



Colocasia esculenta Leaf Extract Mitigates Hippocampal Injury Caused by Lipopolysaccharide in Mice

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ABSTRACT

The hippocampus, a major part of the limbic lobe, is divided into the Cornu Ammonis (CA1-CA4) and the dentate gyrus (DG) and plays major role in memory and learning. It has been determined that the leaves of *Colocasia esculenta* are rich in minerals, vitamins B complex, E, and ascorbic acid, as well as important amino acids. The study evaluated the therapeutic influence of *Colocasia esculenta* leaf aqueous extract (CELE) on hippocampal injury elicited by Lipopolysaccharide (LPS) in mice. Six groups of forty-eight mature male albino mice in good condition, weighing between 20 and 24 grams each, were formed. Group I consist of mice that are in good health, while groups II–VI received intraperitoneal injections of lipopolysaccharide at a dose of 0.5 mg/kg for a period of seven days in order to cause neurodegeneration. Group II was given two milliliters of distilled water, Group III was given CELE (400 mg/kg/day), Group IV was given 600 mg/kg/day, and Group V was given 800 mg/kg/day of CELE. Group VI was given 2.5 mg/kg/day of donepezil-hydrochloride (DPH). When comparing the LPS-exposed groups to group I, there was a statistically appreciable increase in the acetylcholinesterase and indicators of cellular oxidation in the brain ($p < 0.05$). In the group II, Bielschowsky staining revealed neurofibrillary tangles and senile plaques. The delivery of lipopolysaccharide to mice resulted in inflammation, oxidative stress, anxiety-like behaviors, hippocampus injury, and cognitive impairments. CELE reduces degenerative alterations in the hippocampal region and demonstrates antioxidant, anti-inflammatory, and anxiolytic effects

Keywords: Alzheimer disease, *Colocasia esculenta*, Donepezil-hydrochloride, Hippocampus, Lipopolysaccharide.

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Introduction

The brain structure medially situated within the bilateral temporal lobe is called the hippocampus. The dentate gyrus (DG), Cornu Ammonis (CA), and the subiculum are collectively referred to as the hippocampus. The Cornu Ammonis (CA), which symbolizes the many areas of the hippocampal complex, is shaped like a ram's horn. The CA is divided into four regions: CA1, CA2, CA3, and CA4. The dentate gyrus's hilus is surrounded on both sides by CA3 and CA2, with CA3 being the largest subfield.¹ Studies have shown that one area of the brain that is directly impacted by Alzheimer's disease (AD) is the hippocampal region.² The typical size of the hippocampal region steadily decreases with aging. However, some medical conditions cause the hippocampus volume to shrink more quickly.

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Interestingly, among these medical conditions that results in a sharp reduction in hippocampus volume is Alzheimer's disease. As one of the disease's initial clinical indicators, hippocampal volume reduction typically occurs before cognitive impairment symptoms. The accumulation of tau and β -amyloid proteins in the hippocampus causes both a rapid shrinkage of the structure and a significant loss of neurons.³ Pathologically, the hippocampus is the site of the greatest accumulation of neurofibrillary tangles and senile plaques in the brain.² Rough endoplasmic reticulum makes up the dispersed granular structures known as Nissl bodies found in neurons. They exhibit variations in a range of situations; in pathological diseases like as Alzheimer's disease, they may even completely vanish. However, neurons that are able to recover from injury gradually emerge and reposition themselves within the cell according to their usual distribution. In this investigation, lipopolysaccharide induction resulted in the appearance of all the previously indicated pathognomic characteristics of Alzheimer's disease, which were then lessened by the CELE treatment. Research indicates that neuroinflammation has significant role in the pathophysiology of Alzheimer's disease. Various chemicals, including lipopolysaccharide (LPS), are employed to induce neuroinflammation in experimental settings. LPS triggers chemical reactions that change the way many inflammatory mediators present themselves.⁴ Taro, or *Colocasia esculenta*, is a tropical herbaceous perennial crop that grows a big corm that extends slightly above the ground. Its leaves are consistently broad, with lengths ranging from 30 to 200 cm, and nearly uniform in shape. It is mostly cultivated for its roots, but despite its intriguing medicinal potential, the significance of its leaves—aside from their usage as a vegetable—has not yet been thoroughly investigated.

Taro, or *Colocasia esculenta*, is a tropical herbaceous perennial crop that grows a big corm that extends slightly above the ground. Its leaves are nearly love-shaped and consistently broad, measuring between 30 and 200 cm in length. It is mostly grown for its roots, but its leaves are also important and haven't been thoroughly studied beyond eating them as a vegetable, even though they may have some medicinal uses. Compared to other crops in its class, *Colocasia esculenta* is one of the crops having a wide range of medicinal uses. The leaf includes a variety of bioactive components, including tarin, polysaccharides, alkaloids, polyphenols, and saponins, according to several earlier studies. Along with other pharmaceutical uses, these bioactive ingredients have also been linked to anti-hyperglycemia, anti-inflammatory, immunoprotective, and neuroprotective effects.⁵ According to Eneh and associates, this leaf has numerous amount of bioactive components, including saponins, flavonoids, alkaloids, and vital minerals.⁶ This study investigated the potential therapeutic benefit of an aqueous extract of the leaf in ameliorating the symptoms of Alzheimer-like disease generated by lipopolysaccharide in a mouse model, as the leaf is still underutilized despite having a rich pharmacological profile.

Materials and Methods

Materials

The protein analysis kit (ELISA kit) was purchased from eBioscience, Inc. (Carlifornia, USA), and the AChE test kit was obtained from Randox company (Antrim, UK). Chemicals, histology reagents, buffers lipopolysaccharide (LPS) and were bought from Sigma-aldrich, USA. Donepezil-Hydrochloride was procured from Uches Pharmaceuticals in Ondo, Nigeria.

Colocasia esculenta Leaf Extract (CELE) preparation

The Ladoke Akintola University of Technology Ogbomoso (LAUTECH) farm provided the fresh *Colocasia esculenta* leaves that were gathered on February 7, 2022. The leaves were authenticated at LAUTECH Herbarium with the voucher number LHO 840. After being cleaned, the leaves of *Colocasia esculenta* were carefully dried in a shaded area. After, the air-dried leaves were milled into a powder using an electric pulverizer, 600 g of substrate was obtained. This substrate was then steeped in pathogen-free water for 72 h and filtered through muslin fabric to produce a clean filtrate. Whatman filter paper1 was used to filter the clear filtrate one more time before the final clean solution was concentrated in a rotovap set to 20 to 30 degrees Celsius. The concentrated aqueous extract was kept in the freezer before being reconstituted and administered at various doses.⁷

Experimental animals

The Experimental mice were kept in the Laboratory Rodent breeding apartment, University of Medical Sciences (UNIMED), Ondo-State. Forty-eight (48) healthy adult Swiss male albino mice (aged 10–12 weeks), with body weights ranging from 20 to 24 g which were obtained from UNIMED animal breeding habitat were used for the research. The animals had access to water and animal feed (grower marsh pellets) *ad libitum*. Every mouse was given a 14-day acclimatization period, and all procedures were carried out in accordance with the policies set forth by the University of Medical Sciences, Ondo's research-ethical committee (UNIMED-areco/Apv-2/23/036).

