicate that exposure to stressors within the CUMS paradigm triggered NO release (Figure 3), accompanied by a reduction in antioxidant enzyme activity. The activation of stress responses predominantly results in elevated cortisol and NO levels, which disrupt cellular energy production.[42,43] Furthermore, stress-triggered nitric oxide (NO) release can mediate pathological changes in various brain regions, including the hippocampus, amygdala, and prefrontal cortex, contributing to the behavioral alterations seen in animals subjected to chronic stress.[44] Various antioxidants that contain thiol groups, such as N-acetyl cysteine and lipoic acid, have been reported to provide protection against chronic stress.[45] Thus, the protective effect of CL against CUMS-induced cognitive impairment may be linked to the suppression of NO synthesis and the enhancement of thiol group level. A sophisticated antioxidant system maintains cellular redox balance by counteracting both ROS and reactive nitrogen species.[46] CL treatment reduced brain NO levels and MDA level expression in the hippocampal CA1, and prefrontal cortex in mice subjected to CUMS.

Effect of Centella lujica (CL) on acetylcholinesterase levels AChE in chronic unpredictable mild stress (CUMS)

AChE levels in the hippocampus and prefrontal cortex of the mice revealed statistically significant differences among the various groups (Figure 6). CUMS exposure resulted in a significant increase in acetylcholinesterase levels, whereas pretreatment with DNP, CL at 25 and 50 mg/kg decreased AChE to levels comparable to that of the control group.

The cholinergic neuron is responsible for the synthesis of ACh, the neurotransmitter that plays vital roles in learning and memory functions.[47,48] The activity of acetylcholine (ACh) in the brain and other tissues is regulated by cholinesterase (AChE), an enzyme responsible for the breakdown of ACh through hydrolysis.[47] Thus, the degradation of ACh by this enzyme has been linked to cognitive dysfunction and cholinergic neuronal damage.[49] However, medications that enhance brain ACh levels by suppressing AChE activity have been found to enhance cognitive function and support cholinergic neurons.[50,51]

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2955-1226 (Print)
ISSN 2955-123(Electronic)

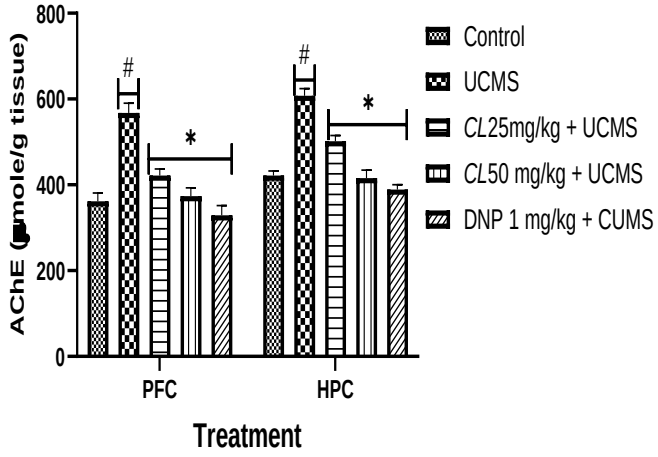

Figure 6: Effect of *Centella lujica* (*CL*) on acetylcholinesterase (AChE) levels in chronic unpredictable mild stress (CUMS) model. Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

The results of the present study demonstrated that mice subjected to CUMS exhibited elevated brain AChE activity, which may have contributed to the memory impairment observed in behavioral assessments. Consequently, the finding that CL mitigated the CUMS-induced increase in brain AChE activity suggests that its memory-enhancing effect could be associated with cholinesterase enzyme inhibition. Brain histological analysis further showed that CL reduced neuronal damage in the hippocampus and lowered the number of dead neuronal cells in CUMS-exposed mice. Several medicinal plants with adaptogenic properties, such as *Gingko biloba* and *Panax ginseng*, have been shown to effectively prevent chronic stress-induced memory impairments in rodents and aid in managing stress-related cognitive decline in humans.[3] Therefore, the beneficial impact of CL on CUMS-induced memory deficits in mice indicates its potential role in stress adaptation. Anxiety, another key behavioral manifestation linked to chronic stress, can present in diverse forms, including worry, fear, apprehension, anhedonia, eating disorders, and suicidal tendencies in humans.[52]