Induction of neurotoxicity

Using Ramrez technique,⁸ the experimental mice were administered 0.5 mg/kg of lipopolysaccharide intraperitoneally (IP) for one week in a row to cause hippocampus injury.

Treatment approach

Treatment was initiated forty-eight hours after LPs administration, and the treatment was given for 21 days consecutively. Mice were distributed into six groups (n = 8); Group I (normal control) was administered 2mls of sterile water daily. Group II (LPS-only) was treated with 2 mL of LPS-only throughout the period of the experiment. Groups III, IV, and V, which represent LPS-injected models, were treated with CELE at doses of 400 mg/kg/day, 600 mg/kg/day, and 800 mg/kg/day, respectively. Group VI (positive control) consisted of LPs-injected mice treated with donepezil-hydrochloride (DPH) at a dose of 2.5 mg/kg/day; 2.5 to 10mg/kg of donepezil has been proven to be safe

and productive in memory loss treatment.⁹ oral gavage was used to administer both the CELE and DPH treatment. The LD₅₀ of *Colocasia esculenta* Leaf extract is around 5000 mg/kg.¹⁰

Behavioral assessments

Behavioral assessments were carried out using; Y-Maze, elevated plus-maze, open-field, and novel object recognition tests, to evaluate the extent of neuroinflammation as well as the effect of CELE to attenuate hippocampal injury. The assessments were performed seventy-two hours before induction of neurotoxicity; twenty-four hours after LPs injection, day 14 of CELE treatment and day 19 into CELE treatment.

Collection of samples

The Sacrifice of mice was done by cervical dislocation the following day after treatment termination. Brain tissues were extracted and dropped into an isotonic saline and placed in the freezer. The brain region of interest (hippocampus) was extracted out and then homogenized for biochemical analyses.

Morphometric analysis

Animal weights and the total brain weight (TBW) were measured with Infitek weighing scale, while the total brain volume (TBV) was estimated using the Erlenmeyer volumetric flask.

Histological analysis

The Hippocampal tissue with 10% Neutral Buffered Formalin (10%NBF) and frontal sectioning was done on the tissue. The tissues were then processed to generate paraffin wax blocks histological staining. The Histological staining techniques used include Hematoxylin-Eosin, Cresyl violet and silver impregnation (Bielschowsky's silver stain).

Acetylcholinesterase (AChE) and Nitric oxide (NO) assay

An Acetylcholinesterase activity assay kit based on the Ellman method,¹¹ was used and the value was estimated using an established procedure,¹² to determine the AChE level while Nitric oxide level was estimated by the protocol described by Fujii and Berliner (1999).¹³

Malondialdehyde (MDA) and Glutathione peroxidase assay

The assay was done according to Varshney procedure.¹⁴ MDA quantity was estimated according to the Adam-Vizi procedure.¹⁵ while GPx activity was determined with the procedure outlined by Rotruck.¹⁶

Superoxide dismutase (SOD), Reduced Glutathione (GSH), and Glutamate Dehydrogenase (GDH) assay

Reduced glutathione was determined by the Moron technique,¹⁷ Superoxide Dismutase was calculated by protocol of Misra and associate.¹⁸ and GDH activity was determined using the procedure of Strecker (1953).¹⁹

Determination of inflammatory markers

Tumor Necrosis Factor- α and Interleukin-6 levels were determined by sandwich enzyme-linked immunosorbent assay (ELISA) following the instructions of the manufacturer.

Photomicrography

Sections were observed with a bright-field microscope. Non-overlapping micrographs were obtained at $\times 40$ and 400 magnifications.

Statistical analysis

Data were presented as Mean \pm Standard Error of Mean (SEM). GraphPad-9 Software was used for data analysis while one-way analysis of variance was used to compare the means amongst the test groups and the level of significance was considered at $p < 0.05$.

Results and Discussion

Effect of CELE on Mice Weight and Their Brain Weights

The positive control group (Group I) maintained increased body weight and significantly ($p < 0.05$) high mean brain weight. Group II experienced significant ($p < 0.05$) reductions in body and brain weight in contrast to the healthy group (Group I) [Figures 1 and 2], indicating disease-induced weight loss and brain damage. Groups III, IV, and V, showed improved body and brain weights. Body and brain weights were

also greater in group F, which was treated with DPH than in the normal group (Group I) [Table1], suggesting an ameliorative effect similar to that of the most effective dose in CELE treatment groups.

groups (Groups III, IV, V, and VI, respectively), showed a significant improvement in these behaviors when contrasted to the mice in group II ($p < 0.05$). Line crossing and walling decreased across all groups compared to those in group I, but the values in the CELE-treated groups were statistically higher than those in the group II ($p < 0.05$), as shown in Table 2. This suggested that exposure to LPS caused a significant reduction in exploratory behavior compared to Group I. However, various doses of CELE improved the behavior, suggesting a potential therapeutic effect in mitigating the adverse behavioral effects caused by LPS.

Impact of CELE on LPS-induced behavioral alterations in open field test

The open field test showed a significant reduction in exploratory behavior (rearing, grooming, line crossing, and walling) of mice in Group II contrasted to Group I ($p < 0.05$). Meanwhile, the other treatment

Table 1: Effect of CELE on body weight and brain weight of mice with LPS- Induced weight loss

Treatment group	Initial Body Weight (g)	Final Body Weight (g)	Brain Weight (g)
I: Control: Normal	18.29 ± 0.6061	25.24 ± 0.423*	0.3740 ± 0.008718
II: LPS-ONLY	22.57 ± 0.4286	14.73 ± 0.5405*	0.2440 ± 0.006782 *
III: LPS+400 mg CELE	21.43 ± 0.5281	19.13 ± 0.3763*	0.3100 ± 0.009487* α
IV: LPS+ 600 mg CELE	20.14 ± 0.6701	18.14 ± 0.3961	0.3020 ± 0.01020* $\alpha\beta$
V: LPS+ 800 mg CELE	20.57 ± 0.8959	18.74 ± 0.1309	0.3000 ± 0.01095* α
VI: LPS+DPH	20.14 ± 0.5084	19.78 ± 0.1886	0.3260 ± 0.005099* α

Data expressed as Mean ± S.E.M, n = 8 in each group, *: represents a significant difference from control, α : represents a significantly different from LPS, β : represents a significant difference from DPH at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