Effect of Centella lujica (CL) on the histology and neuronal density of the hippocampus and prefrontal cortex in mice exposed to chronic unpredictable mild stress (CUMS)

Exposure to CUMS over the course of four weeks have been shown to cause permanent loss of neurons and atrophy of hippocampal dendrites in rodents accompanied by cognitive impairment.[53] Moreover, preclinical studies have also shown that CUMS produced hippocampal damage and apoptosis resulting in loss of memory, which further confirmed the speculation that chronic stress is a major risk factor for the pathogenesis of Alzheimer's disease and premature aging of the brain.[8,53] Dendritic remodeling, in the form of reduced dendritic spines was also reported in the CA3 region of the hippocampus after CUMS exposure.[53] CUMS was also shown to decrease the number of synapses in the sub-granular zone of the hippocampal dentate gyrus, which is involved in the formation of new neuronal cells.[30] The results of the present study also confirmed that CUMS produced degeneration of neuronal cells in the pyramidal layer of the CA3 and the sub-granular zone of the dentate gyrus of the hippocampus, the region of the brain that plays vital roles in learning and memory. Histological examination of CUMS-exposed mice pretreated with *Centella lujica* revealed improved structural integrity and neuronal density in the hippocampus and prefrontal cortex (Figures 7 and 8).

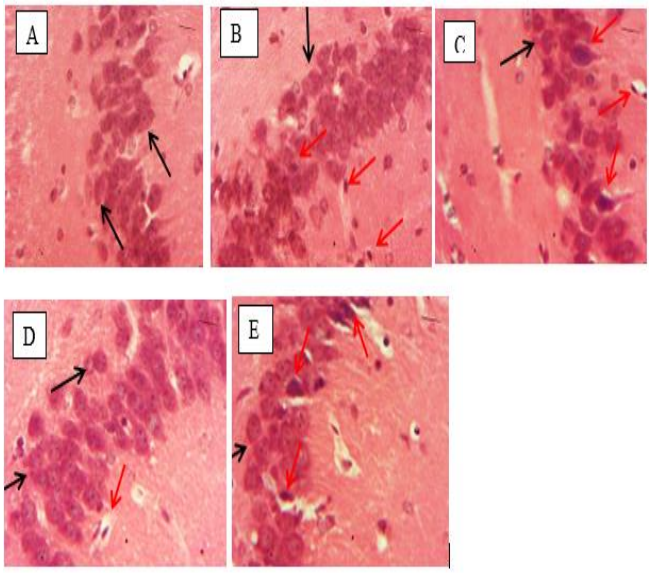

Figure 7a: Representative photomicrograph (H and E-stained section) of the effect of *Centella lujica* (*CL*) on the histology of the hippocampus in mice exposed to chronic unpredictable mild stress (CUMS). Magnification = CV × 40. **A** = Control, **B** = CUMS, **C** = CUMS + CL 25 mg/kg, **D** = CUMS + CL 50 mg/kg, **E** = CUMS + DNP 1 mg/kg.
Slide A: appeared normal (black arrow) and are well structurally organized with multiple laminal arrangement, Slide B: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow), Slide C: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow), Slide D: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow), Slide E: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow).

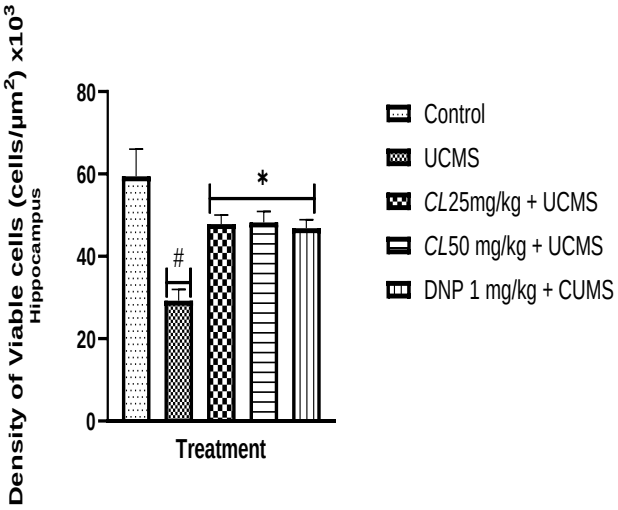

Figure 7b: Effect of *Centella lujica* (*CL*) on histology and neuronal density of the hippocampus of mice exposed to chronic unpredictable mild stress (CUMS)
Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