Table 2: Effect of CELE on LPS-induced behavioral alterations in open field test

Treatment group	Parameter			
	Rearing	Grooming	Line Crossing	Walling
I: Control: Normal	6.20±0.73	6.40±1.17 α	33.40±1.63	3.20±0.97*
II: LPS-ONLY	1.20±0.37*	1.60±0.40*	9.40±1.63*	23.40±1.17
III: LPS+400 mg CELE	4.40±0.51 α	5.20±1.20 α	19.80±2.22* α	11.40±1.57* $\alpha\beta$
IV: LPS+ 600 mg CELE	4.40±1.12 α	3.80±1.11 α	15.20±3.72* α	15.40±1.03* $\alpha\beta$
V: LPS+ 800 mg CELE	3.80±0.73 α	4.00±0.45 α	18.00±2.39* α	13.20±0.37* $\alpha\beta$
VI: LPS+DPH	4.40±0.81 α	5.00±0.71	22.40±1.12* α	5.00±1.30*

Data expressed as Mean ± S.E.M, n=8 in each group, *: represents a significant difference from control, α : represents a significantly different from LPS, β : represents a significant difference from DPH at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

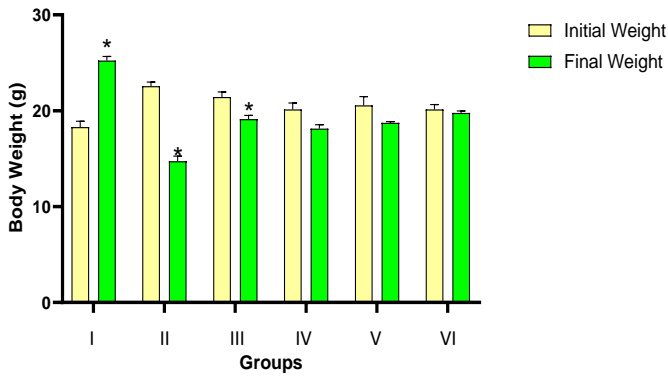


Figure 1: Effect of CELE on the body weight loss induced by LPS: *: represents a significant difference from control at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

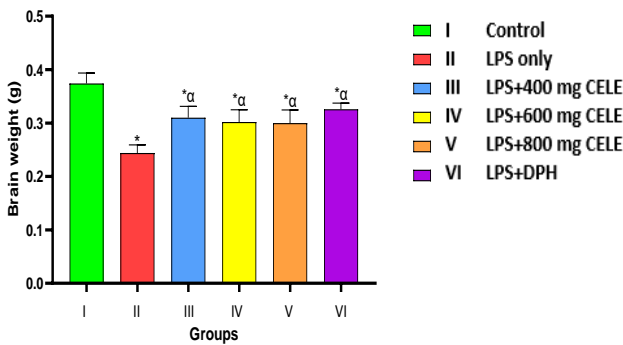


Figure 2: Effect of CELE on the weight of the brain after LPS-Induced weight-loss: *: represents a significant difference from control, α : represents a significantly different from LPS at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

Effect of CELE administration on Y-maze and elevated plus-maze parameters in Lipopolysaccharide-induced neurodegeneration
This study showed a statistically significant reduction ($p < 0.05$) in the percentage of alternation frequency (%ALTN) in Group II compared to that in the normal control group (Group I). Meanwhile CELE treatment groups (Groups III, IV & V) displayed a markedly increase in %ALTN, more than those in Groups I and VI ($p < 0.05$) as shown in [Table 3]. The frequencies of entering the open and closed arms were significantly lower across other treatment groups when compared to that of the normal group ($p < 0.05$), but the treatment groups showed no significant values ($p > 0.05$) for both entries than did Group II. Additionally, LPS-exposed mice displayed significantly decreased duration of stay in the open arm (DOSOA) and increased duration of stay in the closed arm (DOSCA) ($p > 0.05$), while post-treatment with CELE significantly increased DOSOA and decreased DOSCA in a dose-dependent manner when compared to those in Group II and Group VI ($p < 0.05$) [Table 3]. Overall, these findings suggest a potential beneficial impact of CELE on behavioral parameters, mitigating anxiety behavior elicited by Lipopolysaccharide.

Impact of CELE on LPS-elicited behavioral alteration in the Novel Object Recognition Task

The familiar object engagement time (FOET) increased significantly, while the Novel object engagement time (NOET) decreased in the LPS-treated group compared to the normal group ($p < 0.05$). However, in Groups III, IV and V, FOET reduced and NOET increased appreciably

when contrasted to both the normal group (Group I) and Group VI ($p < 0.05$) [Table 4]. When compared with CELE groups, NOET of mice in Groups I and VI significantly increased. These findings suggest that CELE treatment holds promise in the cognition and memory dysfunction treatment.

Impact of CELE on markers of Cellular oxidation and antioxidant indicators

This study showed statistically significant increase in the MDA and NO levels in mice administered with LPS ($p < 0.05$), but after CELE and DPH treatments, there was a significant decrease in the levels of these parameters compared to those in GROUP II ($p < 0.05$). Interestingly, Groups IV and V had no significantly lower values than Group III ($p < 0.05$). While Groups III, IV and V had significantly greater values than group VI ($p < 0.05$). [Figures 3 and 4]. The brain oxidative stress markers levels were statistically lower in the groups exposed to LPS when contrasted to the normal group ($p < 0.05$). But compared with Group II, CELE treatment significantly increased the levels of these antioxidant enzymes ($p < 0.05$). Obviously, the amount in Group VI were significantly greater than those in Groups III, IV and V ($p < 0.05$) [Figure 3]. These results suggest that CELE treatment may protect against cellular oxidation in the brain, as indicated by its ability to modulate cellular oxidation and antioxidant markers.

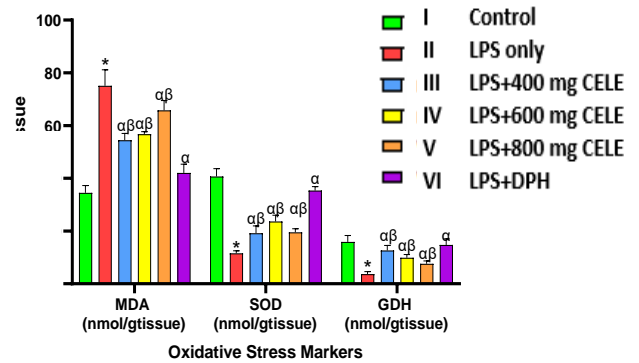


Figure 3: Impact of CELE on MDA, SOD, and GDH in LPS-induced oxidative stress: *: represents a significant difference from control, α : represents a significantly different from LPS, β : represents a significant difference from DPH at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

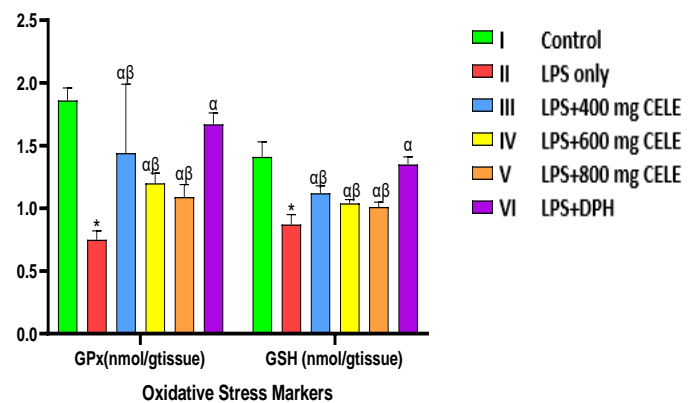


Figure 4: Impact of CELE on GPx and GSH in LPS-induced cellular oxidation: *: represents a significant difference from control, α : represents a significantly different from LPS, β : represents a significant difference from DPH at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

Table 3: Effect of CELE on Y-Maze and Elevated Plus-maze parameters in mice with LP_S-induced neurodegenerative changes

Treatment group	Y-Maze Alternation		Elevated Plus Maze Parameter		
	%ALTN	No Open	No closed	Time OA	Time CA
I: Control: Normal	80.16±2.20 ^α	10.80±1.66	6.00±1.14	219.00±11.00 ^α	81.00±11.00 ^α
II: LP _S -ONLY	41.62±4.66*	4.00±0.54*	5.00±0.71*	62.80±10.08*	237.20±10.08*
III: LP _S +400 mg CELE	77.06±2.39 ^{αβ}	5.20±0.86*	4.00±1.10*	183.60±15.88 ^{αβ}	116.40±15.88 ^{αβ}
IV: LP _S + 600 mg CELE	70.98±3.46 ^{αβ}	4.80±1.02*	3.40±0.51*	175.00±4.68 ^{αβ}	125.00±4.68 ^{αβ}
V: LP _S + 800 mg CELE	60.88±5.78 ^{αβ}	3.60±0.60*	3.80±0.49*	171.40±27.49 ^{αβ}	128.60±27.49 ^{αβ}
VI: LP _S +DPH	75.74±1.92	6.80±1.02*	4.40±0.40*	213.80±10.16	86.20±10.16

Data expressed as mean ± S.E.M, n=8 in each group, *: represents a significant difference from control, α: represents a significantly different from LP_S, β: represents a significant difference from DPH (p< 0.05), One-Way ANOVA followed by Tukey's post hoc test

Table 4: Effect of CELE on LP_S-induced behavioral alteration in the Novel Object Recognition Test

Treatment groups	Parameter	
	FOET	NOET
I: Control: Normal	201.40±14.85	98.60±14.85
II: LP _S -ONLY	150.80±17.63 ^{*β}	149.20±17.63 ^{*β}
III: LP _S +400 mg CELE	187.40±17.56 ^{*β}	142.60±17.56 ^{*β}
IV: LP _S + 600 mg CELE	144.40±11.40 ^{*β}	155.60±11.40 ^{*β}
V: LP _S + 800 mg CELE	157.80±20.43 ^{*β}	142.20±20.43 ^{*β}
VI: LP _S +DPH	226.40±14.91*	73.60±14.91*

FOET: Familiar Objects Engagement Time; NOET: Novel Objects Engagement Time. Data expressed as Mean ± S.E.M, n=8 in each group, *: represents a significant difference from control, α: represents a significantly different from LP_S, β: represents a significant difference from DPH (p< 0.05), One-Way ANOVA followed by Tukey's post hoc test

Impact of CELE on makers of inflammation and neurotransmitters

In the experimental mice exposed to Lipopolysaccharide, there was appreciable increase (p<0.05) in Interleukin-6 as well as Tumor necrosis Factor-α levels. However, post-treatment with plant extract resulted in a significant reduction in elevated brain IL-6 and TNF-α levels compared to those in LPS-exposed and DPH-treated mice (p < 0.05). In the extract treatment groups, no significance was noticed in the IL-6 and TNF-α levels, but groups III and IV showed no significantly greater IL-6 and TNF-α levels group V (p > 0.05). Additionally, AChE and NO levels decreased significantly in Groups III, IV and V compared to Group I and Group VI (p > 0.05). The AChE and NO levels in Groups III and IV showed no significance when contrasted to Group V (p>0.05) [Figures 5 & 6]. These results suggest that CELE treatment effectively reduces markers of inflammation and modulates acetylcholinesterase and nitric oxide levels in the brain of the mice model.

Hematoxylin-Eosin staining of the hippocampal structure

Plate1a and1b Representative Photomicrographs of hippocampal sections from experimental mice show the cornu Ammonis (CA) and dentate gyrus (DG). A higher magnification (X400) image of the CA1 region revealed well-outlined three layers. The positive control group displayed regular outlines and clear nuclei in the CA1 and DG regions (blue and slender blue arrows).

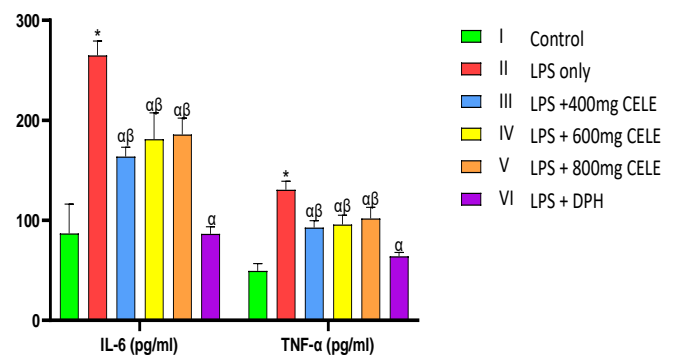


Figure 5: Impact of CELE on markers of inflammation in LPS induced mice: *: represents a significant difference from control, α: represents a significantly different from LP_S, β: represents a significant difference from DPH (p< 0.05), One-Way ANOVA followed by Tukey's post hoc test.

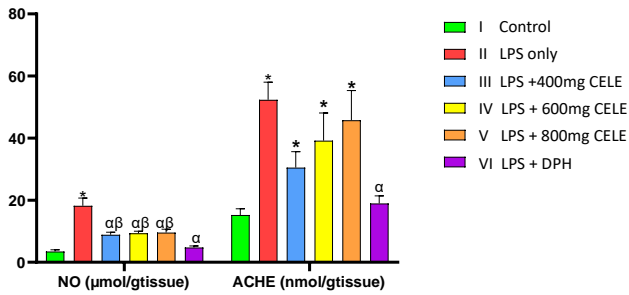


Figure 6: Impact of CELE on Neurotransmitters in LPS-induced mice: *: represents a significant difference from control, α : represents a significantly different from LP_s, β : represents a significant difference from DPH at ($p < 0.05$), One-Way ANOVA followed by Tukey's post hoc test.