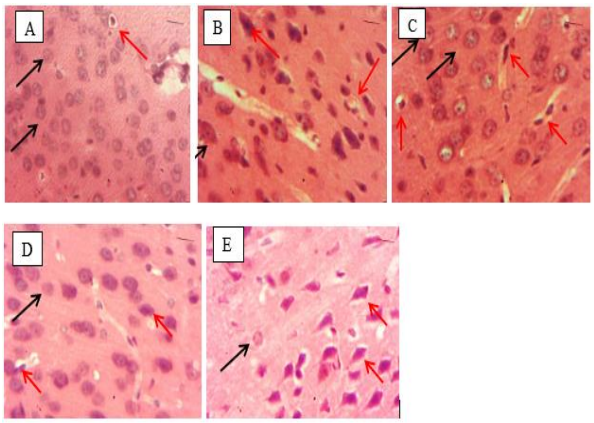

Figure 8a: Representative photomicrograph (H and E-stained section) of the effect of *Centella lujica* (CL) on the histology of the prefrontal cortex in mice exposed to chronic unpredictable mild stress (CUMS). Magnification = CV × 40. **A** = Control, **B** = CUMS, **C** = CUMS + CL 25 mg/kg, **D** = CUMS + CL 50 mg/kg, **E** = CUMS + DNP 1 mg/kg
Slide A: appeared normal (black arrow) and are well structurally organized with multiple laminal arrangement, Slide B: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow) , Slide C: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow), Slide D: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow), Slide E: few neurons appeared degenerated (atrophic) with variable basophilia (red arrow)

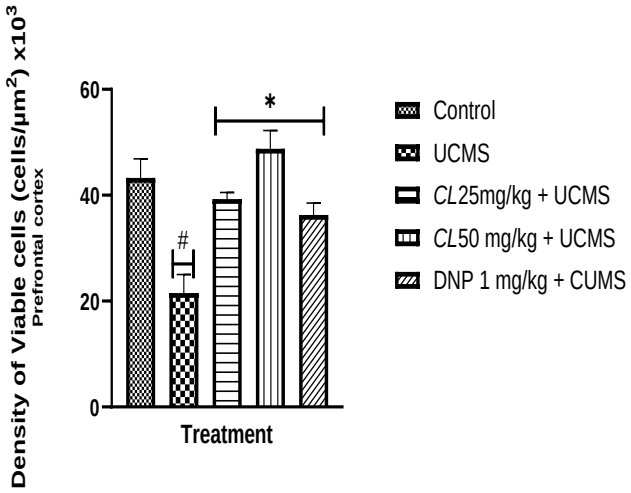

Figure 8b: Effect of *Centella lujica* (*CL*) on histology and neuronal density of the prefrontal cortex of mice exposed to chronic unpredictable mild stress (CUMS)
Values represent the mean ± S.E.M, (n = 6). # $p < 0.05$ compared to the control group, * $p < 0.05$ compared to the CUMS group.

Conclusion

The findings from this study provide evidence suggesting that *Centella lujica* demonstrated memory enhancing effect via mechanisms related to inhibition of oxidative stress, neurodegeneration, and enhancement of cholinergic neurotransmission.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

Acknowledgements

The authors acknowledged the technical staff of the Department of Pharmacology and Therapeutics, Delta State University, for their support.