In contrast, the negative control group (Group II) exhibited an irregular neuronal outline, extended pericellular spaces (red arrows), unclear nuclear structure in the DG (blue-arrows), and signs of necrosis (yellow-arrows). The morphology of the cells in Group III was relatively normal, with minimal enlargement of pericellular spaces (red-arrows) and congestion (yellow-arrows). The Group IV tissue exhibited normal morphology with increased glial cells in the granular layer while group V displayed normal morphology with heavily proliferating of pyramidal cells. Whereas in group VI the neurons were with prominent nucleoli (blue arrows). These findings suggest a potential ameliorative effect of CELE against hippocampal pathology.

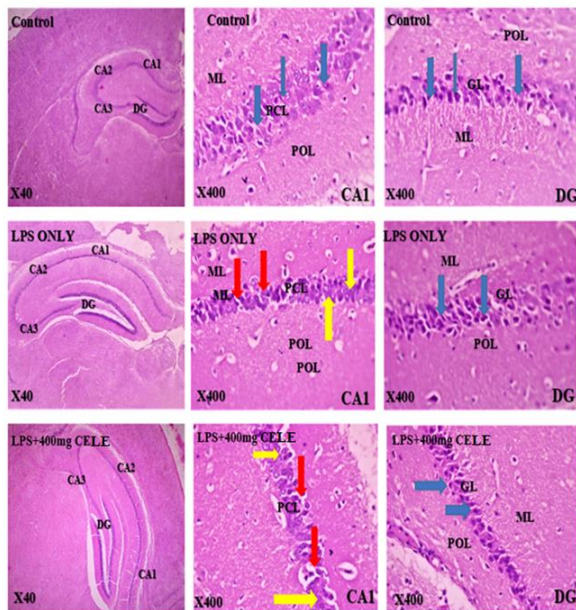


Plate 1a: Photomicrographs of a frontal section of H & E-Stained hippocampal morphology of the Group I, II and III: For the positive control group; thicker-blue arrows indicate clear nuclei and while the slender blue arrows indicate nucleoli. The CA1 region exhibits well-outlined three zones; polymorphic layer (POL), pyramidal cell layer (PCL) and molecular layer (ML) while LP_s ONLY group exhibited irregular neuronal arrangement in the granular layer (GL), an extended pericellular space (red-arrows), faint nuclear structure in the dentate gyrus (DG) - as depicted by blue- arrows - as well as signs of necrosis (yellow-arrows). Whereas in the LP_s+400mg CELE group, there were a few extended pericellular spaces (red-arrows) and few cellular-congestions (yellow-arrows), with well-stained nuclei (blue arrows).

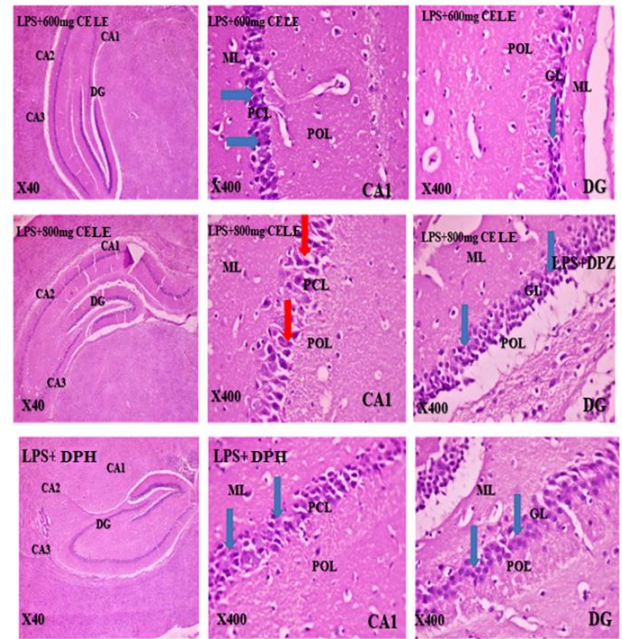


Plate 1b: Photomicrographs of a frontal section of H&E-Stained hippocampal tissue of the Groups IV, V and VI. In the LP_s+600mg CELE group, the hippocampus exhibited a normal morphology with numerous glial cells (blue-arrows) and a proliferation of these glial cells (slender blue-arrows) within the granular layer (GL). Likewise, in the LP_s+800mg CELE group, neurons displayed normal morphology, and numerous proliferating pyramidal cells with slightly abnormal shapes (red-arrows) in both the external granular layer (GL) and the pyramidal layer (POL). Additionally, a sparse presence of nerve cells (blue-arrows) was noted in the molecular layer (ML). While the LP_s+DPH group exhibited intact neurons with distinctive nucleoli (blue-arrows) within the pyramidal layer (POL), featuring pyramidal cells.

Cresyl violet staining of the hippocampus of experimental mice
Plates 2a and 2b representative photomicrographs of cresyl violet-stained hippocampal sections illustrate distinct morphological changes. In the control group, the dentate gyri displayed a regular density of granule cells with well-stained nuclei. The negative control group (Group II) showed signs of degeneration, such as faintly stained granule cells, reactive astrocytes, and cytoplasmic vacuolations. Group III showed large pyramidal neurons, improved cytoarchitectural manifestations, and well-delineated structures while there was improvement in the cytoarchitecture as well as well-stained Nissl-bodies in Group IV. There was no conspicuous evidence of degenerative changes in Group V. Group VI showed reduced degenerative changes with mild astrocyte expression. These findings suggest a dose-dependent ameliorative effect on hippocampal morphology, with the highest dose and positive control exhibiting more notable improvements.

Bielschowsky silver staining of Hippocampal tissue

The photomicrographs of Bielschowsky-stained hippocampal sections of Plates 3a and 3b revealed notable histopathological changes. In Group II, there was an aggregation of senile plaques and leukocytes, along with neurofibrillary tangles. Group III showed a moderate formation of plaques, while Group IV exhibited mild plaque formation and there were no plaques and leukocytes in Group V whereas Group VI displayed scanty plaque formation. These findings suggest varying effects of the plant extract treatment and the positive control on senile plaque formation, indicating potential ameliorative effects. Understanding the pathogenesis of AD is crucial to advancement in the search for newer alternative therapies.

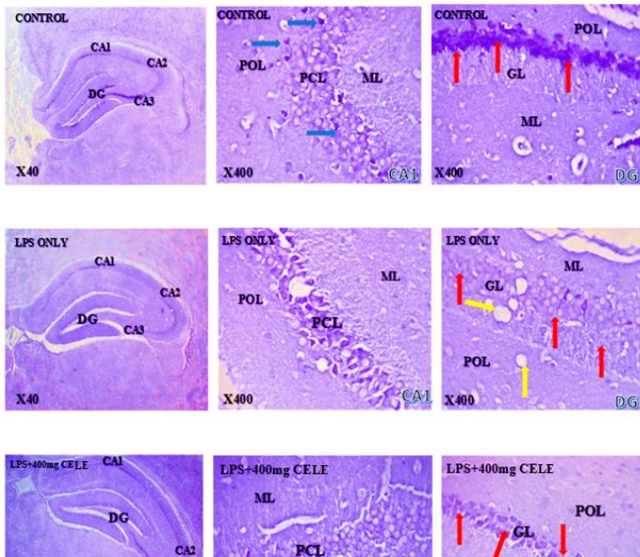


Plate 2a: Photomicrographs of a frontal section of Cresyl Violet-stained hippocampal morphology of the Group I, II and III. The Control group showed properly delineated dentate gyri (DG) (red-arrows) and well-stained nuclei (blue arrow) and also revealed more population of granule cells in the granular layer (GL). The LPS-ONLY group showed poorly stained granule cells in the granular layer of the dentate gyrus as well as poorly stained Nissl bodies (red arrows) and cytoplasmic vacuolations (yellow-arrow) in the polymorphic layer (POL) and granular layer. LPS+400 mg CELE showed characteristically large pyramidal neurons with well outlined cytoarchitectural appearance (red-arrows).

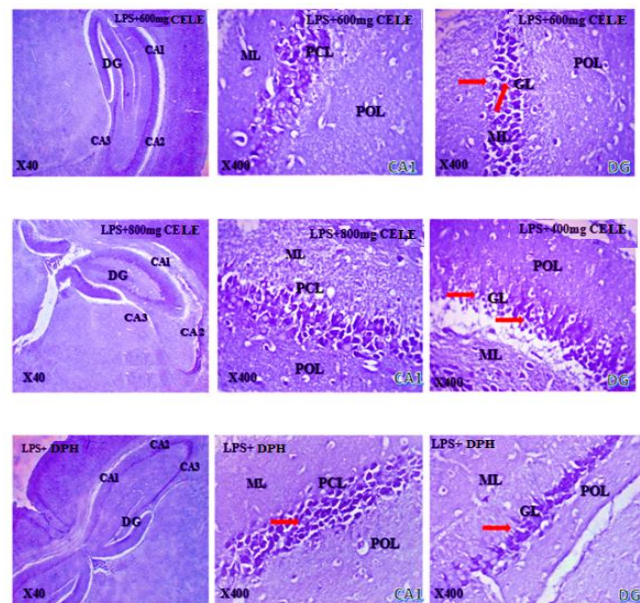


Plate 2b: Photomicrographs of a frontal section of Cresyl Violet-stained hippocampus of the Group IV, V and VI: LPS + 600 mg CELE showed a finely organized and well delineated cytoarchitectural organization with properly stained Nissl-Bodies (red arrows). LPS + 800 mg CELE hippocampus showed no degenerative changes with moderate astrocytes (red arrows) and well stained Nissl-bodies. These pyramidal cells (PCL) are clearly expressed and properly delineated in the CA1 region. The adjacent polymorphic layers (POL) on either side of the cornu Ammonis (CA1) regions have cells expressed with brevity. Each of the pyramidal cells (PCL) stands out distinctly with other one another and have properly delineated cytoarchitectural manifestation (red-arrows). LPS+DPH group showed reduced degenerative changes with mild astrocyte expression (red-arrows).

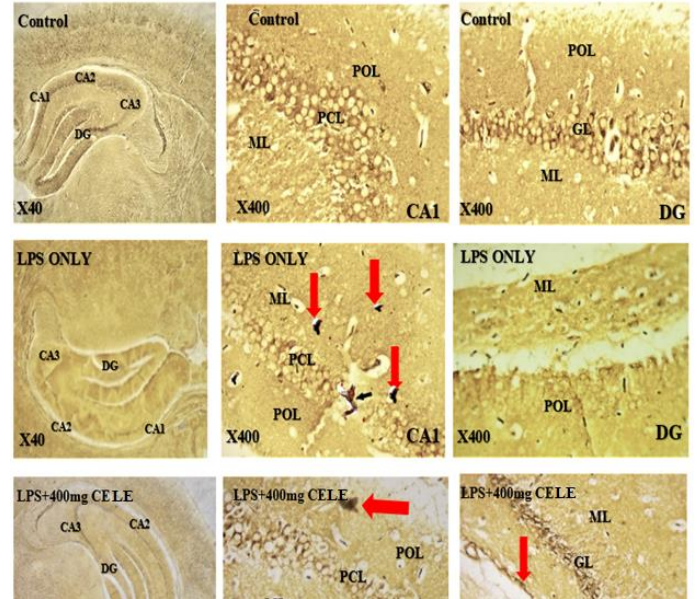


Plate 3a: Photomicrographs of a frontal section of Bielschowsky Silver stained hippocampal tissue of the Group I, II and III: With Bielschowsky's staining the hippocampus showed no pathological evidence in the positive control group whereas in the LPS group, there are aggregation of plaques and leukocytes (red-arrows), along with the presence of neurofibrillary tangles (black-arrow). The LPS+400mg CELE group showed moderate formation of plaques (red-arrows).

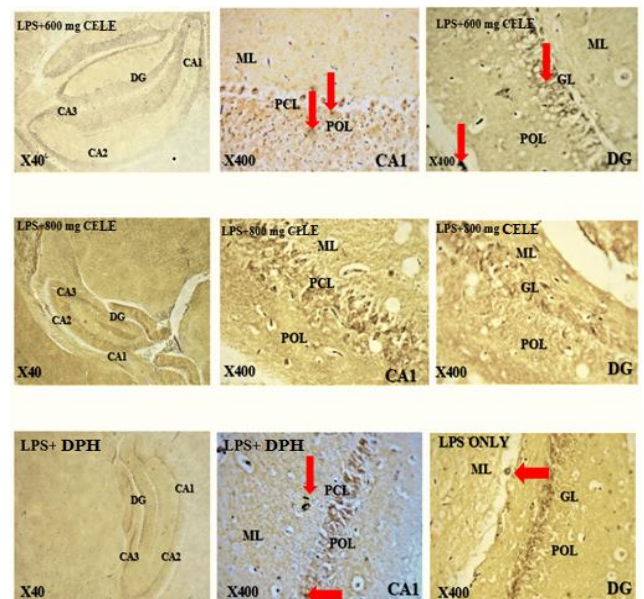


Plate 3b: Photomicrographs of a frontal section of Bielschowsky Silver stained hippocampus of the Group IV, V, and VI: In the LPS+600mg CELE group, there was a mild formation of plaques (red-arrows). There were no evidences of plaques and leukocytes in the LPS+800mg CELE group. Finally, in the LPS+DPH group, there was formation of scanty plaques (red-arrows).