References

1. Taborsky B, English S, Fawcett TW, Kuijper B, Leimar O, McNamara JM, Ruuskanen S, Sandi C. Towards an Evolutionary Theory of Stress Responses. Trends Ecol Evol. 2021; 36:39-48. doi:10.1016/j.tree.2020.09.003.
2. Salehi B, Martorell M, Arbiser JL, Sureda A, Martins N, Maurya PK, Sharifi-Rad M, Kumar P, Sharifi-Rad J. Antioxidants: Positive or Negative Actors? Biomolecules. 2018; 8:124-130. doi:10.3390/biom8040124.
3. Panossian A. Adaptogens in Mental and Behavioral Disorders. Psychiatr Clin North Am. 2013; 36:49-64.
4. Henckens MJ, Hermans AG, Pu EJ, Joels ZM, Fernandez G. Stressed Memories: How Acute Stress Affects Memory Formation in Humans. J Neurosci. 2009; 29:10111-10119.
5. Murphy MP, Bayir H, Belousov V, Chang CJ, Davies KJA, Davies MJ, Dick TP, Finkel T, Forman HJ, Janssen-Heininger Y, Gems D. Guidelines for Measuring Reactive Oxygen Species and Oxidative Damage in Cells and *in Vivo*. Nat Metab. 2022; 4:651-662.
6. Eduviere AT, Umukoro S, Aderibigbe AO, Ajayi AM, Adewole FA. Methyl Jasmonate Enhances Memory Performance Through Inhibition of Oxidative Stress and Acetylcholinesterase Activity in Mice. Life Sci. 2015; 132:20-26.
7. Gould E, Tanapat P, McEwen BS, Flügge G, Fuchs E. Proliferation of Granule Cell Precursors in the Dentate Gyrus of Adult Monkeys Is Diminished by Stress. Proc Natl Acad Sci. 1998; 95:3168-3171.
8. Sapolsky RM and Pulsinelli WA. Glucocorticoids Potentiate Ischemic Injury to Neurons: Therapeutic Implications. Science. 1985; 229:1397-1400.
9. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RMD. Oxidative Stress and Antioxidants in Neurodegenerative Disorders. Antioxidants. 2023; 12:517-522. doi:10.3390/antiox12020517.
10. Potter PE, Rauschkolb PK, Pandya Y, Sue LI, Sabbagh MN, Walker DG, Beach TG. Pre-and Post-synaptic Cortical Cholinergic Deficits Are Proportional to Amyloid Plaque Presence and Density at Preclinical Stages of Alzheimer's Disease. Acta Neuropathol. 2011; 122:49-60.
11. Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S, Rathi B, Kumar D. Oxidative Stress in Cancer Cell Metabolism. Antioxidants. 2021; 10:642.
12. Hao Y, Zhu YJ, Zou S, Zhou P, Hu YW, Zhao QX, Gu LN, Zhang HZ, Wang Z, Li J. Metabolic Syndrome and Psoriasis: Mechanisms and Future Directions. Front Immunol. 2021; 12:711060.
13. Jia KK, Zheng YJ, Zhang YX, Liu JH, Jiao RQ, Pan Y. Banxia-houpu Decoction Restores Glucose Intolerance in CUMS Rats Through Improvement of Insulin Signaling and Suppression of NLRP3 Inflammasome Activation in Liver and Brain. J Ethnopharmacol. 2017; 219:29.
14. Jagadeesan S, Chiroma SM, Baharuldin MT, Taib CN, Amom Z, Adenan MI, Moklas MA. *Centella asiatica* Prevents Chronic Unpredictable Mild Stress-Induced