The minimal side effects and cost-effectiveness of medicinal plants have positioned them as a promising alternative to most medical conditions. This study investigated the possible health benefits of *Colocasia* leaf in managing Alzheimer-like symptoms. Changes in body weight are crucial indicators of health and drug effects. As shown in Table 1, in this study, compared with that in the control group, LPS administration resulted in significant weight loss across the groups,

while treatment with CELE and the standard drug, DPH, led to a significant weight gain. Morphological studies revealed a notable reduction in brain volume of mice in group II in contrast to group I. However, the groups treated with CELE and DPH showed a significant increase in brain volume compared to Group II, indicating potential therapeutic effects in preserving brain morphology and halting progression of weight loss. As represented in Table 2 of this study, the positive control group (Group I) displayed typical healthy mouse behavior but with a low level of walling. Group II mice exhibited decreased rearing, grooming, and line crossing, indicating cognitive and motor deficits, along with increased walling, suggesting heightened anxiety. Groups III, IV, and V showed positive effects following treatment with CELE. Similar to Group VI, the mice Group III displayed significant improvement, indicating potential cognitive and self-care benefits, enhanced locomotor activity, and reduced anxiety. The DPH group (group VI) also showed improvements in rearing, grooming, and line crossing while walling behavior remained relatively high, suggesting persistent anxiety. These findings emphasized the potential of CELE to improve cognitive impairment. In the Y-maze test, there were no evidences of memory or cognitive deficits prior to LPS-induction. Consequent to LPS administration, an appreciable reduction in the frequency alternation, was observed, and this is consistent with findings of Lucas on endotoxin-induced hypolocomotive activity in mice.²⁰ Post-treatment with different doses of CELE improved the cognitive dysfunction, as shown in Table 3. In the elevated plus maze test, Group II mice initially displayed anxiety-like behavior, as evidenced by increased time spent in closed arms (Table 4). Similar to those in Group VI, the CELE-treated mice showed increased time and number of entries into the open arm. This finding aligns with previous studies on *Phyllanthus amarus*, which demonstrated significant anxiolytic activity in rat models.²¹ The findings in this study suggest that CELE may have anxiolytic effects, as indicated by improved behavior. In the Novel Object Recognition (NOR) task, experimental mice in the Group II spend more time with the familiar object than the unfamiliar one when compared with those in Group I. In contrast, mice treated with CELE demonstrated increased choice for the unfamiliar object, translating into an improvement in memory when contrasted to mice in Group II. This result is in tandem with the report of Meng.²² These findings suggest that CELE has the ability to improve recognition memory, making it a promising alternative in the treatment of neurodegenerative diseases. In this study, Group II showed increased level of malondialdehyde (MDA), and reduction in the activities of antioxidant enzymes. The administration of varying doses of CELE notably reduced the levels of MDA and caused an increment in the activities of antioxidant enzymes correlated with those in Group II. This result insinuates that CELE holds promise for alleviating oxidative stress in disease conditions. In the induction model groups, AChE levels significantly increased, reflecting the brain's response to an inflammatory insult. Post-treatment with CELE at varying doses resulted in decreased AChE quantity compared to those in the negative control group (Group II), suggesting a regulatory effect on acetylcholine breakdown. In comparison with the mice in Group I, LPS-treated mice showed raised cytokines level, and subsequent treatment with CELE drastically lowered the cytokine levels. This finding suggests that CELE has anti-inflammatory property, making it a potential therapeutic agent for neuroinflammation, this is consistent with the report of Kesharwani and associates on *Euglena* extract.²³ H&E-stained sections from the normal group displayed regular hippocampal morphology (Plate 1a) while the negative control group (Group II) exhibited irregular neuronal arrangements (Plate 1a). Group III showed some improvement in the neuronal architectural distortion, suggesting potential healing against hippocampal injury. Both Groups IV and V exhibited more normal hippocampal morphology, indicating an ameliorative effect comparable to that of Group VI (Plate 1b). Cresyl fast violet staining of the hippocampus revealed that the positive control group displayed a healthy morphology, while the negative control group (Group II) exhibited significant evidences of neurodegeneration, such as weakly stained granule cells and vacuolations. Group III demonstrated moderate pyramidal neurons with improved morphology (Plate 2a), while groups IV and V exhibited further improvement in hippocampal health, with well-organized cellular structures and well-stained Nissl bodies (Plate 2b). Photomicrographs (Plates 3a and 3b) of the hippocampal

morphology using Bielschowsky's stain showed no pathological evidence in Group I, whereas Group II histopathological analysis revealed plaques, leukocytes, and neurofibrillary tangles. Group III displayed a moderate reduction in the number of senile plaques, while Groups IV and V showed a mild reduction. At varying doses, CELE positively impacted hippocampal morphology, reducing Alzheimer's-related changes, aligning with findings reported by Alagan and colleague on memory impairment in rat models.²⁴

Conclusion

In this study, treatment with lipopolysaccharide caused neurodegenerative changes in the hippocampus of the experimental mice. However, treatment with various doses of CELE alleviated these effects. CELE enhanced cognition and also reduced anxiety-like behavior in the mice. CELE displayed anti-inflammatory, and antioxidant activities, as evidenced by the reduction in the concentration of cytokines and malondialdehyde, as well as the remarkably rise in antioxidant enzyme activities. It regulates acetylcholinesterase (AChE) levels, induces histopathological improvements in the hippocampus, and reduces Alzheimer's disease-related changes. These findings point to the potential of CELE to alleviate the clinical manifestations and pathological evidences of Alzheimer's disease. Previously identified active compounds such as alkaloids, flavonoids, saponins, and vitamins C and E in *Colocasia esculenta* formed a strong basis for the observed pharmacological efficacies; hence, subsequent studies would include further phytochemical analysis using gas chromatography-mass spectrometry, and each compound would be put to test to unlock optimal clinical applications of CELE.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Daugherty AM, Bender AR, Raz N, Ofen N. Age differences in hippocampal subfield volumes from childhood to late adulthood. *Hippocampus*. 2016; 26(2):220-228.
2. Rao YL, Ganaraja B, Murlimanju BV, Joy T, Krishnamurthy A, Agrawal A. Hippocampus and its involvement in Alzheimer's disease: A review. *Biotech*. 2022; 12(2):55. doi: 10.1007/s13205-022-03123-4.
3. Josephs KA, Dickson DW, Tosakulwong N, Weigand SD, Murray ME, Petrucelli L, Liesinger AM, Senjem ML, Spychalla AJ, Knopman DS, Parisi JE, Petersen RC, Jack CR, Jr, Whitwell JL. Rates of hippocampal atrophy and presence of post-mortem TDP-43 in patients with Alzheimer's disease: a longitudinal retrospective study. *Lancet Neurol*. 2017; 16(11):917-924.
4. Khan MS., Ali T, Kim MW, Jo MH, Chung JI, Kim MO. Anthocyanins Improve Hippocampus-Dependent Memory Function and Prevent Neurodegeneration via JNK/Akt/GSK3 β Signaling in LPS-Treated Adult Mice. *Mol Neurobiol*. 2019; 56:671-687. doi: 10.1007/s12035-018-1101-1.