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Behavioral Changes in Rats. Biomed Res Ther. 2019; 6:3233-3243.
15. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J. Chronic Unpredictable Mild Stress for Modeling Depression in Rodents: Meta-Analysis of Model Reliability. Neurosci Biobehav Rev. 2019; 99:101-116. doi:10.1016/j.neubiorev.2018.12.002.
16. Markov K, Manji H, Lu B. New Insights into BDNF Function in Depression and Anxiety. Nat Neurosci. 2022; 10:1089-1093.
17. Algamai M, Pearson A, Hahn-Townsend C, Burca I. Repeated Unpredictable Stress and Social Isolation Induce Chronic HPA Axis Dysfunction and Persistent Abnormal Fear Memory. Prog Neuropsychopharmacol Biol Psychiatry. 2020; 23:410-417. doi:10.1016/j.pnpbp.2020.110035.
18. Zahara K, Bibi Y, Tabassum S. Clinical and Therapeutic Benefits of *Centella asiatica*. Pure Appl Biol. 2014; 3:152-159.
19. Marisa R, Assessor D, Calapai G. Assessment Report on *Centella asiatica* (L.) Urban, Herba. European Medicines Agency. Available From:. https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-centella-asiatica-l-urb-herba-revision-1_en.pdf. Accessed December 8, 2023.
20. Singh S, Gautam A, Sharma A, Batra A. *Centella asiatica* (L.): A Plant with Immense Medicinal Potential But Threatened. Int J Pharm Sci Rev Res. 2010; 4:9-17.
21. Ben-Azu BB, Nwoke EE, Aderibigbe OA, Omogbiya IA, Ajayi AM, Olonode ET, Umukoro S, Iwalewa EO. Possible Neuroprotective Mechanisms of Action Involved in the Neurobehavioral Property of Naringin in Mice. Biomed Pharmacother. 2019; 109:536-546. doi:10.1016/j.biopha.2018.10.055.
22. Eduviere AT, Enaohwo MT, Awhin PE, Otomewo OL, Iwalio O. Gotu Kola Supplement Ameliorates Stress-Induced Liver Injury in Mice. Trop J Nat Prod Res. 2022; 6(6):969-973. doi.org/10.26538/tjnpr/v6i6.25
23. Willner P. The Chronic Mild Stress (CMS) Model of Depression: History, Evaluation and Usage. Neurobiol Stress. 2017; 6:78-93. doi:10.1016/j.ynstr.2016.08.002.
24. Olayinka NJ, Akawa OB, Ogbu EK, Eduviere AT, Ozolua IR, Solima M. Apigenin Attenuates Depressive-Like Behavior via Modulating Monoamine Oxidase A Enzyme Activity in Chronically Stressed Mice. Curr Res Pharmacol Drug Discov. 2023; 5:100161.
25. Wopara I, Modo EU, Adebayo OG, Mobisson SK, Nwigwe JO, Ogbu PI, Nwankwo VU, Ejeawa CU. Anxiogenic and Memory Impairment Effect of Food Color Exposure: Upregulation of Oxido-Neuroinflammatory Markers and Acetyl-Cholinestrase Activity in the Prefrontal Cortex and Hippocampus. Heliyon. 2021;7(3):e06378.
26. Moron MS, Depierre JW, Mannervik B. Levels of Glutathione, Glutathione Reductase and Glutathione S-Transferase Activities in Rat Lung and Liver. Biochim Biophys Acta Gen Subj. 1979; 582:67-78.
27. Ádám-Vizi V and Seregi A. Receptor Independent Stimulatory Effect of Noradrenaline on Na, K-ATPase in Rat Brain Homogenate: Role of Lipid Peroxidation. Biochem Pharmacol. 1982; 31:2231-2236.
28. Mythri RB, Venkateshappa C, Harish G, Mahadevan A, Muthane UB, Yasha TC, Srinivas Bharath MM, Shankar SK. Evaluation of Markers of Oxidative Stress, Antioxidant Function and Astrocytic Proliferation in the Striatum and Frontal Cortex of Parkinson's Disease Brains. Neurochem Res. 2011; 36:1452-1463.
29. Amin SN, Younan SM, Youssef MF, Rashed LA, Mohamady IA. Histological and Functional Study on Hippocampal Formation of Normal and Diabetic Rats. F1000Res. 2013; 2:151-159. doi:10.12688/f1000research.2-151.v1.
30. Umukoro S, Aluko OM, Eduviere AT, Owoeye O. Evaluation of Adaptogenic-Like Property of Methyl Jasmonate in Mice Exposed to Unpredictable Chronic Mild Stress. Brain Res Bull. 2016; 121:105-114. doi:10.1016/j.brainresbull.2015.11.016.
31. Aluko OM and Umukoro S. Methyl Jasmonate Reverses Chronic Stress-Induced Memory Dysfunctions Through Modulation of Monoaminergic Neurotransmission, Antioxidant Defense System, and Nrf2 Expressions. Naunyn Schmiedebergs Arch Pharmacol. 2020; 393:2339-2353. doi:10.1007/s00210-020-01939-6.
32. Ennaceur A. One-Trial Object Recognition in Rats and Mice: Methodological and Theoretical Issues. Behav Brain Res. 2010; 215:244-254.
33. Leger MQ, Bouet A, Haelewyn V, Boulouard B, Schumann-Bard MP, Freret T. Object Recognition Test in Mice. Nat Protoc. 2013; 8(12):2531-2537.
34. Lueptow LM. Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. J Vis Exp. 2017; 126:55718
35. Kim MJ, Kim SS, Park KJ, An HJ, Choi YH, Lee NH, Hyun CG. Methyl Jasmonate Inhibits Lipopolysaccharide-Induced Inflammatory Cytokine Production via Mitogen-Activated Protein Kinase and Nuclear Factor-κB Pathways in RAW 2647 Cells. Die Pharmazie. 2016; 71:540-543.
36. Bakhtiari-Dovvombaygi H, Izadi S, Zare M, Asgari Hassanlouei E, Dinpanah H, Ahmadi-Soleimani SM, Beheshti F. Vitamin D3 Administration Prevents Memory Deficit and Alteration of Biochemical Parameters Induced by Unpredictable Chronic Mild Stress[6] in Rats. Sci Rep. 2021; 11:16271.
37. Rothman SM and Mattson MP. Adverse Stress, Hippocampal Networks, and Alzheimer's Disease. Neuromol Med. 2010; 12:56-70. doi:10.1007/s12017-009-8107-9.
38. Panossian A, Wikman G, Kaur P, Asea A. Adaptogens Stimulate Neuropeptide Y and Hsp72 Expression and Release in Neuroglia Cells. Front Neurosci. 2012; 6:6.
39. Ighodaro OM and Akinloye OA. First Line Defence Antioxidants-Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPX): Their Fundamental Role in the Entire Antioxidant Defence Grid. Alexandria J Med. 2018; 54:287-293. doi:10.1016/j.ajme.2017.09.001.
40. Pitsikas N. The Role of Nitric Oxide Donors in Schizophrenia: Basic Studies and Clinical Applications. Eur J Pharmacol. 2015; 766:106-113. doi:10.1016/j.ejphar.2015.09.045.
41. Wink DA, Miranda KM, Espey MG, Pluta RM, Hewett SJ, Colton CGMB. Mechanisms of the Antioxidant Effects of Nitric Oxide. Antioxid Redox Signal. 2001; 3:203-213.
42. Panossian A and Wikman G. Evidence Based Efficacy of Adaptogens in Fatigue and Molecular Mechanisms Related to their to Stress Protective Activity. Curr Clin Pharmacol. 2011; 4:198-219.
43. Pawar VS and Hugar S. A Current Status of Adaptogens: Natural Remedy to Stress. Asian Pacific J Trop Dis. 2012; 2:S480-S490.
44. Zhu S, Shi R, Wang J, Wang JF, Li XM. Chronic unpredictable mild stress Not Chronic Restraint Stress Induces Depressive Behaviours in Mice. Neuroreport. 2014; 25:1151-1155.
45. Kerksick C and Willoughby D. The Antioxidant Role of Glutathione and N-Acetyl-Cysteine Supplements and Exercise-Induced Oxidative Stress. J Int Soc Sports Nutr. 2005; 2:38-44.
46. Ma Q. Role of Nrf2 in Oxidative Stress and Toxicity. Annu Rev Pharmacol Toxicol. 2013; 53:401-426.
47. Schetinger MR, Morsch VM, Bonan C, Wyse AT. NTPDase and 50 -Nucleotidase Activities in Physiological and Disease Conditions: New Perspectives for Human Health. Biofactors. 2007; 31:77-98.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