5. Aditika, Bhuvnesh Kapoor, Saurabh Singh, Pankaj Kumar. Taro (*Colocasia esculenta*): Zero wastage orphan food crop for food and nutritional security. South Afr J Bot. 2022; 145:157-169.
6. Eneh, G., Okon, O.G., Imabong, F.E., Mfoniso, E.J., & Olajumoke, I. (2018). Phytochemicals, nutraceuticals and antinutritional factors assessment of young leaves of *Colocasia esculenta* (L) Schott. Int. Jour. Sci. Res. Edu., 6(5), 123–129.
7. Rebecca Nakaziba, Sharon Bright Amanya, Crispin Duncan Sesaazi, Frederick Byarugaba, Jasper Ogwal-Okeng, Paul E. Alele, "Antimicrobial Bioactivity and GC-MS Analysis of Different Extracts of *Corchorus olitorius* L Leaves", Sci. W. Jour, vol. 2022, Article ID 3382302, 9 pages, 2022. <https://doi.org/10.1155/2022/3382302>
8. Ramirez, Karol; Quesada-Yamasaki, Daniel and Fornaguera-Trias, Jaime. A Protocol to Perform Systemic Lipopolysaccharide (LPS) Challenge in Rats. Odovtos [online]. 2019, vol.21, n.1, pp.53-66. ISSN 2215-3411. <http://dx.doi.org/10.15517/ijds.v0i0.35510>.
9. Shin CY, Kim HS, Cha KH, Won DH, Lee JY, Jang SW, Sohn UD. The Effects of Donepezil, an Acetylcholinesterase Inhibitor, on Impaired Learning and Memory in Rodents. BiomolTher (Seoul). 2018 May 1;26(3):274-281. doi: 10.4062/biomolther.2017.189. PMID: 29463072; PMCID: PMC5933894.
10. Doriane Camille NyonseuNzebang, Marie Ide NgahaNjila, Emma Fortune Bend, Pierre ClaverOundoumOundoum, Brice Landry Koloko, Calvin BogningZangueu, Philippe Belle Eked, ModesteSameza, DieudonnéMassomaLembè, Evaluation of the toxicity of *Colocasia esculenta* (Aracaceae): Preliminary study of leaves infected by Phytophthoracolocasiae on wistar albinos rats, Biomed. Pharmacol, Volume 99,2018,Pages 1009-1013, ISSN 0753-3322, <https://doi.org/10.1016/j.biopha.2017.12.061>. (<https://www.sciencedirect.com/science/article/pii/S0753332217349740>)
11. Ellman G. L., Courtney K. D., Andres V. Jr., and Featherstone R. M. (1961), A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.
12. Rahman H., Eswaraiah M. (2008). Simple Spectroscopic Methods for Estimating Brain Neurotransmitters, Antioxidant Enzymes of Laboratory Animals like Mice: A Review. Pharmatutor Art, 1244. [Google Scholar]
13. Fujii H, Berliner LJ. Ex vivo EPR detection of nitric oxide in brain tissue. MagnReson Med. 1999 Sep;42(3):599-602. doi: 10.1002/(sici)1522-2594(199909)42:3<599:aid-mrm24>3.0.co;2-y. PMID: 10467306.
14. Varshney R and Kale RF (1990). Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Radiation Biol. 58: 733-743
15. Adam-vizi V., Seregi M., (1982): Receptor dependent stimulatory effect of noradrenaline on Na⁺/K⁺ ATPase in rat brain homogenate: Role of lipid peroxidation. Biochem. Pharmacol., 31: 2231-2236
16. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973 Feb 9;179(4073):588-590. doi: 10.1126/science.179.4073.588. PMID: 4686466.
17. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BiochimBiophysActa. 1979 Jan 4; 582(1):67-78. doi: 10.1016/0304-4165(79)90289-7. PMID: 760819.
18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972 May 25; 247(10):3170–3175. [PubMed] [Google Scholar]
19. Harold J. Strecker, Glutamic dehydrogenase, Archives of Biochemistry and Biophysics, Volume 46, Issue 1,1953, Pages128-140,ISSN:00039861.[https://doi.org/10.1016/0003-9861\(53\)90176-3](https://doi.org/10.1016/0003-9861(53)90176-3).<https://www.sciencedirect.com/science/article/pii/0003986153901763>
20. Lucas Silva Tortorelli, Engelke, D. S., Lunardi, P., Souza, T. M. E., Santos-Junior, J. G., Gonçalves, C. A., et al. (2015). Cocaine counteracts LPS-induced hypolocomotion and triggers locomotor sensitization expression. Behav. Brain Res., 287,226-229. <https://doi.org/10.1016/j.bbr.2015.03.054>.
21. Hashim, A., Ahmed, M. G., Rahiman, N. B. A., Manikkoth, S., & Leena Pramod, K. (2022). Evaluation of the neuroprotective activity of *P. Amarus* in attenuating arsenic-induced neurotoxicity – an in vivo study. Phytomed. Plus, 2(3), 100316. <https://doi.org/10.1016/j.phyplu.2022.100316>
22. Meng, H. W., Kim, J. H., Kim, H. Y., Lee, A. Y., & Cho, E. J. (2023). Paeoniflorin Attenuates Lipopolysaccharide-Induced Cognitive Dysfunction by Inhibition of Amyloidogenesis in Mice. Int. jour. Mol. sci, 24(5), 4838. <https://doi.org/10.3390/ijms24054838>
23. Keshewani, R., Kumar, R., Minhas, U., & Rizvi, S. I. (2021). Euglena tuba extract provides protection against lipopolysaccharide-induced inflammatory response and oxidative stress in mice. Biologia, 76(2), 793–798. <https://doi.org/10.2478/s11756-020-00623-7>
24. Alagan, A., Jantan, I., Kumolosasi, E., Ogawa, S., Abdullah, M. A., & Azmi, N. (2019). Protective effects of *Phyllanthus amarus* against lipopolysaccharide-induced neuroinflammation and cognitive impairment in rats. Front.Pharm, 10, Article 632. <https://doi.org/10.3389/fphar.2019.00632>