48. Lovinger DM. Neurotransmitter Roles in Synaptic Modulation, Plasticity, and Learning in the Dorsal Striatum. Neuropharmacol. 2010; 58:951-961.
49. Loizzo M, Tundis R, Menichini F. Natural Products and Their Derivatives as Cholinesterase Inhibitors in the Treatment of Neurodegenerative Disorders an Update. Curr Med Chem. 2008; 15:1209-1228.
50. Knopman DS, DeKosky ST, Cummings JL, Chui H, Corey-Bloom J, Relkin N, Small GW, Miller B, Stevens JC. Practice Parameter: Diagnosis of Dementia (An Evidence-Based Review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurol. 2001; 56:1143-1153.
51. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I. Effects of Imperatorin on Scopolamine-Induced Cognitive Impairment and Oxidative Stress in Mice. Psychopharmacol. 2015; 232:931-942.
52. Viana AG, Trent ES, Conroy HE, Raines EM. Fear and Anxiety. Dev Psychopathol. 2021;182-218.
53. Moghimian M, Azin S, Alavi–Kakhki SS, Kourosh-Arami M, Gholami M, Beheshti F. Preventive Impacts of Vitamin C on Memory Damage Caused by Chronic unpredictable mild stress in Relation to Biochemical Parameters in the Hippocampus of Male Rats. Nutr Neurosci. 2023; 26:1222-1231.

© 2025 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License